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by

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# Risk Factors for Cervical HPV Infection in Montreal University Students

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## ABSTRACT

The objectives of this study were to estimate the point prevalence of cervical HPV infection in asymptomatic women and to identify risk factors for overall HPV infection and HPV infection by type. The study was a cross sectional survey at the McGill university student health clinic. Endocervical and ectocervical cell scrapings were collected from students presenting themselves at the clinic for a routine pap smear. Cytology results from the clinic were provided and epidemiological data on potential risk factors including sexual behaviour was obtained from a detailed, self-administered questionnaire. The presence of HPV DNA was detected in specimens by polymerase chain reaction (PCR) using consensus primers (MY09/MY11) targeting a 450 base pair segment in the L1 gene. Amplified products were hybridized with generic and type-specific probes using Southern blot and dot blot techniques, respectively, to determine overall HPV prevalence and HPV prevalence by type.

A total of 489 women agreed to participate in the study, of which 375 were eligible for final analysis. Overall cervical HPV prevalence was 21.8%. Among those women infected with HPV, 6.2% had a low-risk HPV infection, 11.8% had a high-risk HPV infection, 7.1% had an unknown HPV type infection and 2.7% had a multiple type infection.

Two profiles emerged for sexual activity and risk of HPV infection according to oncogenic risk after multivariate analysis. Sexual activity (frequency of sex and lifetime number of oral sex partners) was associated with high oncogenic-risk HPV infections. However, HPV infection with low oncogenic-risk types were mostly invariant with respect to markers of sexual activity, suggesting that there may be differences in epidemiological correlates of transmission between low-risk and high-risk HPV types on the basis of their oncogenicity. In view of our findings, we suggest that low oncogenic-risk HPV types may be less mucosotropic and therefore be transmitted by modes other than sexual activity.

## RÉSUMÉ

Les études épidémiologiques effectuées au cours des trente dernières années ont invariablement démontré que les marqueurs d'activité sexuelle étaient les principaux déterminants du risque de cancer du col de l'utérus, suggérant ainsi qu'un agent microbien transmissible sexuellement pourrait causer ce type de cancer [zur Hausen, 1976 #191]. Les données biologiques et épidémiologiques accumulées montrent de façon convaincante que l'infection du col utérin par certains types de HPV est un événement précurseur dans la genèse des néoplasmes cervicaux [zur Hausen, 1991 #50; Schiffman, 1993 #94; Muñoz, 1992 #146; Franco, 1995 #260]. Par conséquent, l'activité sexuelle devrait pouvoir prédire encore plus effectivement les infections par le HPV, étant donné le rôle étiologique démontré du HPV dans la genèse du cancer du col utérin.

Ceci n'a pas toujours été facile à démontrer, principalement à cause de techniques de laboratoire insuffisamment sensibles ou spécifiques pour la détection des infections latentes au HPV qui sont présentes dans la population générale. Avec l'avènement du PCR et de l'union interdisciplinaire de la biologie moléculaire et de l'épidémiologie, un certain nombre d'études récentes d'épidémiologie moléculaire utilisant le PCR pour la détection du virus ont révélé que l'infection du col utérin par le HPV était transmise sexuellement [Ley, 1991 #9; Bauer, 1993 #162] Cependant, ces résultats n'ont pas été reproduits uniformément par toutes les études utilisant le PCR réalisées dans des populations différentes. Selon les études, l'association entre l'activité sexuelle et la prévalence du HPV peut être forte [Ley, 1991 #9; Bauer, 1993 #162], modérée [Franco, 1995 #260; Wheeler, 1993 #8], faible [Rohan, 1991 #7; Hildesheim, 1993 #262] ou même absente [Kjaer, 1993 #144]. De nouvelles données suggèrent une explication de cette disparité des observations épidémiologiques : la répartition entre divers types de HPV ayant une transmissibilité sexuelle inégale varierait d'une population à une autre [Franco, 1995 #260]. Le risque relié

à l'infection au HPV semble être influencé de façon indépendante par des facteurs tel que la parité, l'usage de contraceptifs oraux et le tabagisme [Bauer, 1993 #162]. Toutefois, le principal déterminant du risque d'infection au HPV est l'âge, la plupart des études ayant rapporté une forte diminution de la prévalence chez les femmes âgées de plus de trente ans [Bauer, 1993 #162; Wheeler, 1993 #8].

Le présent travail visait à estimer le taux de prévalence des infections au HPV du col utérin chez des étudiantes universitaires de Montréal et à identifier les facteurs de risque d'infection au HPV généraux, ainsi que les facteurs de risque propores aux infections à faible risque oncogène et des infections à risque oncogène élevé. Cette recherche consistait en une étude épidémiologique transversale d'étudiantes consultant la clinique médicale de l'université McGill. Des cellules endocervicales et ectocervicales ont été prélevées par frottis vaginal chez des femmes asymptomatiques se présentant à la clinique pour un prélèvement vaginal ou « test de Pap » de routine. La clinique nous a fourni les résultats cytologiques. Nous avons obtenu les données épidémiologiques à partir d'un questionnaire auto-administré portant sur l'âge, le statut socio-économique, le niveau de scolarité, l'usage du tabac, les habitudes sexuelles, l'usage de contraceptifs, les antécédents médicaux et l'hygiène personnelle des participantes.

Les échantillons étaient d'abord amplifiés par PCR à l'aide de la paire d'amorce GH20/PC04. Cette amplification visait une région de 268 paires de bases du gène de la b-globine, qui servait d'étalon interne dans le but d'assurer une quantité et qualité adéquates d'ADN. La présence de l'ADN du HPV était également détectée par amplification au PCR en utilisant les amorces consensus MY09/MY11, ces amorces s'hybridant à un fragment d'ADN de 450 paires de bases dans la région L1 du génome viral [Ley, 1991 #9]. Les produits d'amplification, après un transfert de type Southern, étaient hybridés à une sonde générique pour déterminer la prévalence globale du HPV. Les échantillons positifs étaient

ensuite déposés sur une membrane de nylon et hybridés avec des sondes types spécifiques pour identifier les différents types de HPV. Les ADN viraux identifiables par électrophorèse mais ne réagissant pas avec la sonde générique ont été clonés et séquencés.

Des 489 femmes participant à l'étude, 411 (84%) ont complété et retourné le questionnaire. Les échantillons cellulaires de 40 (8,2%) de ces femmes n'ont pu être amplifiés pour la b-globine. Des infections au HPV à un ou plusieurs types ont été détectées chez 98 femmes (21,8%). Parmi ces femmes infectées, 6,2% avaient un HPV à faible risque oncogène, 11,8% avaient un HPV à risque oncogène élevé, 7,1% avaient un HPV de type indéterminé et 2,7% avaient une infection mixte. Les types de HPV les plus prévalents étaient les types indéterminés (7,1%), le HPV-16 (4,7%), le HPV-51 (2,2%), le PAP-155 (2,0%), le HPV-66 (1,6%), les HPV-6,11,31,33,et 58 (1,1% chacun), le HPV-18 (0,9%), le HPV-53 (0,9%), le HPV-56 (0,4%) et les HPV-35,68,W13B et 238A (0,2% chacun). Les résultats de cytologie ont révélé que 55 des 449 femmes éligibles (12,2%) présentaient des « test de Pap » anormaux. Parmi celles-ci, 37 (67,3%) avaient des cellules squameuses atypiques de signification indéterminée ("ASCUS") et 18 (32,7%) avaient de faibles ou de fortes lésions squameuses intraépithéliales (SIL).

Les différences brutes de prévalence du HPV par catégorie d'exposition ont été évaluées par le test du chi-carré de Pearson. Les rapports de cotes ou "odds ratios" (OR) bruts et ajustés pour l'âge ont été calculés pour estimer le risque d'infection au HPV selon les groupes démographiques et les habitudes de comportement. Les types de HPV ont été regroupés selon leur potentiel oncogène. Le OR a été utilisé pour quantifier le degré d'association entre l'exposition et chacune des différentes variables. Suivant des critères de sélection prédéterminés précis, les variables identifiées comme facteurs de risque et variables confondantes potentiels dans les analyses univariées ont été intégrées à un modèle de régression logistique multivariée. Des modèles multivariés ont été élaborés pour les

infections globales, et séparément pour les infections au HPV à faible risque oncogène et à risque élevé. Les résultats cytologiques constituaient la variable dépendante, tandis que l'infection au HPV constituait la variable prédictive de résultats cytologiques anormaux après ajustement pour l'âge et pour différents indicateurs d'activité sexuelle.

L'association entre l'infection au HPV et les anomalies cytologiques était statistiquement significative, que ce soit pour les infections à risque oncogène élevé ou à risque faible. Cependant, l'infection au HPV prédisait mieux les lésions SIL (OR=10) que la présence de cellules ASCUS (OR=2.5). De plus, le risque de présenter une anomalie cytologique ASCUS ou SIL ne variait pas de façon tangible en fonction du type de HPV.

Les analyses multivariées ont montré que l'association entre le profil d'activité sexuelle et le risque d'infection au HPV était modifiée selon que le risque cancérogène de l'infection était faible ou élevé. Le risque d'infection au HPV était associé avec le degré d'activité sexuelle - la fréquence des rapports sexuels et le nombre total de partenaires de sexe oral dans le passé - si le risque oncogène était élevé. Par contre, le risque d'infection au HPV ne variait pas en fonction de l'activité sexuelle lorsque le risque oncogène était faible. En outre, tandis que l'usage du condom semblait prévenir les infections à risque oncogène élevé, il était au contraire associé avec le risque d'infection au HPV à faible risque oncogène. Enfin, le lavage des parties génitales après les rapports sexuels semblait prévenir les infections au HPV à faible risque oncogène.

Nos résultats suggèrent que les facteurs épidémiologiques de transmission des infections au HPV différent selon que le type d'infection est à risque oncogène élevé ou à faible risque oncogène. À la lumière de ces résultats, nous avançons une hypothèse suivant laquelle les types de HPV comportant un plus faible risque oncogène seraient moins mucosotropiques et que, par conséquent, leur transmission se ferait davantage par d'autres voies que par l'activité sexuelle.

## ABBREVIATIONS

| | |
|---|---|
| **AP-1:** | Ubiquitous Factor |
| **ASCUS:** | Atypical Squamouus Cells of Undetermined Significance |
| **bp:** | Base Pair |
| **CIN:** | Cervical Intraepithelial Neoplasia |
| **EGF:** | Epithelial Growth Factor |
| **FCS:** | Fetal Calf Serum |
| **HG-SIL:** | High Grade - Squamous Intraepithelial Lesions |
| **HPV:** | Human Papillomavirus |
| **HSV:** | Herpes Simplex Virus |
| **IU:** | International Units |
| **LCR:** | Long Control Region |
| **LES:** | Lower Economic Status |
| **LG-SIL:** | Low Grade - Squamous Intraepithelial Lesions |
| **NFA:** | Novel Factor |
| **oct1:** | oct1 Factor |
| **OR:** | Odds Ratio |
| **PCR:** | Polymerase Chain Reaction |
| **pRB:** | Retinoblastoma Gene |
| **PVF-1:** | Novel Transcription Factor |
| **SIL:** | Squamous Intraepithelial Lesions |
| **SSC:** | Standard Saline Citrate |
| **SSPE:** | Standard Salt Phosphate EDTA |
| **STD:** | Sexually Transmittted Disease |
| **TE:** | Tris-HCl & EDTA |
| **Tm:** | Melting Temperature |
| **URR:** | Upper Regulatory Region |
| **VLP:** | Virus-Like Protein |

# CHAPTER 1

# INTRODUCTION

## INTRODUCTION

Cervical cancer is still one of the most common neoplastic diseases affecting women, with a combined worldwide incidence that is second only to breast cancer, despite the advent of cytological screening. Epidemiologic research conducted over the last three decades has consistently shown that markers of sexual activity are the most important determinants of a woman's risk of developing cervical cancer, suggesting a sexually-transmissible microbial agent as the cause (zur Hausen, 1976). There is now overwhelming evidence, both biologic and epidemiologic, that cervical infection by certain human papillomavirus (HPV) types is a precursor event in the genesis of cervical cancer (Franco *et al.*, 1995; Muñoz *et al.*, 1992; Schiffman *et al.*, 1993; zur Hausen, 1991).

Clinical and subclinical HPV infections are the most common sexually-transmitted diseases today (von Krogh, 1991). There are over 70 different HPV types defined on the basis of DNA homology. Two major groups are defined according to their epithelial affinity: those infecting the dry skin (cutaneotropic) and those infecting the moist mucosal areas of the body (mucosotropic). Genital types are typically divided into three groups based on the frequency of association with malignant tumours and are thus ranked according to oncogenic potential (i.e. low oncogenic-risk, intermediate oncogenic-risk and high oncogenic-risk) (Bauer *et al.*, 1993; de Villiers, 1994).

Since sexual activity and cervical HPV infection are two of the strongest predictors of cervical cancer, it follows then, that sexual activity should be an even stronger predictor of cervical HPV infection, considering HPV's apparently strong etiological role in cervical carcinogenesis. In the past, however, it has not been easy to demonstrate this, in large part because laboratory techniques were not sufficiently sensitive or specific enough to detect latent HPV infections within the general population. With the advent of the polymerase chain reaction (PCR) and the interdisciplinary marriage of molecular biology and epidemiology, a

number of more recent molecular epidemiological surveys using PCR to detect the virus have unveiled the sexually transmitted profile of cervical HPV infection (Bauer *et al.*, 1993; Ley *et al.*, 1991). However, not all PCR based studies conducted in different populations have been able to reproduce these results uniformly (Kjaer *et al.*, 1993; Rohan *et al.*, 1991). There is some new evidence to suggest that the variation within the literature might be caused by differences across populations in the relative prevalence of HPV types with greater or lesser transmissibility by the sexual route (Franco *et al.*, 1995).

In an attempt to clarify the role of sexual behaviour and the transmission of HPV infection, this study aimed to determine the point prevalence of cervical HPV infection in young, asymptomatic women attending university in Montreal and to identify risk factors for low oncogenic-risk HPV infection and high oncogenic-risk HPV infection.

# CHAPTER 2

# REVIEW OF THE LITERATURE

# REVIEW OF THE LITERATURE

## 1 The Evidence for a Relationship Between HPV and Cervical Cancer

### *1.1 Epidemiology of cervical cancer*

Cervical cancer is one of the most common neoplastic diseases affecting women, with a combined worldwide incidence (437,000 new cases annually), that is second only to breast cancer (Pisani *et al.*, 1993). While cervical cancer ranks as the tenth most common malignant disease in Western developed nations, it is the most important cancer in developing countries, even when considering both sexes. Yearly incidence rates may be as low as 3-4 new cases per 100,000 women, as in Israel, or as high as 80 new cases per 100,000 women, as in Recife, Brazil, (which can be equated to a lifetime risk of approximately 8%) (Franco, 1991a). In Canada, an estimated 1,500 new cases are diagnosed annually, and with a survival rate that is far worse than that for breast cancer, over 400 deaths per year are attributed to cervical cancer (Canadian Centre for Health Information, 1992) (Figure 1).

The past several decades have seen a decrease in morbidity and mortality due to cervical cancer in the industrialized nations. Improved perceptions about health and greater knowledge of associated risks have undoubtedly contributed to this observed decline, but it is the introduction of the Papanicolaou (Pap) test and the adoption of large-scale cytological screening that has contributed most significantly to the decline. Unfortunately, this decline has not been observed in most Third World countries where availability of Pap smear screening is limited to a small portion of the population (Franco, 1993). Consequently, the cervical cancer mortality rate is still increasing every year in non-industrialized nations, with 203,000 deaths presently reported annually (Pisani *et al.*, 1993). Alarmingly, this trend is also observed in some undeserved sub-populations in more developed countries. For example, while the incidence of cervical cancer for most of the Canadian female population is less then 3%, both the Inuit and Indian female population have much higher incidence rates

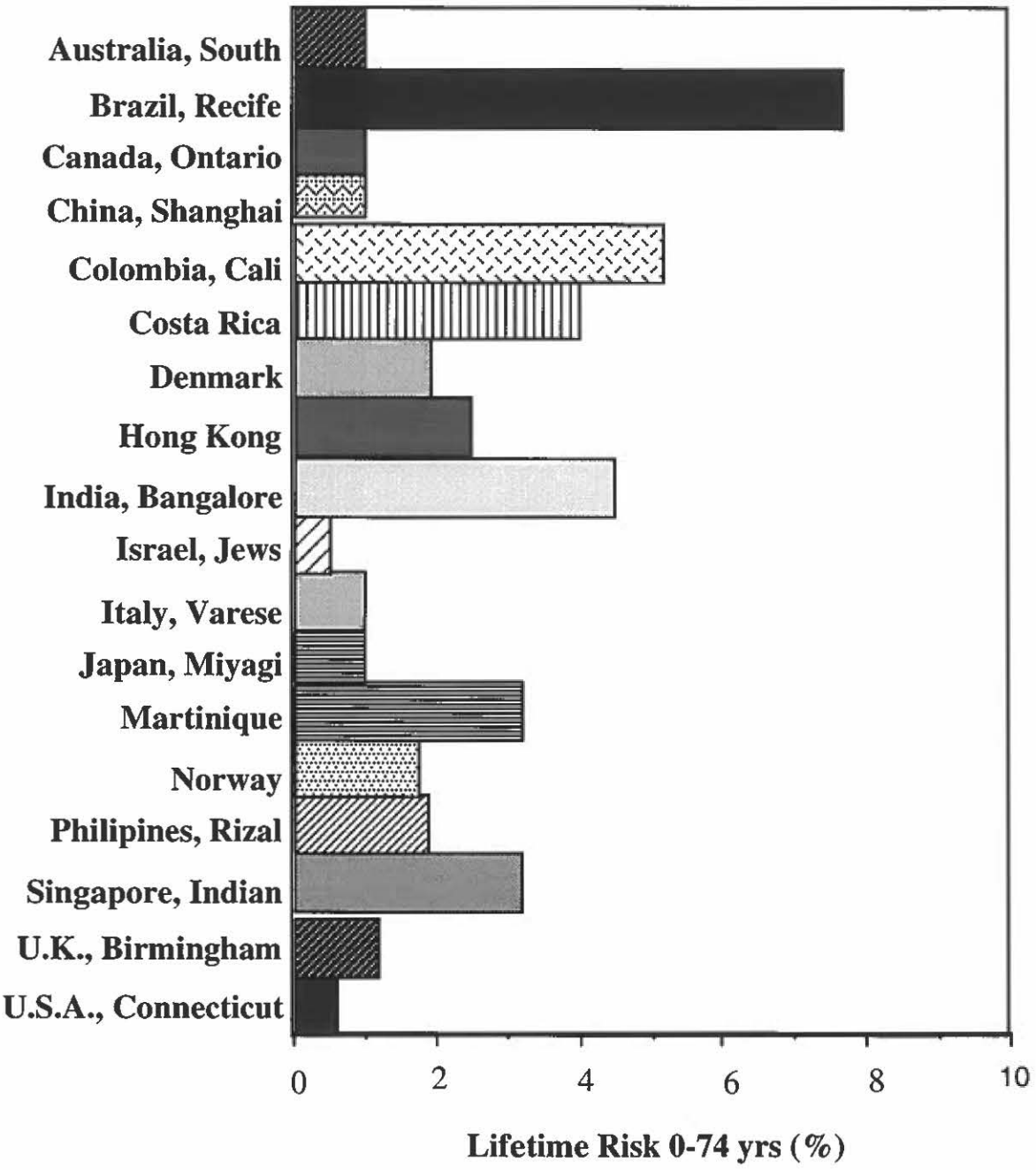


**Figure 1:**
Cumulative Risk of Cervical Cancer

Age-standardized incidence rates of invasive cervical cancer in selected countries or areas served by population-based registries. Rates are expressed as lifetime (0-74 years) cumulative risk of acquiring disease. (Adapted from Muir et al., 1987)

of 15% and 29% respectively (Canadian Centre for Health Information, 1991). In fact, incidence of cervical cancer is more frequent than incidence of breast cancer in both these populations, which is a very different epidemiological profile then that observed in most North American women, where breast cancer is by far the most common malignancy. Improved health education and screening participation in these high risk groups for cervical cancer could undoubtedly help reduce the distressingly high incidence rates (McNicol and Young, 1995).

### *1.2 Etiology of cervical cancer*

As early as 1743 a French eighteenth century physician by the name of Jean Astruc hypothesized that uterine cancer (including cancer of the cervix) may be a sexually transmitted disease, postulating that a putative cause included "injection of semen tainted with lues" and "venereal virus" (Skrabanek, 1988). Two hundred years later, Gagnon observed that cervical cancer was extremely rare in virgins and nuns (Gagnon, 1950). Other early studies looking at environmental factors of cervical cancer, observed that women with cervical cancer had lower socio-economic status, were married earlier and had their first sexual encounter at an earlier age than controls (Boyd and Doll, 1964; Jones *et al.*, 1958; Wynder *et al.*, 1954). Since then, the association of cervical cancer and sexual activity has been the most consistent epidemiological finding, irrespective of study design and methods of analysis. In the last two decades, with better-controlled studies, the effect of sexual activity has been targeted to two critical determinants of risk: age at first intercourse and lifetime number of sexual partners (Brinton *et al.*, 1987; Brinton, 1992; Brinton *et al.*, 1989; Brisson *et al.*, 1994; Brisson *et al.*, 1988; Kessler *et al.*, 1974; La Vecchia *et al.*, 1986a; Martin, 1967; Pridan and Lilienfeld, 1971; Rotkin, 1967; Terris *et al.*, 1967) (Table I). Case-control studies examining the role of the 'male factor' have identified a significant

**Table I**

**Historical Outline of Epidemiological Research on Cervical Cancer**

**Before 1980**

**Sexual activity → Microbial agent → Cervical cancer**

- *Astruc, 1743:* Putative causes of uterine cancer included "injection of semen tainted with lues" and "venereal virus"
- *Gagnon, 1950:* Cervical cancer rare in nuns
- *Rotkin , 1967:* Adolescent coitus associated with cervical cancer
- *Kessler et al., 1976:* Is cervical cancer a venereal disease? Male partner is investigated
- *Harris et al., 1980:* Number of male sex partners associated with cervical dysplasia and carcinomas

**1980-1987: Use of modern epidemiological study designs**

**Sexual activity → Viral agent → Cervical cancer**

- *Brinton & Fraumeni, 1986:* HSV*, HPV putative candidates as infectious agent
- *La Vecchia et al., 1986a:* HSV and warts associated with CIN**, age first coitus and number of sex partners associated with cervical cancer
- *Brinton et al., 1987:* Age first coitus and number of sex partners associated with cervical cancer
- *zur Hausen, 1989:* HPV associated with cervical cancer

**1988-1990: Use of DNA hybridization techniques to detect HPV**

**Sexual activity → HPV → Cervical cancer**

- *Villa and Franco, 1989:* Sexual activity not associated with HPV infection in Brazilian asymptomatic women
- *Brinton et al., 1989:* Husbands of cases and controls tested for HPV. HPV positivity not associated with risk of cervical cancer.
- *Kjaer et al., 1990:* Sexual activity not associated with HPV infection in Danish and Greenlandic asymptomatic women.

* Herpes simplex II virus; ** Cervical intraepithelial neopolasia

## Table I

### Historical Outline of Epidemiological Research on Cervical Cancer (Continued)

**1991-1995: Use of PCR techniques to detect HPV in epidemiology studies**

**Sexual activity → HPV → Cervical cancer**

| | |
|---|---|
| • *Ley et al., 1991:* | Number of sexual partners strongly associated with HPV infection. Decreased age, oral contraceptives and ethnicity moderately associated with HPV infection |
| • *Schiffman 1992:* | Failure of association between sexual activity and HPV infection |
| • *Bauer et al., 1993:* | Number of lifetime sex partners strongly associated with HPV infection. Low economic status moderately associated with HPV infection |
| • *Wheeler et al., 1993:* | Number of lifetime sex partners moderately associated with HPV infection |
| • *Hildesheim et al., 1993:* | Number of recent sex partners strongly associated with HPV infection. Number of lifetime sex partners mildly associated with HPV infection. Current use of oral contraceptives associated with HPV infection |
| • *Franco et al., 1995:* | Number of sexual partners strongly associated with high-risk HPV infection |

increase in the risk of cervical cancer with the number of sexual partners reported by the male partner/husband. In all of these studies, the husbands of cases reported significantly more sexual partners than husbands of controls (Bosch *et al.*, 1995; Brinton *et al.*, 1989; Buckley *et al.*, 1981; Kjaer *et al.*, 1991; Zunzunegui *et al.*, 1986). Poor hygiene of the male partner has also been thought to play a role in the etiology of cervical cancer, with special attention given to the effects of circumcision. Despite early reports of a protective effect associated with circumcision of the partner (Terris and Oalmann, 1960; Wynder *et al.*, 1954) most studies have shown no substantial difference between case and control husbands (Boyd and Doll, 1964; Brinton *et al.*, 1989; Jones *et al.*, 1958; Rotkin, 1967). However, in light of a recent investigation that showed wives of circumcised men were at a significantly low relative risk (0.3) (Kjaer *et al.*, 1991), coupled with the consistently low incidence rates of cervical cancer in countries that practice circumcision, (Middle East), the question still warrants further examination. Finally, mortality and morbidity data have revealed strong associations between cervical and penile cancer in correlation studies (Franco *et al.*, 1988; Li *et al.*, 1982) and there is evidence from cohort studies showing wives of patients with penile cancer at an increased risk of developing cervical cancer later in life (Graham and Schotz, 1979; Smith *et al.*, 1980).

In recent years, cigarette smoking has also emerged as a potential etiological factor. However, there is still much debate as to the role of tobacco in cervical carcinogenesis. Nicotine derivatives have been found in the cervical mucus of smokers (Brinton, 1990; Schiffman *et al.*, 1987) and early investigations have found that the proportion of smokers among patients with cervical cancer was greater than that among the control subjects (Naguib *et al.*, 1966; Thomas, 1972). However, smoking has often been associated with sexual behaviour, and while an independent role for tobacco smoking in the risk of cervical cancer has been found in some studies that attempted to control for the confounding effect of age at first intercourse and the number of sexual partners, (Brinton and Fraumeni, 1986; Clarke *et*

*al.*, 1982; Peters *et al.*, 1986) other researchers have not observed such an independent effect (Celentano *et al.*, 1987; Stellman *et al.*, 1980). A further need in evaluating smoking effects is consideration of confounding by dietary factors (Brinton, 1990). Studies suggest that diets low in either carotenoids or vitamin C may predispose to high risk of cervical cancer and smokers have been shown to have lower levels of plasma β-carotene than nonsmokers (Brock *et al.*, 1988; La Vecchia *et al.*, 1986b; Verreault *et al.*, 1989). Thus, future investigations of smoking and cervical cancer need to carefully evaluate both confounding and effect modifying effects of nutritional factors.

Other risk factors for cervical cancer include use of oral contraceptives, conditions leading to immunosuppression and infrequent Pap smear testing (Brinton, 1992; Franco, 1991a; Muñoz *et al.*, 1992). There is also growing evidence that multiparity may significantly relate to cervical cancer risk (Brinton *et al.*, 1987; Brinton, 1992; Brinton *et al.*, 1989; Parazzini *et al.*, 1989). A four-fold excess risk of cervical cancer was found associated with 12 or more births compared to 0-1 births in Latin America, even after adjustment for a variety of socio-economic and sexual factors (Brinton *et al.*, 1989). Possible explanations for the association include cervical trauma during parturition and hormonal or nutritional influences of pregnancy (Brinton, 1992). A selection of epidemiological research on cervical cancer is summarized in Table I.

### *1.3 HPV and cervical carcinogenesis*

The consistently strong association between cervical neoplasia and sexual behaviour prompted the suggestion that a sexually transmitted, infectious agent may have an etiological role in cervical carcinogenesis. Although the possible chemical carcinogenicity of semen and sperm were proposed as possible etiological agents of cervical cancer (Coppleson, 1970; Malhotra, 1971), along with many micro-organisms including bacteria (*Treponema pallidum*, *Neisseria gonorrhoe*, *Chlamydia trachomatis*), and protozoa (*Trichomonas vaginalis*), very

little evidence was generated to support these putative agents. Focus quickly shifted to herpes simplex virus type 2 (HSV-2) and to human papillomavirus (HPV) as potential viral etiological agents. HSV was proven to be carcinogenic *in vitro* and *in vivo*, however, clinical studies eventually demonstrated that only a fraction of cervical carcinomas contained traces (viral DNA) of HSV infection and epidemiological studies failed to demonstrate an association between HSV and cervical cancer (Franco, 1991a). In 1976, zur Hausen observed that cervical cancer and papillomavirus induced condyloma acuminata (genital warts), shared similar epidemiological profiles, and postulated that a papillomavirus may be involved in the development of cervical cancer (zur Hausen, 1976). The last two decades have since, seen an explosion of experimental and epidemiological research, all implicating the human papillomaviruses (HPV) as the most likely cause of cervical cancer (Table II).

Papillomaviruses were already known to cause tumours in a variety of mammalian species (Campo *et al.*, 1980). With improved methods for viral nucleic acid detection, more than 90% of human cervical carcinomas, including condylomata, intraepithelial neoplasms and invasive tumours have now been found to harbor HPV DNA sequences (Muñoz *et al.*, 1992; Park *et al.*, 1994). Cytopathologists began to observe morphological abnormalities, such as koilocytosis, in dysplastic lesions which were traditionally associated with HPV induced condylomas only (Meisels and Morin, 1976). This was followed by findings that there existed many different HPV types with different tropisms (zur Hausen, 1976) and specific HPV types were discovered in carcinomas of epidermodysplasia verruciformis patients and in cervical carcinomas (Boshart *et al.*, 1984; Durst *et al.*, 1983). The physical state of the viral DNA has been shown to be mostly integrated in invasive tumors and extrachromosomal in benign and premalignant lesions (Lehn *et al.*, 1988; McCance, 1988). Specific viral transcripts in cervical cancer cells and specific modes of viral DNA integration have also been identified (Schwarz *et al.*, 1985; Yee *et al.*, 1985). It has further been demonstrated that HPV DNA can induce malignant transformation when transfected into

**Table II**

**Biological Evidence for a Carcinogenic Role of HPV**

- Papillomaviruses associated with tumours in other species
  *Campo et al. (1980)*
- Transcriptionally active viral genome frequently found in cervical tumors
  *Schwarz et al. (1985)*
- HPV found in both early and advanced tumour lesions
  *Schneider et al. (1987a)*
- Malignant transformation upon transfection of viral DNA into cultured cells
  *Bedell et al. (1987)*
- Cooperation between HPV and oncogenes in transformation
  *Matlashewski et al. (1987)*
- Viral DNA integrated in malignant lesions and episomal in premalignant lesions
  *McCance et al. (1988)*
- HPV E7 protein binds to the retinoblastoma anti-oncogene product
  *Dyson et al. (1989)*
- HPV E6 binds to the p53 tumor-suppressor gene product
  *Werness et al. (1990)*
- Cervical cells immortalized *in vitro* with HPV DNA differentiate dysplastically *in vivo* after being implanted in nude mice
  *Waggoner et al. (1990)*

mammalian cells in culture, and integration of viral DNA was identified in the vicinity of proto-oncogenes, e.g., *c-myc* (Durst *et al.*, 1987). The malignant phenotype of human cervical carcinoma cells and malignant transformation of rodent cells were found to depend on HPV E6/E7 gene expression (Crook *et al.*, 1989; Von Knebel-Doeberitz *et al.*, 1988). Finally, the proteins encoded by the E7, E6 and E5 domains of the HPV genome have recently been shown to bind to the products of the retinoblastoma, (Dyson *et al.*, 1989; Münger *et al.*, 1989), p53 tumour-suppressor genes, (Vousden, 1993; Werness *et al.*, 1990), and the epithelial growth factor (EGF) receptor (Banks and Matlashewski, 1993), respectively. HPV appears to have the ability to modify the activity of these 3 important cellular molecules, resulting in loss of control at essential points in the cell cycle and amplification of certain cell signal transductions.

A consensus panel convened by the World Health Organization's International Agency for Research on Cancer (IARC) has recently concluded that there is now compelling evidence, both from biologic and from epidemiological standpoints, to consider HPV infection as a cause of cervical cancer (IARC, 1995). A variety of case-control and cohort studies conducted in the last five years have consistently shown that HPV infection is the strongest risk factor for cervical cancer, with relative risks (RR) in the range of 20-70. Such a magnitude is higher than that for the association between smoking and lung cancer and is second only to that of the association between the chronic carrier state of hepatitis B infection and liver cancer, causal relations in cancer that are no longer challenged (Franco, 1994).

### *1.4 Screening for cervical cancer*

Cytological screening (Pap smear) has proven to be a very powerful tool, instrumental in reducing the incidence of cervical cancer in North America and Western Europe where it is used most routinely. The cervical smear test for the detection of premalignant lesions and/or cervical cancer was first introduced by Papanicolaou

(Papanicolaou, 1954). Commonly referred to as the Pap test, it is based on the recognition of cytomorphological signs indicating disruption in the maturation of the cervical squamous epithelium (Koss, 1989). The initial grading system of cervical lesions was proposed by Richart and was based on the concept of cervical intraepithelial neoplasia (CIN) and dysplasia (Richart, 1968). Cervical lesions were classified into three grades of CIN according to the severity of disturbances of cellular maturation, stratification and cytological atypia. CIN I was defined as a well differentiated intraepithelial neoplasm, often associated with HPV 6 and 11, while CIN II and III were defined as poorly differentiated intraepithelial neoplasms, most often associated with HPV 16 and 18. In 1989, the CIN classification was replaced by the Bethesda classification system. The three CIN grades were re-classified as low- and high-grade squamous intraepithelial lesions (LG-SIL and HG-SIL). Low-grade SIL represents CIN grade 1 corresponding to those lesions with a presumably low risk of progression to carcinoma and high-grade SIL includes both CIN grade II and III corresponding to lesions with increased risk of progressing to carcinoma (Solomon, 1989) (Table III).

If HPV infection is an early biologic precursor of cervical neoplasia, should HPV testing be used routinely in screening for cervical cancer? Opponents to HPV testing have claimed that cytology screening alone has been very successful in decreasing invasive cervical cancer morbidity and mortality in many countries. An additional criticism is that cervical HPV infection is found in many women of reproductive age and that it would be impractical to modify follow-up guidelines for them (Nuovo and Nuovo, 1991). On the other hand, proponents of using HPV testing in combination with other cervical cancer screening methods claim that it would represent a scientifically sound approach for secondary prevention and that any pending issues could be evaluated by intervention trials (Reid and Lorincz, 1991).

## Table III

### Bethesda Classification of Epithelial Cell Abnormalities

| Designation | Abnormalities |
|---|---|
| **Atypia** | 1. Atypical squamous cells of undetermined significance (ASCUS), excludes preneoplastic changes. |
| **Low-Grade SIL*** | 1. Cellular changes associated with HPV (without other abnormalities) |
| | 2. Mild dysplasia (originally termed CIN I**) |
| **High-Grade SIL** | 1. Moderate dysplasia (originally termed CIN II) |
| | 2. Severe dysplasia (originally termed CIN III) |
| | 3. Carcinoma in situ (originally termed CIN III) |
| | 4. One of the latter abnormalities and HPV associated changes |

*SIL Squamous intraepithelial lesion
**CIN Cervical intraepithelial neoplasia

The issue of whether HPV testing should be used in managing cervical cancer screenees was raised in the recent IARC consensus meeting. Its participants concluded that there is enough justification to evaluate HPV testing as an adjunct to Pap smear screening in cervical cancer (Franco, 1992b). There are however, several questions that need to be addressed by prospective studies. For instance, what are the conversion rates (from LG-SIL to HG-SIL) according to different HPV types? Can HPV testing predict more accurately the occurrence of advanced lesions relative to Pap smear cytology?

In Canada, two separate consensus papers have summarized the position of authorities in preventive practices. The earlier evaluation recommended that information on HPV infection not be used to guide patient management (Miller, 1992). A more recent position paper by the Canadian Task Force on Periodic Health Examination concluded that it is still premature to adopt large scale HPV testing in our country (Johnson, 1995). However, the Task Force established some research priorities as follows: (i) refinement of diagnostic methods, (ii) precise definition of HPV incidence in the population, (iii) assessment of risks associated with certain HPV genotypes for cancer progression, (iv) identification of cofactors influencing HPV transmission and carcinogenesis, (v) treatment of HPV infection, (vi) development of vaccines, and (vii) assessment of efficacy and cost-effectiveness of screening for HPV infection (Johnson, 1995).

## 2 Characterization of HPV

### *2.1 HPV structure*

The human papillomavirus (HPV) is a small (45-55 nm), non-enveloped, icosahedral DNA virus that replicates in the nucleus of squamous epithelial cells. The papillomavirus particles have a sedimentation coefficient ($S_{20}$, W) of 300 and their cytosine:guanosine content is approximately 42%. The DNA constitutes approximately 12% of the virion, accounting for the density in cesium chloride of 1.34 g/ml. HPV consists of a single

molecule of double-stranded circular DNA approximately 8000 base pairs (bp) long with a molecular weight of 5 x $10^6$ daltons, contained within a capsid composed of 72 capsomeres. Electron microscopy has revealed 12 five-coordinated and 60 six-coordinated capsomeres arranged on a T = 7 surface lattice (Finch and Klug, 1965; Klug and Finch, 1965).

The capsid consists of at least two structural proteins encoded by the L1 and L2 open reading frames of the viral genome. The major capsid protein has a molecular weight of approximately 55,000 daltons and represents about 80% of the total viral protein. The minor capsid protein has a molecular weight of 70,000 daltons (Favre *et al.*, 1975; Gissman *et al.*, 1987; Pfister *et al.*, 1977). The HPV genome can exist in three different forms within virions and in infected cells: a closed supercoiled circular form, an open circular form, and a linear form (Crawford, 1965). HPV usually exists as non-integrated episomal plasmids in benign and premalignant lesions, but frequently integrates into the host cell genome in malignant lesions (zur Hausen, 1989).

### *2.2 HPV classification*

Papillomaviruses, members of the *Papovaviridae* family, are widespread in nature inducing warts (or papillomas) primarily in higher vertebrates. Within the genus papillomavirus, there exist several highly related viruses that cause species-specific infections in animals. These characterized papillomaviruses include several bovine papillomaviruses (BPV), canine oral papillomaviruses (COPV), cotton tail rabbit (Shope) papillomaviruses, equine papillomaviruses (EPV), and approximately 70 human papillomaviruses (HPV) (de Villiers, 1994; Koutsky *et al.*, 1988).

HPV has traditionally been described on the basis of DNA homology as determined by cross-hybridization (Pfister and Fuchs, 1994). Independent isolates from one species were defined as different types if there was less than 50% cross-hybridization and regarded as subtypes if cross-hybridization exceeded 50%. Presently, however, complete DNA

sequences of almost all HPV types have been determined, thereby adjusting the HPV classification criteria on the basis of the available sequence data instead (Pfister and Fuchs, 1994). HPV genotypes are now considered new when the nucleotide homology of specific regions of the viral genome (L1, E6 and E7 sequences) differ by more than 10%. A subtype is specified as a type which exhibits between 90% and 98% homology while HPV variants share less than 2% DNA sequence variation in their E6, E7 and L1 regions (de Villiers, 1994).

### *2.3 HPV genome organization*

The viral genomes of all papillomaviruses are divided into an early region (E1-E8), encoding proteins required for viral replication, transcription and transformation, a late region (L1 to L2) coding for viral structural proteins and a non-coding region ('long control region' (LCR) or 'upstream regulatory region' (URR), which harbours the origin of replication and transcription control signals and are essential for regulatory functions of the genome (Koutsky *et al.*, 1988). Since HPV comprises a small genome with all open reading frames (ORF) on the same DNA strand it requires very efficient template usage. This is in part accomplished by the distribution of the ORFs over all three reading frames with considerable overlap. In addition, a considerable degree of alternative splicing gives rise to truncated (e.g. E2C) or fused (e.g. E1/E4) products, allowing to encode a number of proteins which clearly exceeds the number of ORFs (de Roda Husman, 1995). Because of the difficulties in propagating HPV *in vitro*, most research on the functions of the various ORFs have been conducted on BPV-1 which can transform rodent cells and be maintained stably as a plasmid, allowing for "genetic dissection" (Koutsky *et al.*, 1988).

#### *2.3.1 Open reading frames (ORF)*

The entire E1 appears to be involved in transcriptional repression. The E1 ORF encodes two proteins that are involved directly with extrachromasomal DNA replication: One

that serves as a positive modulator and one that serves as a negative regulator (Lusky and Botchan, 1985; Roberts and Weintraub, 1986). It has been recently shown that both BPV and HPV E1 proteins have helicase activity (Hagensee *et al.*, 1993; Hughes and Romanos, 1993). Bream and collaborators (1993) have shown that HPV 11 E1 can form a heterodimer with E2 binding to DNA sequences which contain the viral origin of replication and possess both ATPase and GTPase enzymatic activity. The HPV 11 E1M^E2 splice variant exhibits transcriptional repressor activity triggered by binding to an E2 responsive element (Chiang *et al.*, 1991).

The E2 ORF is involved in maintenance of the episomal state of the virus and in transcriptional regulation. The E2 gene regulates the expression of the viral long control region (LCR) promoters by encoding two proteins that regulate an enhancer element in LCR (Spalholz *et al.*, 1985). The full length protein encodes a transactivator, whereas the COOH-terminal end encodes a transrepressor (Lambert *et al.*, 1987). In the absence of either the enhancer or the E2 product, the viral promoter has minimal activity and transformation efficiency is greatly reduced (Broker, 1987). The abolished expression of the E2 gene results in the deregulated expression of the viral E6 and E7 genes that encode transforming functions (Howley and Schlegel, 1988).

At present, the E4 proteins resemble late structural proteins rather than early regulatory proteins. The E4 polypeptides translated from the major spliced E1^E4 mRNA have been shown to be abundant in HPV 1 induced skin warts and, late in the viral life cycle, E4 proteins have been shown to be involved in the collapse of the cytokeratin network, suggesting that E4 proteins may enhance virus release from the productively infected cell (Chow *et al.*, 1987; Doorbar *et al.*, 1991).

It has been hypothesized that HPV E5 plays a role in early tumour development (Banks and Matlashewski, 1993). HPV E5 proteins have the ability to transform

immortalized rodent cells (Chen *et al.*, 1994; Leechanachai *et al.*, 1992) and the HPV 16 E5 gene was found to cooperate with the E7 gene in stimulating proliferation of primary rodent epithelial cells (Bouvard *et al.*, 1994). In these assays, the oncogenic activity of HPV 16 E5 was augmented in the presence of epidermal growth factor (EGF). The E5 ORF has been shown to encode a hydrophobic protein that plays a role in cell transformation, altering the phenotypic characteristics of the infected cell by increasing its growth potential (Banks and Matlashewski, 1993; Schiller *et al.*, 1986; Yang *et al.*, 1985). This increased growth potential appears to be mediated by the down regulation of activated growth factor receptors via E5's association with ductin, the 16 KDa pore-forming protein component of the vacuolar ATPase (Schlegel, 1995; Straight *et al.*, 1995).

HPV E6, a major oncoprotein encoded by the high-risk HPV types, is capable of inhibiting an important tumour-suppressor gene, p53. In stressed cells, e.g. after UV or gamma irradiation, p53 expression induced by DNA damage may result in cell cycle arrest, allowing for DNA repair before mutations are incorporated into newly synthesized DNA. In cases of extreme DNA damage, p53 may even trigger an apoptotic response (Lane, 1992). *In vitro* studies have demonstrated that E6 binding to p53 results in the rapid proteolytic degradation of the p53 proteins through a ubiquitin dependent pathway (Vousden, 1993). This interference with p53 appears to play a significant role in cellular transformation.

Like E6 oncoproteins, the E7 oncoproteins are clearly involved in cellular transformation, capable of inhibiting a second important tumour-suppressor gene, the retinoblastoma gene (pRb) (zur Hausen and de Villiers, 1994). Like p53, pRb is known to be involved in the control of the G1 cell cycle checkpoint. In its hypophosphorylated form, pRb negatively regulates cell growth by complexing with the transcription factor E2F. This complex inactivates E2F, which, when activated stimulates transcription of several genes relevant for cells to enter into the cell cycle and progress through G1 into S phase. Although

low-risk HPV E7 proteins can complex with pRb, their affinity for binding is approximately tenfold lower than is the case for the high-risk HPV E7 proteins. The observed association of E7 with cyclin A and cyclin dependent kinase cdk2 has led to further indication that E7 expression may also stimulate cells to proceed through G2 phase to cell division. This interaction could disturb the normal control of entry into mitosis, regulated by cyclin dependent kinases, and may contribute to the development of HPV-associated cancers (Hamel *et al.*, 1992; Münger *et al.*, 1989; Tommasino *et al.*, 1993; Vousden, 1993).

The L1 ORF gene product is the major capsid protein of mature PV virions with a molecular weight of 54,000 daltons, and is highly conserved among all papillomaviruses, containing the genus-specific epitope (Broker, 1987; Koutsky *et al.*, 1988). An icosahedral array of major (L1) and minor (L2) capsid proteins forms the virus particle. Five L1 molecules form a pentameric capsomere of which 72 are necessary for the particle structure, arranged in a T=7 symmetry (Baker *et al.*, 1991).

The L2 ORF encodes a minor capsid protein of about 76,000 daltons. This ORF is quite variable among HPVs and the L2 protein might serve as a distinguishing target for immunocytochemical typing. However, it is not yet clear whether or not these type-specific epitopes could form the basis for vaccines, since its localization within the virion is still unclear (Broker, 1987). Its function seems to be in the stabilization of the capsid structure, based on *in vitro* studies analyzing the self-assembly of HPV 1 capsids into virus-like particles (VLPs) (Hagensee *et al.*, 1993).

### *2.3.2 Long control region*

The non-coding region (LCR or URR) contains the viral origin of replication and viral gene promoter and enhancer sequences. Responsive elements for numerous cellular transcription factors are also localized in the URR, such as the progesterone and glucocorticoid responsive elements, and some nuclear binding sites including: ubiquitous

factors (AP-1), a novel factor (NFA), a novel transcription factor (PVF-1) and the oct1 factor (Chan *et al.*, 1990; Chong *et al.*, 1991; Gloss *et al.*, 1989). It was recently shown that the URR also contains an oct2 binding motif which upon binding in cervical cells can transactivate the viral promoter activity. However, in non cervical cells, promoter activity is inhibited, indicating a role for oct2 in tissue specificity of URR driven gene expression (Morris *et al.*, 1993).

### *2.3.3 Capsid assembly*

HPV infection remains latent in the basal cells. As progeny cells start to differentiate, the first signs of active expression occur within the parabasal layer. Transforming proteins encoded by the "early" genes act as mitogenic stimuli causing increased cell division (acanthosis) and capillary overgrowth (papillomatosis). In the upper layers, the late events in the viral life cycle produce koilocytic atypia characterized by degenerative clumping of host-cell chromatin, nuclear collapse and the formation of cytoplasmic vacuoles. Assembly of the viral capsid (virion formation) usually appears to take place in this fully differentiated layer, since the L1 and L2 mRNA that codes for capsid proteins are usually only present in the cytoplasm of the keratinocytes (Reid, 1993).

## *2.4 HPV DNA detection*

### *2.4.1 DNA hybridization techniques*

Most studies have employed nucleic acid hybridization techniques to detect HPV, since classical virus detection methods such as virus cultivation cannot be used because of the difficulty in propagating HPV *in vitro*. Nucleic acid hybridization, based on the ability of DNA probes to hybridize with complementary sequences, offers a specific and sensitive approach for detection of DNA sequences. The stability of the formed hybrid depends on the melting temperature (Tm) at which half of the hybrids are dissociated: Tm = 81.5°C + 16.6 (logM) + 0.41 (%G+C) - 500/n - 0.61 (% formamide) where M is the ionic strength (mol/l),

(%G+C) is the composition of guanine and cytosine and n is the shortest chain in the duplex. The expected stringency can be reached by altering 1) the salt concentration, 2) temperature, 3) formamide concentration and 4) the conditions in the post hybridization washes. The following is a description of the advantages and disadvantages of the hybridization techniques most commonly used (Sambrook *et al.*, 1989).

Dot blot hybridization: DNA is denatured, neutralized and applied to a filter and then hybridized with type specific HPV probes. Many samples can be tested on a single filter. Dot blot hybridization functions reliably only under high stringency conditions because of the increasing rates of false positive reactions due to cross-hybridization with the cellular DNA when the stringency conditions are relaxed (de Roda Husman, 1995).

Southern blot: This method, regarded by many as the "gold standard" for detecting and typing HPV, was first described by Southern in 1975 (Lorincz *et al.*, 1986; Nuovo and Richart, 1989; Southern, 1975). Cellular DNA of high molecular weight is extracted, digested with nucleotide sequence-specific restriction endonucleases and separated by gel electrophoresis. The DNA is then denatured, neutralized and transferred to a filter (nylon or nitrocellulose). The DNA can be hybridized with HPV probes under low stringency conditions to determine overall HPV DNA prevalence and then washed under high stringency conditions to determine the specific HPV types. The results are interpreted by exposing the membrane to autoradiography. Information on HPV integration events and rearrangements can be easily detected and the assay is very reliable due to its sensitivity and specificity. However, this method is too laborious for routine diagnostic purposes, requiring large quantities of DNA (5-10 μg) which are not always available. In addition the Southern blot hybridization is subject to remarkable variations in both HPV detection rates and HPV types reported among different laboratories, primarily because interpretation of obtained hybridization results can be quite complex and subjective (Brandsma *et al.*, 1989).

*In situ* hybridization: The major advantages of the *in situ* hybridization technique is the possibility for direct visualization of the cells containing the target HPV DNA signals and the preservation of the tissue morphology. Tissue sections are pre-treated, (digested with proteinase K), and then labeled directly with HPV DNA probes. Both RNA and DNA can be targeted, detecting an estimated 20 HPV copies per cell with non-radioactive probes (de Roda Husman, 1995).

Liquid hybridization (Hybrid Capture): Liquid hybridization is a highly sophisticated method which hybridizes denatured DNA to RNA probes in solution. The RNA-DNA complexes are transferred to a tube coated with an antibody directed against the RNA-DNA hybrids. An alkaline phosphatase conjugated antibody against RNA-DNA hybrids is added to the captured RNA-DNA hybrids. A chemiluminescent substrate then reacts with the alkaline phosphatase and the light that is produced is subsequently measured. A great advantage of this test is that it is quantitative and can be applied to crude cell suspensions. The quantity of produced light represents the amount of detected HPV DNA (de Roda Husman, 1995). However, it is still a very costly method of analysis, but is presently the most likely candidate to be employed for diagnostic purposes, should HPV testing as an adjunct to cytology become routine in screening for cervical premalignant lesions (Schiffman and Schatzkin, 1994).

### *2.4.2 Polymerase chain reaction (PCR)*

PCR is an enzymatic *in vitro* DNA amplification reaction. DNA PCR consists of three steps which include DNA denaturation, annealing of primers (complementary oligonucleotides) which provide free 3'-OH ends for DNA polymerase mediated chain elongation in the last step. The process exploits a thermostable *Taq* polymerase allowing the steps to be repeated 30 to 40 times in the described order (Saiki *et al.*, 1988). Theoretically, a million fold increase in target DNA can be obtained resulting in a sensitivity of 1 HPV copy

per sample. Very low amounts of input DNA (25-500 ng) are sufficient to generate detectable amounts of target DNA and this system can be easily applied to crude cell suspensions, eliminating the tedious task of DNA purification (Bauer *et al.*, 1991; Saiki *et al.*, 1988). PCR can also be applied to diagnose paraffin embedded tissue, although samples stored for prolonged periods of time (>7 years) have shown a marked reduction in PCR sensitivity (Greer *et al.*, 1990). The combination of PCR and DNA hybridization has gradually become the gold standard in HPV detection techniques. However, PCR's greatest strength is also its greatest liability. Its superior sensitivity gives rise to increase risk of laboratory contaminations and excessive laboratory anticontamination measures must be followed to ensure reliable results (Bauer *et al.*, 1993).

## 3 Pathogenesis of Cervical HPV Infection

### *3.1 HPV type and cancer risk*

Papillomaviruses are found exclusively in squamous cell epithelium. This specificity is further defined by their tropism for cutaneous squamous cell epithelium or for mucosal squamous cell epithelium. For the most part, HPV types are either mucosotropic or cutaneotropic, having a specificity for the anatomic site that they infect (Table IV). Of the over 70 HPV types identified thus far, 27 have been associated with the genital tract (mucosotropic). They are divided into three groups based on their oncogenic potential. The low risk types are common in low grade squamous intraepithelial lesions (SIL) but rarely are they found in high grade SILs or carcinomas. They include types 6, 11, 42, 43 and 44. The intermediate risk types are more common in high grade SILs but less so in cancer and include types 31, 33, 35, 51 and 52. The high risk types are strongly associated with carcinomas but have a diverse behaviour with respect to high grade SILs. The most common HPV types noted in cases of cervical neoplasia are 16 and 18 followed by 45 and 56. However, types 18 and 45 are also often associated with high-grade SILs (Bauer *et al.*, 1993; Franco, 1991a; Koutsky *et al.*, 1988).

## Table IV

### HPV Types, Site of Infection and Clinical Manifestations

| HPV type | Site of infection | Usual lesions |
|---|---|---|
| 1 | Soles of feet | Deep plantar warts |
| 2, 4, 7 | Hands | Common wart |
| 3, 10 | Arms | Flat and juvenile warts |
| 5, 8, 9, 12, 14, 15, 17, 19-25, 36, 38 | Forehead, arms and trunk | Skin cancer, flat and macular warts |
| 26-29, 34 | Forehead, arms and trunk | Common warts in the immunosuppressed |
| 6, 11 | Anogenital tract, larynx and nasal cavity | Condylomata, papillomas |
| 16, 18, 31, 33, 35, 39, 41-45, 51-59, 66, 68, 73, PAP155, PAP291, W13B | Genital tract and anus | Genital tract and anal cancers. Atypical condylomata |
| 13, 32 | Oral cavity | Focal epithelial hyperplasia |

Howley & Schlegel, 1988; Hildesheim *et al.*, 1994

### *3.2 Viral entry*

Infection of basal layer cells from a wound or an abrasion of an appropriate epithelial surface appears to play a major role in contracting HPV. Junctions of different types of epithelia also appear to be highly vulnerable to HPV infection, possibly because of the proximity of proliferating cells to the surface of such sites (zur Hausen and de Villiers, 1994). Wound healing may facilitate viral growth by acceleration of basal or parabasal cell division and by stimulation of an augmented blood supply at the site of the abrasion (Taichman and LaPorta, 1987). Anogenital infections are primarily sexually transmitted, although there is also evidence that poor hygienic conditions may be a factor in transmission, particularly in early childhood infections (Koutsky *et al.*, 1988). The male anogenital tract is recognized as the silent reservoir for sexual transmission of high-risk HPV types (Barrasso *et al.*, 1987; Levine *et al.*, 1984; Rosemberg, 1985; Sedlacek *et al.*, 1986).

### *3.3 Tissue damage*

The outer surface of the uterine cervix and the vagina are lined with a non-keratinizing, stratified squamous epithelium. The endocervical canal and crypts are covered with a mucus-secreting columnar epithelium. Normally, the junction between these two epithelia coincides with the external cervical os but with age, changes in the shape and volume of the cervix can alter the location of this so-called squamo-columnar junction. At puberty the columnar epithelium extends into the ectocervix relocating the squamo-columnar junction to the ectocervix, resulting in a phenomenon known as cervical ectropion. With age, the columnar epithelium of the ectropion is gradually replaced by metaplastic squamous epithelium, and is recognized as *the transformation zone*. In younger women the transformation zone lies on the ectocervix whereas in older women it withdraws into the endocervical canal. The transformation zone is the area from which the majority of intraepithelial and invasive neoplasms of the cervix develop. When cervical lesions are

identified (during colposcopic examination), the transformation zone is biopsied and histologically analyzed (de Roda Husman, 1995; Koutsky *et al.*, 1988).

There appear to be two modes of viral DNA replication that in turn are responsible for the variety of clinical manifestations observed with HPV infection. One mode is described as vegetative or productive viral DNA replication, and occurs in the highly differentiated cells of the epidermis. Productive viral replication is associated with a burst of viral DNA synthesis generating progeny virions in the keratinocytes or upper most epidermal layer, where cellular DNA synthesis does not occur (Ji, 1991), and is responsible for both "clinical" HPV infections, which can usually be visualized with the naked eye, and "sub-clinical" HPV infections, which can be diagnosed with the aid of colposcopy. These infections are characterized by the appearance of hyperplastic proliferations of basal and parabasal cells (acanthosis), degenerative cytoplasmic vacuolization (koilocytosis), and variable thickening of the superficial cell layers (hyperkeratosis, parakeratosis) (Reid, 1993).

In the genital tract, certain HPVs may cause papillary proliferations such as condyloma acuminata or flat condylomas. Condyloma acuminata (venereal warts) are usually associated with HPV-6 (65%) or HPV-11 (20%), and frequently occur in the external genitalia and adjacent and perianal area of both sexes. It is a cauliflower-like, papillary proliferation of the anogenital epithelium or mucosae, which generally grows in multiple clusters. In females, condylomata may regress spontaneously, persist for years or undergo a rapid increase in size during pregnancy. Fortunately, genital warts of long duration are almost always benign and relatively easy to cure (Ji, 1991; Reid, 1993). Flat condylomas, invisible to the naked eye, are the most frequent clinical manifestations of genital HPV infections, but were only recognized in 1976 (when they could be visualized under colposcopic examination). Flat condylomas show acetowhite epithelium with or without asperites after the application of acetic acid and irregular iodine uptake with satellite lesions.

In contrast to condyloma acuminatum, flat condylomas are frequently associated with intraepithelial lesions. Similarly, high-risk HPV types such as HPV 16 and 18 are also detected more often in flat condylomas than in exophytic lesions such as condyloma acuminatum. It has been suggested that the different morphological features between the two forms of condyloma, and the increased likelihood that flat condylomas are at a higher risk to progress may be due to the different HPV types involved (Ji, 1991; Reid, 1993).

The majority of invasive cervical cancers are associated with increasingly oncogenic genital HPV types such as HPV 16, 18, 45 and 56 which can all cause squamous intraepithelial lesions (SIL) or invasive carcinomas, characterized by abnormal cellular growth of the prickle cell layer and excess production of keratin where the virus is believed to replicate. The development of cervical cancer appears to proceed through a continuum of progressive cellular changes from low-grade SIL to high-grade SIL to severe dysplasia and/or carcinoma *in situ*. Most condylomas and low-grade SILs will either persist or regress, whereas the majority of high-grade SILs will either persist or progress (Howley, 1991).

A second mode of HPV DNA replication occurs in basal cells of the lower epidermis where viral DNA is maintained as a stable, multicopy plasmid. In the proposed model, the viral genomes replicate on an average of once per cell cycle and may be faithfully partitioned to the daughter cells. This replication ensures a persistent and latent infection in the stem cells of the epidermis (Howley, 1991). These latent infections have become an important focus in epidemiological studies, not only because they may be responsible for persistent and recurrent infections, serving as reservoirs for transmission, but also because as many as 40% of apparently healthy, asymptomatic women with cytologically normal cervical epithelia are HPV positive (Bauer *et al.*, 1993; Hildesheim *et al.*, 1994; Ley *et al.*, 1991; Wheeler *et al.*, 1993). There is evidence to suggest, however, that a large proportion of these women

infected with HPV eliminate the infection over a short period and are at low or no risk of developing disease. In a recent study that followed a cohort of women who were HPV infected upon accrual, only 46% of the infected women remained consistently or intermittently positive over a two year follow-up, and less than 10% remained positive for the original HPV type (Moscicki *et al.*, 1993). Those "truly" persistent infections with high-risk HPV types are the most likely to develop into malignant lesions. Unfortunately the clinical manifestations are often asymptomatic, and can only be detected with DNA hybridization techniques (Schneider and Koutsky, 1992). Detection becomes even more challenging when either multiple sites of the genital tract are simultaneously infected by HPV or when multiple viral types are present. Multiple infections are associated with an increased risk of cytologically evident low-grade SIL (Morrison *et al.*, 1991; Schiffman *et al.*, 1993) but the explanations remain uncertain. Also unclear is whether the distribution of HPV types in multiple infections is random or reveals a tendency of certain types to favour or exclude each other's presence (Schiffman and Schatzkin, 1994).

### *3.4 Host immune response*

The plurality of HPV serotypes and the lack of purified viral type-specific antigens have hindered the assessment of cellular and humoral immune responses to HPV infection. Cellular immunity associated with regression of lesions appears to be HPV type-specific as does the humoral response to cutaneotropic HPV infections. The dendritic Langerhan's cells that bridge the multiple layers of cervical epithelium and are HLA DR+ are thought to be the primary antigen presenting cells. Other immunoreactive cells including T- and B-cells as well as macrophages have been identified during analysis of the inflammatory cell infiltrates in genital HPV lesions (Vayrynen *et al.*, 1984). The local cervical immune response remains detectable for about 30 days and is predominantly of the IgA isotype (Roche and Crum, 1991). There is also a humoral response to HPV. Patients with chronic and/or multiple warts are much less likely to have IgG against HPV and studies have shown that cutaneous warts

were more likely to have spontaneous regression in patients with both elevated IgG and IgM antibodies than in patients with only IgM (Galloway and Jenison, 1990). However, the role of serologic tests in evaluating HPV infections is hampered by a number of observations that genital HPVs (and related neoplasms) can be recovered from a variety of non-genital sites, including the conjunctiva, subungual region, oropharynx and larynx (McDonnell *et al.*, 1989; Moy *et al.*, 1989). Coupled with the recovery of HPV-16 nucleic acids from the buccal mucosae of normal children, these observations imply a widespread distribution of HPV. Thus, the serological response may not indicate genital exposure (Dillner *et al.*, 1989). Study of the local immune response in the cervix may prove to be more fruitful. If isotype-specific immunoglobulins could be identified in cervical secretions for each specific HPV type, assays of local secretions could then become a unique predictor of virus-associated genital disease. However, there would be substantial issues to be addressed in the collection and testing of cervical secretions for evaluating virus-specific mucosal immunity, including sample volume and purity (e.g., absence of serum contamination), optimal phase of the menstrual cycle for sample collection, and standardization of amounts of immunoglobulin and protein in cervical secretions to be tested (Cowsert, 1994).

The immune response to HPV type-specific epitopes may, in part, be determined by specific HLA class II alleles and may influence the risk of cervical neoplasia in HPV infected individuals. Specific DR-DQ haplotypes have been shown to be significantly associated with either susceptibility or resistance to cervical carcinoma. DR-DQ analysis of patients with low-grade cervical dysplasia coupled with HPV typing of dysplastic lesions may help predict regression or progression of dysplasia to invasive carcinoma (Apple *et al.*, 1994).

## *3.5 Treatment*

### *3.5.1 Current therapies*

Evaluation of women with cervical HPV infection is largely dependent on the detection of cytological abnormalities in cervical scrapings (Pap smear), followed by colposcopy and target-directed biopsies to: 1) determine the location, size and distribution of the cervical lesions clinically and 2) determine whether the lesioin is a low-grade or high-grade SIL or cancerous by histopathology (Reid *et al.*, 1982; Shu *et al.*, 1987; Syrjanen, 1989; Syrjanen *et al.*, 1985; Toki and Yajima, 1987). Treatment of HPV-associated lesions of the vagina and external genitalia, such as condyloma acuminatum, can generally be eradicated by a variety of methods including chemodestruction with trichloroacetic acid, fluoro-uracil, podophyllin and/or interferon therapy. When the lesion extends into the endocervical canal and cannot be visualized by colposcopy, diagnostic endocervical curettage and/or conization is necessary. Treatment of HPV-associated lesions of the cervix is usually accomplished by obliteration of the entire transformation zone, usually by either cryotherapy or by laser, because up to 95% of invasive cancers of the anogenital tract develop within this T-zone (Cowsert, 1994). While this treatment has a good success rate, as great as 86%, the causative HPV appears to persist as a latent infection for the life of the individual. Finally, treatment of invasive cancer depends on the stage of disease, location in the anogenital tract, and includes radical surgery and radiotherapy (Cowsert, 1994). The multicentric nature of the disease has important implications in diagnosis and in the design of therapeutic treatment. Diagnostic tools must be capable of detecting multiple HPV types from one assay and development of treatment designed to be used locally or against one specific type might not prove effective. Instead of the currently used locally destructive treatments, a systemic therapy with future antiviral agents might have a better chance for complete eradication of the virus (Ji, 1991).

### *3.5.2 Prospects for the future*

While cytological screening with the Pap smear has contributed significantly in lowering the incidence of cervical cancer in the developed countries, such large scale screening has not proven possible in many of the third world countries where incidence of cervical cancer is still alarmingly high. Vaccination offers potential prevention and/or cure for HPV-associated papillomas of the mucosal epithelium. However, effective vaccination will have to utilize either type-specific structural or nonstructural antigens targeted to anogenital lesions that are at risk of malignant transformation. The majority of cervical carcinomas are associated with HPV-16. Thus, any vaccination program directed at preventing cervical cancer would have to include a vaccine against HPV-16 (Koss, 1987; Koutsky *et al.*, 1988; McCance, 1988). Using recombinant proteins, Campo and collaborators (1993) have developed prophylactic and therapeutic vaccines against bovine papillomavirus type 4 (BPV-4), a papillomavirus implicated in cancer of the alimentary canal. Both BPV-4 and HPV-16 infect mucus epithelia and give rise to lesions that can neoplastically transform. Both viruses have major transforming properties encoded by the E7 gene and the two E7 proteins have similar amino acid sequences. The authors demonstrated that immunization of cattle with BPV-4 E7 induced rejection of established tumours, accompanied by a strong cellular immune response. Protection against BPV-4 infection was also achieved by immunizing cattle with the L2 protein before compromising them to BPV-4. These are very encouraging results, illustrating that similar possibilities exist for protection against human mucosal papillomaviruses (Campo *et al.*, 1993).

Genetically based strategies may also play a role in revolutionizing HPV therapeutics. The antisense approach has been successfully applied to control HPV gene expression. The application of antisense RNA strategies to reversal of the transformed phenotype of cells derived from cervical cancers containing high-risk HPV have been successfully extended *in*

*vivo* (Von Knebel-Doeberitz *et al.*, 1992). A second antisense approach has been to develop oligonucleotides complementary to mRNA of critical viral genes. Anti-proliferative effects have been observed in all cases where oligonucleotides were targeted to E6/E7 RNAs (Lappalainen *et al.*, 1994; Smotkin *et al.*, 1989; Steele *et al.*, 1993; Storey *et al.*, 1991).

## 4 Epidemiology of Cervical HPV Infection

### *4.1 Prevalence of cervical HPV infection*

The epidemiological study of cervical HPV infection has been shaped and limited by available methods for diagnosing and defining infection (Schiffman and Schatzkin, 1994). Some inconsistencies emerged in early molecular epidemiology studies of HPV and cervical cancer that used first generation DNA hybridization methods to detect the virus. Contrary to expectations, these epidemiological studies were not able to find an association between cervical HPV infection and predictors of sexual activity (Kjaer *et al.*, 1990; Reeves *et al.*, 1989; Villa and Franco, 1989). These paradoxical findings, considering that cervical cancer risk is strongly associated with sexual behaviour, were subsequently explained by measurement errors of HPV status (Franco, 1991b; Franco, 1992a; Schiffman and Schatzkin, 1994). Techniques to detect the presence of HPV in cervical cells have evolved considerably from simple scoring of cytological signs of HPV to immunocytochemical staining, nucleic acid hybridization methods, and more recently, the polymerase chain reaction (PCR). Modern PCR protocols based on consensus primers have become the preferred methods for epidemiological studies in recent years, because of their high sensitivity and specificity (Gregoire *et al.*, 1989; Manos *et al.*, 1989; Van den Brule *et al.*, 1990).

The prevalence of HPV infection in cytologically normal women, as defined by PCR assays, is still in question (Lorincz *et al.*, 1992). The majority of recent molecular epidemiology studies using PCR have found the prevalence rates to vary from 15% to 40%,

peaking in women 15-25 years of age. Table V is a summary of current worldwide HPV DNA detection rates by PCR among women with negative Pap smears. Colposcopic examinations were not done in the majority of these studies, so there is no evidence on how many patients had actual lesions. Therefore, the reported point prevalence estimates comprise subclinical and latent disease.

Prevalence of HPV varies greatly in different populations, dependent on both demographic and behavioral determinants of infection. In particular, young, sexually active women appear to experience the highest HPV prevalences (Kjaer *et al.*, 1993; Ley *et al.*, 1991; Wheeler *et al.*, 1993). Older monogamous women are much less likely to be HPV positive (Melkert *et al.*, 1993; Muñoz *et al.*, 1993) (Table V). Prevalence also depends on the definition of HPV infection (Schiffman and Schatzkin, 1994). In one study, HPV DNA was detectable in 30%-40% of young sexually active women when consensus primer PCR, capable of detecting a wide spectrum of HPV types, was used to test cervical specimens. Dot blot testing of the same specimens yielded a much lower prevalence. Virtually none of the young women had cytological evidence of HPV infection (Bauer *et al.*, 1991).

Differing estimates may be attributed in part by variation in age distribution and risk factors for genital infection in the cohorts examined. Geographical factors may also play a role but the possibility of interlaboratory variation can not be dismissed (Kjaer and Jensen, 1992; Schiffman, 1992). Therefore, in describing HPV prevalence and the distribution of HPV infections worldwide, the population profile, the method of HPV detection and method of sample collection must also be defined.

The high prevalence of HPV in sexually active women aged 15-25 appears to describe an "epidemic curve", a rapid rise in prevalence following first (sexual) exposure.

**Table V**

**HPV DNA Detection Rates by PCR Among Cytologically Normal Women**

| References | Country | HPV + (%) | Population profile | HPV types tested |
|---|---|---|---|---|
| Van den Brule *et al.*, 1990 | Holland | 25.0 | Hospital | 6, 11, 16, 18, 31, 33, 51 |
| Hallam *et al.*, 1991 | England | 56.0 | Family clinic | 6, 11, 16, 18 |
| Ley *et al.*, 1991 | USA | 46.0 | University students (18-30 yrs) | 6, 11, 16, 18, 31, 33 |
| Rohan *et al.*, 1991 | Canada | 18.1 | University students (18-30 yrs) | 6, 11, 16, 18, 31, 33 |
| Czegledy *et al.*,1992 | Kenya | 19.5 | Family clinic | 6, 11, 16, 18, 31, 33 |
| Engels *et al.*, 1992 | Kenya | 3.7<br>16.5 | Family clinic<br>STD clinic | 6, 11, 16, 18 |
| Evander *et al.*, 1992 | Sweden | 20.3 | Young women | 6, 11, 16, 18, 31,33, 35, 39, 40, 45, 55, 56 |
| Vandenvelde & Vanbeers, 1992 | Belgium | 16.6 | Screening clinic | 6, 11, 16, 18 |
| Bauer *et al.*, 1993 | USA | 17.7 | Family planning clinic | 6, 11, 16, 18, 31, 33, 35, 39, 40, 45, 51, 52 |
| Bosch *et al.*, 1993 (Controls) | Spain<br>Colombia | 4.7<br>10.5 | Family clinics<br>Pap screenees | 6, 11, 16, 18, 31, 33 |
| Hansson *et al.*, 1993 | Sweden | 13.0 | (20-29 yrs) | 6, 11, 16, 18 |
| Hildesheim *et al.*, 1993 | USA | 33.7 | Medical assistance clinics | 6, 11, 16, 18, 31, 33, 35, 39, 40, 45, 55, 56 |
| Kjaer *et al.*, 1993 | Greenland<br>Denmark | 43.4<br>38.9 | General population | 6, 11, 16, 18, 31, 33 |
| Melkert, 1993 | Holland | 14.1<br>4.1 | (15-34 yrs)<br>(35-55 yrs) | 6, 11, 16, 18 |
| Tamayo *et al.*, 1993 | Spain | 5.3 | (13-45 yrs) | 6, 11, 16, 18, 31, 33 |
| Wheeler *et al.*, 1993 | USA | 44.3 | University students (18-30 yrs) | 6, 11, 16, 18, 31, 33, 35, 39, 40, 45, 55, 56 |
| Franco *et al.*, 1995 | Brazil | 18.3 | Asymptomatic women | 6, 11, 16, 18, 31, 33, 35, 39, 40, 45, 55, 56 |
| Present study, 1996 | Canada | 21.8 | University students (18-30 yrs) | 6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 45, 51, 52, 53, 55-59, 66, 68, PAP-155, W13B, 238A |

The peak in cervical HPV prevalence in 15-25 year olds in North America, corresponding to the usual age of initiation of sexual intercourse, might be analogous to the peak occurrence of plantar warts in children exposed to HPV 1 during first exposure to school gymnasium showers. The drop in cervical HPV infection might be due to immunologic clearance or suppression of existing infections, combined with less exposure to new HPV types because of fewer new sexual partners (Schiffman and Schatzkin, 1994).

In earlier studies, geographic differences in HPV prevalence did not correlate consistently with geographic differences in risk of cervical cancer. In two well-done surveys using different HPV test methods, Greenlandic women aged 20-39 had a lower prevalence of high-risk HPV than Danish women of the same age, despite the much higher Greenlandic population risk of cervical cancer (Kjaer and Jensen, 1992). In contrast to the still puzzling Greenlandic-Danish comparison, newer geographic correlation studies using sensitive testing methods to detect a wide spectrum of HPV types have observed HPV prevalence to "fit" the population risk of cervical cancer. In a comparison of Colombia and Spain, the Colombian women had higher HPV prevalence when measured by PCR, in accordance with an eight-fold higher risk of cancer (Muñoz *et al.*, 1992). In a pair of studies in the United States using PCR, a higher risk urban clinic population had higher HPV prevalence than a suburban middle-class population (Bauer *et al.*, 1993; Hildesheim *et al.*, 1993). More population surveys of cervical HPV prevalence in regions of known high or low cervical cancer risk are needed to establish whether HPV population prevalence is a reliable correlate of subsequent cervical cancer risk among that population. If the geographic correlations turn out to be weak (as in Greenland), it would suggest that variations in the geographic distribution of (unknown) etiologic cofactors, in addition to the prevalence of HPV infection itself, might be a key population determinant of cervical cancer risk (Schiffman and Schatzkin, 1994).

### *4.2 Risk factors for HPV infection*

Sexual activity and cervical HPV infection are two of the strongest predictors of cervical cancer and should seemingly be closely associated. In addition, considering the compelling evidence that HPV is the primary cause for cervical cancer, it follows that sexual activity should be the stronger predictor of cervical HPV, while cervical HPV infection should be the stronger predictor for cervical dysplasia. In the past, however, the association between sexual activity and HPV infection has not been easy to demonstrate, primarily because laboratory techniques were not sufficiently sensitive or specific enough to detect latent HPV infections within the general population. With the advent of the highly sensitive PCR system and the interdisciplinary marriage of molecular biology and epidemiology, a number of more recent molecular epidemiology surveys using PCR to detect the virus have unveiled the sexually transmitted profile of cervical HPV infection (Bauer *et al.*, 1993; Ley *et al.*, 1991). However, not all PCR-based studies conducted in different populations have been able to uniformly reproduce these results. Their findings indicate that the association between sexual activity and overall HPV prevalence can be either strong (Bauer *et al.*, 1993; Ley *et al.*, 1991), moderate (Franco *et al.*, 1995; Wheeler *et al.*, 1993), mild (Hildesheim *et al.*, 1993; Rohan *et al.*, 1991) or even non-existent (Kjaer *et al.*, 1993). Since misclassification of HPV is less of a concern in these studies (due to the use of the highly sensitive and specific PCR method), it is conceivable that the variability among results might be caused by differences across populations in the relative prevalence of HPV types with greater or lesser transmissibility by the sexual route. A recent cross-sectional study conducted in Northeastern Brazil provided evidence in this regard (Franco *et al.*, 1995). Infection with low oncogenic risk types was only weakly associated with sexual behaviour among women younger than 40, whereas sexual variables were strong predictors of infection with HPV types classified as of high oncogenic risk, regardless of age.

Risk of HPV infection also appears to be independently influenced by other variables, such as parity and oral contraceptive use (Bauer *et al.*, 1993). There is both direct and indirect evidence that hormonal influences (endocrine and exogenous) affect the risk of a woman contracting genital HPV infection. One plausible route for these influences may be through the attenuation of the immune response to the virus, thus facilitating propagation and persistence of cervical infection. It is known, for instance, that genital condylomas, tend to increase in size during the later stages of pregnancy (Koutsky *et al.*, 1988) and that the detection rate of asymptomatic HPV infection of the cervix tends to be higher in pregnant women (Schneider *et al.*, 1987b)). Use of oral contraceptives may affect propagation of the virus by a similar mechanism. Multiparity mayy also influence the acquisition of persistent HPV infection by providing cumulative opportunities for immunosuppression and breaches in the cervical epithelium (Franco, 1993). Hormonal binding may also affect the regulatory functions in the viral gene expression (zur Hausen, 1989).

It is not known whether the small number of HPV-negative tumours have a different etiological route or whether these tumours contain as-yet, unidentified HPV types. One possibility is that HPV-negative cancers are caused by a mechanism entirely dependent upon chemical carcinogens such as those in tobacco smoke. However, some studies have also revealed an association between smoking and risk of HPV infection (Bauer *et al.*, 1993), but it is very difficult to assess whether smoking is a surrogate for sexual activity or a co-factor that can influence the way in which HPV acts.

Diets may also play a role in the steps following acquisition of HPV infection. Vitamins C and A and β-carotene are important dietary components that are thought to counter the action of some carcinogens, either by reducing their electrophilic potential or by inducing normal maturation and differentiation of the cervical epithelium (Franco, 1993).

The evidence for the oncogenicity of HSV is convincing (Franco, 1991; Aurelian, 1989), and a synergistic interaction between HPV and HSV may exist in cervical carcinogenesis (zur hausen, 1982; Hildesheim *et al.*, 1991). HSV may cause mutations and genomic recombinations, much like a chemical carcinogen, which may affect HPV genes and other regulatory elements in host chromosomes (Franco, 1991).

However, by far the most important determinant in risk of HPV infection is age, with most studies indicating a sharp decrease in prevalence after age 30. While age has been shown to be strongly and positively associated with increasing lifetime number of sexual partners in at least one study (Ley et al., 1991), the decrease in HPV infection risk with increasing age has also been shown to be independent of sexual activity (Bauer *et al.,* 1993; Wheeler *et al.,* 1993) and, at least in certain populations, restricted to low oncogenic risk types (Franco *et al.,* 1995). The decrease of HPV prevalence with age could be suggestive that many HPV infections are transient. Alternatively, the decrease in HPV prevalence with increasing age may reflect a cohort effect, in other words, that HPV prevalence is increasing (Ley *et al.*, 1991).

# CHAPTER 3

# OBJECTIVES

## OBJECTIVES

Given that (i) not all studies have been able to show a strong association between sexual activity and cervical HPV infection and (ii) more recent findings have shown that different HPV types appear to have different degrees of association with sexual transmission, the objectives of this study were to:

1. Estimate the point prevalence of cervical HPV infection, overall and type-specific, in Montreal university students.
2. Identify risk factors for overall, low-risk and high-risk HPV infection.

# CHAPTER 4

# MATERIALS AND METHODS

# MATERIALS AND METHODS

## 1 Study Design

### *1.1 Subjects*

The study was a cross-sectional survey at the McGill university student health clinic to determine the prevalence of cervical HPV infection in asymptomatic women. Asymptomatic women presenting themselves at the clinic for a routine Pap smear during a one year period from November 1992 to November 1993 were eligible. All the eligible students were informed about the study and those who agreed to participate were asked to sign a consent form and complete a personal data sheet and questionnaire (Appendices 1 - 3). The questionnaire was based on previous questionnaires used by our research group, designed to probe a detailed list of variables. Access to their Pap smear cytology was made available after the participants agreed to sign a consent form.

### *1.2 Data collection*

Epidemiological data on risk factors such as socio-demographic status and education, smoking history, sexual behaviour, reproductive history, contraceptive and medical history and personal hygiene was obtained from a detailed questionnaire that the women were asked to complete at the clinic while waiting for their appointment or at home, if they preferred, in which case it was then returned by mail (postage prepaid). Those women who did not return the questionnaire within two months were reminded by telephone and then a new questionnaire was sent to them.

### *1.3 Sample collection*

To detect human papillomavirus infection with maximum sensitivity, cells must be collected and assayed for HPV DNA. Cervicovaginal lavage and cervical cell scrapes are two methods employed for sampling exfoliated cervical cells. Cervicovaginal lavage appears to be the more sensitive collection method for exfoliated cervicovaginal cells for the detection of

HPV. However, it has been suggested that the lavage method might also collect vaginal cells. Therefore, epidemiological studies investigating cervical HPV infections generally use the more conservative cervical cell scrape method to ensure no misclassification from the possible detection of vaginal HPV infections (Goldberg *et al.*, 1989; Morrison *et al.*, 1992). The doctors at the McGill clinic employed the scrape technique using a wooden spatula to obtain the ecto-cervical cells and a cytobrush to reach the endo-cervical cells. Both instruments were then placed in 2 ml of TE (10 mM Tris-HCl, pH 7.4, and 1 mM EDTA). The spatula was discarded shortly afterwards while the cytobrush remained in the buffer at 4°C (for no longer than one week, until samples were processed) (Bauer *et al.*, 1991).

## 2 Laboratory Methods

### *2.1 Specimens*

#### *2.1.1 Clinical samples*

The sample with the cytobrush was vortexed and then divided in two 1 ml aliquots into 1.5 ml eppendorf microtubes. Cellular DNA was isolated from the first tube by digestion of cells while the second tube was kept at -20°C as a posterity sample. The cellular suspension was digested with proteinase K (100 μg/ml, final concentration) in the presence of TE (pH 7.8) and sodium dodecyl sulfate (SDS) (0.5%, final concentration). The sample was left to incubate at 56°C for 2-3 hours to permit the digestion of cellular proteins. The proteinase K was then inactivated by heating the sample for 10 minutes at 100°C (Goldenberger *et al.*, 1995).

The drawback to using SDS in the cellular digestion protocol is its strong inhibitory effect on *Taq* polymerase (Gelsand, 1989). Therefore, due to the use of SDS and the presence of blood in certain samples, (blood too can inhibit PCR), the sample DNA was extracted, to ensure the elmination of both inhibitors. Equal volumes of digested sample and phenol were vortexed for 1 minute and then centrifuged for 3 minutes. The aqueous layer

was kept while the organic phase was discarded. A mixture of phenol/chloroform (1:1) was added to the aqueous phase and the mixture was vortexed for 1 minute and then centrifuged for 3 minutes. The aqueous layer was kept and an equal volume of chloroform/isopropanol (24:1) was then added and the mixture was vortexed for 1 minute and, once again, centrifuged for 3 minutes. The extracted DNA was then precipitated from the aqueous phase with the addition of 0.1 volume of 3 M sodium citrate and 2.5 volume of 100% cold ethanol. Precipitation took place at -70°C for 1 hour or overnight at -20°C. The sample was then centrifuged for 30 minutes at 4°C after which the ethanol was decanted. The DNA pellet was washed with 1 ml of 70% cold ethanol and centrifuged for another 15 minutes at room temperature. The ethanol was then discarded and the pellet was left to dry overnight on the lab bench. The DNA was then resuspended in 50 µl of TE and stored at -20°C (Sambrook *et al.*, 1989). The DNA could not be quantified because neither a fluorometer nor the new BRL quantification system, which could measure a highly diluted sample volume, was available for laboratory use.

### *2.1.2 Controls*

The two cell lines that served as controls were HUT 78 (ATCC TIB-161) and the SiHa cell line (ATCC HTB-35). The HUT cells are derived from the peripheral blood of a 50 year-old patient with Sezary syndrome. This line has properties of a mature T cell line with inducer/helper phenotype (Chen, 1992; Gootenberg *et al.*, 1981) and was used as a positive control for the detection of a 268 base pair (bp) human DNA fragment, part of the gene coding for β-globin.

The SiHa cells (squamous carcinoma, cervix) were established from fragments of a primary tissue sample obtained after surgery from a patient in Japan. SiHa cells have been reported to contain an integrated HPV 16 at 1 to 2 copies per cell (Baker *et al.*, 1987). This cell line was used as a positive control for the detection of a 450 bp HPV DNA fragment.

### *2.1.3 Cell cultures*

Both cell lines had to be cultured and harvested. Lymphocytes were cultured in RPMI 1640 (Gibco BRL) supplemented with fetal calf serum (FCS, 10%) (Gibco BRL) streptomycin (50 µg/ml) and penicillin (100 IU/ml). SiHa cells were grown in Dulbecco's modified eagle medium (DMEM; Gibco BRL), containing FCS (10%), streptomycin (50 µg/ml) and penicillin (100 IU/ml). Both cell lines were incubated at 37°C in an atmosphere of 5% $CO_2$ and divided every 4-5 days. The HUT cells were split by diluting 10 ml of the cell suspension with 40 ml of fresh medium in a 75 $cm^2$ flask. Because SiHa cells adhere strongly to the surface of the flask, they had to be trypsinized before splitting them. The growth medium was removed and the cell monolayer was washed with 5 ml of phosphate buffer saline (pH 7.4) (PBS), both $Ca^{2+}$ and $Mg^{2+}$ free. Two ml of 0.25% trypsin (Gibco BRL) was then added to the flask for a few minutes at 37°C, enabling the cells to detach from the surface of the flask. One-fifth of the volume of the cell suspension was then transferred to a new flask containing 40 ml of fresh complete medium (DMEM) (Sambrook *et al.*, 1989).

Cells were counted with a hemocytometer and resuspended to a concentration of $10^6$/ml. One ml of the resuspended cells was then centrifuged at 12,000 rpm (IEC, Micromax Centrifuge) for 2 minutes. The cell pellet was digested and extracted in the same manner as the samples, and then used to refine the sensitivity of the PCR protocol while also serving as a control.

## *2.2 HPV DNA amplification*

### *2.2.1 PCR*

The HPV detection system described here is a highly sensitive DNA amplification method that uses a mixture of degenerate primers, MY09 and MY11, to amplify a broad spectrum of HPV types (Bauer *et al.*, 1991; Manos *et al.*, 1989) (Figure 2). A third primer,

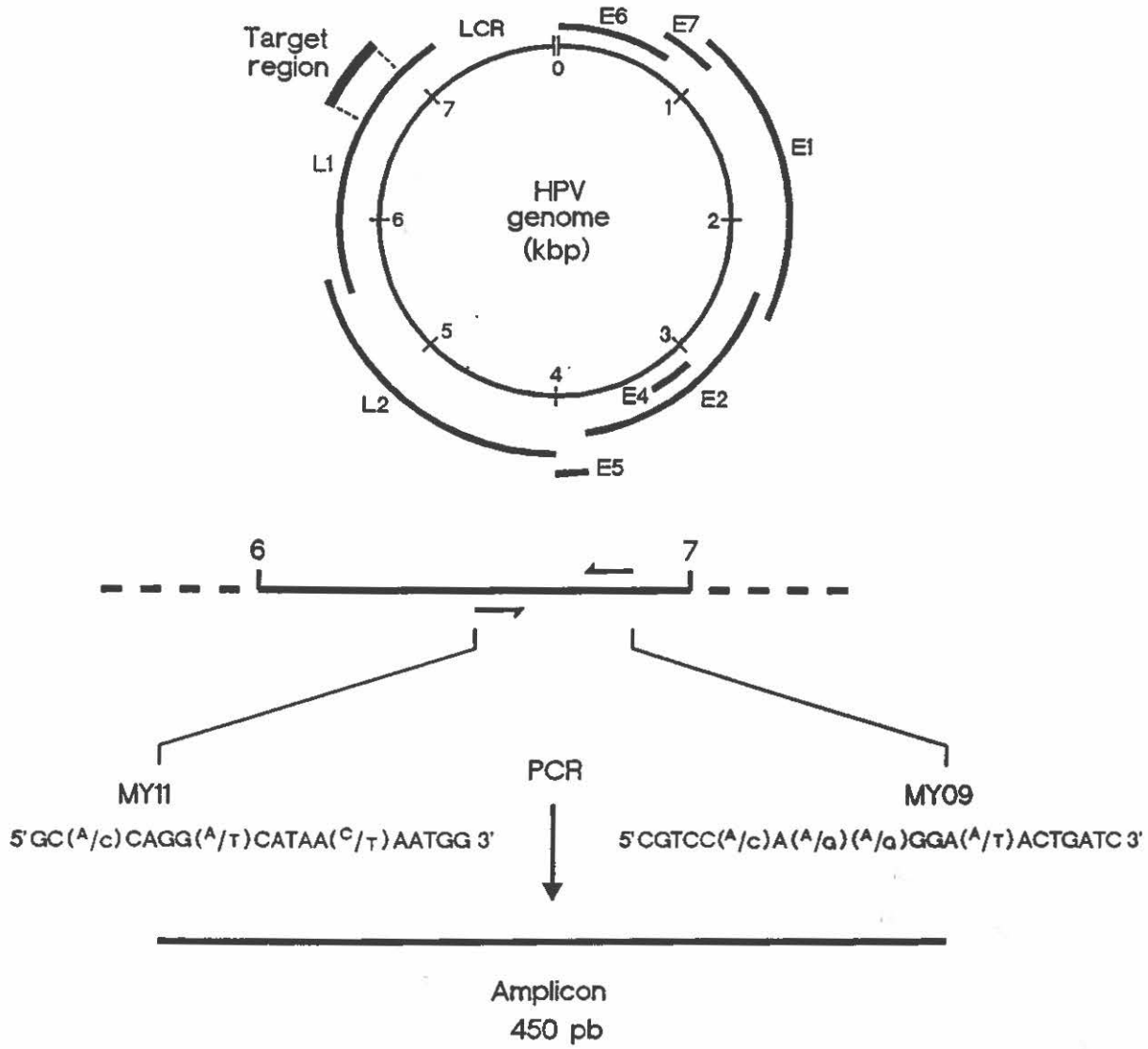


**Figure 2**

Amplification strategy

Genomic organization map of HPV genome showing the 450 bp target fragment within the L1 region; amplified with MY09/MY11 consensus primers

analogous to MY09, HMB01, is added to enable detection of HPV 51 (Hildesheim *et al.*, 1994). These primers target the highly conserved late region 1 (L1) of HPV which encodes a viral capsid protein, amplifying a 450 bp fragment. A β-globin detection system was also employed as an internal control to ensure the quality of the DNA samples. Human β-globin can be detected with specific primers, GH20 and PC04, which target a 268 bp region of the gene (Saiki *et al.*, 1985). If human β-globin could not be detected in the extractedsamples, the DNA sample was considered inadequate for further HPV testing.

Traditionally, PCR is performed by combining all reagents, the DNA template and the *Taq* polymerase. This cocktail then undergoes 35 cycles of DNA denaturation, primer annealing and nucleotide extension with the aid of the enzyme, *Taq* polymerase (Saiki *et al.*, 1988). Unfortunately, if the *Taq* is added at a lower temperature (i.e. room temperature), as is the case with the conventional PCR protocol, there is an increased chance that the hybridized primer will elongate nonspecifically. The nonspecific products can then compete for the formation of the specific products and reduce the sensitivity of the entire reaction (Hébert *et al.*, 1993). Hot start PCR is a recently developed technique that increases the sensitivity and specificity of DNA amplification (D'Aquila *et al.*, 1991; Erlich *et al.*, 1991), because, unlike the traditional PCR method, the *Taq* polymerase is only added once the reaction has reached a stringent temperature. However, hot start PCR requires the preparation of two cocktails instead of one, which means more laboratory manipulations and an increased possibility of DNA crossover contamination. Because of this extreme sensitivity, many precautions had to be taken to avoid contamination such as the use of positive displacement pipette tips, when preparing the PCR cocktails, or the use of plugged tips when pipetting sample DNA and the "second" PCR mixture following hot start. The "first" and "second" PCR cocktails were prepared in a laboratory designated as the "DNA-free lab", where all PCR reagents were mixed but no sample DNA ever entered. The first mixture consisted of 23.5 µl distilled sterilized water, 1 µl of nucleotides (dATP, dCTP, dGTP, dTTP, final

concentration 200 μM) and 0.5 μl Tween 20 (1% final concentration). It was aliquoted into PCR tubes and layered with 2 drops of light mineral oil. The PCR caps were closed and tubes were transported to a second lab where 1 μl (100-500 ng) of the sample DNA was added to its respective tube in a chemical hood. This procedure was repeated until all DNA samples were added to their respective tubes. The tubes were then placed in the thermocycler (Perkin-Elmer Canada Ltd.) and the DNA was denatured at 95°C for 5 minutes ("hot start"). The temperature was then programmed to decrease to 72°C and the second PCR mixture, consisting of 15.75 μl distilled, sterilized water, 5 μl 10x*Taq* PCR buffer (100 mM Tris-HCl, pH 8.5, 500 mM KCl, 15 mM $MgCl_2$), 1 μl MY09 (100 pmoles), 1 μl MY11 (100 pmoles), 1 μl HMB01 (10 pmoles), and 0.25 μl *Taq* DNA polymerase (1.25 units; Bio/Can Scientific, Canada), was added, one tube at a time (closing the cap before opening the following tube). Once the second mixture was added to all tubes, the selected program for the MY09/MY11 protocol was implemented as follows: 35 cycles of 95°C x 1 minute (denaturation), 72°C x 1 minute (annealing), 54°C x 1 minute (extension). This was followed by a primer extension at 72°C for 10 minutes and then left to soak at 4°C (Bauer *et al.*, 1991).

### *2.2.2 Refining the PCR protocol*

Extensive experimentation went into refining the final PCR protocol. There was the initial difficulty of detecting β-globin in the sample DNA followed by the need to increase the sensitivity of both systems (HPV & β-globin) so that as few as 10 SiHa cells or lymphocytes could be detected after hybridization with α-$P^{32}$dCTP labeled probe. Various parameters were examined including the concentration of $MgCl_2$, the concentration of primers in the PCR cocktail, type of *Taq* polymerase, hot start vs traditional PCR, oil versus wax and the addition of Tween 20 (Table VI & Figures 3, 4, 5, 6).

**Table VI**

**Parameters Tested to Refine the MY09/11 PCR Protocol**

| Tested Parameter | Original Protocol | Tested Protocol | Tested Sample | Effect on Sensitivity | Effect on Specificity | Reference |
|---|---|---|---|---|---|---|
| PCR | Traditional | Hot start | SiHa (10-$10^6$ cells) | +++ | | D'Aquila *et al.*, (1991) Erlich *et al.*, (1991) Mullis (1991) |
| Detergent for β-globin amplification | Absent | Tween 20 1% final | Sample DNA | +++ (Tween 20 decreased the background) | | Kawasaki, (1990) Goldenberger *et al.*, (1995) |
| Cocktail evaporation barrier | Light mineral oil | Wax pellets | SiHa cells | - - -* * Wax pellets appeared contaminated | - - -* | Hébert *et al.*, (1993) |
| [$MgCl_2$] + [MY09/11] | $MgCl_2$ **(4 mM)** + MY09/11 **(50 mM)** | $MgCl_2$ **(1.5 mM)** + MY09/11 **(50** mM) | SiHa cells & Sample DNA | - - - | + | Manos *et al.*, (1989) Saiki (1989) |
| | | $MgCl_2$ **(1.5 mM)** + MY09/11 **(200 mM)** | SiHa cells & Sample DNA | + | + | |
| | | $MgCl_2$ **(3 mM)** + MY09/11 **(50 mM)** | SiHa cells & Sample DNA | + + | - - - | Ley *et al.*, (1991) |
| | | $MgCl_2$ **(3 mM)** + MY09/11 **(200 mM)** | SiHa cells & Sample DNA | + + | - - - | |

HPV L1

MY09: CGT CC(A/C) A(A/G)(A/G) GGA (A/T)A CTG ATC (-)
MY11: GC(A/C) CAG G(A/T)C ATA A(C/T)A ATG G (+)
HMB01: GCGACCCAATGCAAATTGGT

β-globin

GH20: GAA GAG CCA AGG ACA GGT AC (+)
PC04: CAA CTT CAT CCA CGT TCA CC (-)

HPV 16

MY74: CAT TTG TTG GGG TAA CCA AC (+)
MY75: TAG GTC TGC AGA AAA CTT TTC (-)

HPV18

MY76: TGT TTG CTG GCA TAA TCA AT (+)
MY77: TAA GTC TAA AGA AAA CTT TTC (-)

HPV31

MY49: TAT TTG TTG GGG CAA TCA G (+)
MY50: CTA AAT CTG CAG AAA ACT TTT (-)

HPV51

CEG03: CAT TTG CTG GAA CAA TCA G (+)
CEG04: TAA ATC TAA AGA AAA TCG TTC CT (-)

HPV66 (PAP88)

MY47: CAT ATG CTG GGG TAA TCA GG (+)
MY48: CAG GTC TGC AGA AAA GCT GT (-)

**Figure 3:**
Oligonucleotide Primers for PCR

Type specific primers used to amplify required fragments for the hybridization generic probe
Source: Bauer *et al.*, 1991

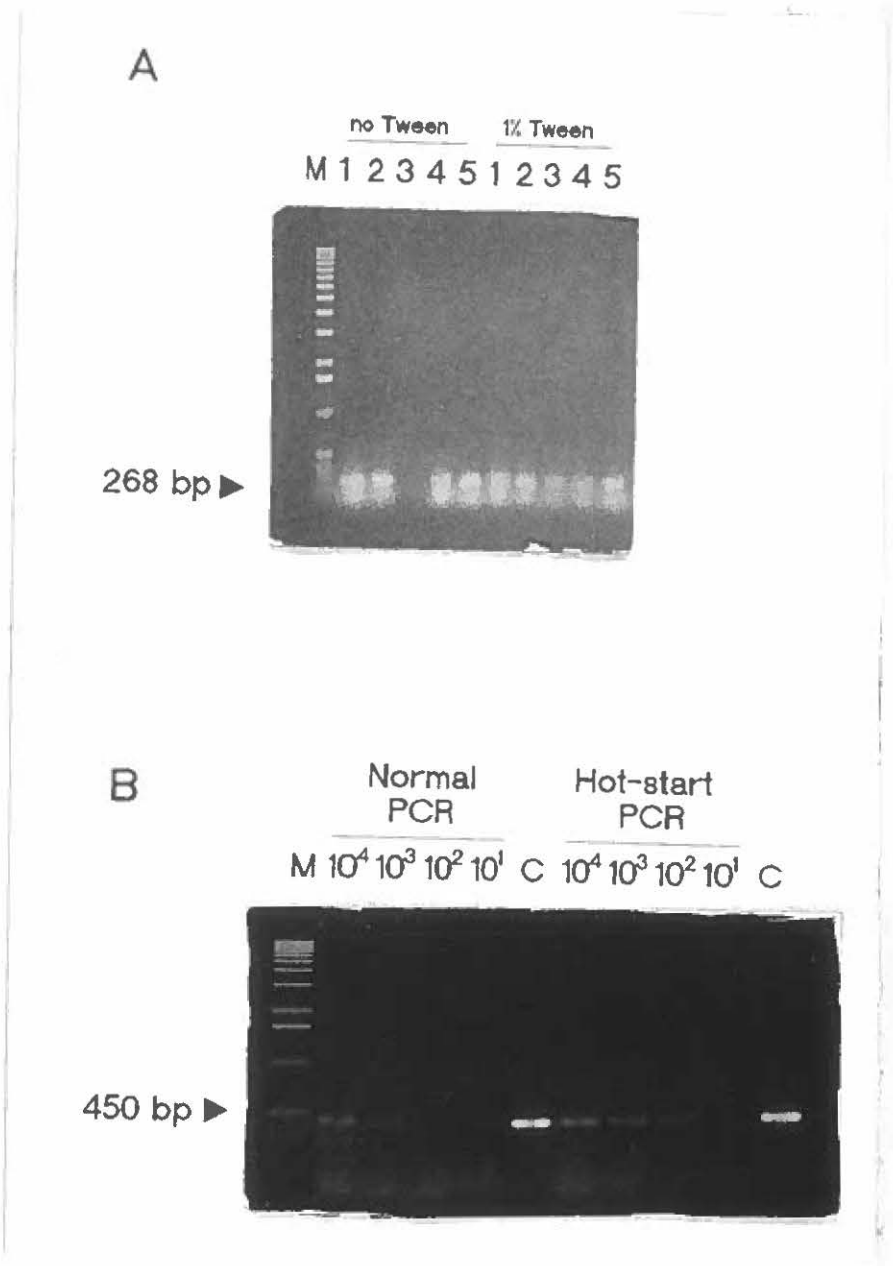


**Figure 4:**
PCR protocol refinement (I)

A) Amplification of β-globin positive samples with and without 1% Tween 20
B) Comparison of normal and hot start PCR, amplifying $10^4$ to $10^1$ SiHa cells with MY09/MY11.

*C=Control (HPV 16 plasmid)*

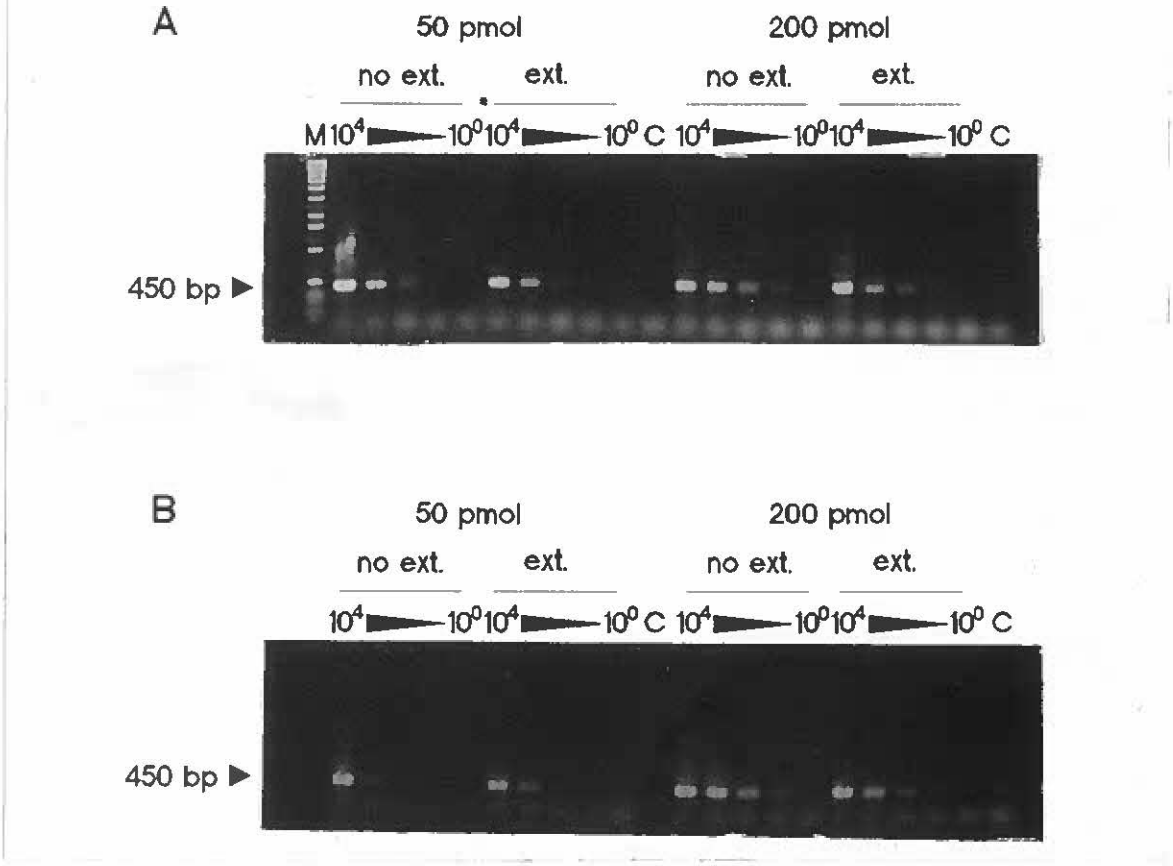


**Figure 5:**
PCR protocol refinement (II)

Extracted (ext.) and non-extracted (no ext.) DNA from $10^4$ to $10^0$ SiHa cells in the presence of 50 pmoles or 200 pmoles of MY09/MY11 and:
A) 1.5 mM $MgCl_2$
B) 3.0 mM $MgCl_2$
*C=Control (No DNA)*

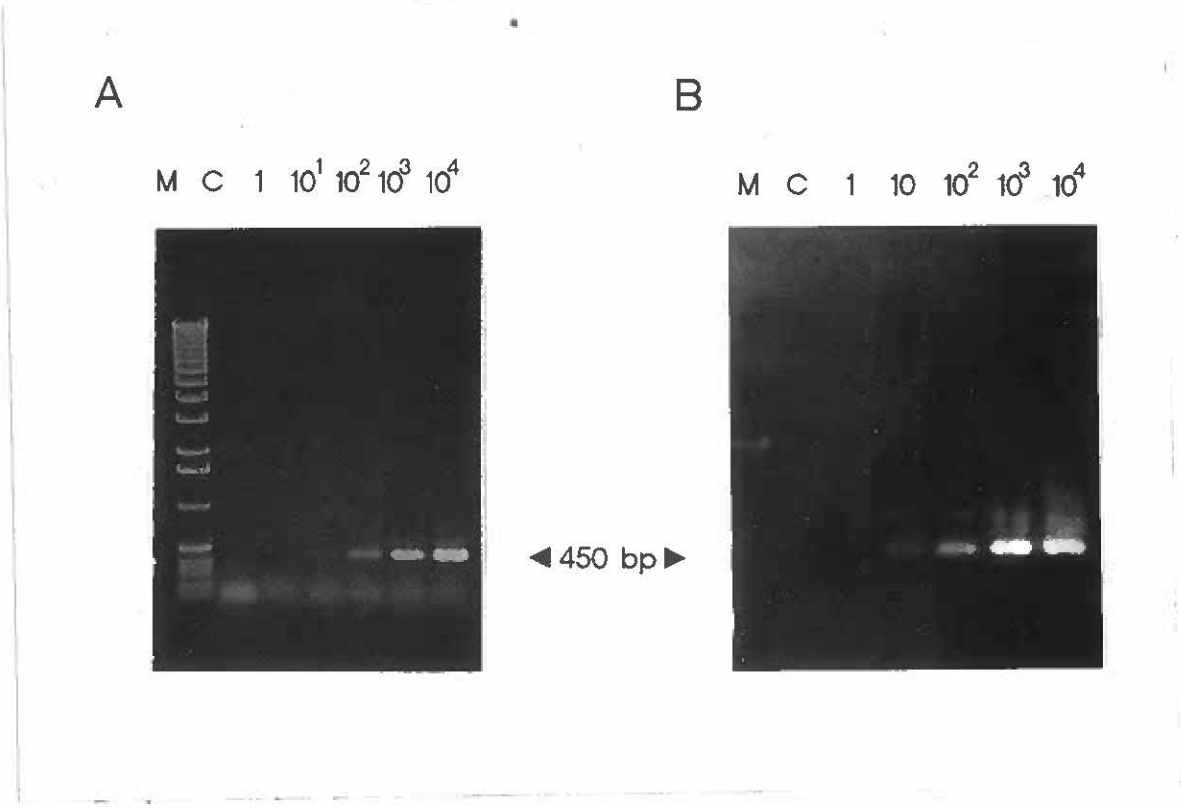


**Figure 6:**
Detection limits of HPV PCR products

Ten µl of PCR product (total volume 50 µl) of $10^4$ to $10^0$ SiHa cells were:
A) loaded onto a 0.8% agarose gel (gel electrophoresis) and then
B) hybridized with $P^{32}$-labeled HPV generic probe (Southern blot)

The system that finally worked best in our laboratory, providing us with an acceptable level of sensitivity (10 copies of HPV), was the combined use of hot start PCR (Hébert et al., 1993) and an enhancer, Tween 20 (1% final concentration) (Kawasaki, 1990; Greenberger *et al.*, 1995). However, while a sensitive system is very important in these types of epidemiological studies, it is also important to achieve high specificity. This, unfortunately, is not possible without some loss of sensitivity. Increasing the concentration of $MgCl_2$ naturally increases the sensitivity of the PCR system because it acts as a cofactor for the *Taq* polymerase. The $MgCl_2$ concentration may be varied from approximately 0.5 mM to 5 mM before obtaining the optimum concentration. $Mg^{2+}$ influences enzyme activity, increases the melting temperature (Tm) of the double-stranded DNA and forms soluble complexes with dNTP which is essential for dNTP incorporation. However, it may do so at the cost of increased background and nonspecific DNA amplification (Saiki *et al.*, 1988). While other investigators have achieved satisfactory results with $MgCl_2$ concentrations of 2.0 mM (Manos *et al.*, 1989) and 4.0 mM (Bauer *et al.*, 1991) with 50 pmoles of MY09/11, our best results were achieved with the combined final concentrations of $MgCl_2$ (1.5 mM) and MY09/11 (200 pmoles), which provided sufficient sensitivity but did not produce high levels of background.

### *2.3 Gel electrophoresis*

PCR products (10 µl) were loaded onto an agarose gel (0.8%) and left to migrate for 30 minutes at 100 volts in Tris acetic EDTA, (1x TAE buffer). The DNA was then stained by soaking the gel in an ethidium bromide (EtBr), solution (0.5 µg/ml) for 30 minutes and subsequently visualized and photographed under ultra-violet light (Sambrook *et al.*, 1989).

### *2.4 Southern transfer*

The DNA was transferred from the gel onto a nylon membrane with the vacu blot apparatus (Pharmacia LKB Biotechnology Inc., Piscataway, N.J., USA). Under vacuum,

buffer was drawn from an upper reservoir, eluting nucleic acids from the gel and depositing them onto the membrane. Briefly, the gel was placed under a vacuum of 50 mBar and saturated with a denaturing solution consisting of NaOH (0.5 N) and NaCl (0.015 M) for 15 minutes. The remaining buffer was then removed and replaced with a neutralizing solution made up of Tris-HCl (1 M, pH 7.4) and NaCl (1.5 M). After 15 minutes the buffer was removed and the gel was saturated with 20x SSC for 45 minutes, after which the gel was discarded and the DNA was immobilized onto the membrane (cross-linked) with ultra-violet irradiation for 5 minutes. A biotrans 66 nylon membrane, 0.45 μm (ICN Biomedicals inc., Irvine, Calif., USA), pre-soaked in 10x SSC was used as the solid support (Sambrook *et al.*, 1989).

### *2.5 Dot blot transfer*

The dot blot transfer of the amplified PCR products was only used for the typing of the HPV samples. The first step in the dot blot transfer was to denature the PCR products into single strands. The PCR product (2 μl) was combined with 100 μl of denaturing solution (NaOH 1.5 N and EDTA 0.1 M). A nylon membrane, pre-soaked in 2x SSC was fitted into the 96 wells manifold (Biorad) and vacuum was applied. As many as 96 samples could be loaded onto the membrane. The denatured PCR products (100 μl) were placed in their respective wells (96 in total) and aspirated, after which, the vacuum was turned off and the excess liquid was absorbed with Whatman 3 MM paper. The DNA was then UV cross-linked for 5 minutes as previously described.

### *2.6 Preparation of probes*

#### *2.6.1 First generation of generic probes*

Cloned HPV plasmids 16, 18 and 31 provided by E.-M. de Villiers, and typed samples 51 and 66, provided by L.L Villa, were amplified with type-specific primers MY74/75, MY76/77, MY49/50, CEG03/04 and MY47/48 respectively (Figure 3). These

primers all targeted a variety of smaller fragments, approximately 400 bp, internal to the L1 450 bp fragment (Bauer *et al.*, 1991). Purified plasmid DNA (1 ng) was amplified in a 100 µl PCR reaction as previously described. The 400 bp amplicons were then purified from the excess primer and dNTPs with Magic PCR prep (Promega), according to the manufacturer's specifications. The concentration of the purified amplicons was estimated with a U.V spectrophotometer (Perkin Elmer Coleman 55) at 260 nm (1 $O.D._{260\ nm}$ = 50 µg/ml). The DNA was diluted with water to a final concentration of 100 ng/µl for further labeling.

The first generation of generic probes consisted of a pool of type-specific 400 bp PCR products of HPV 16, 18, 31, 51 & 66 (Bauer *et al.*, 1991). The probe was $\alpha$-$P^{32}$ labeled with the Random Primer DNA Labeling system (BRL-Life Technologies). The collection of PCR products (100 ng each) was combined and the final volume was completed to 23 µl with distilled water. The DNA was boiled for 5 minutes and then cool on ice for 5 minutes. The denatured DNA was then combined with 15 µl random primers buffer (Life Technologies), 2 µl of each dNTP (dATP, dGTP, dTTP) and 5 µl of $\alpha$-$P^{32}$dCTP (specific activity > 3000 Ci/mmol; 10 µCi/µl) which was added on ice. The Klenow fragment of *E.coli* DNA polymerase I was added last (1 µl) and the reaction took place at 25°C for 60 to 90 minutes.

The $\alpha$-$P^{32}$ incorporation was then estimated with a liquid scintillation analyzer (Tri-Carb 2200 CA, Canberra Packard Canada). Briefly, 1 µl of the labeled probe was precipitated onto a filter with 5% trichloroacetic acid (TCA), the filter was placed in a scintillation tube with 5 ml of scintillation fluid (CytoScinct™ ICN Biomedical, CA.) and then estimated with the liquid scintillation analyzer. Approximately $10^6$/cpm/ml of hybridization solution was required for an adequate signal.

### *2.6.2 Second generation of generic probes*

In refining the hybridization protocol, it became apparent that the first generic probe developed did not detect a wide enough spectrum of HPV types. Some samples that were amplified by PCR and were clearly positive on the gel failed to hybridize with the first generic probe. These amplicons were subsequently cloned and sequenced (section 2.8). Plasmids containing the 450 bp L1 fragment of HPV types 6/11, 33 and PAP155 were re-amplified with MY09/11. After purification with Magic PCR Prep these amplicons were diluted to 100 ng/µl and added to the generic probe prior to labeling. However, because these additional types were only amplified with MY09/11 and not also amplified with type-specific primers amplifying a smaller 400 bp fragment, the generic probe could only be used on a southern blot where the primer dimers had already been separated out from the actual fragment. If this type of generic probe had been used with a dot blot, many of the samples would have appeared positive because of non-specific annealing with the primer dimers not separated out in the dot blot.

### *2.6.3 β-globin probe*

After amplification, samples that were β-globin negative on gel were then hybridized with a β-globin probe. The probe was developed using the same approach as was used for the preparation of the generic probe. In brief, a 268 bp region of the β-globin gene from human lymphocytes was amplified with the primers GH20 and PC04 (fig. 3). The PCR product was then purified with Magic PCR Prep and 100ng was labeled with the Random Primers DNA Labeling system and $\alpha$-$P^{32}$dCTP

### *2.6.4 Type-specific oligonucleotide probes*

A T4 polynucleotide kinase (T4PNK) reaction was established to label the type-specific oligonucleotides (Figure 7). In a microtube, 1 µl of each oligonucleotide (1

| | |
|---|---|
| HPV6/11: | [MY12] CAT CCG TAA CTA CAT CTT CCA |
| | [MY13] TCT GTG TCT AAA TCT GCT ACA |
| | [MY125] ACA ATG AAT CCY TCT GTT TTG G |
| HPV16: | [MY95] GAT ATG GCA GCA CAT AAT GAC |
| | [MY133] GTA ACA TCC CAG GCA ATT G |
| HPV18: | [MY130] GGG CAA TAT GAT GCT ACC AAT |
| | [WD74] GGA TGC TGC ACC GGC TGA |
| HPV26: | [MY186] GCT GAC AGG TAG TAG CAG AGT T |
| | [MY187] GCC ATA ACA TCT GTT GTA AGT G |
| HPV31: | [MY92] CCA AAA GCC YAA GGA AGA TC |
| | [MY143] TTG CAA ACA GTG ATA CTA CAT T |
| HPV33: | [MY16] CAC ACA AGT AAC TAG TGA CAG |
| | [MY64] TCC TTT GGA GGT ACT GTT TTT |
| HPV35: | [MY115] CTG CTG TGT CTT CTA GTG ACA G |
| | [MY117] ATC ATC TTT AGG TTT TGG TGC |
| HPV39: | [MY89] TAG AGT CTT CCA TAC CTT CTA C |
| | [MY90] AGA CAC TTA CAG ATA CCT ACA G |
| HPV40: | [MY176] CCC AAG GTA CGG GAG GAT CC |
| HPV42: | [MY34] GGC TAA GGT AAC AAC GCC |
| | [MY121] CAC TGC AAC ATC TGG TGA T |
| HPV45: | [MY69] ATA CTA CAC CTC CAG AAA AGC |
| | [MY129] GCA CAG GAT TTT GTG TAG AG |
| HPV51: | [MY87] TAT TAG CAC TGC CAC TGC TG |
| | [MY88] CCC AAC ATT TAC TCC AAG TAA C |
| HPV52: | [MY81] CAC TTC TAC TGC TAT AAC TTG T |
| | [MY82] ACA CAC CAC CTA AAG GAA AGG |
| HPV53: | [MY102] TTC TAC CTT ACT GGA AGA CTG G |
| | [MY182] GCA ACC ACA CAG TCT ATG TC |
| HPV54: | [MY160] CAG CAT CCA CGC AGG ATA G |
| | [MY161] GAA TAA TGC CCC TGC AAA G |
| HPV55: | [MY151] GTG CTG CTA CAA CTC AGT CT |
| | [MY171] CCC TGA AAA GGC AAA GCA G |
| HPV56: | [MY197] GCA CAG CTA TAA CAT GTC AAC G |
| | [MY199] CAG TTA AGT AAA TAT GAT GCA CG |
| HPV57: | [MY154] AAT GTC TCT TTG TGT GCC AC |
| | [MY156] GGA TCA GTA GGG GTC TTA GG |
| HPV58: | [MY94] AGC ACC CCC TAA AGA AAA GGA |
| | [MY179] GAC ATT ATG CAC TGA AGT AAC TAA G |
| HPV59: | [MY123] GCC AGT TAA ACA GGA CCC |
| | [MY162] CCT AAT GWA TAC ACA CCT ACC AG |
| HPV66: | [MY83] ATT AAT GCA GCT AAA AGC ACA TT |
| | [MY178] CAT GTC AGA GGG AAC AGC C |
| HPV68: | [MY191] CAT ACC GCT ATC TGC AAT CAG |
| | [MY194] CTA CTA CTG AAT CAG CTG TAC C |
| PAP155: | [MY85] CCA ACA CCG AAT CAG AAT ATA AA |
| | [MY163] GTT GTG CCC CCT CCC TCC A |
| PAP238A: | [MY104] GTA GGT ACA CAG GCT AGT AGC TC |
| | [MY106] AGT TGC CAA CGT CCT CAA C |
| PAP291: | [MY166] GGC TAA TGA ATA CAC AGC CTC |
| | [MY167] TCC TTC CAC CAG CCT TGA T |
| W13B: | [MY164] CTC AAT CTG TTG CAC AAA CA |
| | [MY165] TAA CCT TGC CCC CCT CAG |

**Figure 7:**
Sequences of Oligonucleotide Probes used to Identify Specific HPV Types

Source: Hildesheim *et al.*, 1993

pmole/µl), 2 µl of 10x T4 polynucleotide kinase buffer (Pharmacia), 10 µl of distilled water, 1 µl of T4PNK (8 U/µl) (Pharmacia) and 5 µl of $\gamma$-$P^{32}$ATP (specific activity 4500 Ci/mmol ; 10 µCi/µl) (ICN) were combined. The mixture was then left to incubate at 37°C for 30 to 60 minutes before adding 1 µl of 0.5 M EDTA stop solution (Ausubel *et al.*, 1989). The radioactive incorporation, in a final volume of 21 µl ,was then estimated with the scintillation counter and $10^5$ cpm/ml (final) was used for hybridization.

## *2.7 Hybridization*

### *2.7.1 Hybridization with generic HPV probe*

The following technique is described in Molecular cloning: A laboratory manual (Sambrook *et al.*, 1989). All PCR products were transferred onto nylon membranes with a Southern blot apparatus and incubated with 30 ml pre-hybridization solution (6x SSC, 5x Denhardt's reagent, 0.5% SDS, 100 µg/ml denatured, fragmented salmon sperm DNA and 50% formamide), for a minimum of 2 hours at 42°C. The membrane and pre-hybridization solution was placed in a glass tube and incubated in a rotary hybridizing incubator HI-12000 (Tyler Research Instruments Inc.). The radioactive generic probe was denatured at 100°C for 5 minutes and cooled on ice for another 5 minutes. It was then added ($10^6$ cpm/ml) directly to the pre-hybridization solution and left to incubate overnight at 42°C. The probe was subsequently discarded and the membrane was washed twice with 2x SSC, 0.1% SDS at 60°C, twice with 0.2x SSC, 0.1% SDS at 60°C and finally, twice with 0.1x SSC, 0.1% SDS at 42°C. Each washing took place over a 10-minute period in the glass tube within the hybridizing incubator. The membrane was left to dry momentarily on a piece of Whatman 3MM blotting paper and was subsequently wrapped in cellophane and exposed to X-ray film (Kodak X-Omat) with an intensifying screen. The autoradiographic image was usually detected after 16-24 hours of exposure at -70°C.

### *2.7.2 Hybridization with β-globin probe*

The same protocol as the one above (2.7.1) was also used for hybridizing PCR products that were β-globin negative after Southern blotting onto nylon membranes, but instead, these membranes were probed with the β-globin probe ($10^6$ cpm/ml). After hybridization, the membranes underwent the same washings and exposure time.

### *2.7.3 Hybridization with type-specific oligonucleotides*

PCR products that were HPV positive with the generic probe were transferred to a nylon membrane with the dot blot apparatus, as previously described. The dot blots were incubated at 65°C for a minimum of 15 minutes in a hybridizing solution consisting of 5x SSPE (3.6 M NaCl, 0.2 M $NaH_2P0_4$, 0.2 M EDTA) & 0.1% SDS. The $\gamma$-$P^{32}$ATP-labeled type-specific oligonucleotide was then added to 10 ml ($10^5$ cpm/ml) of the hybridizing solution and the membranes were left to incubate for a minimum of 2 hours at 55°C in the hybridizing incubator. The membranes were subsequently washed twice with 2x SSPE, 0.1% SDS over a 10 minute period at 56°C in a glass tube within the hybridizing incubator. The membrane was left to dry momentarily on a piece of Whatman 3MM blotting paper and was subsequently wrapped in a sheet of cellophane and exposed to X-ray film (Kodak X-Omat) with an intensifying screen. The autoradiographic image was usually detected after 16-24 hours of exposure at -70°C.

### *2.7.4 Re-use of dot blot nylon membranes*

The previous probe was removed within 12 hours from the completion of filming. Blots were treated in 0.2 N NaOH & 0.5% SDS at 56°C for 30 minutes with gentle shaking, followed by boiling the membrane twice for 10 minutes in 500 ml of pre-treatment solution (0.1x SSPE & 0.5% SDS). The membranes were brought to neutral pH by equilibration in 2x SSPE and 0.1% SDS. Stripped blots were then stored dry until re-used.

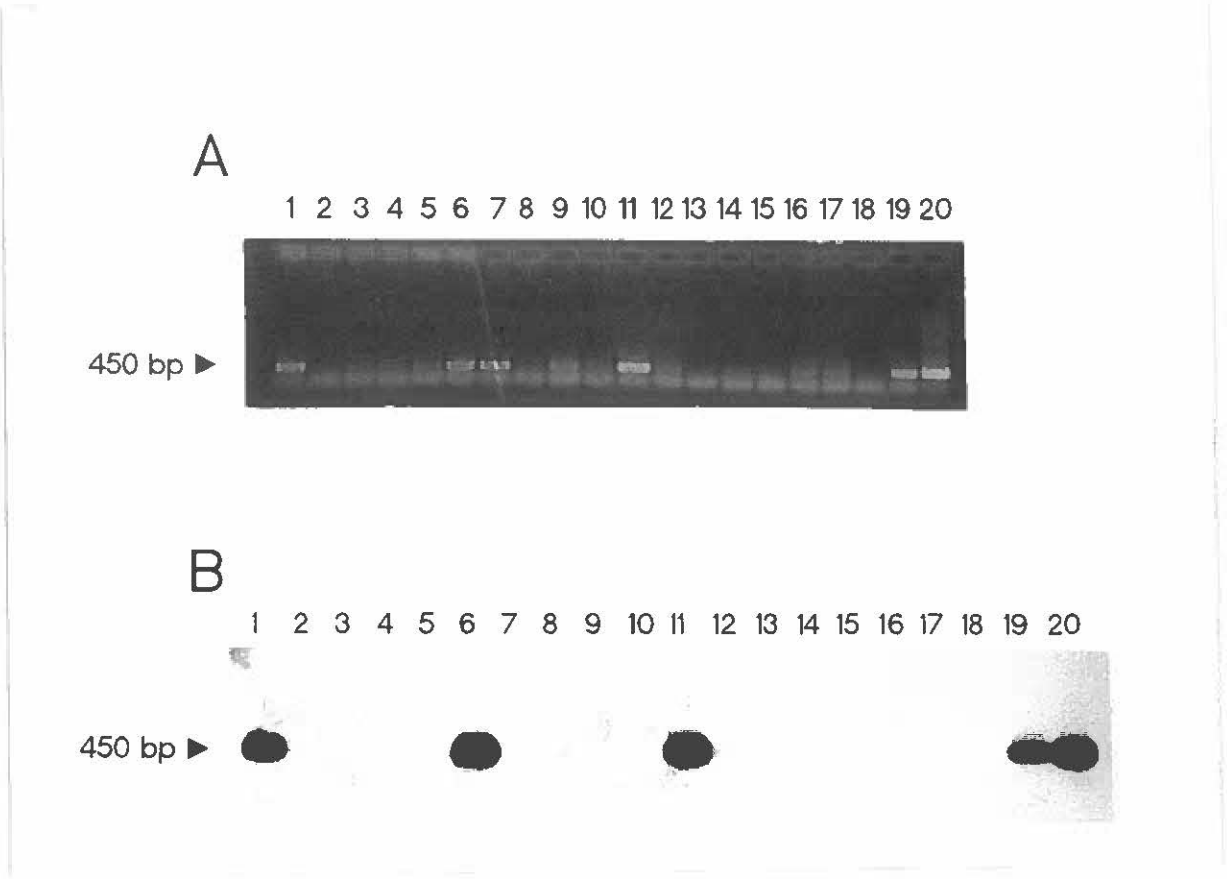


**Figure 8:**
Selection of HPV samples negative after hybridization

A) Gel electrophoresis
B) Southern blot hybridized with $P^{32}$-labeled HPV generic probe

## *2.8 Cloning*

### *2.8.1 Cloning of the 450 base pair amplicon*

Clinical samples that were positive after PCR amplification with the gel electrophoresis but negative after hybridization with the first generation of the HPV generic probe were cloned for subsequent sequencing and typing (Figure 8).

The positive samples (from the gel) that did not hybridize to the generic probe were loaded onto a second gel electrophoresis, and following EtBr staining, the 450 bp fragment was cut from the gel. The DNA was extracted from the agarose slice using the gene clean kit (BioCan) according to the manufacturer's specifications. The DNA concentration was estimated by electrophoresis using a known standard. The purified 450 bp amplicon was then ligated into the PCRII plasmid (TA cloning kit; BioCan). To obtain a molar ratio of 1:3 (vector:insert), the following ligation reaction was assembled: in a final volume of 10 µl, 50 ng of plasmid was added to 25 ng of gel purified amplicon, 1 U of T4 DNA ligase in 1x ligase reaction buffer (BioCan). The ligation took place at 14°C overnight. Following the overnight ligation, $CaCl_2$-competent *E.coli* XL-1 blue cells were transformed with the ligated plasmid in which 5 µl of the ligation reaction was added to 100 µl of XL-1 blue cells and kept at 0°C for 30 minutes. The cells then underwent heat shock at 42°C for 45 seconds and 4.5 ml of 2YT (Bactotryptone 16 g, yeast extract 10 g & NaCl 5 g/l) was added to each tube. The cells were incubated for one hour at 37°C in an orbital shaker (Queue Orbital). Transformed cells (100 µl) were then plated on 2YT-agar plates (2YT & 1.5% Bacto agar) containing ampicilin (50 µg/ml) , IPTG (isopropylthio-β-D-galactoside; 3 µl/ml of 100 mM solution) and X-gal (5-bromo-4 chloro-3-indolyl-β-D-galactoside; 13 µl/ml of 2% solution in Dimethylformamide) and left to incubate overnight at 37°C (Sambrook *et al.*, 1989).

### *2.8.2 Analysis of clones*

Following the overnight incubation, 3 ml of 2YT broth containing 100 µg/ml of ampicilin was inoculated with a white colony and incubated at 37°C overnight. The plasmid DNA was then purified using the Wizard mini prep kit (Promega). Clones were analyzed by digesting 1 µg of plasmid with 5 U of EcoRI in 20 µl of the appropriate buffer. After electrophoresis, the clone containing a 450 bp insert was sent to be sequenced.

### *2.8.3 Typing by sequencing*

At least 2 clones per transformation were sent to the sequencing service at the Armand-Frappier Institute. Sequencing was performed using the dideoxy method described by Sanger *et al.*, (1977), using the T7 sequencing kit (Pharmacia) and T7 and Sp6 fluorescent labeled primers. Sequencing reactions were electrophoresed on an ALF™ automated sequencer (Pharmacia). Sequences were analyzed with the IBI/Pustell sequence analysis programs (IBI/Kodak). A database of all the published HPV sequences was not accessible to us at the time. Instead, a database containing the sequences of all the type-specific oligos was created. A homology search was then conducted by aligning the sequence of the cloned 450 bp L1 fragment with the compiled type-specific HPV oligo sequences in the database. The section of the 450 bp fragment showing 100% homology with 'x' type-specific oligo would then be classified as HPV type 'x' .

## 3 Statistical Analysis

Crude differences in HPV prevalence by category of exposure were compared using the Pearson chi-square statistic. Crude odds ratios (OR) and age-adjusted OR, given that HPV infection appears to be heavily influenced by age in previous studies (Bauer *et al.*, 1991; Schiffman and Schatzkin, 1994), were then calculated to analyze risks of HPV infection by grouped demographics and behavioural characteristics. HPV types were grouped according to oncogenic risk. The OR served to gauge the strength of the association between exposure and single outcome variables. The OR and its 95% confidence interval were computed using unconditional logistic regression techniques (Breslow, 1980) with the multiple logistic regression software program MULTLR (Campos-Filho and Franco, 1988).

A few new variables were created from a number of old variables. Frequency of sexual encounters was computed by combining information elicited on number of encounters either per week, month or year and converted into frequency of sexual encounters per month, only. Tobacco smoking status was calculated from information provided by the women on their past and current smoking status. Lifetime cumulative tobacco exposure was estimated with pack-years of cigarettes smoked. A pack-year was defined as the cumulative exposure equivalent to smoking one pack of cigarettes (20 cigarettes) daily during one year.

In order to obtain a comprehensive set of variables containing all potential risk factors and confounders, we selected all variables that were significantly associated with HPV infection, in the univariate logistic regression analyses, at a conservative 20% level of significance. These variables were further analyzed in multivariate logistic regression models. Three different sets of analyses were performed: for overall HPV infection, high-risk HPV infection, and low-risk HPV infection. A number of multivariate models were constructed using a backward stepwise regression strategy for eliminating variables not independently associated with risk. All initially selected variables were placed in the model.

A p-value of 0.10 was used as a threshold value for considering which variables already present in the model were to be removed. Once out of the model, a variable was re-selected for inclusion if it contributed to the model's goodness of fit statistic at least at the 5% significance level. This was performed in a stepwise fashion. Factors that remained significant ($p \leq 0.10$) despite adjustment for all related and independent variables were included in a final model.

Cytology results from the clinic were provided for further analyses. Cytology was treated as the outcome variable and HPV infection was measured as the predictor of abnormal cytology adjusting for age, selected sexual activity variables and *a priori* confounders.

# CHAPTER 5

# RESULTS

## RESULTS

A total of 489 women agreed to participate in the study, of which 411 (84%) completed and returned the questionnaire. Participants always had the option to skip a question if they did not feel comfortable answering it.The cervical specimens from 40 women (8.2%) failed to be amplified for β-globin (4 of those women also failed to return the questionnaire). The univariate and multivariate analyses refer to the remaining 375 evaluable participants (76.7%). Subjects not included in the analysis, because they did not respond to the questionnaire, did not differ significantly from those who did respond, with respect to age, cytological morphology or overall and type-specific HPV prevalence (Table VII). Similarly, those subjects whose specimens failed to be amplified for β-globin did not differ materially from those subjects whose specimens were β-globin positive (Table VIII).

A limited amount of information was gathered for all 489 subjects who originally agreed to participate. The subject's mean age was 22.2 years (median, 21). There were 87 subjects (18.0%) aged <20 years, 378 (78.4%) aged 20-29 years, 15 (3.1%) aged 30-39 years and 2 (0.4%) aged 40-49 years. Cytology results were made available for all 489 women, of which 20 women had abnormal Pap smears: 17 (3.5%) with low-grade and 3 (0.6%) with high-grade lesions. An additional 39 (8.0%) women had a diagnosis of atypical squamous cells of undetermined significance (ASCUS).

### 1 Laboratory results

Laboratory results were based on 449 samples because 40 did not have adequate DNA for testing. HPV infection with one or more types was detected in 98/449 women (21.8%). Among those HPV infected women, 28 (6.2%) had a low-risk HPV infection, 53 (11.8%) had a high-risk HPV infection, 32 (7.1%)) had an unknown HPV type infection and 12 (2.7%) had a multiple type infection (Table IX). The most prevalent types were unknown HPV types (7.1%), followed by HPV-16 (4.7%), HPV-51 (2.2%), PAP-155 (2.0%), HPV-

## Table VII

### Comparison of Non-Respondents and Respondents with Respect to Age, Cytological Morphology and HPV Prevalence

| | Non-Respondents | | Respondents | |
|---|---|---|---|---|
| **Variable** | **N** | **%** | **N** | **%** |
| **Age (yrs)**[1] | | | | |
| ≤19 | 19 | 25.0 | 68 | 16.7 |
| 20-21 | 26 | 34.2 | 134 | 33.0 |
| 22-23 | 13 | 17.1 | 104 | 25.6 |
| ≥24 | 18 | 23.7 | 100 | 24.7 |
| *p-value*[2] | *0.2694* | | | |
| **Cytology (Pap smear)** | | | | |
| Normal | 66 | 84.6 | 363 | 88.5 |
| Abnormal | 12 | 15.4 | 47 | 11.5 |
| *p-value* | *0.3302* | | | |
| **Overall HPV positivity** | | | | |
| Negative | 61 | 82.4 | 290 | 77.3 |
| Positive | 13 | 17.6 | 85 | 22.7 |
| *p-value* | *0.3318* | | | |
| **HPV positivity by oncogenicity of the type** | | | | |
| Negative | 61 | 82.4 | 290 | 77.3 |
| Low-risk | 6 | 8.1 | 45 | 12.0 |
| High-risk | 7 | 9.5 | 40 | 10.7 |
| *p-value* | *0.5724* | | | |

[1]Note that only 76/78 non-respondents provided the study with their birthdate, while 406/411 respondents provided the study with their birthdate.

[2]Pearson chi-square ($\chi^2$) test

## Table VIII

**Comparison of β-Globin Negative and β-Globin Positive Samples of Subjects with Respect to Age, Ethnicity and Selected Sexual Characteristics**

| Variable | β-Globin Negative | | β-Globin Positive | |
|---|---|---|---|---|
| | N | % | N | % |
| **Age (yrs)** | | | | |
| ≤19 | 5 | 12.8 | 82 | 18.5 |
| 20-21 | 13 | 33.3 | 147 | 33.2 |
| 22-23 | 12 | 30.8 | 105 | 23.7 |
| ≥24 | 9 | 23.1 | 109 | 24.6 |
| *p-value*[1] | 0.7810 | | | |
| **Ethnicity** | | | | |
| Anglophone | 22 | 62.9 | 213 | 59.3 |
| Francophone | 2 | 5.7 | 39 | 10.9 |
| Other | 11 | 31.4 | 107 | 29.8 |
| *p-value* | 0.5100 | | | |
| **Age at first coitus** | | | | |
| ≤16 | 12 | 34.3 | 89 | 25.5 |
| 17-18 | 12 | 34.3 | 139 | 39.8 |
| ≥19 | 11 | 31.4 | 121 | 34.7 |
| *p-value* | 0.3220 | | | |
| **Number of sexual partners** | | | | |
| 1 | 8 | 22.9 | 72 | 19.2 |
| 2-4 | 11 | 31.4 | 142 | 38.0 |
| 5-9 | 12 | 34.3 | 104 | 27.8 |
| ≥10 | 4 | 11.4 | 56 | 15.0 |
| *p-value* | 0.7622 | | | |

[1]Pearson chi-square ($\chi^2$) test

## Table IX

### Prevalence Rates of Overall HPV Infection and According to Oncogenic Risk

| HPV Classification | N | %[1] |
|---|---|---|
| **Overall HPV positivity** | 98 | 21.8 |
| **Low-risk HPV types** | 28 | 6.2 |
| **High-risk HPV types** | 53 | 11.8 |
| **Multiple infections[2]** | 12 | 2.7 |
| **Unknown HPV types** | 32 | 7.1 |

[1] Total number of samples adequate for HPV testing: n=449

[2]*All multiple infections had at least one high-risk type. Breakdown is as follows:*

| | |
|---|---|
| Double infection: | (16, 6), (16, 31), (16, 33), (16, 53), (16, 58), (18, 56), (18, 66), (31, 73), (51, 56) |
| Triple infection: | (6, 16, 51) |
| Quadruple infection: | (51, 53, 66, PAP-155), (16, 66, PAP-155, W13B) |

66 (1.6%), HPV-6, 11, 31, 33 and 58 (1.1% each), HPV-18 and 53 (0.9%), HPV-56 (0.4%), HPV-35, 68, W13B and 238A (0.2% each) (Table X). None of the amplified products hybridized with probes for types 26, 39, 40, 42, 45, 52, 54, 55, 57, 59 or 291.

## 2 Epidemiological profile

The epidemiological profile of the 411 respondents revealed a population of women very close in age, between 18 and 24 years of age. One quarter of the population was over 24 but only 3% were over 30 years of age. Most women were from Ontario (32.1%). The second largest group came from Quebec (18.2%) followed closely by women from the Prairies and British Columbia (B.C.) (14.1%). There were more American women (10.9%) than women from the Maritime provinces (6.6%), Europe (7.1%) or elsewhere (9.7%), including Africa, South America, India, Australia, Asia or the Caribbean. The majority of women were anglophone (57.2%) and single (82.2%). The low percentage of women from Quebec is not necessarily representative of the McGill student population, but rather is a profile of the users of the student clinic. Women from Quebec undoubtedly have easier access to their family physicians or gynecologists from private clinics within the city of Montreal (Appendix 4). Note, however, that the sum of responses for the different variables presented in the tables, do not necessarily total 411. Women were always given the option to skip a given question if the felt too uncomfortable to answer it.

The onset of menstruation for most of the women in the study occurred before the age of 14 (77.3%). Only one quarter of the women had experienced a sexual encounter by the age of 16, but over two thirds of the women had experienced their first coitus by the age of 18. The majority of women (56.7%) had less than 5 lifetime sexual partners and had sex 3 times a week or less (67.7%) throughout their sexual life. However, within the year before entering the study, most women (65.2%) had 1 or no sexual partners and engaged in sex once a week or less frequently (42.6%). Oral sex was another measurement of sexual

## Table X

### Distribution and Prevalence of Individual HPV Types

| HPV type | Oncogenic risk | N | % |
|---|---|---|---|
| HPV-6/11 | Low-risk | 5 | 1.1 |
| HPV-16 | High-risk | 21 | 4.7 |
| HPV-18 | High-risk | 4 | 0.9 |
| HPV-31 | High-risk | 5 | 1.1 |
| HPV-33 | High-risk | 5 | 1.1 |
| HPV-35 | High-risk | 1 | 0.2 |
| HPV-51 | High-risk | 10 | 2.2 |
| HPV-53 | Low-risk | 4 | 0.9 |
| HPV-56 | High-risk | 2 | 0.4 |
| HPV-58 | High-risk | 5 | 1.1 |
| HPV-66 | Low-risk | 7 | 1.6 |
| HPV-68 | Low-risk | 1 | 0.2 |
| PAP-155 | Low-risk | 9 | 2.0 |
| W13B | Low-risk | 1 | 0.2 |
| 73 | Low-risk | 1 | 0.2 |
| Unknown type* | Low-risk | 32 | 7.1 |

**None of the amplified HPV positive specimens hybridized with probes for types:* 26, 39, 40, 42, 45, 52, 54, 55, 57, 59 or 291

activity. The majority of women (71.5%) had 3 or fewer lifetime partners who engaged in cunnilingus on an average of once a week or less (65.7%). The number of sexual partners that the majority of women (81.8%) had for more than 3 months was also 3 or less, following a similar profile to the number of oral sex partners observed. Over two thirds of (0.4%) and HPV 35, 68, W13B and 238A (0.2% each) (Table X). None of the amplified specimens hybridized with probes for types 26, 39, 40, 42, 45, 52, 54, 55, 57, 59 or 291.he women (65.2%) engaged in masturbation or were sexually active during their menstruation (60.3%) but most had never engaged in anal intercourse (76.9%). Only 11.2% of the women had ever been pregnant and the majority of pregnancies (89%) were terminated with an abortion (Appendix 5).

Approximately one third (32.8%) of the participants had previously smoked (former smoker) or still smoke (current smoker). Among these, the majority of women (76.3%) smoked one pack of cigarettes per day for 5 years or less. Use of oral contraceptives appeared quite common with 74.5% women reporting regular use of the pill throughout their lifetime. Most of the women started taking the pill between the ages of 17 to 19 and have been on it for at least 1 year (54.5%). The majority of women (56.0%) also reported using a condom regularly throughout their sexual life, but only 41.1% reported using condoms recently (the year before entering the study). Information on other forms of barrier-like contraceptives such as diaphragms, cervical caps, sponges and spermicide was also collected and grouped together with condoms as a new variable, "use of contraceptive barrier". The majority of women (63.5%) reported using one or more forms of a contraceptive barrier in their lifetime, however, condom use was definitely the most popular form of a barrier contraceptive (Appendix 6).

Information on the participant's hygienic and health characteristics was also obtained. The vast majority of women (86.6%) had never had a vaginal douche. Most women

(73.3%) showered at least once a day, and approximately half the cohort (51.8%) washed their genitals after having sex. Two thirds of the women (66.2%) preferred to wipe themselves using toilet paper with a front-to-back hand motion after each bowel movement.

The frequency of having a sexually transmitted disease was very low, with the large majority of women (83.5%) reporting no knowledge of ever having *Trichomonas*, *Chlamydia*, herpes, syphilis, gonorrhea or genital sores. A slightly lower percentage of women (77.4%) also reported never having genital warts. Most women (82.1%) have had at least two Pap smear tests to screen for cytological abnormalities that may lead to cervical cancer (Appendix 7).

### *2.1 Prevalence of cervical HPV infection*

The prevalence rates of HPV infection were calculated for overall HPV infection and according to oncogenic risk, (high oncogenic-risk HPV infections and low oncogenic-risk HPV infections). Only those women who had completed and returned their questionnaire and who tested positive for β-globin (having an adequate DNA specimen) were included in the analyses. There were 375 such cases.

#### *2.1.1 Prevalence and sociodemographics*

There were no significant associations between any of the demographic variables, including age, and overall HPV infection, high-risk HPV infection, or low-risk HPV infection (Table XI). (Please note that because some women have multiple type infections, the sum of % high-risk and low-risk HPV infections does not necessarily equal to the sum of % overall HPV infection).

#### *2.1.2 Prevalence and sexual activity*

The association between lifetime number of partners and high-risk HPV infection approached significance (p=0.07), with the greatest risk observed for 10 lifetime sexual

## Table XI

**Prevalence of HPV Infection by Demographic Factors: Overall and According to Oncogenic Risk[1]**

| Variable | N[2] | Overall %HPV + | %High Risk HPV + | %Low Risk HPV + |
|---|---|---|---|---|
| **Age (yrs)** | | | | |
| <=19 | 82 | 22.0 | 11.1 | 13.5 |
| 20-21 | 147 | 24.5 | 14.0 | 12.5 |
| 22-23 | 105 | 20.0 | 9.7 | 11.3 |
| 24+ | 109 | 21.1 | 12.2 | 11.3 |
| *p-value* [3] | | 0.8446 | 0.8009 | 0.9451 |
| **Origin** | | | | |
| Ontario & West[4] | 174 | 27.6 | 16.1 | 16.7 |
| Que. & Maritimes | 91 | 16.5 | 4.9 | 11.5 |
| USA | 40 | 17.5 | 13.2 | 5.7 |
| Europe | 28 | 32.1 | 20.8 | 17.4 |
| Other | 37 | 16.2 | 6.1 | 11.4 |
| *p-value* | | 0.1184 | 0.0835 | 0.4626 |
| **Ethnicity** | | | | |
| Anglophone | 212 | 23.6 | 13.2 | 10.8 |
| Francophone | 39 | 12.8 | 7.7 | 5.1 |
| Other | 107 | 26.2 | 13.1 | 12.1 |
| *p-value* | | 0.2343 | 0.4112 | 0.5452 |
| **Marital status** | | | | |
| Single | 311 | 23.5 | 13.2 | 10.6 |
| Married | 14 | 7.1 | | 7.1 |
| Living with partner | 42 | 26.2 | 9.5 | 14.3 |
| *p-value* | | 0.3584 | 0.7776 | 0.4550 |

[1]High-risk HPV: (16,18,31,33,35,39,45,51,52,56,58) Low-risk HPV: (6,11,26,40,42,53-55,57,59,66,68 and unidentified types) (Bauer *et al.*, 1991); [2]Number of women in overall HPV infection category; [3]Chi-square ($\chi^2$) test for the association between each variable and HPV infection; [4]Prairies & B.C.

partners or more (Table XII). The association between lifetime frequency of sexual encounters and overall HPV infection or high-risk HPV infection was highly significant (p=0.009 and p=0.006, respectively). The excess risk was seen among those women who engaged in sex a minimum of 3 times per week. The association between frequency of sexual encounters, within the last year only, and low-risk HPV infection approached significance (p=0.080), with a moderate risk observed among those women who engaged in sex a minimum of 3 times per week. The association between number of oral sex partners (partners performing cunnilingus) and high-risk HPV infection was significant (p=0.023), with the greatest risk observed for 10 oral sex partners or more within the last 5 years. The association between number of oral sex partners with overall HPV infection approached significance (p=0.068). Similarly, the association between number of sex partners for more than 3 months and overall HPV infection or high-risk HPV infection approached significance (p=0.088 and p=0.062, respectively). The excess risk was seen for 10 partners or more. The association between engaging in sexual activity while menstruating and low-risk HPV infection approached significance (p=0.089). The association between masturbation and high-risk HPV or low-risk HPV infection also approached significance (p=0.056 and p=0.058, respectively) (Table XII).

### *2.1.3 Prevalence and contraceptive history*

The association between lifetime oral contraceptive use and low-risk HPV infection approached significance (p=0.084) (Table XIII). Recent use of the pill, within the last year, was moderately associated with high-risk HPV infection (p=0.068) but was strongly associated with overall HPV infection (p=0.014) and low-risk HPV infection (p=0.026). The greatest risk was observed for occasional use of the pill. Lifetime condom use was strongly associated with high-risk HPV infection (p=0.035), with an excess risk observed among women who never used condoms. The association between use of condoms within

## Table XII

### Prevalence of HPV Infection by Reproductive and Sexual Factors: Overall and According to Oncogenic Risk[1]

| Variable | N[2] | Overall %HPV + | %High Risk HPV + | %Low Risk HPV + |
|---|---|---|---|---|
| **Age at menarche** | | | | |
| <=11 | 56 | 23.2 | 15.7 | 10.4 |
| 12 | 125 | 16.8 | 8.0 | 10.3 |
| 13 | 112 | 28.6 | 15.6 | 16.5 |
| 14+ | 82 | 23.2 | 11.4 | 16.2 |
| *p-value* [3] | | 0.1948 | 0.3106 | 0.4572 |
| **Age at first coitus** | | | | |
| <=17 | 158 | 23.4 | 12.2 | 13.5 |
| 18+ | 191 | 21.5 | 10.8 | 13.9 |
| *p-value* | | 0.6630 | 0.6912 | 0.9188 |
| **Lifetime No. sex partners** | | | | |
| 1 | 72 | 22.2 | 11.1 | 13.8 |
| 2-4 | 142 | 18.3 | 9.3 | 10.0 |
| 5-9 | 104 | 25.0 | 10.6 | 20.0 |
| 10+ | 56 | 30.4 | 23.1 | 9.1 |
| *p-value* | | 0.2897 | 0.0703 | 0.1357 |
| **Recent[4] No. sex partners** | | | | |
| 0-1 | 243 | 21.0 | 11.2 | 12.8 |
| 2-3 | 91 | 24.2 | 11.5 | 15.9 |
| 4+ | 41 | 29.3 | 18.9 | 11.8 |
| *p-value* | | 0.4657 | 0.4035 | 0.7507 |
| **Lifetime frequency of sex** | | | | |
| 0-4/month | 107 | 15.0 | 6.2 | 9.9 |
| 5-13/month | 146 | 21.2 | 11.5 | 11.5 |
| 14+/month | 84 | 33.3 | 22.5 | 19.1 |
| *p-value* | | 0.0094 | 0.0058 | 0.1798 |
| **Recent frequency of sex** | | | | |
| 0-4/month | 161 | 24.8 | 11.1 | 7.8 |
| 5-13/month | 134 | 17.9 | 11.3 | 8.3 |
| 14+/month | 78 | 26.9 | 15.9 | 13.4 |
| *p-value* | | 0.2280 | 0.5634 | 0.0797 |
| **No. oral sex partners** | | | | |
| 0-3 | 266 | 19.5 | 9.7 | 12.3 |
| 4-9 | 92 | 29.3 | 15.4 | 17.5 |
| 10+ | 17 | 35.3 | 31.3 | 8.3 |
| *p-value* | | 0.0684 | 0.0232 | 0.4375 |

## Table XII

### Prevalence of HPV Infection by Reproductive and Sexual Factors: Overall and According to Oncogenic Risk (Continued)

| Variable | N | Overall %HPV + | %High Risk HPV + | %Low Risk HPV + |
|---|---|---|---|---|
| **Lifetime frequency of oral sex** | | | | |
| 0-4/month | 248 | 22.6 | 11.9 | 13.1 |
| 5-13/month | 98 | 20.4 | 11.5 | 12.5 |
| 14+/month | 12 | 41.7 | 22.2 | 30.0 |
| *p-value* | | 0.2515 | 0.6346 | 0.2935 |
| **No. of sex partners >3 months** | | | | |
| 0-3 | 309 | 20.7 | 10.3 | 13.2 |
| 4-9 | 64 | 31.1 | 20.4 | 14.0 |
| 10+ | 4 | 50.0 | 33.3 | 33.3 |
| *p-value* | | 0.0875 | 0.0622 | 0.5917 |
| **Sexually active when menstrual** | | | | |
| Never | 145 | 20.7 | 13.5 | 9.4 |
| Ever | 228 | 24.1 | 11.3 | 16.0 |
| *p-value* | | 0.4410 | 0.5406 | 0.0885 |
| **Anal sex** | | | | |
| Never | 288 | 23.6 | 12.4 | 14.4 |
| Ever | 84 | 20.2 | 11.8 | 10.7 |
| *p-value* | | 0.5171 | 0.9057 | 0.4064 |
| **Masturbation** | | | | |
| Never | 131 | 22.9 | 7.3 | 17.9 |
| Ever | 240 | 22.5 | 14.7 | 10.6 |
| *p-value* | | 0.9298 | 0.0562 | 0.0584 |
| **Pregnant** | | | | |
| Never | 330 | 21.8 | 11.0 | 13.4 |
| 1 | 33 | 33.3 | 24.1 | 15.4 |
| 2+ | 12 | 16.7 | 9.1 | 9.1 |
| *p-value* | | 0.2831 | 0.1137 | 0.8765 |
| **Type of pregnancy** | | | | |
| Abortion | 40 | 30.0 | 20.0 | 15.2 |
| Full-term delivery | 5 | 20.0 | 20.0 | 0.0 |
| *p-value* | | 0.5008 | 0.2655 | 0.7031 |

[1]High-risk HPV: (16,18,31,33,35,39,45,51,52,56,58) Low-risk HPV: (6,11,26,40,42,53-55,57,59, 66, 68 and unidentified types) (Bauer, 1991); [2]Number of women in overall HPV infection category [3]Chi-square ($\chi^2$); test for the association between each variable and HPV infection; [4]Within last year.

## Table XIII

**Prevalence of HPV Infection by Smoking and Contraception: Overall and According to Oncogenic Risk[1]**

| Variable | N[2] | Overall %HPV + | %High Risk HPV + | %Low Risk HPV + |
|---|---|---|---|---|
| **Tobacco** | | | | |
| Never | 247 | 21.9 | 11.5 | 13.1 |
| Former | 56 | 32.1 | 17.4 | 20.8 |
| Current | 70 | 18.6 | 10.9 | 9.5 |
| *p-value* [3] | | 0.1642 | 0.5061 | 0.2126 |
| **Packyears** | | | | |
| <1 | 45 | 17.8 | 9.8 | 9.8 |
| 1-5 | 53 | 26.4 | 13.3 | 17.0 |
| 5+ | 13 | 15.4 | 15.4 | 0.0 |
| *p-value* | | 0.7032 | 0.9258 | 0.4356 |
| **Oral contraceptive** | | | | |
| Never | 78 | 25.6 | 12.1 | 17.1 |
| Occasionally | 20 | 35.0 | 13.3 | 27.8 |
| Regularly | 277 | 20.9 | 12.0 | 11.3 |
| *p-value* | | 0.2724 | 0.9891 | 0.0843 |
| **Recent[4] oral contraceptive use** | | | | |
| Never | 145 | 25.5 | 13.0 | 17.1 |
| Occasionally | 11 | 54.5 | 37.5 | 37.5 |
| Regularly | 219 | 19.2 | 10.6 | 10.1 |
| *p-value* | | 0.0138 | 0.0676 | 0.0256 |
| **Age started pill (yrs)** | | | | |
| <=16 | 41 | 24.4 | 16.2 | 11.4 |
| 17-19 | 179 | 22.2 | 12.0 | 13.1 |
| 20+ | 80 | 18.8 | 9.7 | 11.0 |
| *p-value* | | 0.7101 | 0.7364 | 0.7795 |
| **Time on pill (yrs)** | | | | |
| <=1 | 94 | 23.4 | 11.3 | 16.5 |
| 1-2 | 64 | 20.3 | 8.9 | 13.6 |
| 2-3 | 52 | 26.9 | 15.2 | 13.3 |
| 3+ | 87 | 18.4 | 13.4 | 6.6 |
| *p-value* | | 0.7229 | 0.8886 | 0.3338 |

## Table XIII

**Prevalence of HPV Infection by Smoking and Contraception: Overall and According to Oncogenic Risk (Continued)**

| Variable | N | Overall %HPV + | %High Risk HPV + | %Low Risk HPV + |
|---|---|---|---|---|
| **Condom use** | | | | |
| Never | 22 | 31.8 | 28.6 | 6.3 |
| Occasionally | 145 | 22.1 | 13.2 | 12.5 |
| Regularly | 208 | 22.1 | 9.4 | 14.7 |
| *p-value* | | 0.5721 | 0.0354 | 0.5907 |
| **Recent condom use** | | | | |
| Never | 121 | 19.0 | 15.5 | 4.9 |
| Occasionally | 98 | 22.4 | 9.5 | 15.6 |
| Regularly | 156 | 25.6 | 10.8 | 18.3 |
| *p-value* | | 0.4245 | 0.3659 | 0.0076 |
| **Barrier use[5]** | | | | |
| Never | 20 | 30.0 | 26.3 | 6.7 |
| Occasionally | 116 | 21.6 | 11.8 | 13.5 |
| Regularly | 239 | 22.6 | 11.0 | 13.9 |
| *p-value* | | 0.7060 | 0.1458 | 0.7301 |
| **Recent barrier use** | | | | |
| Never | 119 | 18.5 | 14.9 | 4.9 |
| Occasionally | 90 | 20.0 | 7.7 | 14.3 |
| Regularly | 166 | 27.1 | 12.3 | 18.8 |
| *p-value* | | 0.1809 | 0.3206 | 0.0064 |

[1]High-risk HPV: (16,18,31,33,35,39,45,51,52,56,58) Low-risk HPV: (6,11,26,40,42,53-55,57,59,66,68 and unidentified types) (Bauer *et al.*, 1991); [2]Number of women in overall HPV infection category; [3]Chi-square ($\chi^2$) test for the association between each variable and HPV infection; [4]Within last year; [5]Includes foam, cap, diaphram, sponge and condom.

the last year and low-risk HPV infection was highly significant (p=0.008), with the greatest risk observed for women who used condoms occasionally or regularly. Use of a contraceptive barrier within the last year ("recent barrier") was significantly associated with low-risk HPV infection (p=0.006) with the greatest risk observed for women who used contraceptive barriers occasionally or regularly (Table XIII).

#### *2.1.4 Prevalence and hygiene and medical history*

Washing the genital area after sexual intercourse was strongly associated with overall HPV infection (p=0.016) and low-risk HPV infection (p=0.004) with an excess risk observed in women who did not wash after having sexual intercourse (Table XIV). The method of wiping after a bowel movement was associated with low-risk HPV infection (p=0.052), with the greatest risk observed for wiping front-to-back. Genital warts were marginally associated with overall HPV infection (p=0.068) and low-risk HPV infection (p=0.090) (Table XIV).

### *2.2 Univariate analysis*

#### *2.2.1 Risk associated with sociodemographics*

Ontario was chosen as the reference category for place of origin because the greatest number of subjects were from Ontario. In the interest of preserving statistical power, the Prairies (Manitoba, Saskatchewan and Alberta) and B.C. (West) were grouped with Ontario because of their similar OR profile. Only women from Europe were at a greater risk of being overall HPV positive compared to the reference group (OR=1.36) (Table XV). Women from Quebec and the Maritimes, USA, or elsewhere, excluding Europe, had a reduced risk of having any type of HPV infection (age adjusted ORs of 0.52, 0.53 and 0.49, respectively). This profile was also observed when risk was assessed according to HPV positivity by oncogenicity of the type. Women from Europe remained the group at greatest risk, compared to the reference category, for both low-risk HPV infection (OR=1.36) and high-risk HPV

## Table XIV

### Prevalence of HPV Infection by Hygiene and History of Sexually Transmitted Diseases: Overall and According to Oncogenic Risk[1]

| Variable | N[2] | Overall %HPV + | %High Risk HPV + | %Low Risk HPV + |
|---|---|---|---|---|
| **Vaginal douche** | | | | |
| Never | 323 | 22.0 | 11.3 | 13.7 |
| Ever | 52 | 26.9 | 17.0 | 11.4 |
| *p-value* [3] | | 0.4296 | 0.2664 | 0.6658 |
| **No. showers (per day)** | | | | |
| <1 | 99 | 24.2 | 12.8 | 14.8 |
| 1 | 243 | 22.6 | 12.6 | 13.0 |
| 1+ | 33 | 18.2 | 6.9 | 12.9 |
| *p-value* | | 0.7714 | 0.6644 | 0.9120 |
| **Wash after sex** | | | | |
| Never | 176 | 28.4 | 13.7 | 19.2 |
| Ever | 196 | 17.9 | 11.0 | 8.5 |
| *P-value* | | 0.0155 | 0.4674 | 0.0044 |
| **Method of wiping[4]** | | | | |
| Back-to-front | 121 | 19.0 | 12.5 | 8.4 |
| Front-to-back | 247 | 25.1 | 12.3 | 16.3 |
| *p-value* | | 0.1926 | 0.9632 | 0.0519 |
| **History of STD[5]** | | | | |
| Never | 337 | 23.4 | 11.9 | 14.2 |
| Ever | 38 | 15.8 | 13.9 | 6.1 |
| *p-value* | | 0.2855 | 0.7306 | 0.1909 |
| **History of warts** | | | | |
| Never | 289 | 20.1 | 10.1 | 11.8 |
| Ever | 36 | 33.3 | 17.2 | 22.6 |
| *p-value* | | 0.0679 | 0.2386 | 0.0902 |
| **Hormone therapy** | | | | |
| Never | 360 | 23.1 | 12.6 | 13.4 |
| Ever | 15 | 13.3 | 0.0 | 13.3 |
| *p-value* | | 0.3782 | 0.1719 | 0.9908 |
| **Lifetime No. Paps** | | | | |
| 1 | 67 | 28.4 | 14.3 | 18.6 |
| 2-3 | 134 | 17.9 | 7.6 | 12.0 |
| 4-5 | 94 | 22.3 | 12.0 | 13.1 |
| 6-10 | 51 | 25.5 | 15.6 | 13.6 |
| 10+ | 26 | 30.8 | 25.0 | 10.0 |
| *p-value* | | 0.3863 | 0.1499 | 0.7737 |

[1]High-risk HPV: (16,18,31,33,35,39,45,51,52,56,58) Low-risk HPV: (6,11,26,40,42,53-55,57,59,66,68 and unidentified types) (Bauer *et al.*, 1991); [2]Number of women in overall HPV infection category; [3]Chi-square ($\chi^2$) test for the association between each variable and HPV infection; [4]Method of wiping after a bowel movement; [5]Includes *Trichomonas*, *Chlamydia*, herpes, syphillis & gonorrhea.

## Table XV

### Odds Ratios[1] of Overall, High-Risk and Low-Risk HPV Infection According to Demographic Factors

| Variable | Overall HPV Infection | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| | Crude OR | Adjusted OR[2] (95% CI) | Crude OR | Adjusted OR (95%CI) | Crude OR | Adjusted OR (95%CI) |
| **Age (yrs)** | | | | | | |
| ≤19 | 1.00[3] | | 1.00 | | 1.00 | |
| 20-21 | 1.01 | | 0.91 | | 1.11 | |
| 22-23 | 0.68 | | 0.56 | | 0.80 | |
| ≥24 | 0.89 | | 0.93 | | 0.85 | |
| **Origin** | | | | | | |
| Ontario & West[4] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Que. & Maritimes | 0.52 | 0.52 (0.27-0.99) | 0.65 | 0.65 (0.29-1.43) | 0.27 | 0.27 (0.08-0.80) |
| USA | 0.56 | 0.53 (0.22-1.24) | 0.30 | 0.29 (0.07-1.31) | 0.79 | 0.74 (0.26-2.10) |
| Europe | 1.24 | 1.22 (0.51-2.90) | 1.05 | 1.04 (0.32-3.35) | 1.37 | 1.36 (0.46-4.06) |
| Other | 0.51 | 0.49 (0.19-1.25) | 0.65 | 0.64 (0.20-1.99) | 0.34 | 0.31 (0.07-1.38) |
| **Ethnicity** | | | | | | |
| Anglophone | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Francophone | 0.48 | 0.47 (0.17-1.27) | 0.41 | 0.41 (0.09-1.85) | 0.51 | 0.49 (0.14-1.71) |
| Other | 1.16 | 1.12 (0.65-1.91) | 1.15 | 1.09 (0.52-2.27) | 1.01 | 0.98 (0.49-1.96) |
| **Marital status** | | | | | | |
| Single | 1.00 | 1.00 | 1.00 | | 1.00 | |
| Married | 0.25 | 0.22 (0.03-1.82) | 0.56 | 0.47 (0.05-4.07) | 0.00 | 0.0 |
| Living with partner | 1.16 | 1.07 (0.48-2.34) | 1.35 | 1.20 (0.43-3.35) | 0.73 | 0.66 (0.21-2.09) |

[1] By logistic regression; [2] Age adjusted; [3] Reference category; [4] Prairies & British Columbia.

## Table XVI

### Odds Ratios[1] of Overall, High-Risk and Low-Risk HPV Infection According to Reproductive and Sexual Factors

| Variable | Overall HPV Infection | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| | Crude OR | Adjusted OR[2] (95% CI) | Crude OR | Adjusted OR (95%CI) | Crude OR | Adjusted OR[2] (95%CI) |
| **Age at menarche (yrs)** | | | | | | |
| ≤11 | 1.00[3] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 12 | 0.67 | 0.68 (0.31-1.47) | 0.47 | 0.46 (0.17-1.28) | 0.99 | 1.00 (0.33-3.01) |
| 13 | 1.32 | 1.35 (0.64-2.84) | 1.00 | 1.00 (0.39-2.55) | 1.70 | 1.75 (0.60-5.12) |
| ≥14 | 1.00 | 1.00 (0.45-2.24) | 0.69 | 0.69 (0.24-1.99) | 1.67 | 1.71 (0.56-5.24) |
| **Age at first coitus** | | | | | | |
| ≤17 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| ≥18 | 0.89 | 0.94 (0.56-1.56) | 0.87 | 0.90 (0.44-1.83) | 1.03 | 1.09 (0.57-2.10) |
| **Lifetime No. sex partners** | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2-4 | 0.78 | 0.83 (0.41-1.69) | 0.82 | 0.87 (0.32-2.35) | 0.69 | 0.73 (0.29-1.18) |
| 5-9 | 1.17 | 1.30 (0.63-2.69) | 0.95 | 1.07 (0.37-3.12) | 1.56 | 1.71 (0.71-4.13) |
| ≥10 | 1.53 | 1.73 (0.76-3.95) | 2.40 | 2.70 (0.94-7.77) | 0.62 | 0.70 (0.20-2.47) |
| **Recent[4] No. sex partners** | | | | | | |
| 0-1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2-3 | 1.20 | 1.26 (0.71-2.26) | 1.04 | 1.15 (0.50-2.63) | 1.29 | 1.33 (0.64-2.73) |
| ≥4 | 1.56 | 1.61 (0.76-3.42) | 1.86 | 1.97 (0.76-5.10) | 0.91 | 0.91 (0.30-2.82) |

## Table XVI

### Odds Ratios of Overall, High-Risk and Low-Risk HPV Infection According to Reproductive and Sexual Factors (Continued)

| Variable | Overall HPV Infection | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| | Crude OR | Adjusted OR (95% CI) | Crude OR | Adjusted OR (95%CI) | Crude OR | Adjusted OR (95%CI) |
| **Lifetime frequency of sex** | | | | | | |
| 0-4/month | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 5-13/month | 1.53 | 1.62 (0.83-3.18) | 1.96 | 2.17 (0.80-5.89) | 1.18 | 1.20 (0.51-2.83) |
| ≥14/month | 2.90 | 3.08 (1.51-6.28) | 4.49 | 5.22 (1.87-14.55) | 2.19 | 2.24 (0.90-5.61) |
| **Recent frequency of sex** | | | | | | |
| 0-4/month | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 5-13/month | 0.64 | 0.65 (0.37-1.14) | 0.99 | 1.01 (0.46-2.19) | 0.41 | 0.41 (0.19-0.89) |
| ≥14/month | 1.13 | 1.13 (0.61-2.10) | 1.53 | 1.57 (0.68-3.66) | 0.72 | 0.72 (0.31-1.64) |
| **No. of oral sex partners** | | | | | | |
| 0-3 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 4-9 | 1.71 | 1.80 (1.04-3.11) | 1.68 | 1.78 (0.83-3.82) | 1.51 | 1.55 (0.77-3.13) |
| ≥10 | 2.25 | 2.19 (0.77-6.22) | 4.21 | 4.20 (1.32-13.31) | 0.65 | 0.63 (0.08-5.07) |
| **Lifetime frequency of oral sex** | | | | | | |
| 0-4/month | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 5-13/month | 0.88 | 0.93 (0.52-1.67) | 0.96 | 1.04 (0.48-2.28) | 0.95 | 0.99 (0.47-2.10) |
| ≥14/month | 2.45 | 2.65 (0.80-8.80) | 2.12 | 2.54 (0.48-13.40) | 2.85 | 3.09 (0.74-12.88) |

## Table XVI

### Odds Ratios of Overall, High-Risk and Low-Risk HPV Infection According to Reproductive and Sexual Factors (Continued)

| Variable | Overall HPV Infection | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| | Crude OR | Adjusted OR(95% CI) | Crude OR | Adjusted OR (95%CI) | Crude OR | Adjusted OR (95%CI) |
| **No. sex partners >3 months** | | | | | | |
| 0-3 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 4-9 | 1.73 | 1.97 (1.05-3.71) | 2.23 | 2.58 (1.15-5.78) | 1.07 | 1.19 (0.49-2.91) |
| ≥10 | 3.83 | 4.59 (0.61-34.39) | 4.36 | 5.32 (0.44-64.07) | 3.30 | 3.93 (0.33-46.16) |
| **Sexually active when menstrual** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ever | 1.22 | 1.30 (0.78-2.16) | 0.81 | 0.88 (0.45-1.72) | 1.83 | 1.92 (0.95-3.90) |
| **Anal sex** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ever | 0.82 | 0.87 (0.48-1.61) | 0.95 | 1.02 (0.46-2.28) | 0.71 | 0.75 (0.33-1.71) |
| **Masturbation** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ever | 0.98 | 1.01 (0.61-1.69) | 2.17 | 2.26 (1.00-5.11) | 0.54 | 0.56 (0.30-1.06) |
| **Pregnant** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1 | 1.79 | 1.76 (0.81-3.86) | 2.57 | 2.51 (0.97-6.53) | 1.17 | 1.17 (0.38-3.62) |
| ≥2 | 0.72 | 0.69 (0.14-3.29) | 0.81 | 0.76 (0.09-6.35) | 0.65 | 0.63 (0.08-5.24) |
| **Type of pregnancy** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Abortion | 1.54 | 1.50 (0.71-3.15) | 2.02 | 1.96 (0.77-5.03) | 1.15 | 1.31 (0.41-3.16) |
| Full-term delivery | 0.90 | 0.96 (0.10-8.98) | 2.02 | 2.14 (0.22-20.98) | 0.00 | 0.00 |

[1] By logistic regression; [2] Age adjusted; [3] Reference category; [4] Within last year

infection (OR=1.04). Women from the USA were more inclined to be infected with a low-risk HPV infection (OR=0.74) compared to women from Quebec and the Maritimes (OR=0.27) or other countries (OR=0.31) excluding Europe, while the reverse profile was true for women infected with oncogenic HPV types (Table XV). Francophone women were less likely to be HPV positive compared to anglophones, although the difference did not reach statistical significance (OR = 0.47; CI: 0.17-1.27).

### *2.2.2 Risk associated with sexual behaviour*

Table XVI shows the OR for infection with all HPV types and low- and high-risk HPV according to selected sexual activity variables and related characteristics. After controlling for age, the adjusted analyses revealed a moderate profile of a sexually transmitted disease for overall HPV infection. A significantly increased risk for overall HPV infection was seen for women experiencing sex more than 3 times a week (OR=3.08) compared to those experiencing sex once a week or less frequently. Women with 4 or more lifetime oral sex partners were almost twice (OR=1.80) as likely to be overall HPV positive compared to women with 3 or fewer lifetime partners who performed cunnilingus. Women with 4 or more "long-term" sex partners (number of sex partners for more than 3 months) were also at a greater risk (OR=1.97) of having an overall HPV infection compared to those with 3 or fewer long-term sex partners.

Different profiles emerged when the analyses focused on low- and high-risk HPV types. In the analysis of determinants of cervical infection with low-risk HPV types, no excess risks were observed with increased sexual activity. Moderate frequency of recent sexual activity (within the last year) even appeared to be marginally protective (OR=0.41); however, the reduction in risk was not significant. Conversely, a stronger profile of a sexually transmitted infection emerged from the analysis of high-risk HPV types. Women having sex more than 3 times a week were at a significantly increased risk (OR=5.22) for

having a high-risk HPV infection compared to those experiencing sex once a week or less frequently. Women with 10 or more oral sex partners were 4 times (OR=4.20) more likely to be infected with a high-risk HPV infection compared to women with 3 or fewer lifetime partners performing cunnilingus. Women with 4 or more long-term sex partners were at significantly greater risk (OR=2.38) of having a HPV infection with an oncogenic type compared to women with 3 or fewer long-term sex partners. In addition, women who engaged in masturbation appeared to have a two-fold increased risk (OR=2.26).

### *2.2.3 Risk associated with contraceptive history*

Women who used oral contraceptives occasionally within the last year were at a greater risk of having an overall HPV infection (OR=3.38) compared to women who never used the pill (Table XVII). However, this association was not significant after adjustment for age. While the associations were also strong for low-risk (OR=2.77) and high-risk (OR=4.09) HPV infections, neither was statistically significant. Women who reported using condoms regularly throughout their sexual lifetime were much less likely to have a high-risk HPV infection (OR=0.27) compared to those who never used condoms. However, women reporting occasional or regular use of condoms within the last year only were at a much greater risk of having a low-risk HPV infection (ORs of 3.46 and 4.39, respectively) compared to those who did not use condoms within the last year. The same profile was observed for women using contraceptive barriers within the last year, with women reporting occasional or regular use of a contraceptive barrier within the last year only at a much greater risk of having a low-risk HPV infection (ORs of 3.10 and 4.46, respectively) compared to those who did not use a contraceptive barrier within the last year.

### *2.2.4 Risk associated with hygiene and medical history*

Regarding hygiene and history of sexually transmitted diseases, washing the genital area after sex appeared protective for overall HPV infection (OR=0.56) and low-risk HPV infection (OR=0.40) compared to not washing after sex (Table XVIII). Women who

## Table XVII

### Odds Ratios[1] of Overall, High-Risk and Low-Risk HPV Infection According to Smoking and Contraceptive Factors

| Variable | Overall HPV Infection | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| | Crude OR | Adjusted OR[2] (95% CI) | Crude OR | Adjusted OR (95% CI) | Crude OR | Adjusted OR[2] (95% CI) |
| **Tobacco** | | | | | | |
| Never | 1.00[3] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Former | 1.69 | 1.71 (0.90-3.24) | 1.63 | 1.68 (0.70-4.03) | 1.75 | 1.74 (0.78-3.88) |
| Current | 0.82 | 0.77 (0.39-1.51) | 0.95 | 0.91 (0.37-2.24) | 0.70 | 0.65 (0.26-1.66) |
| **Packyears** | | | | | | |
| <1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1-5 | 1.70 | 1.72 (0.64-4.63) | 1.46 | 1.50 (0.39-5.81) | 1.95 | 1.95 (0.53-7.11) |
| >5 | 0.84 | 0.78 (0.14-4.27) | 1.68 | 1.49 (0.24-9.44) | 0.0 | 0.0 |
| **Oral contraceptive** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Occasionally | 1.56 | 1.63 (0.56-4.68) | 1.12 | 1.17 (0.22-6.19) | 1.86 | 1.95 (0.58-6.55) |
| Regularly | 0.77 | 0.80 (0.44-1.44) | 0.99 | 1.07 (0.46-2.50) | 0.62 | 0.63 (0.30-1.32) |
| **Recent[4] oral contraceptive use** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Occasionally | 3.50 | 3.38 (0.97-11.80) | 4.01 | 4.09 (0.88-19.01) | 2.92 | 2.77 (0.61-12.54) |
| Regularly | 0.69 | 0.69 (0.42-1.15) | 0.79 | 0.81 (0.40-1.64) | 0.55 | 0.53 (0.27-1.02) |
| **Age started pill (yrs)** | | | | | | |
| ≤16 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 17-19 | 0.89 | 0.89 (0.40-2.00) | 0.71 | 0.68 (0.25-1.90) | 1.17 | 1.20 (0.38-3.80) |
| ≥20 | 0.72 | 0.77 (0.30-1.98) | 0.56 | 0.58 (0.17-2.00) | 0.95 | 1.06 (0.28-3.97) |

## Table XVII

### Odds Ratios of Overall, High-Risk and Low-Risk HPV Infection According to Smoking and Contraceptive Factors (Continued)

| Variable | Overall HPV Infection | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| | Crude OR | Adjusted OR (95% CI) | Crude OR | Adjusted OR (95% CI) | Crude OR | Adjusted OR[2] (95% CI) |
| **Time on pill (yrs)** | | | | | | |
| ≤1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1-2 | 0.89 | 0.87 (0.43-1.76) | 0.92 | 0.91 (0.33-2.51) | 0.95 | 0.96 (0.41-2.24) |
| 2-3 | 0.74 | 0.81 (0.36-1.81) | 0.71 | 0.82 (0.25-2.72) | 0.76 | 0.79 (0.30-2.11) |
| ≥3 | 1.07 | 1.08 (0.18-2.41) | 1.30 | 1.39 (0.46-4.22) | 0.74 | 0.73 (0.25-2.11) |
| **Condom use** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Occasionally | 0.61 | 0.63 (0.23-1.69) | 0.38 | 0.40 (0.14-1.19) | 2.14 | 2.20 (0.27-17.93) |
| Regularly | 0.61 | 0.62 (0.23-1.62) | 0.26 | 0.27 (0.09-0.79) | 2.58 | 2.61 (0.33-20.66) |
| **Recent condom use** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Occasionally | 1.23 | 1.15 (0.59-2.24) | 0.57 | 0.52 (0.21-1.28) | 3.61 | 3.46 (1.18-10.20) |
| Regularly | 1.47 | 1.43 (0.79-2.58) | 0.66 | 0.62 (0.29-1.32) | 4.39 | 4.39 (1.61-11.98) |
| **Barrier use[5]** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Occasionally | 0.64 | 0.66 (0.23-1.92) | 0.37 | 0.40 (0.12-1.31) | 2.18 | 2.23 (0.27-18.40) |
| Regularly | 0.68 | 0.70 (0.26-1.92) | 0.35 | 0.36 (0.12-1.10) | 2.26 | 2.31 (0.29-18.27) |
| **Recent barrier use** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Occasionally | 1.10 | 1.02 (0.51-2.07) | 0.48 | 0.43 (0.16-1.16) | 3.23 | 3.10 (1.03-9.30) |
| Regularly | 1.64 | 1.59 (0.89-2.85) | 0.80 | 0.75 (0.36-1.57) | 4.49 | 4.46 (1.65-12.10) |

[1] By logistic regression; [2] Age adjusted; [3] Reference category; [4] Within last year; [5] Includes foam, cap, diaphram, sponge and condom.

## Table XVIII

### Odds Ratios[1] of Overall, High-Risk and Low-Risk HPV Infection According to Hygiene Habits

| Variable | Overall HPV Infection | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| | Crude OR | Adjusted OR[2] (95% CI) | Crude OR | Adjusted OR (95% CI) | Crude OR | Adjusted OR[2] (95% CI) |
| **Vaginal douche** | | | | | | |
| Never | 1.00[3] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ever | 1.31 | 1.28 (0.65-2.52) | 1.61 | 1.58 (0.67-3.72) | 0.80 | 0.78 (0.29-2.14) |
| **No. showers (per day)** | | | | | | |
| <1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 1 | 0.91 | 0.91 (0.52-1.58) | 0.98 | 0.97 (0.46-2.06) | 0.86 | 0.86 (0.42-1.75) |
| >1 | 0.69 | 0.75 (0.27-2.04) | 0.51 | 0.54 (0.11-2.61) | 0.86 | 0.92 (0.27-3.09) |
| **Wash genitals after sex** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ever | 0.55 | 0.56 (0.34-0.92) | 0.78 | 0.80 (0.41-1.56) | 0.39 | 0.40 (0.21-0.78) |
| **Method of wiping[4]** | | | | | | |
| Back-to-front | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Front-to-back | 1.43 | 1.41 (0.82-2.42) | 0.98 | 0.98 (0.49-1.97) | 2.12 | 2.08 (0.96-4.51) |

[1] By logistic regression; [2] Age adjusted; [3] Reference category; [4] Method of wiping after a bowel movement.

## Table XIX

### Odds Ratios[1] of Overall, High-Risk and Low-Risk HPV Infection According to Medical History

| Variable | Overall HPV Infection | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| | Crude OR | Adjusted OR[2] (95% CI) | Crude OR | Adjusted OR (95% CI) | Crude OR | Adjusted OR[2] (95% CI) |
| **History of STD[3]** | | | | | | |
| Never | 1.00[4] | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ever | 0.61 | 0.62 (0.24-1.56) | 1.20 | 1.21 (0.43-3.42) | 0.39 | 0.40 (0.09-1.75) |
| **History of warts** | | | | | | |
| Never | 1.0 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ever | 1.99 | 2.21 (1.02-4.80) | 1.86 | 2.08 (0.70-6.16) | 2.18 | 2.42 (0.93-6.28) |
| **Hormone therapy** | | | | | | |
| Never | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| Ever | 0.51 | 0.52 (0.11-2.40) | 0.00 | 0.00 | 0.99 | 1.01 (0.22-4.73) |
| **Lifetime No. of Paps received** | | | | | | |
| 1 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 | 1.00 |
| 2-3 | 0.55 | 0.60 (0.29-1.21) | 0.49 | 0.56 (0.20-1.58) | 0.60 | 0.61 (0.26-1.44) |
| 4-5 | 0.73 | 0.83 (0.39-1.79) | 0.82 | 1.07 (0.37-3.08) | 0.66 | 0.69 (0.26-1.80) |
| 6-10 | 0.86 | 1.11 (0.44-2.78) | 1.11 | 1.81 (0.51-6.39) | 0.69 | 0.76 (0.23-2.50) |
| >10 | 1.12 | 1.46 (0.48-4.41) | 2.00 | 3.38 (0.84-13.71) | 0.49 | 0.54 (0.10-2.93) |

[1] By logistic regression; [2] Age adjusted; [3] Includes *Trichomonas*, *Chlamydia*, herpes, syphillis & gonorrhea; [4] Reference category.

reported ever having a STD (excluding genital warts) appeared to be slightly protected against overall HPV infection (OR=0.62) compared to women who reported never having a STD (Table XIX). When analyzed according to high- and low-risk HPV infection, women reporting history of a STD were marginally at risk of having a high-risk HPV infection (OR=1.21), but were protected from infection with a low-risk HPV type (OR=0.40) compared to women who never had a STD. However, none of these results were statistically significant. Women with a history of warts were twice more likely to have an overall HPV infection compared to women without having a history of genital warts, but this excess risk was not statistically significant when analyzed according to low-risk or high-risk HPV types.

### *2.3 Multivariate analysis*

Multivariate analysis revealed that origin, lifetime frequency of sex and recent oral contraceptive use were all independent predictors of overall HPV infection (Table XX). Women from Europe and Western Canada (Prairies and B.C.) were at greatest risk. Likewise, women that engaged in sexual intercourse more than 3 times a week and used the pill sporadically in the last year had a higher risk of overall HPV infection. Women that engaged in sexual intercourse more than 3 times a week, had more than 4 lifetime partners who performed cunnilingus and used the pill sporadically in the last year had a greater risk of having an HPV infection with a high-risk HPV type. Regular use of a condom during the woman's sexual lifetime also proved to exert an independent effect in this group and play a significantly protective role. The profile was different with low-risk HPV infections. Recent use of oral contraceptives and lifetime condom use remained in the model for low-risk HPV infection but other significant variables in the model were masturbation and habit of washing the genital area after sex. Recent use of condoms did not prove to be protective in women infected with non-oncogenic HPV types. Occasional use of the pill or condom within the last year or regular use of the condom within the last year were independent predictors of low-

## Table XX

**Adjusted Odds Ratios[1] of Overall HPV Infection and High and Low Risk HPV Infection According to Selected Risk Factors**

| | Overall | | High-Risk | | Low-Risk | |
|---|---|---|---|---|---|---|
| **Variable** | **OR** | **(95% CI)** | **OR** | **(95%CI)** | **OR** | **(95%CI)** |
| **Origin** | | | | | | |
| Ontario & West[4] | 1.00 | | | | | |
| Que. & Maritimes | 0.48 | 0.25-0.94 | | | | |
| USA | 0.46 | 0.18-1.14 | | | | |
| Europe | 1.18 | 0.48-2.89 | | | | |
| Other | 0.35 | 0.13-0.95 | | | | |
| **Lifetime frequency sex** | | | | | | |
| 0-4/month | 1.00 | | 1.00 | | | |
| 5-13/month | 1.70 | 0.86-3.37 | 2.22 | 0.81-6.09 | | |
| 14+/month | 3.45 | 1.67-7.15 | 5.09 | 1.80-14.39 | | |
| **No. oral sex partners** | | | | | | |
| 0-3 | | | 1.00 | | | |
| 4-9 | | | 2.41 | 1.09-5.32 | | |
| 10+ | | | 4.62 | 1.26-16.94 | | |
| **Recent oral contraceptive use** | | | | | | |
| Never | 1.00 | | 1.00 | | 1.00 | |
| Occasionally | 3.70 | 1.00-13.74 | 3.25 | 0.61-17.33 | 4.68 | 0.96-22.84 |
| Regularly | 0.60 | 0.35-1.01 | 0.53 | 0.24-1.16 | 0.72 | 0.35-1.48 |
| **Condom use** | | | | | | |
| Never | | | 1.00 | | | |
| Occasionally | | | 0.27 | 0.08-0.88 | | |
| Regularly | | | 0.14 | 0.04-0.47 | | |
| **Recent Condom use** | | | | | | |
| Never | | | | | 1.00 | |
| Occasionally | | | | | 3.50 | 1.18-10.42 |
| Regularly | | | | | 3.56 | 1.24-10.21 |
| **Masturbation** | | | | | | |
| Never | | | | | 1.00 | |
| Ever | | | | | 0.45 | 0.23-0.88 |
| **Wash after sex** | | | | | | |
| Never | | | | | 1.00 | |
| Ever | | | | | 0.38 | 0.19-0.77 |

[1] By Multivariate analyses. Models include only variables independently associated with risk.

risk HPV infection. Masturbating and washing the genital area after sexual intercourse were both independently protective of low-risk HPV infection.

### *2.4 HPV infection as a risk factor for squamous intraepithelial lesions*

The association between HPV infection by oncogenic risk and cervical cytological abnormalities was significant. Cytological abnormalities were classified as low-grade or high-grade squamous intraepithelial lesions (SIL) or atypical squamous cells of undetermined significance (ASCUS). Of the 489 specimens and cytology results available, 449 were eligible for analysis (40 specimens were β-globin negative). Of those 449 women, 55 (12.2%) had an abnormal Pap smear; 37 (67.3%) of them were diagnosed with ASCUS while the remaining 18 (32.7%) had an SIL. The 55 women were grouped together (ASCUS and SIL) and the ORs were calculated according to overall HPV infection and by oncogenic and non-oncogenic HPV types (Table XXI). The risk of having an abnormal cytology result (ASCUS or SIL) did not vary materially according to type of HPV infection. After adjusting for age and other potential confounders including number of sexual partners, age at first intercourse and frequency of sex, the final model revealed ORs for abnormal cytology of 2.71 according to overall HPV infection, 2.55 according to high-risk HPV infection and 3.18 according to low-risk HPV infection. HPV infection was a much stronger predictor of SIL than of an ASCUS. When the outcome considered was SILs only, the ORs did not vary substantially according to HPV oncogenicity. After adjusting for age and other confounders (number of sexual partners, age at first intercourse and frequency of sex), the final model revealed ORs for abnormal cytology (SIL) of 9.46 according to overall HPV infection, 11.16 according to high-risk HPV infection and 11.02 according to low-risk HPV infection.

## Table XXI

### Association Between HPV Infection by Oncogenic Risk and Cervical Cytological Abnormalities

| Cytological Outcome | HPV Infection | *% HPV + | OR (95% CI) Crude | Age adjusted | **Adjusted for age and sexual activity |
|---|---|---|---|---|---|
| **ASCUS & SIL (N=55)** | | | | | |
| | Overall | 36.4 | 2.32 (1.3-2.2) | 2.26 (1.2-4.1) | 2.71 (1.4-5.1) |
| | High-risk | 16.4 | 2.14 (1.0-4.8) | 2.08 (0.9-4.7) | 2.55 (1.1-6.0) |
| | Low-risk | 20.0 | 2.48 (1.2-5.3) | 2.48 (1.2-5.3) | 3.18 (1.4-7.1) |
| **SIL (N=18)** | | | | | |
| | Overall | 66.7 | 8.10 (2.9-22.3) | 8.13 (2.9-22.5) | 9.46 (3.2-27.7) |
| | High-risk | 33.3 | 8.32 (2.6-27.1) | 9.15 (2.7-30.7) | 11.16 (3.1-40.7) |
| | Low-risk | 33.3 | 7.90 (2.4-25.7) | 8.19 (2.5-27.1) | 11.02 (2.8-45.6) |

*Breakdown of HPV status according to abnormal cytology.

**Parameters of sexual activity include age at first intercourse, lifetime number of partners and frequency of sexual encounters.

# CHAPTER 6

# DISCUSSION

# DISCUSSION

## 1 Estimating HPV prevalence

### *1.1 Advantages*

Until now, there has only been one other study, (Rohan *et al.,* 1991), designed to investigate the prevalence and determinants of HPV infection in Canadian university women. Previous studies have demonstrated that it is women aged 18 to 25 years that are most at risk for HPV infection (Ley *et al.,* 1991; Schiffman and Schatzkin, 1994; Wheeler *et al.,* 1993). Thus, female university students are a convenient sub-population to study to determine the most important risk factors for cervical HPV infection. This study attempted to use the most sophisticated HPV detection methods available, employing PCR techniques followed by Southern blot hybridization of the amplified products. A modified generic probe (Bauer *et al.,* 1991) was designed to target all the presently identified mucosal HPV types and 27 type-specific oligonucleotides were then used to identify individual types (Hildesheim *et al.,* 1993). Those samples that hybridized with the generic probe but with none of the type specific oligonucleotides were classified as "type unknown". The sample size of nearly 500 participants permitted a number of statistical analysis to be conducted with sufficient power to estimate ORs of moderate magnitude for overall HPV infection and separately for low-risk and high-risk HPV infection. The lengthy questionnaire elicited detailed information on the sexual behaviour of Montreal university women.

### *1.2 Disadvantages*

There are limits to the contribution such a study can make to the understanding of HPV transmission. The study population is very homogenous, coming from relatively similar socio-economic and educational backgrounds. Thus, while this homogeneity eliminates certain confounding by socio-economic status and education, making it easier to evaluate sexual activity as a risk factor, it is not a representative sample of the whole

population and the results apply only to university women who are generally younger than 30 years of age.

In addition, this study shares many methodological difficulties inherent in the estimation of HPV prevalence. The principal among them are :

1. Problems related to biological sampling (including specimen collection and the influence of the menstrual cycle on HPV detectability)
2. Interlaboratory variability for DNA detection
3. Variability within and between the populations studied

### *1.2.1 Problems related to biological sampling*

Methodological deviations can hinder the comparability between studies. This type of study relies heavily on good biological sampling and molecular biology techniques to ensure comparability. In the last 5 years, epidemiological studies on cervical HPV infection have used a variety of specimen collection methods including the swab, (Ley *et al.,* 1991; Wheeler *et al.,* 1993), the cervico-vaginal lavage, (Bauer *et al.,* 1993; Hildesheim *et al.,* 1993) the spatula (Rohan *et al.,* 1991), the cytobrush (Franco *et al.,* 1995), or in our case, a combination of the spatula and cytobrush. It is plausible that the method of specimen collection can influence the ability to detect cervical HPV DNA, subsequently inflating or deflating the "true" prevalence rate. The swab has been the standard tool used to collect cells for cervical cancer screening. It collects both ecto and endocervical cells and occasionally other vaginal cells. Cervicovaginal lavages collect cervical and vaginal cells in abundance. This method is often used in a research setting but rarely in a clinical environment. The spatula and cytobrush are more specific primarily collecting ecto or endocervical cells, respectively. Most of the prevalence rates fall into the range of 15-40% regardless of what specimen collection method was used and the disparity between the majority of prevalence

rates is most likely explained by the different study populations. It is not clear, however, why the two American university studies (Ley *et al.,* 1991; Wheeler *et al.,* 1993) which used the swab observed prevalence rates that were twice as high as the two Canadian university studies (Rohan *et al.,* 1991 and present study) which used the spatula or a combination of the spatula and cytobrush, (Table V). Contrary to the observations that women from American universities have a higher risk of being HPV positive than women from Canadian universities, findings from this study suggest that women from the USA are less likely to be HPV positive than women from Western Canada or Europe. Can this difference be explained by the method of specimen collection or is it more likely attributable to the fact that Ley and collaborators (1991) collected and analyzed vulvar cells in addition to cervical cells while Wheeler and collaborators (1993) studied a university population from New Mexico with a large proportion of white Hispanic women who are apparently at greater risk for HPV infection. Only future Canadian and American studies of HPV infection in female university students can unravel this point.

The influence of the menstrual cycle on the detection of HPV DNA also remains uncertain with inconsistent results from published studies. Intermittent detection of HPV has been reported by certain investigators (Moscicki *et al.*, 1992; Schneider and Koutsky, 1992), suggesting that hormone levels associated with the menstrual cycle may affect the detection of HPV (Frost, 1974). However, Fairley and collaborators (1994) more recently observed that the pellet volume of a specimen, but not HPV detection, vary during the menstrual cycle (Fairley *et al.*, 1994). Their study found no difference in the prevalence of HPV among women whose samples were obtained at different times during the menstrual cycle. Data on the influence of the menstrual cycle on HPV detectibility was not collected in this study. Nonetheless, future studies should probably include information on when, during the menstrual cycle, samples were collected, in an attempt to clarify the issue.

### *1.2.2 Inter laboratory variability*

HPV DNA preparation is a second methodological difficulty which can affect DNA detectability. An organic DNA extraction can result in a more pure and concentrated product for the PCR mixture, but there is also more risk of cross-contamination with other samples because of increased handling. Other studies have successfully completed HPV prevalence surveys, amplifying the viral DNA immediately after cellular digestion with detergents that do not interfere with PCR such as Tween 20 or NP40, avoiding the step of DNA extraction altogether (Bauer *et al.,* 1993; Hildesheim *et al.,* 1993; Ley *et al.,* 1991; Rohan *et al.,* 1991; Wheeler *et al.,* 1993). However, in this study, approximately 20% of the first 100 samples contained varying amounts of blood which can be a strong inhibitor of PCR. The blood in the samples may be a reflection of one of two things. Either, the doctors conducting the examinations were not particularly skilled with the use of the cytobrush, thereby causing some irritation when collecting the sample, or, women were receiving their Pap smears before their period was terminated. Because of this excess blood, we decided to perform organic extractions on all the present and future samples. As a result, a simple proteinase K and SDS protocol was implemented for cellular digestion, before embarking on a phenol-chloroform extraction with an ethanol DNA precipitation (Franco *et al.,* 1995). The proteinase K and SDS protocol for cellular digestion was less complicated than many other digestion protocols, but the use of SDS, another strong PCR inhibitor, made a subsequent organic (phenol-chloroform) extraction essential. The prevalence rates from Brazil (18.3%) and Montreal (21.8%), studies that organically extracted their DNA, however, did not differ substantially from the range of prevalence rates (15-40%) observed in studies that avoided organic extraction procedure.

### *1.2.3 Variability within and between the populations studied*

The range of HPV prevalence rates observed in the literature (PCR based studies) are, in part, a reflection of the different populations studied. High risk populations, such as young, sexually active, single women are more likely to have higher prevalence rates than middle-aged, monogamous, married women, unless the married woman is from a culture where her husband practices unsafe sex with prostitutes (Bosch *et al.*, 1995). Thus, the difference in prevalence rates between different populations is most likely related to differences in risk factors for HPV infection.

## 2 Determinants of cervical HPV infection

### *2.1 Sociodemographics*

It has been demonstrated in previous studies (Brinton, 1992; de Sanjosé *et al.*, 1995) that low economic status (LES) is a risk factor for HPV infection. However, our study population, being a group of university students, was *de facto*, upwardly mobile and therefore, an evaluation of social class and HPV infection could not be performed. Other important sociodemographic variables that have been negatively associated with HPV infection are marital status and parity. However, very few women in our population were married and even fewer had children. Therefore, we did not have the statistical power to properly evaluate any potential effect due to these variables.

On the other hand, we were able to evaluate a risk factor that has been strongly implicated with risk of infection, namely, place of birth. Place of birth has been considered a potential determinant for HPV infection because of the observed high prevalence rates of cervical HPV infection and incidence rates of cervical cancer in various parts of the world, primarily Third World countries (Kjaer and Jensen, 1992; Schiffman, 1992). Some of the important reasons why developing countries have a higher incidence of cervical cancer, include lower economic status and few if any available screening programs for cervical

neoplasm. However, these are not the only explanations. The Middle East, including Israel, observe very low incidence rates of cervical cancer and not all of these countries have equal access to cytological screening. Can it be explained by certain religious rituals that these cultures practice, such as circumcision, or are they more immunologically protected from HPV infection? There is evidence to suggest that certain individuals in a given population are less immunologically equipped to combat HPV infection which may be genetically linked (Apple *et al.,* 1994). However, can entire (homogenous) populations be more genetically predisposed to HPV infection?

In the present study, place of birth was a significant predictor of overall HPV infection although the effect disappeared when analyzed separately with oncogenic and non-oncogenic HPV infections. Women from Europe and "Western" Canada (west of Quebec) were more likely to be HPV infected than women from "Eastern" Canada, the United States or elsewhere. These results were somewhat surprising since "elsewhere" (other) consisted of some of the most high-risk countries for HPV infection and cervical cancer, including Brazil and India. However, the number of women from "elsewhere" was very small and these women are probably not representative, at least financially, of the majority of women from the less developed countries that are at greater risk.

Sexual activity and hygiene appear to explain the association with place of birth, in part, since the association with HPV infection disappears in the analysis of high-risk HPV infection, when sexual activity becomes a much stronger predictor, or in the analysis of low-risk HPV infection when hygiene becomes a much stronger correlate of risk.

### *2.2 Sexual behaviour and contraceptive use*

#### *2.2.1 Sexual activity*

With the overwhelming evidence that HPV infection is the sexually transmitted cause of cervical neoplasia, the risk of HPV infection should then correlate with the same sexual

variables that have been so strongly associated with the risk of cervical cancer in all previous studies. Accordingly, a number of investigations have attempted to correlate subclinical genital HPV infection with sexual variables such as lifetime number of partners and age at first intercourse, and other risk factors for cervical cancer such as race, smoking and oral contraceptive use. However, studies examining the correlates of cervical HPV infection as measured by viral DNA detection, have produced conflicting results. Not all epidemiological studies using PCR techniques to detect HPV in different populations have been able to unveil the profile of a sexually transmitted HPV infection. Some surveys indicate that the association between sexual activity and overall HPV prevalence can be strong (Bauer *et al.,* 1993; Ley *et al.,* 1991), moderate (Franco *et al.,* 1995; Wheeler *et al.,* 1993), mild (Hildesheim *et al.,* 1993; Rohan *et al.,* 1991) or even non-existent (Kjaer *et al.,* 1993) (Table XXII).

Since misclassification of HPV infection is less of a concern in these studies, (they all employed the much more sensitive and specific technique of PCR), it is conceivable that the variability among results might be caused by differences across populations in the relative prevalence of HPV types with greater or lesser transmissibility by the sexual route (Kjaer *et al.,* 1993). The results from this study suggest that the differences in the degree of sexual transmissibility among types may explain why some prevalence studies, including this one, have failed to demonstrate a strong association between measures of sexual activity and overall HPV infection, despite using reliable viral DNA procedures.

Overall prevalence of HPV infection in this study was not associated with the two main correlates of cervical neoplasia, age at first intercourse and lifetime number of sexual partners. However, it was moderately associated with lifetime frequency of sexual encounters (averaged over number of times per month). By analyzing the determinants of

## Table XXII

### Consistancy of the Evidence of an Association Between Sexual Activity[1] and Overall HPV Infection

| Study | Lifetime No. sex partners | OR[2] | Strength of association |
|---|---|---|---|
| *Ley et al. (1991)* | 6-9 | 5.00 | Significant |
| | ≥10 | 11.20 | Significant |
| *Rohan et al. (1991)* | ≥3 | 1.90 | Not significant |
| *Bauer et al. (1993)*[3] | 6-9 | 7.62 | Significant |
| | ≥10 | 8.50 | Significant |
| *Wheeler et al. (1993)*[3] | 6-9 | 3.79 | Significant |
| | ≥10 | 3.80 | Significant |
| *Hildesheim et al. (1993)*[3] | ≥4 | 1.60 | Not significant |
| *Kjaer et al. (1993)* | 4-9 | 0.80 | Not significant |
| | ≥10 | 1.10 | Not significant |
| *Franco et al. (1995)* | 6-10 | 3.16 | Significant |
| | ≥11 | 2.72 | Not significant |
| *Present study* | 5-9 | 1.30 | Not significant |
| | ≥10 | 1.73 | Not significant |

[1] Using number of lifetime sexual partners as marker for sexual activity

[2] With respect to one partner only

[3] Calculated from prevalence differences (PD) given in the literature

cervical HPV infection after grouping HPV types into low and high oncogenic-risk groups on the basis of their frequency of association with invasive cervical carcinomas (Bauer *et al.*, 1993), it became possible to unveil different profiles of association with sexual activity measures and related variables. In fact, a recent Brazilian study on the transmission of cervical HPV infection by sexual activity revealed similar profiles (Franco *et al.*, 1995). Overall HPV infection was moderately associated with age at first intercourse and lifetime number of sexual partners. When the analysis focused on low and high oncogenic-risk types, the likelihood of infection with low-risk types was mostly invariant with respect to markers of sexual activity. However, the latter variables were very strong predictors of infection with high oncogenic risk types.

While not strong, the association between sexual activity and low-risk HPV infection was, nonetheless, difficult to assess. The only marker of sexual activity that was associated with non-oncogenic HPV infection was masturbation, which played a significantly protective role against infection. There is little in the literature that might help explain this finding and one can only speculate at this present time as to why masturbation is a protective factor for low-risk HPV infection. It is tempting to suggest that the action of masturbation implies less sexual contact with a partner, but, even after adjustment for number of sexual partners, masturbation remained an independent protective factor. One can, however, also speculate on the type of woman who would masturbate. While North American women have become increasingly sexually liberated since the sexual revolution in the 1960's, there still exists a fundamentally conservative attitude about female masturbation, held equally by men and women. Perhaps masturbation is an indicator of a more progressively thinking woman, who is not only more sexually aware of her own body, but is also more discriminating in her choice of sexual partners. Although statistical adjustments are easily made for quantity of partners, it is virtually impossible to adjust for quality of partners. In addition, we can also speculate that masturbation may be an indicator of a more experienced and demanding sex

partner, requiring more foreplay, thus more lubrication and therefore fewer lesions. Once again, this is pure speculation, which nonetheless, illustrates one of the many interesting dimensions of the epidemiology of HPV infection and sexual behaviour which cannot be easily assessed via standard questionnaires.

The association between risk of an oncogenic HPV infection and markers of sexual activity was strong and easy to interpret. The adjusted model of correlates of high-risk HPV infection revealed independently strong risks for women who engaged in sexual intercourse more than 3 times a week or had 4 or more lifetime partners who performed cunnilingus. While these two particular parameters of sexual activity have not been observed frequently in other past studies, such a profile is what would be expected for such an early endpoint of HPV infection. It was surprising that number of lifetime sexual partners was not a predictor of high risk HPV infection in the final analyses. However, the relation between infections by high-risk types and sexual behaviour appears to be quite complicated and the risk of infection by high-risk types may be correlated with other dimensions of sexual behaviour besides lifetime number of partners, the most commonly studied marker. A variety of epidemiological surveys have found varying degrees of association between number of lifetime sexual partners and HPV infection (Table XXII). Some surveys have found stronger associations between recent sexual partners and HPV infection than for lifetime number of sexual partners (Hildesheim *et al.,* 1993). Evidently, there is no one ideal marker of sexual activity. Populations may differ in the strength of association for distinct predictors of sexual activity as observed in recent epidemiological studies. Hildesheim and collaborators (1993) studied a population of inner city Washington D.C. women, considered at high risk because of their lower socio-economic status. Number of recent sexual partners (in the past 5 years) was a better determinant of HPV prevalence than lifetime sexual behaviour (Hildesheim *et al.,* 1993). A study of prostitutes in Denmark (Kjaer *et al.*, 1995) unveiled a similar profile. Age at first intercourse and lifetime number of partners were not associated with risk of HPV

infection. However, number of "commercial" (customers) and "private" sexual partners within the last 4 months were significantly related to HPV detection. Both these findings help to support the theory that HPV infection is a transient phenomena, since sexual behaviour closer to the time of sample collection was a more important determinant of HPV prevalence than behaviour in the past or distant past (Hildesheim *et al.,* 1993).

By contrast, a recent study of middle-class, white suburban women (Bauer *et al.,* 1993), defined as "low-risk", observed lifetime number of sexual partners to be the strongest predictor of HPV infection. The risk to this more monogamous population may, therefore, be better explained by the male partner. This has been suggested by Brinton and collaborators (1989) who observed that the risk of cervical cancer increased significantly with the number of sexual partners reported by the husbands of cases in Central and South America. More recently, Bosch and collaborators (1995) have just linked a key role in male sexual behaviour to cervical cancer incidence in Spain and Colombia. Investigators were initially perplexed at the disparity between incidence rates for cervical cancer in Spain and Colombia. There have not been many cervical screening programs in Spain designed to target the middle-aged female population. Organized screening programs, have, however, met with some success in Colombia. Despite this success, Colombia has one of the highest incidence rates for cervical cancer. Conversely, Spain has very low incidence rates for cervical cancer. In their case-control study, Bosch and collaborators (1995) revealed that this disparity in risk for cervical cancer was partly explained by lower socio-economic status, which may explain fewer Pap tests. Colombian (control) women also had more lifetime sexual partners than Spanish (control) women. In addition to female sexual behaviour, however, the male partner's sexual behaviour was a strong predictor of risk. Colombian control husbands had more lifetime sexual partners and much more frequent contact with prostitutes than the Spanish control husbands. The Colombian male partners were also nearly 6 times more likely to be HPV positive. Evidently, the sexual behaviour of the male

partner is a key determining factor for the disparity in cervical cancer incidence between Spain and Colombia, and it is also very likely to be a key factor in determining risk of HPV infection in other "low-risk" female populations (Bosch *et al.*, 1995). Obviously there is more than one marker for sexual activity and the risk of HPV transmission cannot be reduced to lifetime number of partners only.

### *2.2.2 Oral contraceptives*

Assessing contraceptive use is a more indirect method of determining sexual behaviour and, in the case of barrier contraceptives, can also provide some valuable information on the protective role it may play against HPV acquisition. It has also been suggested that oral contraceptives have a potential of increasing susceptibility to viruses through an interference with the immune system. In addition, the biological plausibility of a hormonal mechanism in cervical carcinogenesis has been supported by the findings of hormone receptor sites in cervical tissue, and by reports of cervical changes resulting from administration of contraceptive steroids. Finally, these hormones have been observed to enhance the transcription activity of the HPV genome (Brinton *et al.*, 1990).

The profile for oral contraceptive use in this study was very similar for all types of HPV infection. Duration of use appeared to be a factor since only occasional use of the pill within the last year was a strong risk factor for overall, low-risk and high-risk HPV infection, although this finding was only significant for overall HPV infection, (undoubtedly, due to the small number of women in the "occasional use" category, which was further reduced when analyzed separately by risk-group). It is not clear why only women who used the pill occasionally within the last year, should be at greater risk. Perhaps the sporadic hormonal changes due to occasional use of oral contraceptives render the woman more susceptible to a transient HPV infection. However, it is also possible that intermittent use of the pill is an indirect predictor of number of casual sex experiences and is measuring the start

and end of new sexual relationships. While preliminary analyses suggest this to be true interpretation, the number of occasional pill users (N=11) in the study was too small to perform any comprehensive statistical analyses. It would, however, be interesting to study this specific behaviour of intermittent oral contraceptive use and its association with number of casual sexual experiences. Women who used the pill regularly within the last year appeared to be protected from any type of HPV infection, suggesting that these women were fairly monogamous with one steady partner, although this finding was not statistically significant.

Contrary to the present findings, while a number of surveys have also observed that oral contraceptive use was an independent risk factor for HPV infection (Bauer *et al.,* 1993; Hildesheim *et al.,* 1993; Ley *et al.,* 1991) or development of cervical neoplasia (Kjaer *et al.,* 1993), duration of use had very little effect. Thiry and collaborators (1993) found that the risk of cancer lesions did not increase with the use of oral contraceptives, and SILs (LG-SIL and HG-SIL) were less frequent in women taking the pill, independent of HPV infection. However, HPV prevalence was twice as great in women with normal cytology who were taking oral contraceptives over a minimum of 6 months, compared to women using diaphragms or condoms. Interestingly, HPV prevalence appeared to vary according to the pill trademark, but not according to pill hormone content. Conversely, Davidson and collaborators (1995) found no association with oral contraceptive use and HPV infection or development of cervical dysplasia in Alaska Native women. Evidently the true effect of oral contraceptives and risk of HPV infection is not yet clear and even less clear is the effect of duration of oral contraceptive use.

### *2.2.3 Barrier contraceptives*

There is very little data on the effectiveness of barrier contraceptives in preventing infection. Thiry and collaborators (1993) observed that condom use was not associated with

risk of cervical dysplasia or HPV infection. A more recent study observed condom use to be associated with higher rates of HPV infection before adjusting for other factors (McNicol and Young, 1995). However, this association was most likely explained by sexual activity, since the association disappeared when adjusted for other factors including sexual activity.

In the present study, contrary to oral contraceptive use, the profile for barrier contraceptive use was substantially different for low-risk and high-risk HPV infection. Regular condom use throughout the woman's lifetime proved to be significantly protective for high-risk HPV types. Conversely, regular and occasional condom use within the last year was not protective for low-risk HPV types. Assessing the risk for condom use is very difficult, not least of all because of the high potential for bias reporting. Reporting of condom use may be particularly biased if women are using condoms for contraceptive purposes but not necessarily as a barrier against sexual transmitted diseases. In this case scenario, one can speculate that the condom may only be used closer to her partners moment of "climax" during sexual intercourse, so that the woman may still be coming into contact with HPV from her partner even if she is reporting condom use. Despite the possibility of bias reporting, the two different transmission profiles that emerge from this study, nonetheless, raise a number of questions about the actual biological differences between oncogenic and non-oncogenic HPV types. Could such differences affect viral colonization or shedding in, or transmission by, both the female and male host, and thus, explain the observed variation in risk factors for infection (Ley *et al.,* 1991)? Are oncogenic HPV types more mucosotropic? Are non-oncogenic HPV types more cutaneo/mucosal tropic and therefore more likely to be present on the shaft of the penis or around the testicular region which may not be protected by the condom? Are low-risk HPV infections less persistent, thus, the association with short duration (within the last year) only? With respect to the last question, there is some preliminary evidence to suggest that oncogenic HPV infections are more persistent while non-oncogenic HPV infections are more transient (Franco *et al.*,

1996). However, more natural history cohort studies of cervical HPV infection with multiple follow-ups are needed to really clarify this issue. In addition, future surveys on female contraceptive history should probe more deeply the woman's reasons for practicing safe sex, if in fact she does. Future questionnaires should include questions about consistency of condom use and at what point of the sexual encounter the condom is used.

### *2.3 Hygiene and Medical History*

There are a few published reports describing the relationship between specific aspects of hygiene and HPV infection or cervical cancer (Brinton *et al.,* 1987). Neither hygiene nor douching was consistently related to risk of cancer. In this study, we not only inquired about bathing and douching but we also probed for information on hygiene practices in relation to sexual activity. The more general measures of hygiene (i.e. numbers of showers per day) showed no relation to likelihood of HPV infection. However, washing the genital area after sex appeared to play a role in the modality of low-risk HPV transmission. The adjusted model for low-risk HPV infection revealed that washing the genital area (within an hour) after sexual intercourse was protective against non-oncogenic HPV infections. It is not clear why this hygienic measure could be more effective against low-risk types than high-risk types. Perhaps the mechanical act of sexual intercourse increases detectability of low-risk transient infections, particularly if infected cells are displaced from the upper genitalia to the lower genitalia. Washing immediately after sex may remove infected cells from the lower genitalia, that were otherwise inaccessible. Alternatively, perhaps low-risk types are more capable of lingering around the external genitalia and are more resistant to ambient temperatures, but easily removed with a wash clothe, adding weight to the speculation that low-risk types are less mucosotropic.

Whatever the explanation, evidently HPV transmission can not be explained completely by number of sexual partners. In fact, the collection of different cervical HPV

infections caused by different types of HPV undoubtedly have an assortment of different transmission profiles, that we are only just now beginning to unsort.

The profile for history of an STD was different for low-risk and high-risk HPV infection. Women with a history of having an STD were slightly more at risk for having a high-risk HPV infection but were protected from having a low-risk HPV infection. However, these results were not significant, and while they suggest that there may be two different modes of transmission for HPV infection according to oncogenecity, the association between history of having an STD and risk of HPV infection will have to be investigated in future studies to determine the significance of these observations.

### *2.4 Cytology*

The strongest association observed was between HPV infection (overall and according to oncogenic risk) and cervical dysplasia (low-grade and high-grade SIL). Women with an HPV infection were approximately 10 times more likely to have an SIL than women without HPV, even after adjustment for age and selected predictors of sexual activity. The link between HPV and cervical dysplasia was consistent with the literature (Koutsky *et al.,* 1988; Ley *et al.,* 1991; Meekin *et al.,* 1992; Reeves *et al.,* 1989). Our results also suggest that HPV infection, regardless of type, is a greater risk factor for SIL (OR=9.46) than for ASCUS (OR=2.71). This is because of greater HPV prevalence and/or a greater viral burden present in the SIL specimen. There was no distinction between type of HPV infection and risk of developing an SIL.

Not all cervical dysplasias progress to *in situ* carcinomas. Unfortunately, it is difficult to accurately estimate the risk of progression due to subjective cytological and pathological interpretations and the potential alteration of the natural cause of disease by an intervening biopsy (Morrison, 1994). However, on-going natural history studies of cervical cancer have begun to provide some insight into the potential chronicity of HPV infection and

into risk factors for persistence of HPV and for neoplastic change (Franco, 1994; Hildesheim *et al.,* 1993).

Consequently, a number of different research groups have carried out several investigations, in the last few years, to assess the potential benefits of including HPV testing as an adjunct to cytological screening, so as to help improve early diagnosis of high-grade SIL (Franco, 1994; Hildesheim *et al.,* 1994; Moscicki *et al.,* 1992). It is currently suspected that far too many ASCUS or low-grade SIL diagnoses are unnecessarily referred for colposcopy rather than for a repeat Pap test, which costs millions of tax dollars for North Americans and Europeans. In North America, a Pap smear costs an average of $17 per test, while a colposcopic examination can range from $50-$300. HPV testing could help reduce screening costs by improving the management of borderline cases. Physicians would have an easier time referring women with an abnormal Pap test for a repeat Pap in 6 months knowing she was only infected with a low-risk HPV type, while women with an abnormal Pap test but harbouring a high risk HPV infection would be immediately referred for a colposcopic examination. Therefore, HPV testing as an adjunct to Pap testing would not only limit the amount of unnecessary discomfort women would have to endure (by reducing the number of women referred for colposcopy) but also cut screening costs dramatically.

# CHAPTER 7

# CONCLUSION

## CONCLUSIONS

This study set out to estimate the overall prevalence of cervical HPV infection in asymptomatic university women. We estimated the overall HPV prevalence to be 21.8% which was in the general range of 15-40% cited in the literature. The second aim of the study was to correlate risk factors with overall HPV infection, low oncogenic-risk HPV infection and high oncogenic-risk HPV infection. Sociodemographic factors have been shown to be related to risk of HPV infection in other studies. Place of birth was the only demographic factor to be positively associated with HPV in our study, but surprisingly, women from less-developed countries were not at risk. Two profiles emerged for sexual activity and risk of HPV infection according to oncogenic risk. Sexual activity was associated with high oncogenic-risk HPV infection. However, HPV infection with low oncogenic-risk types were mostly invariant with respect to markers of sexual activity. Furthermore, women who reported masterbating were protected against low-risk HPV infection, independent of other variables for sexual activity. However, while barrier contraceptives were protective against high oncogenic-risk HPV infection they were associated with low oncogenic-risk HPV infection. Finally, the hygienic act of washing the genitalia after sexual intercourse proved to be protective against low-risk HPV infection. These results suggest that there may be differences in epidemiological correlates of transmission between low-risk and high-risk HPV types on the basis of the oncogenicity. In view of our findings, we suggest that low oncogenic-risk HPV types may be less mucosotropic and therefore be transmitted by modes other than sexual activity.

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# APPENDIX 1

## Personal Data Sheet

## PERSONAL DATA SHEET

**Please fill in this sheet and sign the attached consent form. Give both to the laboratory technician with the tube containing the pap smear specimen. Thank you.**

*The information contained on this sheet will not be linked to the questionnaire information which will remain anonymous. This information is necessary so that we can contact you for a follow-up appointment at the health service in the future.*

**Name:** _____

**Address in Montreal:** _____

_____

**Telephone Number in Montreal:** _____

**Permanent Address:** _____

_____

**Date of Birth:** _____
day/month/year

**Do you expect to be studying/working at McGill during the next 2 years?**

❑ Yes ❑ No

**In which Department/Faculty are you enrolled?** _____

**McGill student ID number:** _____

# APPENDIX 2

## HPV Study - Consent Form

## HPV STUDY - CONSENT FORM

By signing this form I agree to participate in a research project to determine the prevalence and incidence of Human Papillomavirus (HPV) infection in a young adult female population. HPV is a virus (the human papilloma virus) which is normally associated with asymptomatic infections of the genital area.

The presence of HPV infection will be detected by collecting samples of cells from the cervix of the uterus at the same time and by use of the same instrument (spatula) as is used for my "Pap" smear. The sample will then be sent for examination to determine the presence or absence of HPV, and if present, its type. The study is taking place in conjunction with the routine annual pelvic examinations and Pap smears done at the Health Service.

I will be asked to complete a self-administered questionnaire. I have the right to refuse to answer any question, and I also have the right to withdraw from the study at any time. One additional visit may be required during the fall of 1993.

1. I understand that two Pap smears will be taken, and specimens will be examined to determine if there is any evidence of HPV infection. I further understand that the risks are minimal.

2. I understand that the potential benefit of this study will be an increase in our knowledge of a potentially hazardous disease.

3. I understand that all records for this study will be kept confidential and that access to my records will be restricted to members of the research group. I also understand that although my name will be known to members of the research group (so that results of Pap smears and HPV tests can be added to my record), I will not be identified by name in any reports from this study.

| | |
|---|---|
| ..... | ..... |
| Participant (signature) | Witness (signature) |
| ..... | ..... |
| Print Name | Print Name |
| ..... | ..... |
| Date | Date |

# APPENDIX 3

## Women's Health Study Questionnaire

**STUDY #:**

# WOMEN'S HEALTH STUDY QUESTIONNAIRE

**McGILL UNIVERSITY STUDENT HEALTH SERVICES**
**DEPARTMENT OF EPIDEMIOLOGY AND BIOSTATISTICS**

## INSTRUCTIONS FOR THE QUESTIONNAIRE

This questionnaire is composed of the following sections:

- General information
- Smoking history
- Reproductive history
- Sexual history
- Contraceptive history
- Personal hygiene habits
- Medical history

Most questions require that you simply check a box ❑ with an "X" to indicate your choice. Other questions require a specific answer, such as age, date, or another number. Depending on your answer for some questions, you will be told to skip the next questions and go to a different part of the questionnaire. This is to save your time, so that you won't have to go over questions that do not apply to you.

There are no right or wrong answers to any question. Many questions require that you think back over your adult years to recall specific information. Please take the time to reflect. If you prefer, you can answer sections of the questionnaire on different days. If you choose to do so, check your answers from previous days to make sure you agree with them before mailing the questionnaire back to us. (You will be surprised that by being "forced" to recall specific information of one type, some of the answers for other questions may come more naturally to you later on.) If you can't possibly remember the information skip the question, but we would like to encourage you to try to answer all questions. A good guess is always better than no information at all. If you'd like to tell us more about any specific items please use the available space at the end of the questionnaire.

Once you have completed the questionnaire please return it to the person who handed it to you or use the enclosed stamped addressed envelope.

**WE APPRECIATE YOUR COOPERATION WITH THE STUDY**

## GENERAL INFORMATION

*This portion of the questionnaire concerns general information about you and where you live.*

1. What is your date of birth? _____/_____/_____ **(VERY IMPORTANT)**
   D M Y

2. In what country were you born? _____
   ↳ If born in Canada: indicate province: _____

3. Are you living in Montreal only for the duration of your studies/work at McGill?
   ❑ Yes ❑ No

4. What is your **current** marital status?
   ❑ Single
   ❑ Married
   ❑ Unmarried, but living with a partner
   ❑ Widowed
   ❑ Divorced/separated

5. Most Canadian metropolitan areas are made up of many ethnic groups. We would like to know in which community you grew up. Check the appropriate category:
   ❑ French/French Canadian
   ❑ Anglo/English Canadian
   ❑ Italian
   ❑ Jewish
   ❑ Hispanic/Portuguese
   ❑ Black
   ❑ Asian/Oriental
   ❑ Other:_____

6. How are you presently enrolled at McGill?
   ❑ Undergraduate - Regular Student (State year: _____ )
   ❑ Undergraduate - Special Student / Continuing Education
   ❑ Graduate studies - Master's or Diploma Program
   ❑ Graduate studies - Doctoral Program
   ❑ Other (e.g. Trainee, Postdoctoral Studies, Sabbattical)

## SMOKING HISTORY

*The following questions are about your tobacco smoking habits.*
*Please try to be as specific as possible in your answers.*

**7.** Have you ever smoked cigarettes **regularly**, that is, **one** cigarette or **more** each **day** for a **year** or more?

❑ Yes ❑ No

**8.** Have you smoked a total of at least 100 cigarettes in your lifetime?

❑ Yes ❑ No

↳ **If No**, go to question 12

**9.** At what age did you start to smoke? _____years

**10.** Do you still smoke? ❑ Yes ❑ No

↓

**If No**, at what age did you stop?_____years

**11.** On average, how many cigarettes do/did you smoke a day? _____cigarettes per day

## REPRODUCTIVE HISTORY

*In this section of the questionnaire we would like to know about your reproductive health including all your pregnancies as well as miscarriages and abortions.*

**12.** At what age did you have your first menstrual period? _____ years

**13.** To the best of your knowledge, are you currently pregnant?

❑ Yes ❑ No ❑ Don't know

**14.** Have you ever been pregnant before?

❑ Yes ❑ No

**If No**, go to question 17

**If Yes**,
how many times? _____ times

**15.** How many of your pregnancies resulted in:

**a)** livebirths: _____

**b)** stillbirth: _____

**c)** miscarriage: _____

**d)** abortion _____

**16.** How many of your full-term pregnancies resulted in:

**a)** vaginal deliveries: _____

**b)** cesarean sections: _____

## SEXUAL HISTORY

*The next questions are about your sexual history. We realize this is a personal subject, but it is very important to the study. Please take the time to recall this information as accurately as possible. Note that some questions in this section refer to your entire life as an adult, whereas others refer only to your recent experience. We would like to remind you that all the information you give us will be kept entirely confidential.*

**17.** Have you ever engaged in sexual intercourse?

❑ Yes ❑ No

**If No**, go to question 25

**If Yes**,

how old were you when you first had sexual intercourse? _____years

**18.** Throughout your life, what is the number of men with whom you have had sexual intercourse?

❑ one
❑ two
❑ 3 or 4
❑ between 5 and 9
❑ between 10 and 19
❑ between 20 and 29
❑ between 30 and 49
❑ 50 or more

**19.** With how many of these men did you have a sexual relationship involving intercourse on a regular basis for **three months or longer**?

Number _____ ❑ None

**20.** For MOST OF YOUR SEXUALLY ACTIVE LIFE, how often on the **average**, did you have sexual intercourse? Please give your answer in number of times per week, month, or year, whichever is easiest:

Number of times per week _____

OR

Number of times per month _____

OR

Number of times per year _____

OR

Less than once a year ❑

**21.** In THE LAST YEAR ONLY, how often on the **average**, did you have sexual intercourse? Please give your answer in number of times per week or month, whichever is easiest:

Number of times per week _____

OR

Number of times per month _____

OR

Number of times last year _____

OR

None in the past year ❑

**22.** During LAST YEAR ONLY, with how many men have you had sexual intercourse?

_____ Number ❑ None in the past year

**23.** When you are/were having your menstrual periods, do/did you have sexual intercourse?

❑ Yes ❑ No

**24.** Have you ever had anal intercourse?

❑ Yes ❑ No

↓

**If yes,**

would you say that you have had anal intercourse:

❑ frequently ❑ sometimes ❑ rarely ❑ once in lifetime

**25.** Do you ever insert your fingers or objects into the vagina to masturbate?

❑ Occasionally ❑ Rarely ❑ Never

**26.** Has anyone ever performed oral sex on you?

❑ Yes ❑ No

**If No**, go to question 28

**If Yes**,

In THE LAST 5 YEARS, how often on **average**, did this happen? Please give your answer in number of times per week, month, or year, whichever is easiest:

Number of times per week _____

OR

Number of times per month _____

OR

Number of times per year _____

OR

Less than once a year ❑

OR

Never in the past 5 years ❑

**27.** During the LAST 5 YEARS, how many people have performed oral sex on you?

_____ Number ❑ None

## CONTRACEPTIVE HISTORY

*Here we would like to know about methods of birth control or family planning that you and your husband/partner used. It would be important to indicate all the methods you've used since you became sexually active.* ***If you answered "No" to Q 17 you may skip this section entirely and go to Q 32.***

**28.** The following is a list of common birth control methods. Read along the list and check if you and a sex partner have ever used any of them (**check all that apply**) either occasionally or regularly.

**BY REGULARLY WE MEAN AT LEAST 3 MONTHS CONSECUTIVELY**

| | | |
|---|---|---|
| **a)** oral contraceptive (birth control pill) | ❑ Regularly | ❑ Sometimes |
| **b)** condom (rubber) | ❑ Regularly | ❑ Sometimes |
| **c)** foam, jelly, cream, or suppository | ❑ Regularly | ❑ Sometimes |
| **d)** loop, coil, or other intrauterine device | ❑ Regularly | ❑ Sometimes |
| **e)** diaphragm | ❑ Regularly | ❑ Sometimes |
| **f)** cervical cap | ❑ Regularly | ❑ Sometimes |
| **g)** sponge | ❑ Regularly | ❑ Sometimes |
| **h)** vaginal douche | ❑ Regularly | ❑ Sometimes |
| **i)** rhythm, calendar, or natural method | ❑ Regularly | ❑ Sometimes |
| **j)** withdrawal/pulling out | ❑ Regularly | ❑ Sometimes |

**29.** If you have used oral contraceptives or birth control pills, please indicate how old you were when you first took them

age: _____ years ❑ Never used oral contraceptives

└→ Go to question 31

**30.** Considering only the times when you were taking the pill, for how long have you been relying on this method of birth control (add together all periods during which you took any oral contraceptives) ?

___ months

OR

___ years

OR

❑ all periods combined were less than 3 months

**31.** Now, considering ONLY THE LAST YEAR on the **average,** which of the following birth control methods have you or your partner come to rely upon? (**check all that apply**)

**BY REGULARLY WE MEAN AT LEAST 3 MONTHS CONSECUTIVELY**

| | | |
|---|---|---|
| **a)** oral contraceptive (birth control pill) | ❑ Regularly | ❑ Sometimes |
| **b)** condom (rubber) | ❑ Regularly | ❑ Sometimes |
| **c)** foam, jelly, cream, or suppository | ❑ Regularly | ❑ Sometimes |
| **d)** loop, coil, or other intrauterine device | ❑ Regularly | ❑ Sometimes |
| **e)** diaphragm | ❑ Regularly | ❑ Sometimes |
| **f)** cervical cap | ❑ Regularly | ❑ Sometimes |
| **g)** sponge | ❑ Regularly | ❑ Sometimes |
| **h)** vaginal douche | ❑ Regularly | ❑ Sometimes |
| **i)** rhythm, calendar, or natural method | ❑ Regularly | ❑ Sometimes |
| **j)** withdrawal/pulling out | ❑ Regularly | ❑ Sometimes |

## PERSONAL HYGIENE HABITS

*This section concerns your personal hygiene habits. As with other topics in this questionnaire, this is also a personal subject of great importance to the study. Again, rest assured that we will treat your answers confidentially. Please take the time to recall this information as accurately as possible. A good guess is always better than leaving the question blank.*

**32.** How many times per day or per week do you usually bathe or shower?

___ per day
OR
___ per week

**33.** **Aside from** those baths and showers, do you ever wash your genital area?

❑ Yes ❑ No

**If No**, go to question 34

**If Yes,**
in the **last year on the average**, how many times per day, week, or month did you wash your genital area?

___ per day
OR
___ per week
OR
___ per month
OR
❑ less than once a month

**34.** Have you ever used a vaginal douche?

❑ Yes ❑ No

**If No**, go to question 36

**If Yes,**
in the **last year on the average**, how many times per day, week, or month did you use a vaginal douche?

___ per day
OR
___ per week
OR
___ per month
OR
❑ less than once a month

**35.** If **you answered yes to question 34**, what kind of douche did you use?

- ❑ Water only
- ❑ Water and vinegar
- ❑ Other (what ingredients or brand name: _____)

**36.** Have you ever used a feminine genital spray or vaginal deodorant?

❑ Yes ❑ No

**If No**, go to question 37

**If Yes,**
in the last year on the average, how many times per day, week, or month did you use a vaginal deodorant?

___ per day
OR
___ per week
OR
___ per month
❑ less than once a month

**37.** When you are/were having your menstrual periods, what do/did you use to collect the blood? (check all that apply)

- ❑ sanitary pads/napkins
- ❑ tampons
- ❑ other

**38.** Following sexual intercourse, do you usually wash your genital area within 1 hour? Choose the category that best reflects your behavior during most of your adult life: (skip this question if you never had sexual intercourse)

- ❑ always
- ❑ sometimes
- ❑ rarely
- ❑ never

**39.** What is your preferred hygiene practice after each bowel movement? (Check all that apply)

- ❑ Use toilet paper with a BACK-TO-FRONT hand motion for wiping
- ❑ Use toilet paper with a FRONT-TO BACK hand motion for wiping
- ❑ Wash with water only
- ❑ Wash with water and soap

## MEDICAL HISTORY

*The next questions are about the frequency with which you have taken PAP smears and about some medical problems including sexually transmitted diseases. We realize that this is a sensitive subject but, again, it is very important to the research. We appreciate your honesty and want to remind you that all information you give us is kept private and confidential.*

**40.** Thinking back over your adult years, how many times have you had a PAP smear? Choose one category below:

- ❑ this is my first PAP smear
- ❑ 2-3 times
- ❑ 4-5 times
- ❑ 6-10 times
- ❑ more than 10 times

**41.** What is the month and year of the last PAP smear you had? _____/_____
Month Year

**42.** Did a doctor ever tell you that you had one of the following conditions? Check all that apply, if you are in doubt check the "don't know" column.

| | | | |
|---|---|---|---|
| a) Vaginal yeast infections: | ❑ Yes | ❑ No | ❑ Don't know |
| b) Trichomonas vaginal infections: | ❑ Yes | ❑ No | ❑ Don't know |
| c) Venereal warts, condylomas, or papilloma virus infections: | ❑ Yes | ❑ No | ❑ Don't know |
| d) Chlamydia: | ❑ Yes | ❑ No | ❑ Don't know |
| e) Genital herpes: | ❑ Yes | ❑ No | ❑ Don't know |
| f) Syphilis: | ❑ Yes | ❑ No | ❑ Don't know |
| g) Gonorrhea: | ❑ Yes | ❑ No | ❑ Don't know |
| h) Ulcers or genital sores: | ❑ Yes | ❑ No | ❑ Don't know |

**43.** Thinking back over **all your adult life**, have you experienced other genital conditions such as a vaginal discharge, itching or irritation?

- ❑ Never
- ❑ Less than once a year
- ❑ More than once a year

**44.** Now, **only during the last year**, have you experienced other genital conditions such as a vaginal discharge, itching or irritation?

- ❑ Never during last year
- ❑ Once or twice
- ❑ More than 3 times last year

**45.** Sometimes women are given female hormones by their doctors because of a variety of reasons (alleviate acne, regulate or eliminate painful periods, menopausal symptoms, reduce discomfort during intercourse due to vaginal dryness, prevent miscarriage, among others). To the best of your recollection, were you ever prescribed any female hormones by your doctor?

❑ Yes ❑ No

**If No**, go to question 48

**If Yes**,
in what month and year did you start taking them and also, in what month and year did you last take them?

Start: _____/_____ (Month Year)

End: _____/_____ (Month Year)

**46.** Between the above two dates, for how long (number of months) did you take the female hormone medication, altogether?

_____months

**47.** Was the female hormone medication in the form of (check all that apply):

- ❑ pills
- ❑ shots
- ❑ creams or suppositories

**48.** Would you please indicate the date when you finished filling in the questionnaire?

_____/_____/_____
DAY MONTH YEAR

USE THE SPACE BELOW IF YOU HAVE ANY ADDITIONAL INFORMATION THAT YOU FEEL WOULD BE IMPORTANT FOR US TO KNOW:

This is the end of the questionnaire. Now we would like you to take a few seconds to review your answers in all sections of the questionnaire. If you answered the sections on different days, take a moment to reflect whether you now agree with your answers from the previous days. Again, try to answer all questions; a good guess will be more useful to the study than leaving the question blank.

**THANK YOU VERY MUCH FOR YOUR COOPERATION**

# APPENDIX 4

## Distribution of Socio-Demographic Characteristics of the Study Population

## Appendix 4

### Distribution of Socio-Demographic Characteristics of the Study Population

| Variable | Number | % |
|---|---|---|
| **Age (yrs)** | | |
| <=19 | 68 | 16.7 |
| 20-21 | 134 | 33.1 |
| 22-23 | 104 | 25.6 |
| 24+ | 100 | 24.6 |
| **Origin** | | |
| Ontario & West[1] | 190 | 46.8 |
| Quebec & Maritimes | 102 | 25.1 |
| USA | 45 | 11.1 |
| Europe | 29 | 7.1 |
| Other | 40 | 9.9 |
| **Ethnicity** | | |
| Anglophone | 235 | 59.6 |
| Francophone | 41 | 10.4 |
| Other | 118 | 30.0 |
| **Marital status** | | |
| Single | 338 | 83.7 |
| Married | 16 | 4.0 |
| Living with partner | 50 | 12.3 |

[1] Prairies & B.C

# APPENDIX 5

## Distribution of Reproductive and Sexual Characteristics of the study Population

## Appendix 5

### Distribution of Reproductive and Sexual Characteristics of the Study Population

| Variable | Number | % |
|---|---|---|
| **Age at menarche (yrs)** | | |
| ≤11 | 61 | 14.8 |
| 12 | 132 | 32.1 |
| 13 | 125 | 30.4 |
| ≥14 | 93 | 22.7 |
| **Age at first coitus** | | |
| ≤17 | 175 | 45.6 |
| ≥18 | 209 | 54.4 |
| **Lifetime No. sex partners** | | |
| 1 | 80 | 19.6 |
| 2-4 | 153 | 37.4 |
| 5-9 | 116 | 28.4 |
| ≥10 | 60 | 14.6 |
| **Recent No. sex partners (within last year)** | | |
| 0-1 | 268 | 65.4 |
| 2-3 | 98 | 23.9 |
| ≥4 | 44 | 10.7 |
| **Lifetime frequency of sex** | | |
| 0-4/month | 117 | 31.9 |
| 5-13/month | 161 | 43.9 |
| ≥14/month | 89 | 24.2 |
| **Recent frequency of sex (within last year)** | | |
| 0-4/month | 175 | 42.9 |
| 5-13/month | 150 | 36.8 |
| ≥14/month | 83 | 20.3 |
| **No. of oral sex partners** | | |
| 0-3 | 294 | 71.5 |
| 4-9 | 99 | 24.1 |
| ≥10 | 18 | 4.4 |

## Appendix 5

### Distribution of Reproductive and Sexual Characteristics of the Study Population (Continued)

| Variable | Number | % |
|---|---|---|
| **Lifetime frequency of oral sex** | | |
| 0-4/month | 270 | 68.9 |
| 5-13/month | 110 | 28.1 |
| 14+/month | 12 | 3.0 |
| **No. of sex partners >3 months** | | |
| 0-3 | 336 | 82.2 |
| 4-9 | 69 | 17.6 |
| 10+ | 4 | 0.2 |
| **Sexually active when menstrual** | | |
| Never | 160 | 39.2 |
| Ever | 248 | 60.8 |
| **Anal sex** | | |
| Never | 316 | 77.6 |
| Ever | 91 | 22.4 |
| **Masturbation** | | |
| Never | 139 | 34.2 |
| Ever | 268 | 65.8 |
| **Pregnant** | | |
| Never | 365 | 88.8 |
| 1 | 34 | 8.3 |
| 2+ | 12 | 2.9 |
| **Type of pregnancy** | | |
| Never | 365 | 88.8 |
| Abortion | 41 | 10.0 |
| Full-term delivery | 5 | 1.2 |

# APPENDIX 6

## Distribution of Smoking and Contraceptive Characteristics of the Study Population

## Appendix 6

### Distribution of Smoking and Contraceptive Characteristics of the Study Population

| Variable | Number | % |
|---|---|---|
| **Tobacco** | | |
| Never | 273 | 66.9 |
| Former | 61 | 15.0 |
| Current | 74 | 18.1 |
| **Packyears** | | |
| <1 | 50 | 42.4 |
| 1-5 | 53 | 44.9 |
| 5+ | 15 | 12.7 |
| **Oral contraceptive** | | |
| Never | 84 | 20.4 |
| Occcasionally | 21 | 5.1 |
| Regularly | 306 | 74.5 |
| **Recent oral contraceptive use** | | |
| Never | 153 | 37.2 |
| Occcasionally | 13 | 3.2 |
| Regularly | 245 | 59.6 |
| **Age started pill (yrs)** | | |
| <=16 | 44 | 13.3 |
| 17-19 | 195 | 59.1 |
| 20+ | 91 | 27.6 |
| **Time on pill (yrs)** | | |
| <=1 | 103 | 31.5 |
| 1-2 | 71 | 21.7 |
| 2-3 | 54 | 16.5 |
| 3+ | 99 | 30.3 |
| **Condom use** | | |
| Never | 24 | 5.8 |
| Occcasionally | 157 | 38.2 |
| Regularly | 230 | 56.0 |
| **Recent condom use** | | |
| Never | 133 | 32.4 |
| Occcasionally | 109 | 26.5 |
| Regularly | 169 | 41.1 |
| **Barrier use[1]** | | |
| Never | 22 | 5.4 |
| Occcasionally | 128 | 31.1 |
| Regularly | 261 | 63.5 |
| **Recent barrier use** | | |
| Never | 130 | 31.6 |
| Occcasionally | 102 | 24.8 |
| Regularly | 179 | 43.6 |

[1] Includes foam, cap, diaphram, sponge and condom

# APPENDIX 7

## Distribution of Hygienic and Health Characteristics of the Study Population

## Appendix 7

### Distribution of Hygienic and Health Characteristics of the Study Population

| Variable | Number | % |
|---|---|---|
| **Vaginal douche** | | |
| Never | 356 | 86.6 |
| Ever | 55 | 13.4 |
| **No. showers (per day)** | | |
| <1 | 110 | 26.8 |
| 1 | 267 | 65.0 |
| 1+ | 34 | 8.2 |
| **Wash genitals after sex** | | |
| Never | 194 | 47.7 |
| Ever | 213 | 52.8 |
| **Method of wiping[1]** | | |
| Back-to-front | 132 | 32.7 |
| Front-to-back | 272 | 67.3 |
| **History of STD[2]** | | |
| Never | 343 | 83.5 |
| Ever | 68 | 16.5 |
| **History of warts** | | |
| Never | 318 | 89.1 |
| Ever | 39 | 10.9 |
| **Hormone therapy** | | |
| Never | 395 | 96.1 |
| Ever | 16 | 3.9 |
| **Lifetime No. of Paps received** | | |
| 1 | 71 | 17.4 |
| 2-3 | 147 | 36.0 |
| 4-5 | 101 | 24.8 |
| 6-10 | 62 | 15.2 |
| 10+ | 27 | 6.6 |

[1] Method of wiping after a bowel movement [2] Includes *Trichomonas*, *Chlamydia*, herpes, syphillis & gonorrhea