Sexual and reproductive health risk factors and risk of cervical cancer in developing countries

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Submitted in fulfilment for the process of PhD.

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Abstract

Background: Invasive cervical cancer (ICC) is the second most common cancer among women in developing countries where early age at first sexual intercourse (AFSI) and first pregnancy (AFP) are prevalent events. The epidemiological evidence of how these sexual and reproductive health (SRH) factors impact the natural history of human papillomavirus (HPV) and ICC remain inconclusive. It has been debated that a woman's risk for ICC will depend more on the "high-risk" sexual behaviour of the male partner than of her own behaviour. Passive smoking in the context of couples is unclear. The aim is to study SRH factors in relation to ICC risk in developing countries.

Methods: *Study 1* evaluated the risk of ICC and its association with AFSI and AFP in a pooled analysis of IARC case-control studies of ICC from eight developing countries. *Study 2* assessed these SRH factors and risk of HPV persistence in a population-based natural history cohort study in Guanacaste, Costa Rica. *Study 3* characterised the male role in the aetiology of ICC among couples in a pooled analysis of five ICC case-control studies and two cervical carcinoma in situ (CIS) case-control studies.

Results: The ICC risk was 2.4-fold among those who reported AFSI and AFP ≤16 years compared with AFSI and AFP ≥21 years. Decreasing AFP, not AFSI, was associated with an increased risk of 2-year persistence. Lifetime number of sexual partners of the husband was the strongest predictor of CIS and ICC risk. The absence of circumcision was significantly associated with an increased risk of CIS. A 2-fold increased risk of ICC was also found among couples with both ever smoking men and women.

Conclusion: These data confirm AFSI and AFP as risk factors for ICC, but any independent effects could not be distinguished. The association of AFP with HPV persistence suggests that AFP may play a more relevant role in cervical carcinogenesis. The combined effects of exposure to active and passive smoking suggest its potential adverse role in cervical carcinogenesis.

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Abbreviations & acronyms

ADC	Adenomcarcinoma
AFM	Age at first marriage
AFP	Age at first pregnancy
AFSI	Age at first sexual intercourse
CIN-3, CIN-2, or CIN-3	Cervical intraepithelial neoplasia grade 1, 2, or 3
CIS	Carcinoma in situ
HIV	Human immunodeficiency virus
HPV	Human papillomavirus
HSIL	High-grade intraepithelial neoplasia
LSIL	Low-grade intraepithelial neoplasia
PEG	Projyecto Epidemiológico Guanacaste
PCR	Polymerase chain reaction
IARC	International Agency for Research on Cancer
ICC	Invasive cervical cancer
ICO	Catalan Institute of Oncology
LSHTM	London School of Hygiene and Tropical Medicine
NCI	National Cancer Institute
Pap	Papanicolaou
SCC	Squamous cell carcinoma
STI	Sexually transmitted infection
WHO	World Health Organization
YLL	Year of lost life

Acknowledgements

The completion of this thesis has been a long journey with many challenges along the way. Without the patience, support and guidance of the many people who have travelled with me along the way, this work would not have been possible. To them, I would like to extend my deepest gratitude.

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I would also like to acknowledge F Xavier Bosch, Xavier Castellsagué, the HPV Information Centre team and other colleagues at the Catalan Institute of Oncology. My motivation for working on HPV and cervical cancer research would have never begun if I had not been challenged and inspired by their work during my years at ICO. I am grateful for their generous support and making hard work also fun. I want to thank Peter Sasieni for his advice and support, especially during the final days of this thesis as I tried to balance both work and study.

Thank you to all the women (and men) who participated in the IARC multi-centric case-control studies on cervical cancer and the IARC Multicenter Cervical Cancer Study Group for supporting this research. I thank Mark Schiffman, Ana Cecilia Rodriguez and the team at the Proyecto Epidemiólogio Guanacaste for their collaboration.

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Supervision and publications

Supervision

The research was carried out under the supervision of Dr Philippe Mayaud at the London School of Hygiene and Tropical Medicine (LSHTM), London UK and Dr Silvia de Sanjose at the Catalan Institute of Oncology in Barcelona, Spain. Additional supervision was provided by Dr Maribel Almonte at Cardiff University (formerly at the Wolfson Institute of Preventive Medicine, Queen Mary University of London) and Dr Helen Weiss at LSHTM.

Publications

The main publications arising from this thesis or from work-related to this thesis are given below. Reprints of these articles are included in Appendix 1-3. Where work included in the thesis has been published in joint names, the role of each author is outlined below.

Chapter 2 – Literature Review (Appendix 1)

Louie KS, de Sanjose S, Mayaud P. The burden of human papillomavirus (HPV) infection and cervical cancer in sub-Saharan Africa: prevention opportunities and challenges. Tropical Medicine & International Health 2009; 14 (10): 1287-1302.

KSL was the primary author, conducted the comprehensive literature review, data extraction and wrote the manuscript. SS and PM contributed to the interpretation of data and revising the draft manuscript critically for important intellectual content.

Chapter 3 – *Study 1* (Appendix 2)

Louie KS, de Sanjose S, Diaz M, Castellsagué X, Herrero R, Meijer CJL, Shah K, Franceschi S, Muñoz N, Bosch FX, for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Early age at first sexual intercourse and early pregnancy are risk factors for cervical cancer in developing countries. British Journal of Cancer 2009; 100 (7):1191-7.

KSL was the primary author, designed and planned the study with SS, XC, FXB, conducted the statistical analysis, interpreted the results, and wrote the manuscript. FXB and NM were the Principal Investigators of the case-control studies who provided the data. All authors critically reviewed the draft of the manuscript.

Chapter 5 – *Study 3* (Appendix 3)

Louie KS, Castellsague X, de Sanjose S, Herrero R, Meijer CJ, Shah K, Munoz N, Bosch FX, for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Smoking and passive smoking in cervical cancer risk: pooled analysis of couples from the IARC multi-centric case control studies. Cancer Epidemiology, Biomarkers & Prevention 2011; 20(7):1379-1390.

KSL was the primary author, designed and planned the study with SS, XC, FXB, conducted the statistical analysis, interpreted the results, and wrote the manuscript. FXB and NM were the Principal Investigators of the case-control studies who provided the data. All authors critically reviewed the draft of the manuscript.

1 Introduction

Cervical cancer is the third most common cancer among women worldwide (Ferlay *et al*, 2008). However, the problem disproportionately affects women in developing countries and ranks as either the first or second most common cancer. Human papillomavirus (HPV), a sexually transmitted infection, is a causal factor for virtually all cases of invasive cervical cancer (ICC). Overall, the poorest women that appear to initiate sex at an early age can subsequently become pregnant at an early age, and have a large number of children, which are factors that have been associated with an increased risk of HPV infection and ICC. These factors tend to cluster in poor settings. Evidence suggests that these early sexual and reproductive health (SRH) factors are associated with a greater risk of persistent HPV infection and cervical cancer development because of the biological predisposition of the immature cervix and cervical trauma experienced during delivery of birth; however, the epidemiological evidence of how these factors influence a woman's risk remain inconclusive. Besides the sexual behaviour of the women, a women's risk may also be heavily dependent on the "high-risk" sexual behaviour of the male partner rather than their own sexual behaviour, particularly among lifetime sexually monogamous women, since men in general, report more lifetime numbers of sexual partners than women.

1.1 Research aims

The overall aim of the work presented in this thesis is to study sexual and reproductive health risk factors in relation to risk of cervical cancer in developing countries.

The specific objectives are:

I. To characterise and provide robust estimates of the risk of cervical cancer and its association with age at first sexual intercourse (AFSI), interrelated characteristics such as age at first pregnancy (AFP) and age at first marriage (AFM) in a pooled analysis of case-control studies on ICC from eight developing countries (Algeria, Morocco, India, Philippines, Thailand, Brazil, Colombia, Paraguay, and Peru)

II. To evaluate the association between sexual and reproductive behaviour factors (AFSI, AFP, and parity) and the prospective risk of HPV viral persistence (as defined by type-specific HPV DNA) among women with prevalent HPV infection in the Guanacaste, Costa Rica cohort

III. To characterize in depth the male role in the aetiology of cervical cancer in different developing geographical settings in a pooled analysis of five case-control studies (Brazil, Colombia, Philippines, Spain, and Thailand) involving ICC and two case-control studies (Colombia and Spain) involving cervical carcinoma in situ (CIS), of couples in which husbands or stable partners of ICC and CIS case and control women participated.

1.2 Thesis outline

The PhD thesis is comprised of a literature review and three studies. This thesis is presented in the style of "research papers" where some chapters or parts of chapters are presented as published manuscripts, or work prepared for peer-reviewed publication. There is often a word limit for manuscripts published in peer-reviewed journals. However, to provide coherence to this thesis, additional details and extended discussions are included as an extension to the publications.

Study 1 provides robust estimates of the risk of ICC and its association with SRH factors (AFSI, AFP, and parity) in a pooled analysis of International Agency for Research on Cancer (IARC) case-control studies of ICC from eight developing countries (Figure 1.1). *Study 2* subsequently evaluates these SRH factors and risk of HPV persistence in a population-based cohort study of HPV and cervical pre-cancer in Guanacaste, Costa Rica. Thus, *Study 1 and Study 2* provide a more comprehensive natural history picture of SRH factors and their associations with the three of the four major steps of cervical carcinogenesis from HPV acquisition, persistence, pre-cancer (not evaluated), and ICC. *Study 3* characterises in depth the male role in the aetiology of ICC in a pooled analysis of five case-control studies involving ICC and two case-control studies involving cervical carcinoma in situ (CIS), of couples in which husbands of ICC and CIS case and control women participated. This research is presented in the following chapters described below.

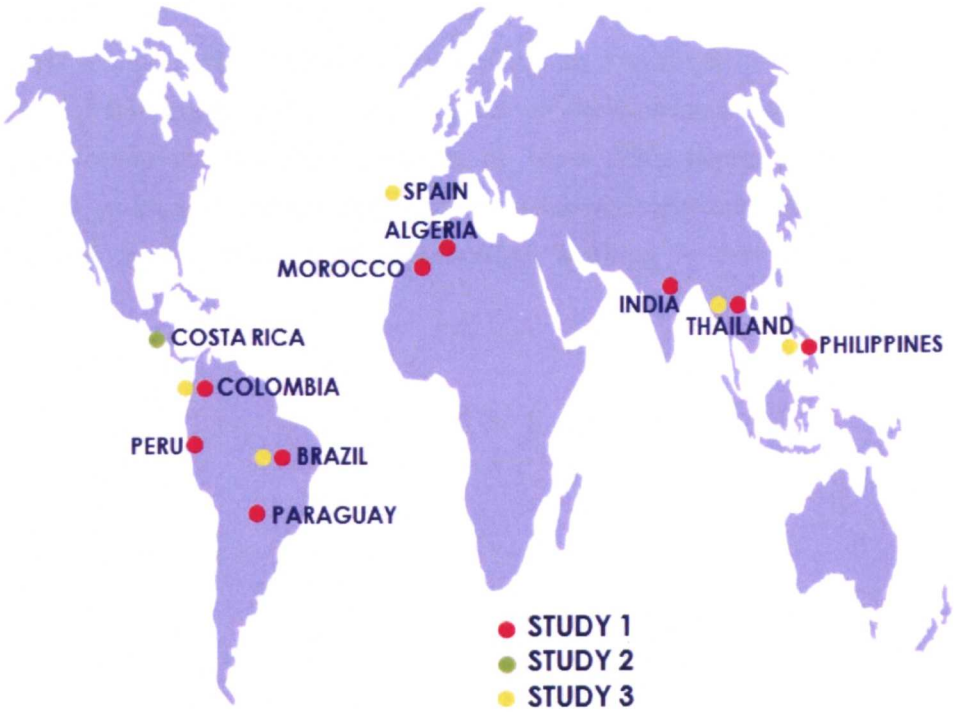

Figure 1.1 Study 1, 2, and 3 country study sites.

The present **Chapter 1** summarises the rationale, the overall objectives, of this thesis as well as outline the structure of this thesis. **Chapter 2** provides a comprehensive literature review to provide background for this research, summarising the burden and epidemiology of HPV and cervical cancer in developing countries in order to put into context the relevance of this research. **Chapter 3** presents *Study 1*, a pooled analysis of case-controls studies on cervical cancer from eight developing countries to investigate the roles of AFSI, AFP and AFM and cervical cancer risk. The results from *Study 1* have been published (Louie *et al*, 2009b). Based on the findings of *Study 1*, *Study 2* aimed at evaluating the impact of sexual and reproductive health factors (AFSI, AFP and parity) on persistence of HPV in a prospective cohort of women from Guanacaste, Costa Rica. *Study 2* results are presented in **Chapter 4** and this second study was used to confirm whether our findings in *Study 1* were also observed in a longitudinal study, completing another major step of HPV and cervical carcinogenesis. **Chapter 5** presents *Study 3*, a pooled case-control study of couples in which husbands or stable partners of women with cervical carcinoma in situ or cervical cancer case and control women participated in. This study

aimed at characterising the male role in the aetiology of cervical cancer in order to clarify its contribution to the consequent risk of HPV infection and of cervical cancer in their female partner. **Chapter 6** presents an overall discussion of the main findings of this thesis and the implications on cervical cancer prevention. **Chapter 7** summarises the dissemination of these PhD thesis results at conferences, as published manuscripts in peer-reviewed journals, and other publications and reports relevant to stakeholders working in cervical cancer prevention.

Cover sheet for each 'research' paper included in a research thesis

NB: Chapter 2 Background has been published partially in 2009, with a specific focus on the sub-Saharan Africa developing region (Louie *et al*, 2009b) and is appended in Appendix B. A global review with more updated information is presented in this Background chapter.

Louie KS, de Sanjose, Mayaud P. The burden of human papillomavirus (HPV) infection and cervical cancer in sub-Saharan Africa: prevention opportunities and challenges. Tropical Medicine & International Health 2009; 14 (10): 1287-1302.

1. For a 'research paper' already published
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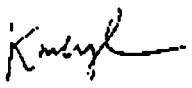
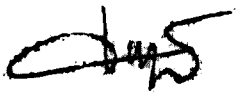
Candidate's signature 

Supervisor or senior author's signature to confirm role as stated in (3) 

2 Background

2.1 Introduction

Cancer of the cervix uteri ranks as the second most common cancer in the developing world (HPV Information Centre, 2010). The magnitude of the problem has been under recognised and prioritised compared to other competing health priorities such as diarrhoeal diseases, HIV/AIDS, malaria, and tuberculosis because of lack of epidemiological data and poor awareness, lack of human financial resources, non-existent cancer service policies and lack of political will to address the complex problem (Denny *et al*, 2006; Parkin *et al*, 2008).

Organised screening and early treatment programmes have been effective in preventing cervical cancer in developed countries but they are costly and difficult to implement in resource-constrained settings. Despite our understanding of the causal relationship of the human papillomavirus (HPV) and cervical cancer (Bosch *et al*, 2002) and the availability of effective HPV vaccines to prevent infection and disease (Lowy *et al*, 2008), the opportunity of these vaccines to have an effective impact in developing countries will not materialise until they become affordable and integrated within the framework of national immunisation programmes (Kane *et al*, 2006).

This review aims to summarise the current epidemiology of HPV and cervical cancer and the complexity of implementing prevention in developing countries; to identify gaps of knowledge and to highlight the challenges and opportunities for controlling cervical cancer.

2.2 Methods

A comprehensive synthesis review of peer-reviewed literature in databases of the World Health Organization and Institute Català d'Oncologia (WHO/ICO) Information Centre on HPV and Cervical Cancer (http://www.who.int/hpvcentre), the International Agency for Research on Cancer (IARC) Screening Group (http://www.screning.iarc.fr), PubMed/Medline, and reports from the World Health Organization (WHO). The following medical subject heading (MESH) and text words were used alone or in combination: 'HPV', 'cervical cancer', 'cervical screening', and 'HPV vaccination'. This review

summarises the evidence from recent systematic reviews, meta-analyses, narrative reviews (non-systematic reviews), epidemiological studies, modelling and related analyses and practice guidelines.

2.3 Global burden of cervical cancer

Cancer of the cervix is the third most common cancer among women worldwide with an estimated 529,409 new cases and 274,883 deaths in 2008 (Ferlay *et al*, 2008). However, about 86% of cases occur in developing countries, making it rank as the first or second most common cancer, and represents 13% of all female cancers. The highest incidence rates are found in regions of sub-Saharan Africa (age-standardised incidence rate, ASR of 31.7 per 100,000 women) Southern Asia (ASR=25.0), Melanesia (ASR=23.7), Latin America and the Caribbean (ASR=23.5), and South East Asia (ASR=15.8) (Figure 2.1). In contrast, the ASRs in Europe and North America are 10.6 and 5.7, respectively. However, better cancer incidence data are needed to characterise the burden of disease in developing regions. For example, at present, only a few population-based cancer registries exist in Africa, covering 11% of the population and fewer produce quality incidence data (Parkin *et al*, 2008). In the period 1998-2002, only two cancer registries in the region, Kyadondo County in Uganda and Harare in Zimbabwe produced high quality data and reported ASRs of 45.8 and 47.3 per 100,000, respectively (IARC, 2008).

When comparing the rates between developing vs. developed regions, the incidence is about two-fold higher (ASR=17.8 in developing *vs.* ASR=9.0 in developed, respectively). Globally, the mortality rates of cervical cancer are substantially lower than incidence with a ratio of mortality to incidence of 52%; similarly, the ratio in developing regions is 55.1% whereas in developed countries, the ratio is 35.6%. The majority of cervical cancer cases are squamous cell carcinoma (SCC) and adenocarcinomas (ADC) are less common. Cervical cancer, is an important cause of lost years of life, which is responsible for 2.7 million (age-weighted) years of life lost (YLL) world and it is the biggest cause of lost of YLL in the developing world (Yang *et al*, 2004).

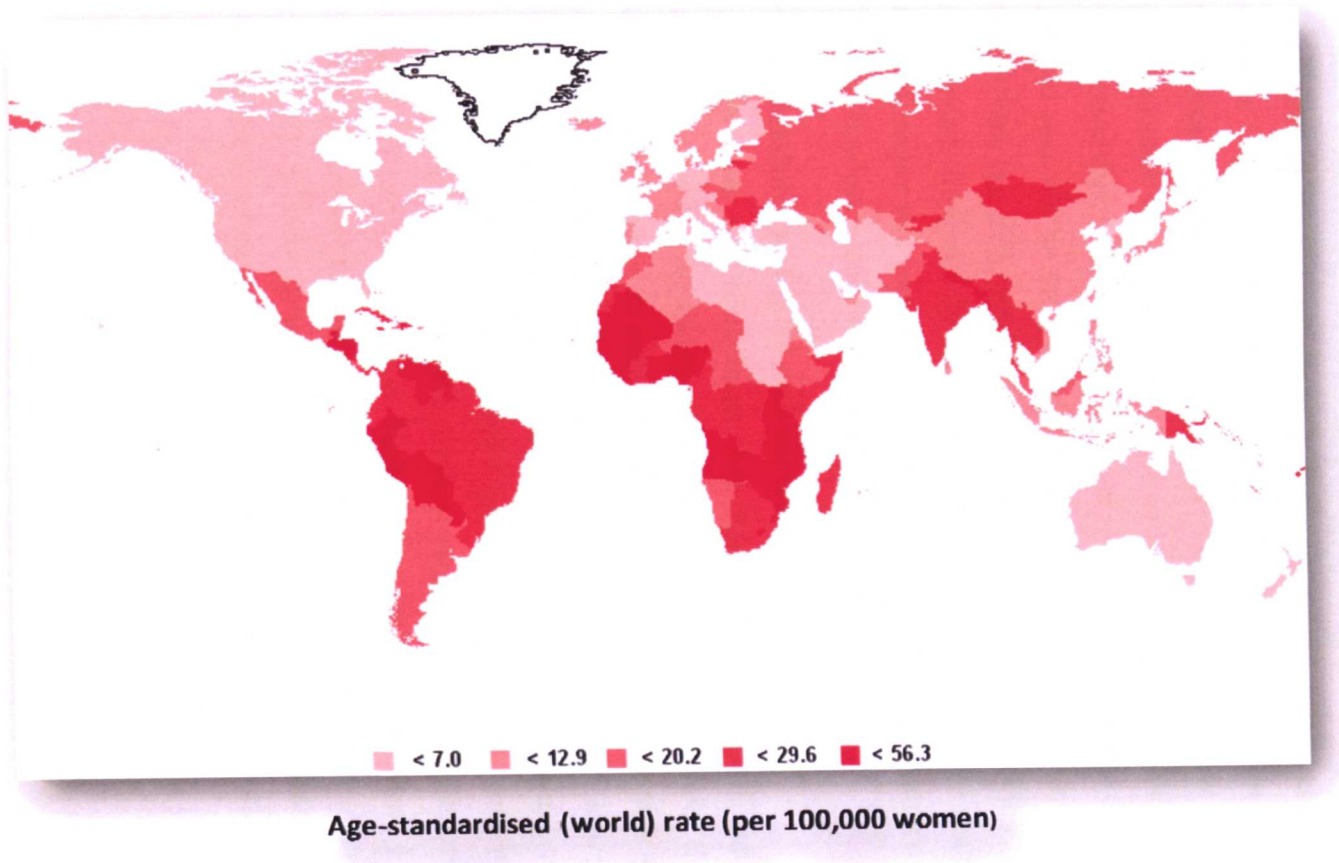


Figure 2.1 World age-standardised incidence rates of cervical cancer (Ferlay *et al*, 2008)

Organised cervical screening programmes have been responsible for significant declines in incidence of cervical cancer in many developed countries. In contrast, the majority of developing countries have none or minimal opportunistic screening services as seen in Africa or ineffective screening programmes as seen in Latin America, leaving incidence rates unchanged. Future projections highlight that developing countries will continue to bear a disproportionate burden of cervical cancer in the world. If current incidence rates of cervical cancer are constant over time and are applied to population forecasts, it is estimated that by 2025, the global estimated number of new cases of cervical cancer per year will be 720,060 with developing regions representing 93% of the burden, which is a 36% increase from 2008 (Ferlay *et al*, 2008; HPV Information Centre, 2010). Similarly, the global estimated number of cases of deaths per year of cervical cancer will increase to 395,095, with developing regions representing 96% of the burden, which is also a 43% increase from 2008.

2.4 Epidemiology of HPV and cervical cancer

It has been well-established that infection with human papillomavirus (HPV) is responsible for nearly all cases (99%) of cervical cancer (Bosch *et al*, 2002). The major steps in cervical carcinogenesis include infection with one of the 15 high-risk (HR) oncogenic types of HPV, viral persistence, and progression of persistent infection to cervical pre-cancer and cancer (Figure 2.2) (Moscicki *et al*, 2006). The prevalence of HR-HPV increases with disease severity, reaching nearly 100% in cases of invasive cervical cancer. An underestimation of HPV prevalence in cervical cancer is likely due to the limitations of HPV testing methodologies. Since identifying HPV as the primary cause of invasive cervical cancer (ICC), primary (prophylactic HPV vaccines) and secondary preventions (including HPV testing in cervical screening programmes) now exist.

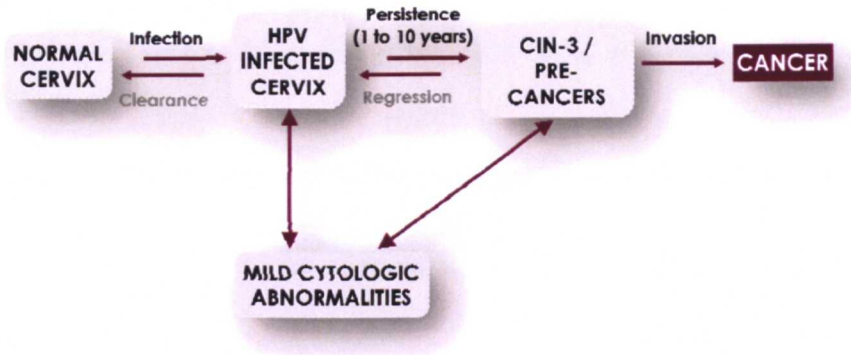


Figure 2.2. Major steps in cervical carcinogenesis. The cervix becomes infected with one of the carcinogenic HPV types. The vast majority of these infections are highly transient and will spontaneously clear. The remaining infections will become persistent, which can potentially lead to cervical pre-cancer and invasion with the possibility that these abnormalities could also regress in the process. Adapted from (Schiffman & Kjaer, 2003).

Over 100 HPV types have been identified, of which 40 types infect the genital tract, primarily through sexual transmission. Of the HPV types, 16 and 18, the two vaccine preventable types, are the most common types found in cervical cancer representing about 70% of all ICC cases, between 41-67% of high-grade cervical lesions and 16-32% of low-grade cervical lesions (Clifford *et al*, 2006). HPV 16 and 18 show a greater risk of progression to pre-cancerous lesions than other HR types (Khan *et al*, 2005; Schiffman *et al*, 2005). Local HPV type-specific data are therefore useful for estimating the impact of HPV vaccines and cervical cancer screening. In countries that lack HPV type-specific data, they can be reassured that HPV vaccines would prevent against the majority of ICC cases (67-70.8%) as HPV-16/18 were consistently shown to be the most prevalent types in a retrospective cross-sectional study of 22,661 cases of invasive cervical cancer from 38 countries across regions of Europe, North America, Central America, Africa, Asia and Oceania (de Sanjose *et al*, 2010)

2.4.1 Prevalence of HPV in the general population

It is estimated that at any given point in time, about 11.4% of women with normal cytology worldwide are positive for HPV infection (Table 2.1); however, this is variable across regions with prevalence ranging from 22.5% in sub-Saharan Africa, 22.3% in Eastern Europe and 17.6% in Latin America and the Caribbean (Bruni *et al*, 2010; de Sanjose *et al*, 2007; HPV Information Centre, 2010). Notably, the prevalence of HPV is highest in developing regions where rates of

cervical cancer are also the highest. Overall, about 80% of sexually active women will have been exposed to genital HPV infection at one point in their lifetime (Myers *et al*, 2000).

Table 2.1 Overall HPV prevalence among women with normal cytology

	Overall HPV prevalence	
	No. of women tested	**HPV prevalence, % (95% CI)**
World	**436,430**	**11.4 (11.3-11.5)**
Developing regions	120,008	14.3 (14.1-14.5)
Developed regions	315,573	10.3 (10.2-10.4)
Africa	**8568**	**21.3 (20.5-22.2)**
Eastern Africa	751	33.6 (30.2-37.1)
Middle Africa	-	-
Northern Africa	863	10.9 (8.9-13.2)
Southern Africa	2485	21.0 (19.4-22.6)
Western Africa	4469	21.5 (20.3-22.8)
Americas	**112,675**	**14.5 (14.3-14.7)**
Latin America & Caribbean	42495	17.6 (17.3-18.0)
Caribbean	212	35.4 (29.1-42.2)
Central America	24783	20.6 (20.1-21.1)
South America	17500	13.2 (12.7-13.7)
Northern America	64504	12.5 (12.3-12.8)
Asia	**84710**	**10.9 (10.7-11.1)**
Central Asia	-	-
Eastern Asia	55365	12.6 (12.3-12.9)
Southern Asia	23061	7.9 (7.5-8.2)
South-Eastern Asia	4849	8.4 (7.6-9.2)
Western Asia	1435	2.2 (1.5-3.1)
Europe	**229,628**	**9.7 (9.6-9.9)**
Eastern Europe	4053	22.3 (21.0-23.6)
Northern Europe	97242	10.8 (10.6-11.0)
Southern Europe	41726	9.2 (8.9-9.4)
Western Europe	77445	7.3 (7.1-7.5)
Oceania	-	-
Australia & New Zealand	-	-
Melanesia	-	-
Micronesia	-	-
Polynesia	-	-

*Adapted from (HPV Information Centre, 2010).

2.4.1.1 Age distribution of HPV prevalence

The global peak age distribution of HPV prevalence (24%) is found at younger ages (<25 years), which occurs soon after sexual debut, and then there is a declining plateau in the middle ages (Figure 2.3) (Bruni *et al*, 2010; HPV Information Centre, 2010). This peak is more pronounced in developing regions (24.4%) than developed regions (19.3%) and is variable by geography. Among women <25 years, Africa (32.1%), Asia (19.7%), and the Americas (24.5%), mainly Latin America (17.6%) have a much higher estimate of HPV prevalence than Europe (17.4%).

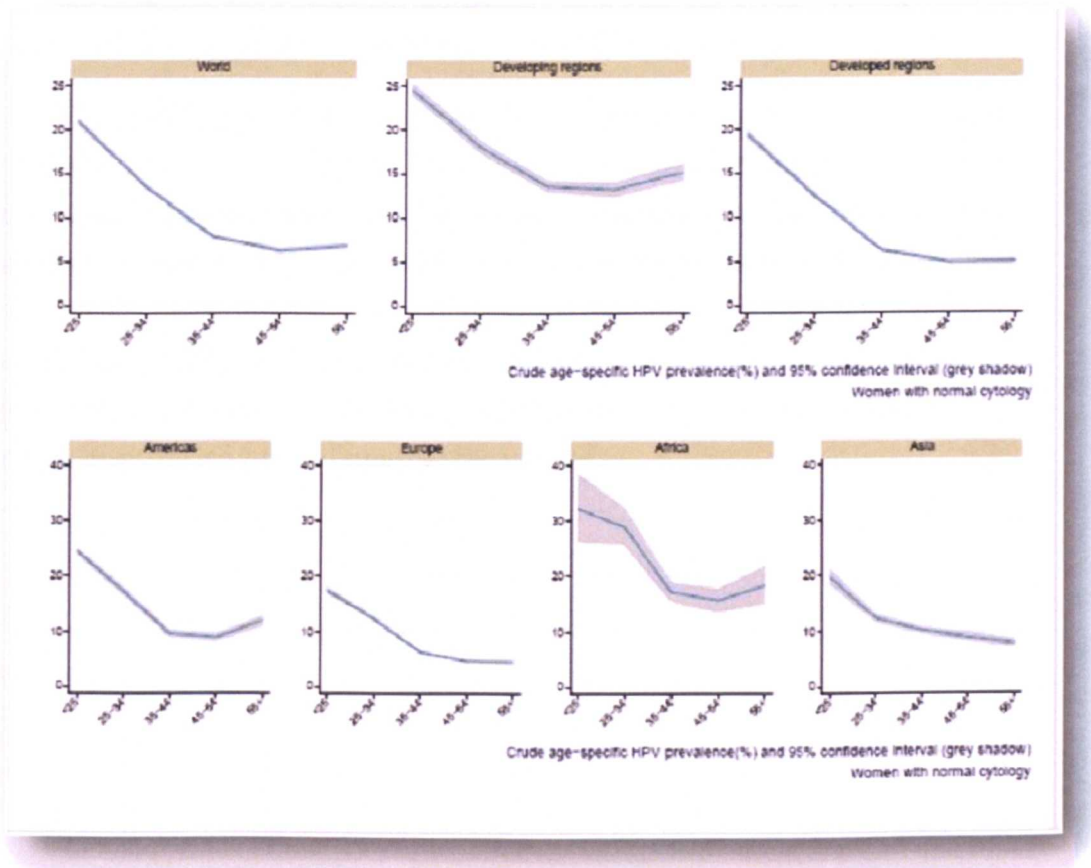
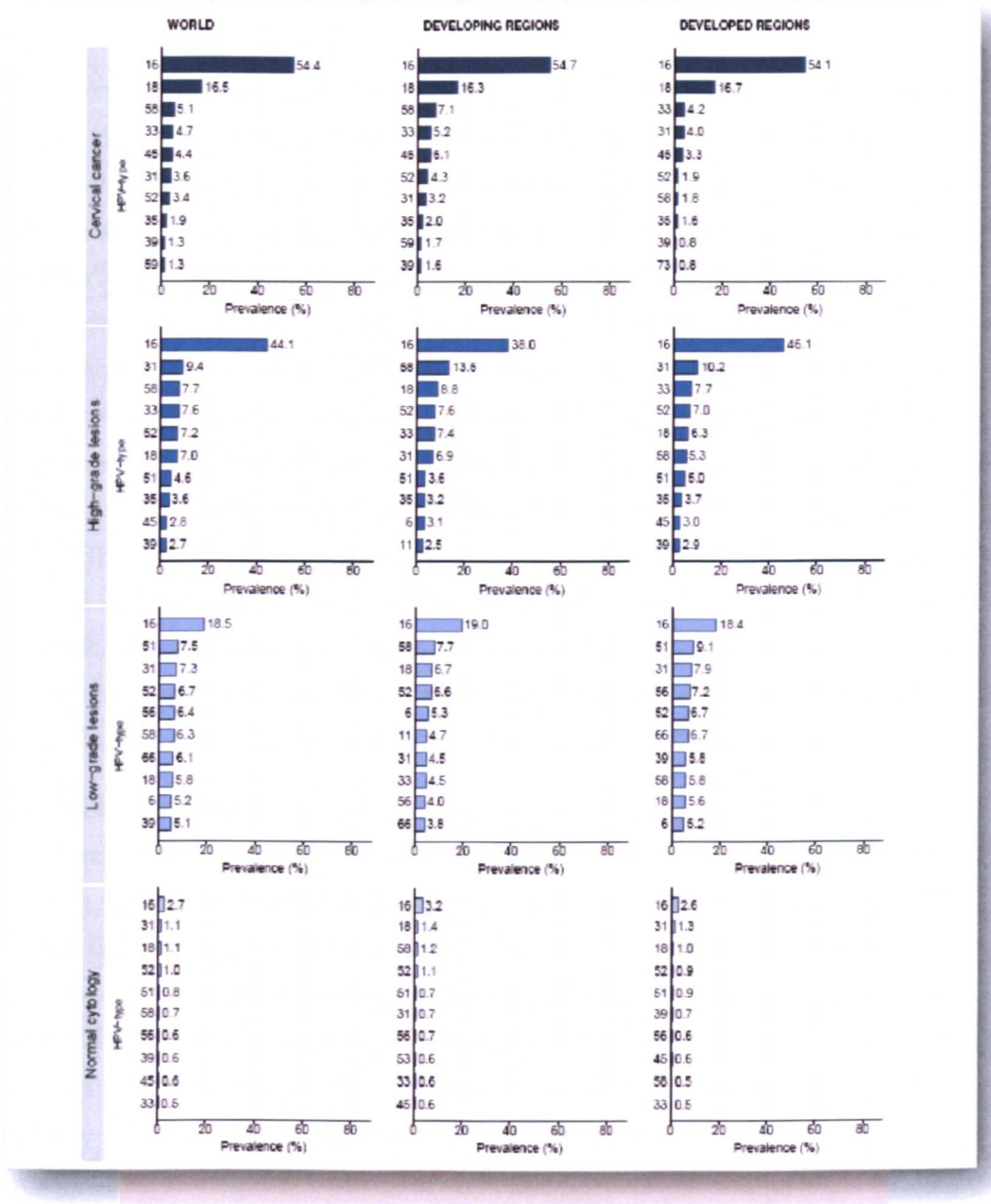


Figure. 2.3 Age-specific HPV prevalence (crude) and 95% confidence interval (grey shade) in women with normal cytology in the World compared to developing and developed regions and five continents. Reproduced from (HPV Information Centre, 2010).

2.4.2 HPV type-specific prevalence

Figure 2.4 shows the ten most common high-risk HPV (HR) types among women with and without cervical lesions in the world from a meta-analysis (HPV Information Centre, 2010). After HPV 16/18, the six most common HPV types among women with ICC are the same worldwide, namely 58, 33, 45, 31, and 52 in descending prevalence; which account for an additional 20% of cervical cancers worldwide (HPV Information Centre, 2010). The distribution between developed and developing regions does not differ significantly but differ in ranking in prevalence, which reflect the geographic variation of HPVs in the regions. However, HPV-16 is the most common type across the spectrum of cervical disease, ranging from normal cytology, to low-grade squamous intraepithelial lesions (LSIL), high-grade intraepithelial lesions (HSIL) and ICC.

While HPV prevalence and type-distribution have been aggregated and summarised for regions of the world, there are, however, large gaps in this epidemiological picture with virtually no data available for Middle Africa; Central and Western Asia; and Melanesia, Micronesia, and Polynesia regions. These data would ideally be required to better understand the burden of HPV infection and ICC in each country to inform local cervical cancer prevention strategies, particularly to provide baseline data prior to the introduction of HPV vaccines in order to measure long-term impact of the vaccine.



NB: The samples for HPV testing come from cervical specimens (fresh/fixed biopsies or exfoliated cells).

Figure 2.4 Ten most frequent HPV types among women with and without cervical lesions in the World compared to developing and developed regions. Reproduced from (HPV Information Centre, 2010).

2.4.3 Sexual and reproductive health factors and risk of HPV infection and cervical carcinogenesis

Sexual behaviour has important implications on a woman's risk of HPV infection. Initiation of sexual intercourse is an important indicator of exposure to the risk of sexually transmitted infections (STIs). Age at first sexual intercourse (AFSI) is an important determinant of exposure to HPV infection and pregnancy as a determinant of risk for ICC. This is highly relevant in developing countries in which young women initiate these two events at an early age and experience high parity. Table 2.2 shows data on the total population of women and selected sexual and reproductive behaviour factors for less developed countries where cervical cancer rates are highest in the world. These indicators are proxy measures that could be used to inform on cervical cancer prevention strategies (i.e. appropriate age for HPV vaccination). Median AFSI ranges from 15.8 to 22 years in Africa, 16.1-20.8 in Latin America and the Caribbean, 15.7-21.6 years in Asia, and 15.6-21.5 years in Oceania. In countries where there are no data on AFSI, age at first marriage may reflect AFSI as sexual initiation for women occurs within marriage, such as that in South Asia, Central, West, and East Africa (Wellings *et al*, 2006).

2.4.4 Biological immaturity of the cervix

Besides a woman's history of lifetime number of sexual partners, AFSI has long been suggested to be associated with an increased risk of cervical cancer, but previous findings have been inconsistent. There are several hypotheses on how these behavioural factors may impact a woman's risk of progression to cervical cancer. It has been speculated that women who initiate first coitus at a young age are at an increased risk of HPV because of a biological predisposition of the immature cervix during adolescence that may be more susceptible to persistent HPV infections and therefore have a greater risk of cancer development.

The vulnerability of the cervix may be attributed to the maturational period of the epithelial topography of the cervix (Hwang *et al*, 2009). The cervix consists of both columnar and stratified non-keratinising squamous epithelia. The squamocolumnar junction (SCJ) is where these two meet and is located on the ectocervix in neonates. The epithelium remains relatively dormant until menarche, at which time squamous metplasia occurs and transforms basal cells of the columnar epithelium into squamous cells. This transformation occurs in the transformation zone. During the maturational process, a new SCJ is formed more

proximally at the endocervix as the ectocervix changes from a predominantly columnar epithelium (considered immature) to a predominant squamous epithelium (considered more mature). Columnar epithelium is a target site for STIs and the transformation zone is the target site for HPV cell proliferation and nearly all cervical neoplasia occurs here. This dynamic process is thought to be triggered by the influence of oestrogens. Since the peak activity of metaplasia occurs during adolescence and first pregnancy, it is highly plausible that early AFSI and pregnancy can increase risks of cervical cancer.

A number of studies have identified an increased risk of high-grade lesions and/or cervical cancer with early AFSI, whereas others have not (IARC, 2007). However, many of these studies were conducted before HPV assessment was feasible, and therefore, the association remains inconclusive. Age at first marriage (AFM) is often used as a proxy measure for AFSI, particularly in societies where the onset of sexual activity occurs within marriage, and those who engage in early sexual intercourse may also consequently become pregnant at an early age. Early childbearing has also been linked as a risk factor for cervical carcinogenesis and attributed to the cervical trauma experienced during early age at first pregnancy (AFP), or subsequently, by high parity births (IARC Monographs 2007). AFP and cervical cancer have been less investigated, although AFSI and AFP are strongly interrelated in most developing countries. The interpretation of the mechanisms by which these sexual and reproductive health events occurring early in life might affect ICC risk is not clear.

Table 2.2. Sexual and reproductive health and cofactors in the aetiology of cervical cancer in less developed countries [a]

		Timing of age at first sexual intercourse (AFSI)			Cofactors of cervical cancer			
Country	**Total population, women (1000s)**	**Median AFSI**	**Female average AFM**	**Difference in average AFM between husband and wife**	**Total fertility**	**Current female tobacco smoking, %**	**Oral contraceptive use, %**	**Estimated number of women 15+ years living with HIV (1000s)**
Africa								
Algeria	17540	-	23.8	7.5	2.4	0.3	45.9	6000
Angola	9631	-	19.4	5.1	6.9	-	2.2	110000
Benin	4560	-	19.9	-	5.8	-	1.8	37000
Botswana	988	-	26.9	2.5	3.3	-	14.3	170000
Burkina Faso	8149	-	18.9	2.6	6.2	8.2	2.2	61000
Burundi	4340	-	22.2	2.4	5.6	-	2.4	53000
Cameroon	9978	20.7	20.2	2.8	5.2	2.7	1.6	300000
Cape Verde	267	-	25.7	2.6	4.2	-	18.2	-
Central African Republic	2292	-	19.4	1.8	5.2	-	4.8	91000
Chad	5786	22	18	2	6.6	2.2	0.5	110000
Comoros	344	19.3	23.6	3.2	5.1	10.4	8.3	<100
Congo	1882	17.8	18.4	-	4.7	0.8	2.3	43000
Cote d'Ivoire	10595	17.5	20.9	8.6	4.7	1.7	3.5	250000
Democratic Republic of the Congo	34208	-	16.6	6.8	7.3	1.6	1	-
Djibouti	440	-	-	2.8	4.2	-	13.6	8700
Egypt	41998	-	22.2	1.8	3.2	4.1	9.9	2600
Equatorial Guinea	349	-	21.7	0.3	5.6	-	-	5900
Eritrea	2653	18.6	19.6	2.4	5.2	1.1	1.4	21000
Ethiopia	42694	19.5	20.5	3	5.7	0.6	3.1	530000
Gabon	751	-	24.3	2.6	4.3	-	4.8	27000

Gambia	882	-	19.2	2.2	6	2.3	3.9	4500
Ghana	12000	-	20.5	-	4.6	0.7	5.5	150000
Guinea	5110	17.6	18.8	3.9	5.6	-	1.6	48000
Guinea-Bissau	831	15.9	-	5	6.8	-	0.3	8700
Kenya	20432	20.2	21.7	2.5	5	1.6	7.5	-
Lesotho	1099	-	21.3	1.7	4.1	-	10.9	150000
Liberia	2063	16.1	20.4	7.2	5.2	-	3.3	19000
Libyan Arab Jamahiriya	3165	16.4	29.2	6.5	4.1	-	9.6	-
Madagascar	10116	16.8	20.6	8.2	5.4	-	3.4	3400
Malawi	7890	15.8	18.6	8.6	6.1	4	2.4	490000
Mali	6743	-	18.4	1.3	6.9	2.5	2.8	56000
Mauritania	1659	18.4	20.5	3.4	4.6	3.7	2.6	3900
Mauritius	655	18.3	23.8	4.9	1.9	1.1	15.8	3800
Morocco	16484	-	26.3	2.4	2.6	0.3	40.1	5900
Mozambique	12006	-	18	2.9	5.6	3.2	4.9	810000
Namibia	1121	-	26.4	3.6	4.2	9.3	8.2	110000
Niger	7931	-	17.6	2.3	7.1	-	3	17000
Nigeria	78916	-	20.3	3.9	5.7	0.9	1.8	1400000
Réunion	429	-	28.2	-	2.4	-	42.6	-
Rwanda	5296	-	22.7	3.8	5.9	-	2.4	78000
Saint Helena	-	18.1	-	3.5	-	-	-	-
Sao Tome and Principe	83	-	17.8	4	4.7	10.6	16.7	-
Senegal	6486	19	20	3.1	5.2	1.3	3.6	38000
Seychelles	-	-	23.8	5.7	-	7	-	-
Sierra Leone	2992	17.9	24.3	5.7	6.6	-	2.5	30000
Somalia	4717	16.1	-	5.1	5.7	-	0.2	6700
South Africa	25590	-	27.1	2.8	2.9	8.9	11.1	3200000
Sudan	21442	-	22.7	-	4.5	-	4.3	170000
Swaziland	613	16.1	25.9	3.9	4.4	2.1	8	100000
Togo	3423	-	21.3	3.7	5.4	-	2.5	69000
Tunisia	5158	18.2	26.6	5.7	2.1	1.7	10.9	1000

Uganda	16864	16	18.2	7.3	6.8	2.5	2.9	480000
United Republic of Tanzania	22574	-	20.5	2.8	5.7	3.3	5.9	760000
Zambia	6641	-	20.3	9.2	5.9	3.5	11.9	560000
Zimbabwe	6526	-	21	-	3.9	2.9	43	680000
Latin America and the Caribbean								
Anguilla	-	-	-	5.2	-	-	-	-
Antigua and Barbuda	-	-	31.5	3.5	-	-	26.2	-
Argentina	20719	18.3	23.3	2.5	2.5	24.6	30.4	0.5
Bahamas	177	-	27.2	3.3	1.9	-	31.5	3.0
Barbados	132	-	31.8	2.5	1.8	3.3	26.2	1.2
Belize	155	-	28.2	2.3	3.4	-	15.6	2.1
Bolivia	5028	-	22.7	2	4.1	-	3.6	0.2
Brazil	99224	18.2	22.7	3.4	2.1	-	20.7	0.6
British Virgin Islands	-	18.1	28.4	4.5	-	-	-	-
Cayman Islands	-	18.5	25.2	3.7	-	-	-	-
Chile	8661	17.6	23.4	4.7	1.9	33.3	23.3	0.3
Colombia	23515	-	22.4	3.4	2.4	-	9.7	0.6
Costa Rica	2284	-	22.2	3.8	2.6	7.3	25.6	0.4
Cuba	5588	-	19.8	1.3	1.6	29.6	3.6	0.1
Dominica	-	-	31.5	1.5	-	-	16.5	-
Dominican Republic	5090	-	22.5	3.4	3	11	13.5	1.1
Ecuador	6877	20.8	21.8	2.3	3.3	5.4	13.3	0.3
El Salvador	3276	17.6	22.3	4.6	3	-	5.8	0.8
French Guiana	116	20.4	29	2.5	4	-	-	-
Grenada	52	20.4	30.9	-	2.5	-	15.2	-
Guadeloupe	243	-	29.5	3.1	2.2	-	-	-
Guatemala	7370	-	21.3	0.9	4.5	3.9	3.4	0.8
Guyana	370	-	27.8	3.3	2.7	-	12.2	2.5
Haiti	5155	-	22.2	4.5	4	-	3.3	2.2
Honduras	3809	-	20.4	-	3.4	3.3	11.3	0.7

Jamaica	1394	-	33.1	5.3	1.9	8.9	18	1.6
Martinique	216	16.2	31	6.5	1.9	-	-	-
Mexico	56179	-	22.4	2.8	3.3	12.4	4.7	0.3
Montserrat	-	-	22.9	-	-	-	-	-
Netherlands Antilles	108	-	29.2	2.6	1.9	-	-	-
Nicaragua	2941	18.6	19.8	4.2	3.3	-	14.6	0.2
Panama	1740	-	21.9	1.7	2.4	-	-	1.0
Paraguay	3200	-	21.5	-	3	14.9	15	0.6
Peru	14715	17.5	23.1	-	2.5	-	7.1	0.5
Puerto Rico	2081	-	23.5	4.1	1.8	-	9.7	-
Saint Kitts and Nevis	-	19.6	31.3	2.2	-	-	-	-
Saint Lucia	89	-	-	2.7	1.8	11	18.4	-
Saint Vincent and the Grenadines	54	16.1	30.9	7.5	2.4	5.2	24.3	-
Suriname	262	-	-	1.1	2.5	-	24.5	2.4
Trinidad and Tobago	691	-	26.8	-	1.6	7.3	10.2	1.5
Turks and Caicos Islands	-	-	-	-	-	-	-	-
United States Virgin Islands	58	16.1	29.9	4.6	3.1	-	-	-
Uruguay	1745	17	23	7.7	2.1	25.8	23.5	0.6
Venezuela (Bolivarian Republic of)	14468	-	22.1	8.3	2.3	27.8	21.1	-
Asia								
Afghanistan	14038	19.4	17.8	4.3	6.8	-	5	-
Armenia	1650	-	22.4	3.6	1.4	4	0.8	0.1
Azerbaijan	4563	-	23.5	-	1.8	0.9	1	0.2
Bahrain	344	-	25.6	3.8	3.1	2.7	10.9	-
Bangladesh	81292	-	18.1	2.1	3	2.9	26.2	-
Bhutan	335	20.3	-	-	4.7	-	3.4	0.1
Brunei Darussalam	197	-	25.1	4	2.4	-	-	-
Cambodia	7679	-	-	6.4	3.4	12.4	12.6	0.8
China	651304	18.4	22.1	8.1	1.4	4.2	1.5	0.1
Cyprus	451	-	23.1	2.8	1.5	-	-	-

Democratic People's Republic of Korea	12139	-	-	-	2.2	-	3.7	-
Georgia	2236	-	22.3	3.8	1.6	5.8	3.2	0.1
Hong Kong Special Administrative Region of China	3721	-	28.6	3.3	1	-	17.1	-
India	587266	18.7	19.3	3	2.8	2.8	3.1	0.3
Indonesia	116455	-	21.6	-	2.6	4.2	13.2	0.2
Iran, Islamic Republic of	36924	-	21	5.3	2.2	4.5	18.4	0.2
Iraq	15557	-	21.7	-	2.8	1.9	14.6	-
Israel	3670	18	24.3	3.2	2.9	17.6	13	0.1
Jordan	3155	-	24.5	2.6	3.7	7.9	7.5	-
Kazakhstan	8257	-	22.2	4.2	2	9.8	2.4	0.1
Kuwait	1239	-	25	-	2.1	-	23.4	-
Kyrgyzstan	2811	15.7	21.4	6.1	2.6	2.2	1.7	0.1
Lao People's Democratic Republic	3223	16.5	21.2	5.6	4.6	14.5	12.9	0.2
Lebanon	2172	-	23.2	2.4	1.9	6.9	10	0.1
Macao Special Administrative Region of China	287	-	26.4	2.4	0.8	-	-	-
Malaysia	13744	-	24.6	-	3	2.5	13.4	0.5
Maldives	155	21.6	19.1	1.3	2.1	9.2	13	-
Mongolia	1366	-	-	-	2.3	5.3	11	0.1
Myanmar	25839	-	26.4	2.4	2.6	11.7	9.8	0.7
Nepal	15028	19.1	18.8	3	3.3	22.6	3.5	0.5
Occupied Palestinian Territory	2165	-	21.7	3.7	4.7	-	7	-
Oman	1269	-	20.7	3	3.6	1	6.1	-
Pakistan	89638	-	21.6	3.6	4	5.2	1.9	0.1
Philippines	46467	17	23.8	4.5	3.6	9.1	13.2	-
Qatar	371	16.4	22.7	4.3	2.8	-	15.8	-
Republic of Korea	24485	-	26.1	2.2	1.2	5.6	1.8	<0.1
Saudi Arabia	11891	20.1	21.7	2.3	3.1	3.2	19.6	-
Singapore	2408	-	27	3.6	1.3	-	10	0.2

Sri Lanka	10368	-	25.3	3.3	2.3	2.5	6.7	-
Syrian Arab Republic	11142	-	21.5	3.5	3.8	-	12.9	-
Tajikistan	3583	-	20.7	1.7	3.6	-	2.1	0.3
Thailand	34639	-	23.5	1.3	2	3.4	30.9	1.4
Timor-Leste	575	-	-	2.6	4.7	-	0.8	-
Turkey	37689	-	22	5.1	2.4	20.5	4.7	-
Turkmenistan	2626	-	22.5	4.1	3	-	1.2	<0.1
United Arab Emirates	1550	-	23.1	4	4.1	2.4	11.9	-
Uzbekistan	13979	18.4	21	1.8	2.9	1.3	2.3	0.1
Viet Nam	45018	16.8	23.2	4.7	1.9	2.1	6.3	0.5
Yemen	11994	18.6	20.8	4.6	6.2	-	6.3	-
Oceania								
American Samoa	-	-	25.7	2	-	-	-	-
Cook Islands	-	-	29.6	4.4	-	20.8	22.6	-
Fiji	421	17.3	22.5	5	2.5	5.1	-	0.1
French Polynesia	133	-	-	3.3	2.2	-	-	-
Guam	88	19.3	24.4	-	2.7	-	-	-
Kiribati	-	-	21.7	2.5	-	-	-	-
Marshall Islands	-	15.6	21	6.3	-	-	-	-
Micronesia, Federated States of	54	16.2	-	6.9	4.4	-	-	-
Nauru	-	17.8	23.4	-	-	53.3	-	-
New Caledonia	127	-	28.4	-	2.3	-	-	-
Niue	-	17	-	3.3	-	-	-	-
Northern Mariana Islands	-	-	-	7.3	-	-	-	-
Palau	-	-	-	4	-	10.1	-	-
Papua New Guinea	3388	-	20.8	4.7	4.8	-	4.4	1.5
Samoa	86	-	23.2	3.5	4.3	23.8	-	-
Solomon Islands	258	19.1	21.2	2.5	4.8	-	-	-
Tokelau	-	21.5	-	2.5	-	-	-	-
Tonga	51	-	25.5	-	3.9	15.7	-	-
Tuvalu	-	-	25.4	-	-	-	-	-

Vanuatu	120	-	22.5	2	4.8	8	-	-
Wallis and Futuna Islands	-	-	23.4	-	-	-	-	-

[a] Data extracted from (HPV Information Centre, 2010).

2.4.5 Male role in the aetiology of cervical cancer

Understanding the burden of cervical cancer in women requires recognising the role of men in sexual transmission of HPV.

A review of sexual behaviour highlighted that women who initiate coitus during adolescence tended to have older male sexual partners (Table 2.2), particularly in African countries, such as that seen in Cote d'Ivoire (average age difference at first marriage between husband and wife=8.6 years), Malawi (8.6 years), and the Zambia (9.2 years). Similarly, the Demographic Health Surveys found that the mean age gap differences between spouses were high in Africa (ranging from 6.7-14.7 years) which significantly differed with developed countries (1.9 in Australia and 2.2 in USA) (Wellings *et al*, 2006). These age gap differences underscore the contribution of the male role in cervical carcinogenesis as they act as carriers of HPV infection, and thus a woman's risk could be dependent on their "high-risk" male partner rather than their own sexual behaviour. In general, men report more lifetime sexual partners than women, who are predominantly monogamous (Wellings *et al*, 2006). These sexual behaviour patterns suggest that men are more likely than women to be the source of STI transmission in marital relationships and sex within marriage is not necessarily safe. It has been reported that condom use is not frequently reported in such marital relationships except for other non-barrier methods of contraception (Cleland *et al*, 2006). The prevalence of condom use in developing countries is low (4.4%) (United Nations, 2007). If unprotected sex is practiced among couples with monogamous women and uncircumcised men, in particular, this may increase the risk of HPV infection and genital disease in their female partners (Castellsague *et al*, 2002).

As HPV is involved in the cervical carcinogenic process, it is central to understand patterns of sexual behaviour in HPV transmission including the behaviours of both men and women. It has been debated that a woman's risk for cervical cancer will depend more on the full sexual history of the male partner than of her own behaviour (Skegg *et al*, 1982). This is particularly relevant in societies where most women are virgins at marriage and monogamous thereafter,

where the incidence of cervical cancer for a population may vary depending on the behaviour of the male partner.

2.4.5.1 Male role hypothesis

Skegg *et al.* hypothesised that in an unscreened population, the risk of cervical cancer will depend on three different sexual behaviour patterns (Skegg *et al*, 1982) :

- Pattern A: a non-promiscuous society in which both women and men are lifetime monogamous
- Pattern B: a society in which women are expected to be lifetime monogamous and men have many sexual partners (e.g. less developed countries as observed in Latin America, Africa and Middle East)
- Pattern C: a permissive society where both men and women have several sexual partners (e.g. more developed countries in North America and Europe)

It has been predicted that the lowest risk would be found in "Pattern A" communities and the highest risk would be found in "Pattern B" communities. A number of studies mostly involving monogamous women have observed an association between lifetime number of sexual partners and history of contact with sex workers of the husband and his wife's risk of cervical cancer while others have not (Bosch *et al*, 1996; Brinton *et al*, 1989; Buckley *et al*, 1981; Munoz *et al*, 1996; Pridan & Lilienfeld, 1971; Thomas *et al*, 2001). In support of this hypothesis, in Spain and Colombia, where women are predominantly monogamous and the cervical cancer incidence rates are different by five-fold, men from Colombia reported a three-fold higher number of sexual partners and a frequency of use of sex workers than men from Spain (Bosch *et al*, 1994). The subpopulation of women practicing prostitution might act in these settings as a "sexual network" of HPV and as a focal point for HPV exposure and spread. The association between the number of sexual partners of the husbands and the risk of cervical cancer in their wives was first shown in a study among mostly monogamous Jewish women (Pridan & Lilienfeld, 1971). In two subsequent studies among monogamous women, the risk of cervical cancer was reported to be two to eight times for women with husbands who had multiple partners (Brinton *et al*, 1989; Buckley *et al*, 1981).

2.4.5.2 Passive smoking in the couples context

Besides their sexual behaviour, other behavioural factors of the male sexual partner, such as tobacco smoking have been less explored despite it being a well-established risk factor for female cervical precancer and cancer (Munoz *et al*, 2006; Plummer *et al*, 2003). Several reviews have summarised the epidemiological and biological association of passive smoking on the risk of cervical cancer (IARC, 2004; National Cancer Institute, 1999), however, the evidence has been suggestive rather than sufficient to implicate the role of passive smoking in the aetiology of cervical cancer among lifetime non-smokers. Among the studies identified, most had small sample sizes of non-smoker controls and cases of cervical cancer, specific information on HPV and sexual behaviour were not available, and most studies obtained spousal history of smoking through questioning of the women rather than the men.

Overall, the extent to which the male role contributes to a woman's risk of cervical cancer has not been fully evaluated.

2.4.6 Natural history of HPV

The most relevant step in cervical carcinogenesis is not acquisition of the infection, but the step in which infection will lead to relevant clinical lesions. Current evidence from longitudinal studies indicate that the majority of HPV infections are transient with about 90% of women, particularly young women, clearing infection within two years, and only a minority of women will harbour persistent HPV infection and develop cervical pre-cancer (Moscicki *et al*, 2006). Persistent infection with high-risk oncogenic HPV types is strongly linked to cervical pre-cancer and progression to invasive cervical cancer (Moscicki *et al*, 2006).

Although infection with HPV is necessary for the development of cervical cancer, infection alone is not sufficient. It has been well-established that a number of cofactors, long-term oral contraceptive use (≥5 years), smoking, high parity, and co-infection with HIV along with HPV infection are necessary for the carcinogenic process to invasive cervical cancer (Munoz *et al*, 2006). The prevalence of these cofactors in developing countries is described in Table 2.2. High parity is particularly relevant as developing countries have some of the highest levels of fertility in the world (Table 2.2) and adolescent childbearing is

common. Total fertility ranges from 0.8 to 7.3 in less developed countries. Besides these exogenous and endogenous factors, sexual and reproductive behaviour factors (International Collaboration of Epidemiological Studies of Cervical Cancer, 2009; Louie *et al*, 2009a), such as early AFSI and early AFP, have also been associated with invasive cervical cancer. It is unclear at what stage of the carcinogenic process do these factors play a role.

Prospective cohort studies have evaluated the effects of oral contraceptive use (Brisson *et al*, 1996; Castle *et al*, 2002b; Richardson *et al*, 2005) and smoking (Giuliano *et al*, 2002; Ho *et al*, 1998; Richardson *et al*, 2005; Silins *et al*, 2005), and their associations with persistent HPV infections, but the results have been inconclusive. The effects of HIV appear more conclusive (Ahdieh *et al*, 2000; Palefsky *et al*, 2006; Sun *et al*, 1997; van der Burg & Palefsky, 2009) although most of the data come from developed rather than developing regions, such as Africa, where the burden of HIV is endemic. Parity as a cofactor of HPV persistence has also been studied but mostly among study populations in developed countries where high parity is less prevalent (Brisson *et al*, 1996; Castle *et al*, 2002b; Giuliano *et al*, 2002; Richardson *et al*, 2005). However, one prospective study in a less developed area of Colombia did find that the rate of HPV persistence was increased among parous women who were positive for HPV infection and had normal cytology at enrolment (Molano *et al*, 2003). Cumulatively, the associations between persistent HPV infections, AFSI, and AFP have been less studied.

2.4.7 Determinants of HPV viral persistence

The viral features of HPV, such as infection by specific types (high-risk *vs.* low-risk), coinfection with multiple HPV types, and HPV viral load could also influence HPV persistence. For instance, high-risk HPV 16 and 18 types show a greater risk of progression to pre-neoplastic lesions compared to other types. The 10-year cumulative incidence rate of developing high-grade lesions is 17% among HPV-16 positive women and 13% among HPV-18 positive women. By contrast, women positive for high-risk types other than HPV-16 or -18 have a 10-year risk of 3.0% to develop high-grade lesions (Khan *et al*, 2005). In addition, the amount of HPV DNA in the cervical epithelium, which is defined by the number, size, and state of HPV-associated disease, is also associated with development of lesions.

Low viral loads are associated with a normal cervix and low risk of developing pre-cancer/cancer; whereas the association of high viral loads and cervical cancer is less clear (Carcopino *et al*, 2011; Cuzick *et al*, 2006; Rousseau *et al*, 2007). Logically, the association of high viral loads is expected to be associated with an increased risk of developing pre-ccancer/cancer, but some of the highest viral loads are also associated with regression of low-grade cervical lesions (Lorincz *et al*, 2002). The measurement of viral load can also be complicated with coinfection with multiple HPV types. About 20% of women with cervical infections are infected with more than one HPV type (Bruni *et al*, 2010). It is also unclear whether these multiple HPV coinfections modify the persistence of a given HPV type or with progression. Given the variable interpretations of viral cofactors in the natural history of HPV, their roles remain uncertain. Studies have evaluated viral HPV factors and cofactors separately, and only one cross-sectional study has evaluated cofactors with HPV infection in the development of cervical cancer (Wang *et al*, 2009). This study identifies current smoking to be associated with type-specific HPV-16 cervical intraepithelial neoplasia grade 2 and 3 (CIN-2/3) and multiparity to be associated with CIN-3 without HPV type specificity. Both HPV viral cofactors and sexual and reproductive health risk factors need to be considered prospectively in order to determine the risk associations in modulating HPV persistence and cancer development.

2.5 Summary

Cervical cancer is the second most common cancer among women in the developing world. Overall, the poorest women appear to initiate sex at an early age, and can subsequently become pregnant at an early age, and have a large number of children, putting them at an increased risk of HPV infection and cervical cancer. Data suggest that women who initiate first coitus and first pregnancy at a young age are at an increased risk of HPV because of a biological predisposition of the immature cervix and cervical trauma experienced during pregnancy delivery that may be more susceptible to persistent HPV infections and therefore have a greater risk of cancer development. The epidemiological evidence of how these sexual and reproductive health factors impact the natural history of HPV and cervical carcinogenesis is not clear since previous studies were conducted before HPV assessment was feasible. This thesis hypothesized that the biological associations of these sexual and reproductive health factors and ICC can be confirmed with strong epidemiological evidence that fully addresses the role of HPV. The objective of this PhD thesis was to characterize and provide robust estimates of the risk of cervical cancer and its association with AFSI, its interrelated characteristics such as AFP and AFM, as well as characterize the contribution of male factors to a woman's risk. Findings from this research may provide clues to additional biological mechanisms and molecular events that are important for progressive disease in the natural history of HPV and cervical cancer, as well as make suggestions in improving the design and assessment of cervical cancer prevention measures.

Cover sheet for each 'research' paper included in a research thesis

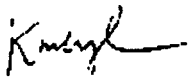
NB: Chapter 3 has already been published:

Louie KS, de Sanjose, Diaz M, Castellsagué, Herrero R, Meijer CJL, Shah K, Franceschi S, Muñoz N, Bosch F, for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Early age at first sexual intercourse and early pregnancy are risk factors for cervical cancer in developing countries. British Journal of Cancer 2009; 100 (7):1191-7.

1. For a 'research paper' already published
    a. Where was the work published? **British Journal of Cancer**
    b. When was the work published? **2009**
        i. If the work was published prior to registration for your research degree, give a brief rationale for its inclusion. **N/A**
    c. Was the work subject to academic peer review? **Yes**
    d. Have you retained the copyright for the work?
2. **No, refer to Appendix 5 for permission from copyright holder.**
3. For a 'research paper' prepared for publication but not yet published
    a. Where is the work intended to be published? N/A
    b. List the paper's authors in the intended authorship order N/A
    c. Stage of publication N/A

4. For multi-authored work, give full details of role in the research included in the paper and in the preparation of the paper.

I, Karly S Louie, was the primary author, designed and planned the study with SS, XC, FXB, conducted the statistical analysis, interpreted the results, and wrote the manuscript.



Candidate's signature

Senior author, F Xavier Bosch, signature to confirm role as stated in (3)

3 Study 1. Early age at first sexual intercourse (AFSI), marriage (AFM), and first pregnancy (AFP) and risk of cervical cancer

3.1 Introduction

Early age at first sexual intercourse (AFSI) has been associated with an increased risk of high-risk human papillomavirus (HPV) infection, a sexually transmitted infection, that in susceptible women, is responsible for virtually all cases of invasive cervical cancer (ICC) (Bosch *et al*, 2002). Since sexual behaviour determines exposure to HPV, AFSI is of particular interest as it has been associated with riskier sexual behaviour, such as having unprotected sex, having multiple sexual partners, as well as a woman's partner having multiple partners. It has also been speculated that the increased risk of HPV is due to a biological predisposition of the immature cervix during adolescence that may be more susceptible to persistent HPV infections and therefore have a greater risk of cancer development (Kruger-Kjaer *et al*, 1998). A number of studies have identified an increased risk of high-grade lesions and/or cervical cancer with early AFSI while others have not (IARC, 2007). However, several of these studies were conducted before HPV assessment was feasible and different statistical methodologies were used to evaluate HPV, and therefore, the associations remain inconclusive. Age at first marriage (AFM) is often used as a proxy measure for AFSI, and those who engage in early sexual intercourse may also consequently become pregnant at an early age. Besides early AFSI, early childbearing has also been linked as a risk factor for cervical carcinogenesis and attributed to the cervical trauma experienced during early age at first pregnancy (AFP), or subsequently, by high parity births (IARC, 2007). The interpretation of the mechanisms by which these sexual and reproductive events occurring early in life might affect ICC risk three or more decades later is not straightforward. The objective of this study was to further characterise and provide robust estimates of the risk of cervical cancer and its association with AFSI, interrelated characteristics such as AFP and AFM in a series of studies that fully considered the association of HPV with cervical cancer.

3.2 Methods

The program of HPV and cervical cancer studies has been coordinated by the International Agency for Research on Cancer (IARC) in Lyon, France and the Institut Català d'Oncologia (ICO) in Barcelona, Spain. They included a series of case-control studies on invasive cervical cancer from eight developing countries with a broad range of rates of incidence of cervical cancer that were pooled for analysis. Regions covered include Morocco (Chaouki *et al*, 1998) and Algeria (Hammouda *et al*, 2005) in Africa; the Philippines (Ngelangel *et al*, 1998) Thailand (Chichareon *et al*, 1998) and Madras, India in Asia; and Brazil (Eluf-Neto *et al*, 1994), Colombia (Munoz *et al*, 1993), Paraguay (Rolon *et al*, 2000), and Peru (Eluf-Neto *et al*, 1994) in South America. Although Spain (Munoz *et al*, 1993) was part of the series of case-control studies, the sexual and reproductive behaviour of this population was heterogeneous to the other countries (late AFSI and low parity) and the study site was therefore excluded from this analysis.

Methods of each study have been described elsewhere. Briefly, women with histologically confirmed incident invasive squamous cell carcinoma, adenocarcinoma or adenosquamous-cell carcinoma were recruited from reference hospitals before treatment. Written informed consent was obtained from those who agreed to participate. Hospital-based controls were frequency-matched to case patients by five-year age groups.

3.2.1 Study questionnaire

A standardized questionnaire was administered to participants by a trained interviewer that included questions about socio-demographic factors, sexual and reproductive behaviour, smoking habits, Pap screening history, hygienic practices, and history of sexually transmitted diseases.

3.2.2 Specimen collection and testing

Two samples of cervical exfoliated cells were collected with wooden spatulae and endocervical brushes. After preparation of one Papanicolaou smear, the remaining cells were eluded in saline, centrifuged and frozen at -70 °C until shipment to the central laboratory for HPV DNA testing. A tumor-biopsy sample was obtained from cases and frozen. Cytology and histology diagnosis were reviewed and confirmed by a panel of expert pathologists that agreed on a diagnosis by consensus or majority.

3.2.3 HPV DNA testing

Detailed descriptions of the polymerase-chain-reaction (PCR) assays used in these studies have been described elsewhere. HPV DNA detection was detected by PCR amplification of a small fragment of the *L1* gene using MY09 and MY11 consensus primers for the study in Colombia (Hildesheim *et al*, 1994)and GP5+/6+ general primer system for the other studies (de Roda Husman *et al*, 1995; Jacobs *et al*, 1995; Walboomers *et al*, 1992a). Beta-globin primers were used to amplify the beta-globin gene to assess the quality of the DNA in the specimen. HPV DNA in PCR products were analyzed with the use of a cocktail of HPV-specific probes and genotyped by hybridization with type-specific probes for 33 HPV types. Samples that tested positive for HPV DNA but did not hybridize with any of the type-specific probes were labelled as HPV X.

3.2.4 Statistical analysis

Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (95% CI).

To assess the association of AFSI with the risk of invasive cervical cancer, three different statistical models to adjust for HPV DNA detection were computed and compared: 1) one model included all patients and controls, and it was not adjusted for HPV DNA status, 2) a second model included all patients and controls, and included a variable to adjust for HPV DNA status, and 3) a third model was restricted to HPV DNA positive cases and controls. As described earlier, results from previous studies have been inconclusive since studies were conducted before HPV assessment was feasible. These three models were used to clarify previous inconsistent associations.

To control for potential confounding, final models were adjusted for age, country, lifetime number of sexual partners (1, >1), parity (0, 1-4, ≥5), and educational level (never, primary, secondary or higher). Each variable included in the adjustment models was assessed for interaction with AFSI. Test for trend was performed when appropriate using the log likelihood ratio test. Only subjects who reported ever having been married and/or ever having had children were included in the analyses of AFM and AFP.

We evaluated other potential confounding factors such as smoking (never, ever), oral contraceptive use (never, 1-4 years, ≥5 years), history of pap smears

excluding those in the 12 months prior to enrolment (never, ever), having had first coitus before menarche and the timing of first coitus relative to age at menarche (Table 3.1), but they were not adjusted for in the final analysis since they did not contribute any change to the odds ratio estimates for AFSI in the adjusted models.

Table 3.1 Univariate analysis of risk factors associated with AFSI and risk of ICC

	Cases/Controls	Odds ratios (95% CI)*
History of first sexual intercourse before first menarche		
No	2090/2079	1.00
Yes	223/147	1.88 (1.36-2.61)
Time from first sexual intercourse from first menarche		
>=5 yrs		1.00
3-4 yrs	946/1299	1.93 (1.63-2.28)
1-2 yrs	489/376	2.37 (1.98-2.83)
0 yrs	466/298	2.70 (2.07-3.52)
<0 years	189/106	3.61 (2.42-5.38
Missing	87/40	

*Age and centre adjusted

3.2.4.1 Sub-analysis of male factors

A sub-analysis was conducted among women (from Brazil, Morocco, Algeria, Philippines, and Thailand) who had information on the age of their first male sexual partner.

3.2.5 Ethical approval

The multi-centric case-control study protocol was approved by the IARC and local ethics and research committees in each study country. Additional approval for this research was obtained from the Lead Principal Investigator, F Xavier Bosch and the Steering Committee of the IARC Cervical Cancer Study Group. PhD Co-Supervisor, Silvia de Sanjose, for this thesis is also part of this steering committee. A letter granting access to the data for collaborative analyses is attached (Appendix 6). This research was also approved by the ethical review boards at the London School of Hygiene and Tropical Medicine (Appendix 7).

3.3 Results

3.3.1 Patient characteristics

Table 3.2 describes some characteristics of the 1864 invasive cervical cancer cases and 1719 corresponding controls that entered the final analysis. Ninety-five percent of case patients and 17% of controls tested positive for HPV DNA. The majority of cases (92%) had squamous cell carcinoma (SCC). Case patients were older than controls with a median age of 49 (range 20-84 years) vs. 48 (18-82 years), respectively. Median AFSI was earlier in case patients (17; range 6-45 years) compared to controls (19; range 6-42 years) and this was consistently found in each country.

Table 3.2 Characteristics of cases of invasive cervix carcinoma and controls.

	HPV Tested		HPV Positive				Age*		Age at Sexual Debut*	
	Cases	Controls	Cases	%	Controls	%	Cases	Controls	Cases	Controls
Country	**1864**	**1719**	**1769**	**94.9**	**285**	**16.6**	**49**	**48**	**17**	**19**
Algeria	142	145	132	93.0	18	12.4	53.5	52	16	18
Morocco	188	176	182	96.8	38	21.6	49	40	16	18
Madras (India)	187	184	180	96.3	51	27.7	48	46.5	17	18
Philippines	364	380	349	95.9	35	9.2	47.5	47	19	21
Thailand	378	259	363	96.0	41	15.8	49.5	50	18	20
Brazil	187	190	181	96.8	32	16.8	51	52	18	19
Colombia	110	124	87	79.1	21	16.9	46	45.5	17	18
Paraguay	112	86	109	97.3	18	20.9	48.5	45.5	16	19
Peru	196	175	186	94.9	31	17.7	48	48	16	18

*Median

3.3.2 AFSI and risk of cervical cancer

Table 3.3 shows the risk of ICC by AFSI according to the three different adjustment models. An increase risk of ICC was consistently observed with decreasing AFSI (p-trend<0.001). Compared to AFSI ≥21 years, the odds ratios of ICC was 1.80 (95%CI: 1.50-2.16) for AFSI 17-20 years, and 2.31 (95%CI: 1.85-2.87) for AFSI ≤16 years, after adjusting for age, centre, lifetime number of sexual partners, parity, and education level in the HPV-unadjusted model. According to the different model adjustments, women reporting AFSI ≤16 years of age had a 2.3 to 2.5-fold risk of ICC and 1.8 to 2.1-fold risk for AFSI 17-20 years of age (Table 3.3). Given the consistent association of AFSI and the risk of ICC across the different models, HPV-unadjusted models were used for the remainder of the results.

Table 3.3. Impact of different strategies of multivariate model adjustments on the association between age at first sexual intercourse and risk of ICC (from IARC case-controlled studies)

			Age and centre adjusted	HPV unadjusted*	HPV adjusted*	HPV-positive only*
	Cases n (%)	Controls n (%)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)	Odds ratio (95% CI)
Sexual Debut						
>=21 years	341 (16.9)	656 (35.4)	1.00	1.00	1.00	1.00
17-20 years	813 (40.2)	667 (36.0)	2.44 (1.07-2.87)	1.80 (1.50-2.16)	1.78 (1.32-2.39)	2.10 (1.49-2.97)
≤16 years	710 (35.1)	396 (21.4)	4.09 (3.38-4.94)	2.31 (1.85-2.87)	2.09 (1.48-2.96)	2.48 (1.65-3.73)
p-trend				<0.001	<0.001	<0.001

* Adjusted for age, study centre, lifetime number of partners (1, ≥2), parity (0, 1-4, ≥5), and education (never, primary, secondary)
† p-heterogeneity between age at first sexual intercourse and country=0.58

We calculated the risk of ICC for each country study, and, in general, each study country showed an increasing risk of ICC with decreasing AFSI (Table 3.4). There was no evidence of heterogeneity with respect to study country (p=0.58).

Table 3.4 Association between age at first sexual intercourse and risk of ICC by country

Country	No. of cases/controls	Odds ratio (95% CI)*	Odds ratio (95% CI)†
Brazil			
≥21	38/83	1.00	1.00
17-20	86/69	2.73 (1.66-4.49)	2.35 (1.36-4.05)
≤16	63/38	3.62 (2.08-6.32)	2.65 (1.41-4.98)
p-trend			0.002
Morocco			
≥21	7/44	1.00	1.00
17-20	50/58	4.11 (1.66-10.17)	1.52 (0.46-4.97)
≤16	131/74	7.39 (3.08-17.74)	2.08 (0.63-6.90)
p-trend			0.16
Paraguay			
≥21	11/25	1.00	1.00
17-20	41/45	2.24 (0.97-5.18)	0.52 (0.17-1.59)
≤16	60/16	9.11 (3.66-22.67)	1.29 (0.38-4.44)
p-trend			0.46
Philippines			
≥21	122/210	1.00	1.00
17-20	165/136	2.09 (1.52-2.88)	1.67 (1.18-2.37)
≤16	77/34	3.92 (2.47-6.22)	2.32 (1.40-3.86)
p-trend			<0.001
Thailand			
≥21	92/126	1.00	1.00
17-20	220/108	2.80 (1.90-3.99)	2.15 (1.47-3.16)
≤16	66/25	3.62 (2.12-6.17)	2.24 (1.25-3.99)
p-trend			<0.001
Peru			
≥21	24/51	1.00	1.00
17-20	71/64	2.36 (1.31-4.26)	1.94 (1.04-3.64)
≤16	101/60	3.58 (2.00-6.40)	2.46 (1.26-4.82)
p-trend			0.01
Madras, India			
≥21	17/30	1.00	1.00
17-20	88/98	1.59 (0.82-3.08)	1.27 (0.61-2.63)
≤16	82/56	2.60 (1.31-5.17)	1.41 (0.66-3.04)
p-trend			0.39

Algeria			
≥21	16/46	1.00	1.00
17-20	50/47	3.33 (1.62-6.86)	2.12 (0.94-4.80)
≤16	76/52	4.76 (2.32-9.78)	2.41 (1.01-5.74)
p-trend			0.08
Colombia			
≥21	14/41	1.00	1.00
17-20	42/42	2.93 (1.39-6.16)	2.53 (1.13-5.64)
≤16	54/41	3.86 (1.85-8.08)	2.43 (1.07-5.52)
p-trend			0.04

*Adjusted for age and study centre

† Adjusted for age, study centre, lifetime number of partners (1, ≥2), parity (0, 1-4, ≥5), and education (never, primary, secondary)

We stratified the analysis according to the established risk factors for ICC and the positive association of ICC with decreasing AFSI remained at each level of exposure for each of these characteristics (Table 3.5). Similar associations were observed for AFP. No interaction was observed between any of the examined risk factors and AFSI. Although not statistically significant, the risk linked to AFSI appeared stronger among parous women compared to nulliparous women.

Table 3.5. Age at first sexual intercourse and risk of cervical cancer according to various characteristics

	Number of Cases/Controls	Odds ratio (95% CI)*	Odds ratio (95% CI)†	p-trend
Parity				
Nulliparous				
≥21 yr	14/40	1.00	1.00	
17-20 yr	7/14	1.88 (0.53-6.66)	1.61 (0.40-6.57)	
≤16 yr	7/6	3.60 (0.83-15.52)	1.50 (0.17-13.55)	0.56
Ever Parous				
≥21 yr	327/614	1.00	1.00	
17-20 yr	804/645	2.47 (2.07-2.94)	1.97 (1.63-2.36)	
≤16 yr	703/387	4.10 (3.36-4.99)	2.59 (2.08-3.21)	<0.001
p-heterogeneity between AFSI and ever parous=0.64				
Parity (1-4 births)				
≥21 yr	171/399	1.00	1.00	
17-20 yr	273/287	2.58 (1.98-3.35)	1.99 (1.51-2.62)	
≤16 yr	169/115	4.72 (3.41-6.54)	2.71 (1.89-3.87)	<0.001
Parity (≥5 births)				
≥21 yr	156/215	1.00	1.00	
17-20 yr	531/358	2.01 (1.57-2.59)	1.71 (1.32-2.23)	
≤16 yr	534/272	2.88 (2.19-3.78)	2.08 (1.55-2.78)	<0.001
p-heterogeneity between AFSI and parous groups (nulliparous, 1-4 births, and ≥5 biths)*=0.90*				
Oral contraceptive use				
Never				
≥21 yr	218/400	1.00	1.00	
17-20 yr	453/364	2.33 (1.87-2.90)	1.77 (1.40-2.25)	
≤16 yr	376/184	4.28 (3.29-5.55)	2.45 (1.82-3.29)	<0.001
1-4 years				
≥21 yr	64/162	1.00	1.00	
17-20 yr	117/114	2.64 (1.76-3.94)	1.67 (1.07-2.61)	
≤16 yr	111/89	3.94 (2.53-6.13)	1.95 (1.15-3.30)	0.01

≥5 years				
≥21 yr	36/52	1.00	1.00	
17-20 yr	124/72	3.26 (1.85-5.72)	2.46 (1.36-4.46)	
≤16 yr	96/49	4.48 (2.39-8.40)	2.80 (1.38-5.65)	0.006
Smoking				
Never				
≥21 yr	272/569	1.00	1.00	
17-20 yr	587/552	2.34 (1.93-2.83)	1.68 (1.36-2.06)	
≤16 yr	550/348	3.76 (3.03-4.67)	2.06 (1.61-2.64)	<0.001
Ever				
≥21 yr	67/85	1.00	1.00	
17-20 yr	221/113	2.73 (1.81-4.13)	2.32 (1.50-3.60)	
≤16 yr	152/43	5.63 (3.42-9.27)	3.62 (2.10-6.26)	<0.001
Lifetime number of sexual partners				
Monogamous				
≥21 yr	270/569	1.00	1.00	
17-20 yr	529/499	2.33 (1.92-2.83)	1.80 (1.46-2.22)	
≤16 yr	349/214	3.89 (3.05-4.96)	2.38 (1.83-3.11)	<0.001
Partners >1				
≥21 yr	69/74	1.00	1.00	
17-20 yr	280/151	2.03 (1.37-3.01)	1.74 (1.15-2.63)	
≤16 yr	352/156	2.75 (1.84-4.11)	2.14 (1.39-3.28)	0.001
		p-heterogeneity=0.36		
Education				
Never go to school				
≥21 yr	54/57	1.00	1.00	
17-20 yr	255/155	1.68 (1.09-2.60)	1.46 (0.93-2.30)	
≤16 yr	402/173	2.41 (1.55-3.75)	2.09 (1.31-3.35)	0.001
Primary School				
≥21 yr	164/238	1.00	1.00	
17-20 yr	404/311	1.99 (1.54-2.56)	1.62 (1.24-2.12)	
≤16 yr	236/167	2.51 (1.87-3.39)	1.71 (1.24-2.36)	0.001

Secondary School				
≥21 yr	118/359	1.00	1.00	
17-20 yr	150/198	2.68 (1.95-3.68)	2.29 (1.65-3.20)	
≤16 yr	72/55	4.79 (3.07-7.47)	3.36 (2.07-5.47)	<0.001
p-heterogneity=0.08				
Ever have a pap smear 12 months prior to study enrolment				
Never				
≥21 yr	183/320	1.00	1.00	
17-20 yr	434/371	2.20 (1.74-2.79)	2.29 (1.65-3.20)	
≤16 yr	416/205	3.65 (2.79-4.78)	3.36 (2.07-5.47)	<0.001
Ever				
≥21 yr	158/336	1.00	1.00	
17-20 yr	379/296	2.65 (2.02-3.46)	1.92 (1.44-2..57)	
≤16 yr	294/191	4.49 (3.32-6.06)	2.30 (1.63-3.24)	<0.001

*Adjusted for age and study centre
† Adjusted for age, study centre, lifetime number of partners (1, ≥2), parity (0, 1-4, ≥5), and education (never, primary, secondary)

3.3.3 AFSI, AFM, and AFP

AFP and AFM were both directly correlated with AFSI in these populations ($p<0.001$). Approximately 92% of women reported AFSI to be the same as AFM. One-quarter of women reported AFP to be the same as AFSI. Cumulatively, 62.4% of women reported giving birth within the first year of AFSI. Among women with AFSI ≤16 years, 52.4% were pregnant within the first year of coitus. Figure 3.1 shows the high correlation of AFSI and AFP and the similar decreasing risk of ICC with increasing age of AFSI/AFP. Given the high correlation between the two variables, we did not adjust for AFSI in the AFP final model and vice versa.

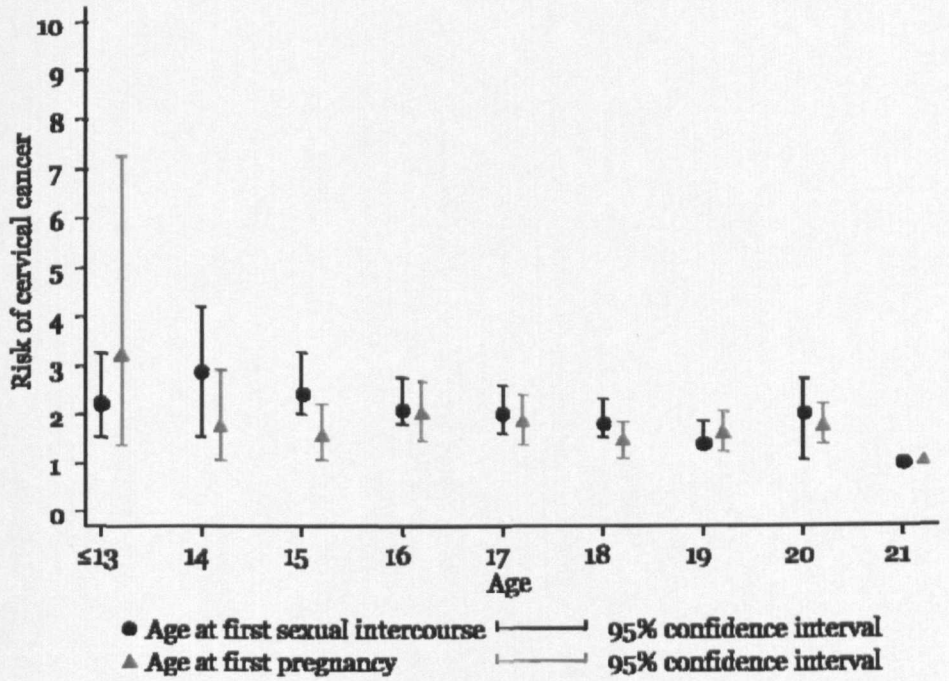


Figure 3.1. Age at first sexual intercourse and age at first pregnancy are highly correlated with the risk of invasive cervical cancer (p-trend<0.001). Models were adjusted for age, centre, lifetime number of sexual partners (1, >1), parity (0, 1-4, ≥5), and education (never, primary, secondary).

We further evaluated the combined effect of AFP and AFSI on the risk of cervical cancer (Table 3.6). An increased risk emerged in subsequent strata of decreasing AFP with decreasing AFSI.

Table 3.6. Interaction between age at first pregnancy and age at first sexual intercourse in the risk of cervical cancer

	Age at first pregnancy		
Age at sexual debut	≥21	17-20	≤16
≥21 yr	1.00		
17-20 yr	1.58 (1.22-2.03)	1.93 (1.58-2.36)	
≤16 yr	2.17 (1.35-3.47)	2.28 (1.74-2.99)	2.36 (1.82-3.07)

† Adjusted for age, study centre, lifetime number of partners (1, ≥2), parity (0, 1-4, ≥5), and education (never, primary, secondary).

Given this combined effect, we assessed the latency period (AFP – AFSI) between these two events to clarify whether it affected cervical cancer risk. Although there was no statistical difference across strata, the data suggested that women with AFSI ≤16 years and a latency period for a subsequent pregnancy of less than 2 years may be at a slight increased risk compared to women with a larger time gap (Table 3.7).

Table 3.7 Association between AFP-AFSI and risk of cervical cancer

No. of years difference between AFP-AFSI	Cases/Controls	Odds ratio (95% CI)*
AFSI ≥21		
≥2	94/202	1.00
1	117/225	0.97 (0.68-1.39)
0	115/187	1.17 (0.81-1.69)
AFSI 17-20		
≥2	268/226	1.00
1	285/247	1.01 (0.78-1.32)
0	250/175	1.15 (0.87-1.52)
AFSI ≤16		
≥2	330/187	1.00
1	235/132	1.11 (0.82-1.50)
0	134/68	1.06 (0.73-1.53)

AFP, age at first pregnancy; AFSI, age at first sexual intercourse
*Adjusted for age, study centre, lifetime number of partners (1, ≥2), parity (0, 1-4, ≥5), and education (never, primary, secondary)

3.3.4 Sub-analysis of age difference with the first male partner

Age difference with the first male sexual partner at the time of the women's first sexual intercourse was identified as a risk factor for ICC (Table 3.8). Among the subset of women reporting the age of her first male sexual partner, we observed a decrease in age difference with the first male sexual partner with increasing AFSI (Figure 3.2), highlighting the fact that younger women tend to have older partners at sexual initiation or at first marriage.

Table 3.8. Age at first sexual intercourse (AFSI) and risk of cervical cancer according to age difference with the first male sexual partner

AFSI	No. of cases/controls	Odds ratio (95% CI)*	Odds ratio (95% CI)†	p-trend
Age difference of ≤2 years				
≥21	164/301	1.00	1.00	
17-20	113/175	2.78 (2.05-3.78)	1.87 (1.34-2.61)	
≤16	26/47	3.58 (2.10-6.12)	1.64 (0.91-2.95)	0.002
Age difference of 3-7 years				
≥21	139/60	1.00	1.00	
17-20	161/210	3.03 (2.08-4.42)	2.52 (1.68-3.79)	
≤16	91/148	4.55 (2.97-6.97)	3.31 (2.04-5.39)	<0.001
Age difference of ≥8 years				
≥21	66/49	1.00	1.00	
17-20	137/180	1.84 (1.19-2.85)	1.54 (0.96-2.48)	
≤16	103/207	3.27 (2.04-5.23)	2.32 (1.37-3.94)	0.001
p-heterogeneity=0.55				

*Adjusted for age and study centre

† Adjusted for age, study centre, lifetime number of partners (1, ≥2), parity (0, 1-4, ≥5), and education (never, primary, secondary)

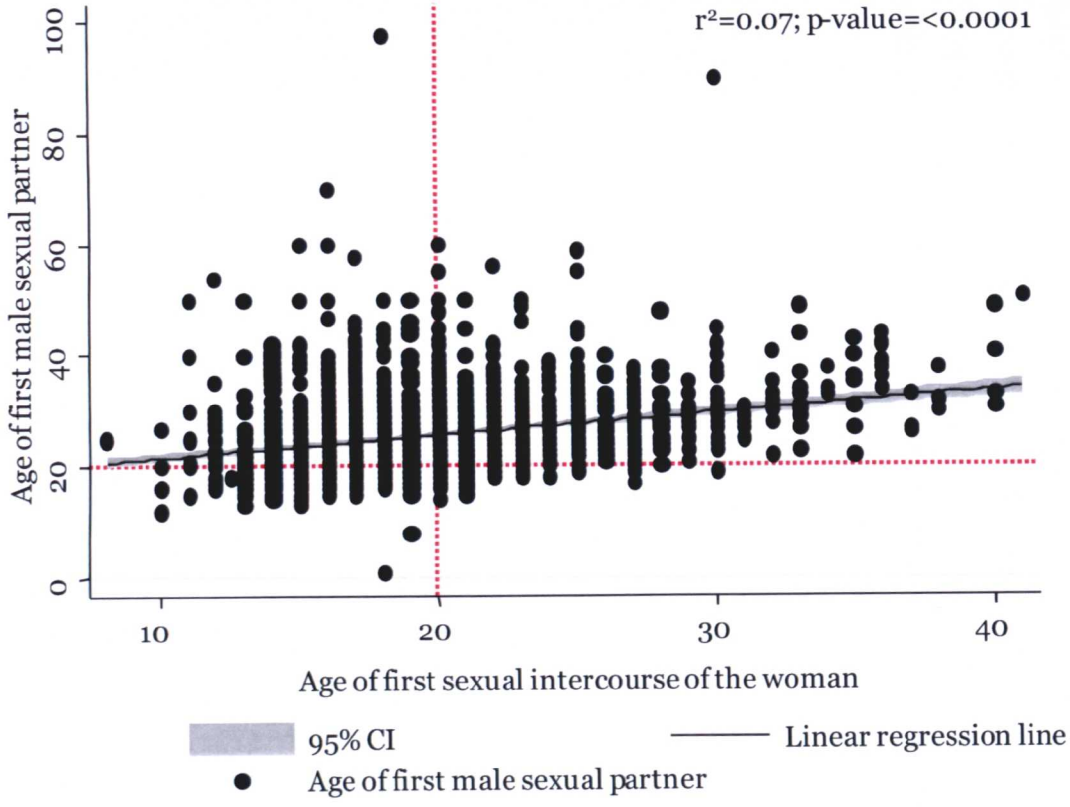


Figure 3.2. Age at first sexual intercourse of the woman vs. age of corresponding first male sexual partner

About half of the cases and one-third of the controls (47.5% and 33.7%, respectively) with an age gap of ≥8 years with their first male sexual partner initiated sexual intercourse at ≤16 years. In contrast, about 12% of cases and 6.0% of controls who had a smaller age gap of ≤2 years initiated first intercourse at ≤16 years (Table 3.8). No heterogeneity was observed between the age gap difference among monogamous women and women with more than one lifetime number of sexual partners (p=0.18). The odds ratios vs. age difference with the first male sexual partner of ≤2 years was 1.11 (95%CI: 0.90-1.37) among women with an age difference of 3-7 years, and 1.44 (95%CI: 1.15-1.80) for ≥8 years, after adjusting for age, centre, AFSI, lifetime number of partners, parity, and education.

3.4 Discussion

The IARC/ICO series of case-control studies remain the largest set of aetiological investigations on invasive cervical cancer that fully addresses the role of HPV DNA and of the independent established cofactors. This is probably also the largest dataset reporting on invasive cervical cancer in the developing world where early AFSI, AFP and high parity are prevalent phenomenons. The results show that early age at first sexual intercourse and early age at first pregnancy are risk factors for cervical cancer, irrespective of other known risk factors for the disease. The data presented show a possible additional increase in risk when the early event of first sexual intercourse is shortly followed by a pregnancy.

The mechanism by which the early experience of first coitus and first pregnancy could influence the risk of cervical carcinogenesis may be explained by the steroid hormonal influence on HPV infection and on the host's immune response to HPV during preadolescence and adolescence. The transformation zone of the cervical epithelium has been recognized as the site for which HPV infection tends to cause cancer and the susceptibility of this area is believed to be related to its desnudation of the stratified epithelium, thus facilitating exposure of the basal layer to HPV with minimal trauma. Biological immaturity during adolescence has also been proposed as an additional susceptibility factor (Elson *et al*, 2000; Moscicki *et al*, 1989; Singer & Monaghan, 2000). During adolescence and pregnancy, the cervix is exposed to augmented levels of hormonal changes (Singer & Monaghan, 2000), in which oestrogen stimulation facilitates acidification of the vaginal cavity, a determinant of squamous metaplasia when the endocervical epithelial everts (Elson *et al*, 2000). When this estrogen-stimulated metaplastic transformation occurs in the presence of HPV, the probability of cell transformation increases, resulting in neoplastic changes (Elson *et al*, 2000; Hwang *et al*, 2009; Shai *et al*, 2007; Shai *et al*, 2008). This phenomenon is dependent primarily on parity, and is more likely to occur during the first pregnancy rather than subsequent pregnancies (Singer & Monaghan, 2000). Although it has been postulated that these metaplastic changes are also influenced by the trauma and repair experienced during delivery, no increased risk for cervical carcinoma was observed in this same dataset when traumatic partition was evaluated (Munoz *et al*, 2002).

Increased risks of cervical carcinoma have been identified in women with long-term use of hormonal steroids (Moreno *et al*, 2002)and those who are highly parous (Munoz *et al*, 2002). In addition, HPV-16 transgenic mouse models have shown that those treated with longer durations of oestrogen were more likely to develop larger tumours and have a significantly higher number of tumours than those treated with a shorter duration (Arbeit *et al*, 1996; Brake & Lambert, 2005; Elson *et al*, 2000; Mitrani-Rosenbaum *et al*, 1989), supporting the human observations of a susceptible cervix to carcinogenic progression by continuous exogenous oestrogen exposure (e.g. oral contraceptive use) or increased endogenous oestrogen levels (e.g. pregnancy). If indeed oestrogen is needed for cervical carcinogenesis, close follow-up of young women and of their early pregnancies may be relevant to further understanding the role of steroids in the acquisition and persistence of HPV infections.

The influence of oestrogens on immune response may offer another explanatory effect (Arbeit *et al*, 1996; Mitrani-Rosenbaum *et al*, 1989), particularly during the follicular phase of the ovarian cycle and pregnancy, when levels of estrogens are increased up to 3-8 fold normal levels (Duncan *et al*, 1994; Jabbour *et al*, 2006; Marzi *et al*, 1996). Immune response against early HPV16 E2 and E6 oncoproteins has been shown to be severely impaired or be absent in patients with cervical cancer (de Jong *et al*, 2004).The higher density of oestrogen receptors and their expression in the transformation zone may synergize with the effects of HPV oncoproteins, decreasing levels of cytotoxic cytokines that may down-regulate the cervical cell-mediated immune response, which favour persistent HPV infections instead of clearance (de Jong *et al*, 2004; Giannini *et al*, 1998; Giannini *et al*, 2002; Jacobs *et al*, 2003; Marzi *et al*, 1996). During pregnancy, oestrogens appear to suppress Th1 (T helper 1) cell-mediated immune responses and stimulate Th2 humoral immune response that may contribute to the increase rate of HPV proliferation and the decrease rate of apoptosis (Marzi *et al*, 1996). It has been suggested that development of squamous intraepithelial lesions is associated with decreased levels of cytotoxic cytokines that may down-regulate the cervical cell-mediated immune response during pregnancy, favouring persistent HPV infections instead of clearance (Giannini *et al*, 1998). Additional research is needed to further understand the

interaction between oestrogens and the regulation of immunomodulators, which may contribute to anti-tumour immunity.

The varying results between the studies concerning the role of AFSI and AFP may reflect the true differences between the study populations in relation to sexual and reproductive behaviours. In our study populations, the similar increased risks shown for AFSI and AFP may reflect, in general, the behaviour patterns of most developing countries where women initiate both sex and childbearing at an early age and experience high parity, making the effects of these two events difficult to distinguish from one another. In contrast, studies in more developed countries where there is a longer latency period between sexual initiation and AFP, like in Spain, the United States or in Italy (Brinton *et al*, 1987; Parazzini *et al*, 1989), the results tend to show an increased risk with early AFSI but not with AFP as first pregnancies tend to occur much later. Interestingly, in countries like the UK, where the rates of teenage pregnancies are high, women with AFSI of ≤17 years had a 2-3 fold increased risk for cervical cancer compared to those with AFSI ≥20 years. Consistently, women with an early AFP 15-19 years had a 2-fold increased risk for cervical cancer compared to those with AFP ≥25 years (Green *et al*, 2003). These observations merit further exploration but in aggregate, tend to indicate a significant increase in risk of neoplastic transformation and progression when early sexual initiation occurs (surrogate of early HPV exposure and a period of increased cervical susceptibility) and is followed closely by an early pregnancy (surrogate of early exposure to high oestrogen levels).

3.4.1 Sexual behaviour

Irrespective of their lifetime number of sexual partners, women have a similar increased risk of ICC with early AFSI as shown by the 2.4-fold risk among monogamous women with AFSI ≤16 years as compared to the 2.2-fold risk among women with >1 lifetime number of sexual partners. These results support that among lifetime monogamous women, their risk may be more dependent on the sexual history of their male partner in addition to their own behaviour. This is particularly relevant in conservative societies where most women are virgins at marriage and monogamous thereafter, where the incidence of cervical cancer for a population may vary depending on the behaviour of the male partner. Seventy-

percent of the women in our study were monogamous. As summarized earlier (**Chapter 2**), several studies among monogamous women, the risk of cervical cancer was reported to be two to eight times for women with husbands who had multiple partners (Brinton *et al*, 1989; Buckley *et al*, 1981; Pridan & Lilienfeld, 1971).

3.4.2 Male role factors

The sexual history of the male partner was not evaluated in this analysis, however, promiscuity, history of other STIS, and lack of male circumcison are factors that have been associated with the male role in cervical carcinogenesis (Castellsague *et al*, 2003). The full evaluation of the male role is presented in *Study 3* (**Chapter 5**). However, the results presented in Figure 3.2 and Table 3.4 contribute to a different angle to this interesting association and clearly show that women with an early sexual debut tend to have partners who are older and this age gap difference was an independent predictor of risk for ICC. In our study, the median age of the male partner among women with AFSI ≤16 was 26 years, whereas the median age was 23 for women with AFSI 17-20 and 22 for women with AFSI ≥21. When analysed by country, the age difference was particularly relevant in Brazil, where the age gap was the widest in this analysis (data not shown). Age of the first partner is likely to be another descriptor to the male role in cervical carcinogenesis in addition to the already established factors (Castellsague *et al*, 2003). The age gap between partners at the time of first sexual intercourse may act as a surrogate estimate of the probability of HPV exposure. The variability of the age-specific HPV prevalence among men has not been adequately described. In some high-risk countries, it has been shown to be consistently high (51-61%) across all age groups (18-70 years) (Giuliano *et al*, 2008), whereas in other countries, it appears to follow a similar age-related profile as is consistently observed in women (de Sanjose *et al*, 2007). It thus seems that these associations are highly population-dependent and generalisation of these observations should be done with caution.

3.4.3 Study caveats

In the interpretation of our results, we cannot emphasize the difficulty in fully disentangling a woman's sexual and reproductive health profile and her cancer risk (Schroder *et al*, 2003). We cannot exclude the possibility of

misclassification bias if AFSI and the number of sexual partners were inaccurately reported, leading to some residual confounding, However, the presence of established risk factors for ICC, use of oral contraceptives, smoking, and Pap smear history did not appear to significantly affect the strength of the association we found between AFSI, AFP, and risk of ICC.

3.4.4 Different HPV adjustment methodologies

We examined the different stratified methodologies (unadjusted, HPV-adjusted, and HPV-positive restricted) used to evaluate the association of AFSI and risk of ICC traditionally employed in the literature. This was done to exclude any spurious association related to statistical adjustment and to clarify inconsistent findings of the association found in previous studies, Although in strict terms, restriction of analyses to HPV positive cases and controls seemed preferable, the consistency of the results across the three different methods provide convincing evidence of the risk associated with AFSI. The magnitude of the association was shown to be a 2-fold risk of ICC among women with AFSI ≤16 years. Furthermore, these results indicate that for the evaluation of other risk factors, adjusting for HPV status is not necessary as the adjustments do not contribute to remove any confounding effect.

3.4.5 Social implications

Sexual practices in the world indicate that very early intercourse might be occurring in adolescents with 44%, 45% and 52% of girls between the ages of 13-19 years reporting being sexually experienced in Argentina, Botswana and Nigeria, respectively (Brown *et al*, 2001). In a number of case studies among young females, first sexual intercourse has been reported as forced in 5% to 15% of cases, and in some extreme cases worldwide, the estimates range from 21% among out-of-school adolescents in Botswana, 20% among secondary schools in Peru, and 41% among young urban females attending night schools in Peru. Among 15-30% of sexually active girls between 15-19 years report forced first sexual intercourse (Brown *et al*, 2001). Previous studies assessing the risk for STIs after coercive sexual intercourse found that sexually abused children to be at risk for Chlamydia, gonorrhoea (Beck-Sague & Solomon, 1999), HIV (Lindegren *et al*, 1998) and HPV (Gutman *et al*, 1992; Kahn *et al*, 2005; Siegfried *et al*, 1998). Sexual abuse is unfortunately common also in developed countries (Bechtel,

2010) and has been shown to be a predictor of HR-HPV infection (Wingood *et al*, 2009). It is likely that the partners of these adolescents who report sexual coercion are adult males who are sexually experienced (Wellings *et al*, 2006) and at high-risk to HPV exposure. Globally, these exposures might affect a high proportion of very young girls in areas of human strife, and thus, adding to the burden of child sexual abuse above the burden of a lifetime increased risk of genital cancer.

3.5 Conclusions

This study shows that women who initiate first sexual intercourse and experience their first pregnancy at a young age are at an increased risk of cervical cancer. The implications of this study are particularly important for populations, where age at first sexual intercourse and pregnancy occur early. The importance of HPV vaccination programmes targeting young adolescents prior to first sexual intercourse can have great impact in decreasing the incidence of cervical cancer. Cervical cancer prevention measures require additional efforts in the fields of family planning and sexual education adapted to the extremely variable sociocultural contexts in the world.

4 Study 2. Sexual and reproductive health factors and risk of HPV persistence in the Guanacaste Cohort

4.1 Introduction

Persistent infection with high-risk oncogenic human papillomavirus (HPV) types is strongly linked to cervical pre-cancer and progression to invasive cervical cancer (Moscicki *et al*, 2006). Although infection with HPV is necessary for the development of cervical cancer, infection alone is not sufficient. Long-term oral contraceptive use (≥5 years), smoking, and high parity are well-established cofactors for the carcinogenic process to invasive cervical cancer (Munoz *et al*, 2006). Few studies have tried to distinguish the risk factors for viral persistence from neoplastic progression. The two stages of the cervical carcinogenic process are not identical and need to be separated. Long-term persistence without progression to cervical neoplasia is believed to be uncommon. The intermediate stage, HPV persistence, between HPV acquisition and cervical neoplasia is not well understood.

HPV persistence is the most important stage in cervical carcinogenesis as it is a precursor to progressing to relevant clinical lesions. Longitudinal studies indicate that the majority of HPV infections are transient with the majority of women clearing infection within two years, and only a minority of women will harbour persistent HPV infection to develop cervical pre-cancer (Moscicki *et al*, 2006). Inconclusive associations with HPV persistence have been found for oral contraceptive use (Brisson *et al*, 1996; Castle *et al*, 2002b; Richardson *et al*, 2005) and smoking (Giuliano *et al*, 2002; Ho *et al*, 1998; Richardson *et al*, 2005; Silins *et al*, 2005). Parity as a cofactor of HPV persistence has also been studied but mostly among study populations in developed countries where high parity is less prevalent (Brisson *et al*, 1996; Castle *et al*, 2002b; Giuliano *et al*, 2002; Richardson *et al*, 2005).

Sexual and reproductive health (SRH) behaviour factors, such as early age at first sexual intercourse (AFSI) and first pregnancy (AFP), have been associated with invasive cervical cancer as described in *Study 1* (**Chapter 3**). However, it is unclear whether AFSI and AFP continue to play a relevant role throughout the

different stages of the natural history of HPV and cervical cancer, specifically HPV persistence. The objective of this study was to evaluate the role of AFSI and AFP on 2-year persistence of prevalent HR-HPV infections and to confirm the role of these established factors for cervical carcinogenesis in a longitudinal cohort.

4.2 Methods

4.2.1 Study population

This study was conducted in Guanacaste (Figure 4.1), a rural province in Costa Rica with 240,000 inhabitants in 1993, which represented 8% of the country's population, of whom 58% were at least 18 years old. Detailed study design and methods of the Guanacaste Cohort have been reported (Bratti *et al*, 2004; Herrero *et al*, 1997). The main objective of the population-based cohort was to study the natural history of HPV infection and cervical neoplasia in an area with a high incidence of cervical cancer (age-standardized incidence rate of 33 per 100,000 women). Incidence in this region was higher than the national average in Costa Rica and four- to five-fold higher than that found in the United States (Herrero *et al*, 1997), highlighting the disparities between a less developed country compared to a developed country. Despite having a national health care system that provided cervical cancer screening services, coverage was low and mainly only women in the target population were screened if they were attending family planning and prenatal clinics. In addition, the lack of high quality cytology laboratories and adequate follow-up, referrals and treatment of women with cervical abnormalities may partially explain the sustained high rates of cervical cancer in Guanacaste throughout the decade prior to study commencement.

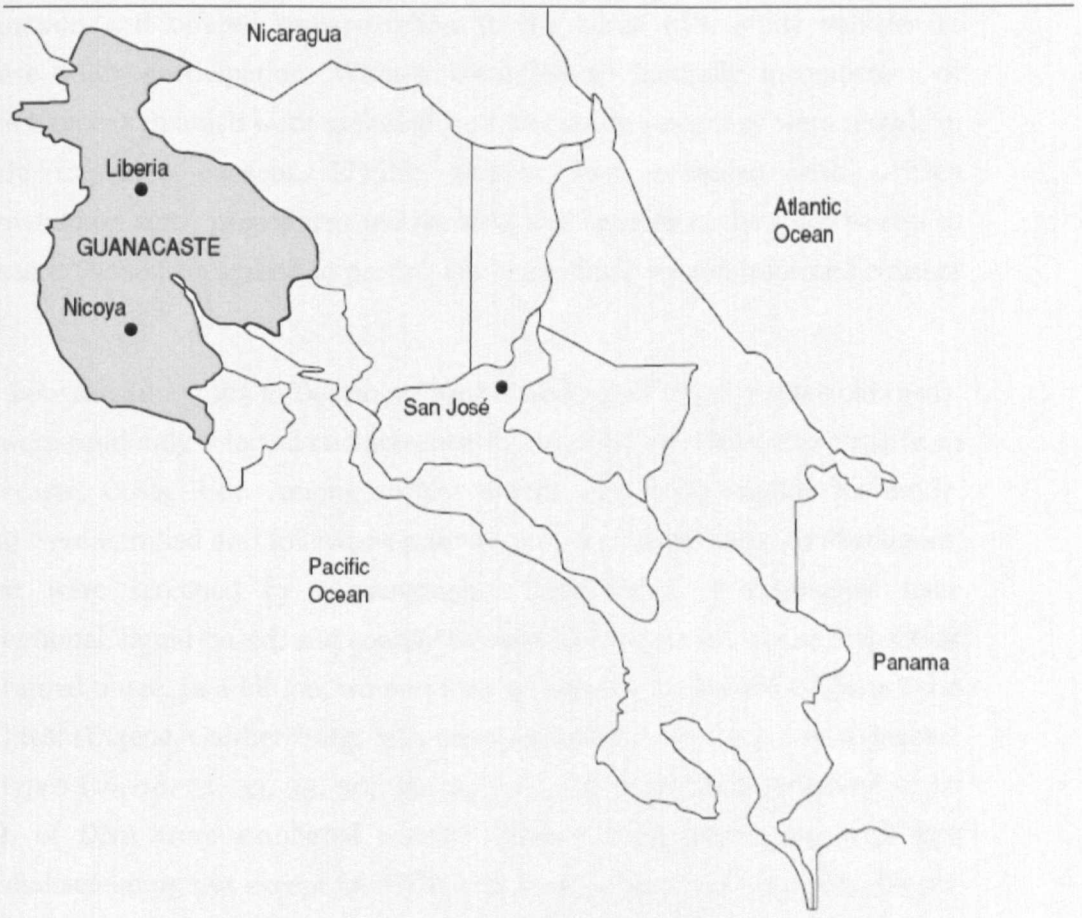


Figure 4.1 Map of Costa Rica. The study was carried out in the northwest province of Guanacaste with two field offices in Liberia and Nicoya, where two regional hospitals were located. Central headquarters of the Guanacaste natural history cohort study was in San José, the capital of Costa Rica. Reproduced from (Herrero *et al*, 1997)

Prior to the start of the study, population enumeration was conducted between February and March 1993 by visiting every house in a random selection of 178 of 1038 censal segments in Guanacaste, which would provide the target sample size of 10,000 women (Bratti *et al*, 2004; Herrero *et al*, 1997). Population characteristics (age group, province of birth, nationality, social security affiliation, province of residence in the previous 5 years, educational level obtained, marital status, employment, and number of live children) of the study censal segments were comparable to the census. Women aged 18 years or older by 1 July 1993 were invited by mail or personal visits were made to invite women to participate in the study. Women were given appointments to attend the closest government clinic to participate in the study which included cervical screening. Those who did not attend their first appointment were visited at home, provided with more

information and offered transportation to the clinic with study vehicles to increase study participation. Women identified as mentally incompetent or unable to speak Spanish were excluded from the study since they were unable to provide informed consent. Eligible women were provided with written information on study procedures and the risks and benefits of the study were also discussed. Those who agreed to participate in the study signed informed consent forms.

Between June 1993 to December 1994 women aged 18 to 97 years old (n=11, 742) were randomly selected and screened in June 1993 to December 1994 from Guanacaste, Costa Rica. Among 10,769 women who were eligible for study, 10,049 were enrolled and followed-up for seven years up to 2001. At enrolment, women were screened by cervicography, three types of cytological tests (conventional, liquid-based, and computer-assisted Papnet) and visual inspection by a trained nurse. In addition, women were screened with Hybrid Capture Tube (HCT) test (Digene, Gaithersburg, MD, now Qiagen) which detected 11 oncogenic HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, and 58) at a threshold of 10 pg/mL of DNA from exfoliated cervical cells. Women presenting with any abnormal screening test except for HCT were referred for colposcopy and biopsy of the worse appearing lesion and were censored from study follow-up.

Diagnosis at enrolment categorized the study participants into two groups for follow-up based on their perceived risk for developing cervical intraepithelial neoplasia grade ≥2 (CIN ≥2): active or passive follow-up (Figure 4.2). Women with cytological evidence of LSIL or histological diagnosis of CIN-1 were followed-up every 6 months (n=492). Annual follow-up was done for women who had ASCUS or Cervigrams suggestive of minor lesions (n=980), positive for HCT (n=303), or reported having had five or more sexual partners (n=344). A random sample of women with normal cytology (n=537) and 410 virgins who were ≤26 years at enrolment were also followed-up annually. The remaining group of women with normal cytology were assigned to a passive cohort and were referred to routine screening. However, at five to six years after enrolment, a random sample of this passive cohort was invited for follow-up screening.

The active follow-up group returned for screening every 12 months. At any point during follow-up, if a woman presented with LSIL, she was switched to a

more intense 6-months screening interval. In addition, if at any point during follow-up, a woman presented with CIN-2+, she was censored from the study and referred for appropriate follow-up. The passive cohort were screened at 5-7 years and if they presented with any cytological abnormalities, they would also switch to a more intense 6-months screening interval until they completed 7 years of follow-up. To ensure their safety, those women who continued to have cytological abnormalities at the end of study were followed outside the study.

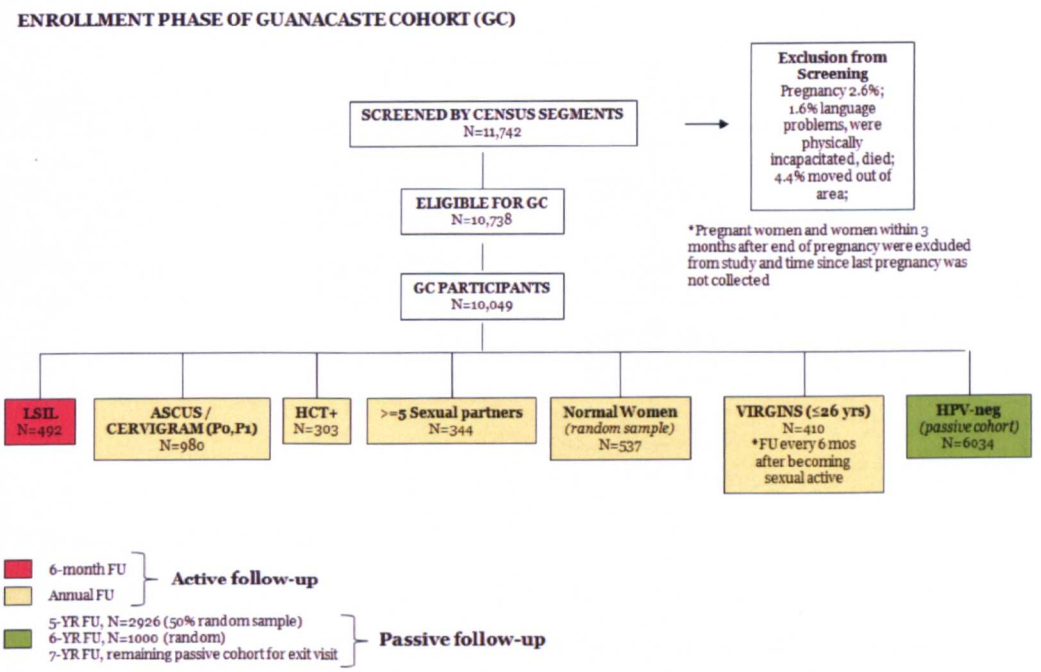


Figure 4.2 Enrolment phase of the Guanacaste Cohort (GC). The active follow-up cohort was followed-up at either 6 months or annually which included women with low-grade intraepithelial lesions (LSIL)/cervical intraepithelial neoplasia grade 1 (CIN-1), ASCUS or Cervigram positive (Po or P1), Hybrid-Capture Tube (HCT) positive, women with >5 sexual partners, a random sample of women with normal cytology, and virgins (≤26 years). The passive follow-up cohort was women with normal cytology and HCT-negative who were followed up at Year 5, 6, or 7 of the study.

4.2.1.1 Selected *Study 2* sub-population

For the purposes of this study objective, a sub-population was further selected for analysis (Figure 4.3). At enrolment, 955 women (with hysterectomy, histologically confirmed cervical cancer, and CIN2+) were censored from follow-up. Women with no follow-up exam (i.e. virgins at enrolment who did not become sexually active during the study) or did not return for at least one follow-up visit were excluded (n=1327). In addition, women with no adequate baseline (except for virgins) or at least one adequate follow-up HPV DNA PCR result were

excluded (n=143). 7624 women (n=2785, active cohort and n=4839, passive cohort) would be available for follow-up analysis. For this thesis, we will focus on the active cohort who was sexually active at time of study enrolment. Therefore, virgins were further excluded (n=248). Of the n=2537 sexually active women, 613 (24.2%) were infected with prevalent HR-HPV at time of enrolment.

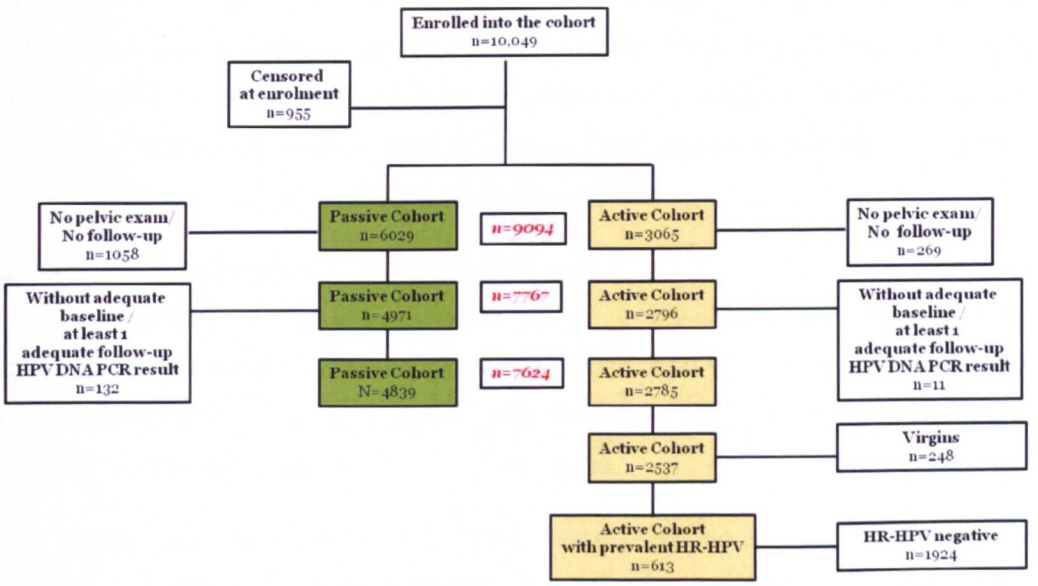


Figure 4.3 Consort diagram

4.2.2 Ethical approval

Ethical approval for this study was obtained from the Institutional Review Boards of Costa Rica and the United States (US) National Cancer Institute (NCI) (Appendix 8). *Study 2* planned secondary data analyses were approved by the US Principal Study Investigator, Mark Schiffman, MD MPH. A letter of data transfer agreement was submitted to the NCI for study approval on 9 February 2010 and data was transferred to K. Louie, the investigator of this project on 1 July 2010 (Appendix 9). Additional ethical approval was also granted by LSHTM for this PhD project (Appendix 7).

4.2.3 Visit procedures

4.2.3.1 Study questionnaire

At enrolment, a standardised detailed questionnaire on demographics (*age*), sexual and reproductive health history (*age at first menarche, age at first sexual intercourse, lifetime number of sexual partners, history of pregnancy, age at*

first pregnancy, number of pregnancies and live births, history of stillbirths, miscarriages, tubal or ectopic pregnancies, or caesarean sections), medical history (*history of sexually transmitted infections (STIs)*) were collected by trained interviewers (Bratti *et al*, 2004; Herrero *et al*, 1997). At subsequent follow-up visits, a shortened version of the enrolment questionnaire was administered to capture the risk factor history since the previous visit. All interviews were coded and double-entered with the Keyentry III Program at the central office in San José. Databases were sent electronically to WESTAT Inc. in Rockville, Maryland for data quality checks. Hard copies of the questionnaires were reviewed if there were discrepancies to resolve.

4.2.3.2 Pelvic examination and cytological screening

All sexually active women were asked at enrolment and follow-up to undergo pelvic examination for cytological evaluation (by conventional, liquid-based (ThinPrep; Cytyc Corporation, Marlborough, MA), or computer-assisted PapNet) and visual inspection by a trained nurse.

Specifically, exfoliated cervical cells were collected using a Cervex brush (Unimar, Conecticut) that was rotated five times in the ecto- and endocervical area to prepare the conventional smear. After preparation of the smear, the Cervex brush was swirled in 20 mL of methanol-based PreservCyt solution. This solution was refrigerated and kept until it was sent to the US to prepare the liquid-based Pap smear. In addition, a Dacron swab over the ectocervix and rotated in the endocervical canal was used to collect cervical cells for HPV DNA testing. The swabs were stored in Virapap DNA transport medium (Digene, Gaithersburg, MD, now Qiagen) tubes and were frozen at -30°C at the regional offices on the day of sample collection, and then transported weekly to the central office in San José to be stored at -70°C. Periodic batch shipments of the specimens on dry ice were made to the NCI repository. After these cytological specimens were collected, the cervix was rinsed with 5% acetic acid and two photographic images of the cervix (Cervigrams) were taken. The undeveloped film was sent to US National Testing Laboratories in Fenton, Missouri for evaluation.

Conventional slides were stained and interpreted by local cytotechnologists and cytopathologists in Costa Rica. After smear interpretation, slides were sent to Neuromedical Systems in New York for computerised screening and preparation

of PapNet videos and interpretation were done at Johns Hopkins Hospital in Baltimore, Maryland. The PreservCyt samples were sent weekly to Tufts University in Boston, Massachusetts for ThinPrep smear preparation and interpretation. All three cytology results were reported and classified with the Bethesda System (National Cancer Institute Workshop, 1989). Cytological diagnosis was reported as normal; normal with reactive changes; atypical squamous cells of unknown significance (ASCUS); low-grade intraepithelial neoplasia (LSIL); high-grade squamous intraepithelial neoplasia (HSIL); and further classified as cervical intraepithelial neoplasia grade 2 or 3 (CIN-2 or CIN-3); squamous carcinoma; and adenocarcinoma. Cervigrams were categorised as negative (no lesion found); atypical (lesion found but colposcopy was not recommended based on the site/morphology); positive (colposcopy recommended); and technically defective (not positive and inadequate evaluation). Positive cervigrams were further classified as P0 (normal), P1-P1A (low-risk disease but colposcopy recommended as lesion extended into the canal), P2 (compatible with CIN-2 or CIN-3), or P3 (compatible with cancer).

4.2.3.3 Colposcopy and treatment

Women were referred for colposcopy or biopsy if they had abnormal results upon Pap smear (ASCUS or worse) or positive for Cervigram (P0+). Any women with HSIL or worse with any screening test were censored from the study and referred to Social Security health services for further local clinical management. Women who presented with cancer were referred for appropriate treatment (i.e. hysterectomy or radiotherapy) as needed. At any point during follow-up, women presenting with low-grade intraepithelial neoplasia (LSIL) or histological abnormalities were more intensely screened at 6 months rather than 12 months.

Histology diagnosis was reviewed locally in Costa Rica. At the end of study follow-up, all slides were reviewed by one of two US pathologists who were blinded to all other study data. A final diagnosis was given by the majority diagnoses and if there was disagreement, a joint review was held in the US to reach a consensus.

4.2.3.4 HPV DNA testing

Initial HPV DNA testing was done with HCT as described earlier. However, since HCT only detected 11 carcinogenic HPV types, specimens were retested with polymerase chain reaction (PCR) test at Albert Einstein College of Medicine in New York. Detailed descriptions of the PCR assays have been previously described (Castle *et al*, 2002a; Herrero *et al*, 2005). DNA was extracted from exfoliated cells and HPV DNA was detected by PCR amplification of the *L1* gene using My09/MY11 primer system with AmpliTaq Gold polymerase (TaqGold; Perkin-Elmer-Cetus, Norwalk, CT). HPV DNA in PCR products were analysed using type-specific probes for the following types: HPV 2, 6, 11, 13, 16, 18, 26, 31-35, 39, 40, 42-45, 51-59, 61, 62, 64, 66-74, 81-85, and 89. Thirteen HPV types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68 were considered carcinogenic (Bouvard *et al*, 2009; IARC, 2007; Munoz *et al*, 2003).

4.2.4 Field experience

In July 2011, a field visit was made to the Projyecto Epidemiológico Guanacaste (PEG) Centre in Liberia, Costa Rica. I worked with the Local Investigator Dr. Ana Cecilia Rodriguez on identifying the appropriate sub-group from this analysis as well as data cleaning of the longitudinal data.

Although the natural history cohort study ended in 2001, the regional office in Liberia continues to be the central Centre for coordinating the long-term follow-up of women immunised in the Phase III Clinical Trial of the bivalent HPV vaccine against HPV 16 and 18 (Herrero *et al*, 2008) and carry out other cancer-related research in Guanacaste. For these reasons, much of the complex organisational infrastructure established during the natural history study has been maintained. Briefly, about 100 staff members currently operate PEG and were also required to follow-up 10,000 women in the natural history study. There were three field teams for data collection, operating out of one of the Liberia, Nicoya or San Jose field offices. A PEG Investigator, Siliva Jimenez gave me a tour of the study clinic in Liberia. Each study clinic is staffed with a field supervisor, a registered nurse, a nurse's aide; three or more interviewers, a driver and an office manager. To motivate participation, all the nurses, nurse's aides and interviewers were women. Each office is equipped with a four-wheel drive vehicle, a -30°C freezer for specimen storage, a computer, and a -20°C freezer for storage

of ice packs. At the time of the study, there was also a mobile colposcopy clinic equipped with a Zeiss 2000 colposcope and computer image system that was run by a colposcopist, a nurse's aide and two interviewers. Each day, the field teams would visit the selected censal segments of Guanacaste to recruit and enrol women into the study and/or transport participants to their clinic visits. Typically, field teams would return to the Liberia central office each day to turnover data (i.e. questionnaire data and biological specimens) collected to other teams for data entry, sample processing, storage and analysis.

4.2.5 Statistical analysis

4.2.5.1 Definition of prevalent infections and HPV persistence

Prevalent infection was defined as the infection that was detected at baseline enrolment. Persistence of HPV infection was defined as detection of the same HPV type at 1-year and 2-years.

4.2.5.2 Classifying follow-up time

In order to standardise time for each prevalent infection, each clinic visit was assigned three time-bins (0 (baseline), 1, and 2 years). For baseline prevalent infections, time-bin was 0.

If a type-specific HPV infection was tested more than once in the same time-bin because she had a more intense 6-month follow-up because of cytological abnormalities, and the HPV DNA result was discordant, the overall result was assumed to be positive to assume the possibility of measurement error. In addition, when classifying persistent infection, if a woman missed a study visit in time-bin 1 but attended the following study visit in time-bin 2 and her prevalent HPV infection from baseline was still positive, then we assumed that she was persistent at time-bin 1 and time-bin 2. A total of 226 type-specific changes were made for the 2-year study period.

Similarly for the risk factor data, if the woman responded to two questionnaires in the same time-bin, the overall result was assumed to be the "worse" response. For example, for the question, "have you had a history of an STI since the last study visit?" the discordant response was assumed to be yes.

4.2.5.3 Descriptive analyses

First, each continuous potential risk factor (e.g. age at first sexual intercourse, lifetime number of sexual partners, number of pregnancies, etc.), was converted into quantile groups where possible, otherwise into tertile groups. Then the proportion of women with HPV infections and HR-HPV infections was calculated as well as the number of infections in each category.

4.2.5.4 Generalised estimating equation models

The associations of SRH factors (independent variables) were evaluated for persistence at 1-year and 2-years time-points (dependent variables). Persistence was defined as a binary outcome (yes and no). Robust variance models were constructed using logistic generalised estimating equation (GEE) with an independent correlation structure to evaluate whether baseline SRH factors was a predictor of HPV persistence at 1 year and 2 years (Liang & Zeger, 1986). Age-adjusted odds ratios (OR) and 95% confidence intervals (CI) between persistent HPV at visit (t) and persistence at visit (t-1) were calculated to measure the magnitude of the longitudinal associations.

The individual history of each HR-HPV infection was considered separately for HPV alpha species 7 (Appendix 10.1), 9 (Appendix 10.2), and alpha species 5 and 6 (Appendix 10.3) so a woman could contribute more than one HPV-type if she was co-infected with multiple types. Persistence of each HR-HPV infection was considered to be independent of other co-infected types since previous analyses have shown no interaction between types when a woman was infected with multiple types (Plummer *et al*, 2007; Rodriguez *et al*, 2010; Schiffman *et al*, 2005). However, since the sample size of individual HR-HPV infections was small this precluded an analysis at the type-specific level that would provide informative results.

To assess the association of selected SRH factors and risk of HPV persistence, the following three statistical models were computed and compared for prevalently detected infections:

(i) persistence of all pooled 13 HR-HPV infections (HPV-16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68);

(ii) persistence of pooled HPV-16/18 infections (the two most common types found in invasive cervical cancer and found to have the greatest risk for progression to invasive cervical cancer); and

(iii) persistence of other HR-HPV infections (non-HPV-16/18)

4.3 Results

Of the 2785 women in the active cohort available for analyses, 42.1% (n=1398) were positive for any HPV, 24.2% (n=613) were positive for HR-HPV at enrolment. These women represented 805 prevalent HR-HPV infections. A quarter of women were co-infected with more than one HPV-type (Figure 4.4)

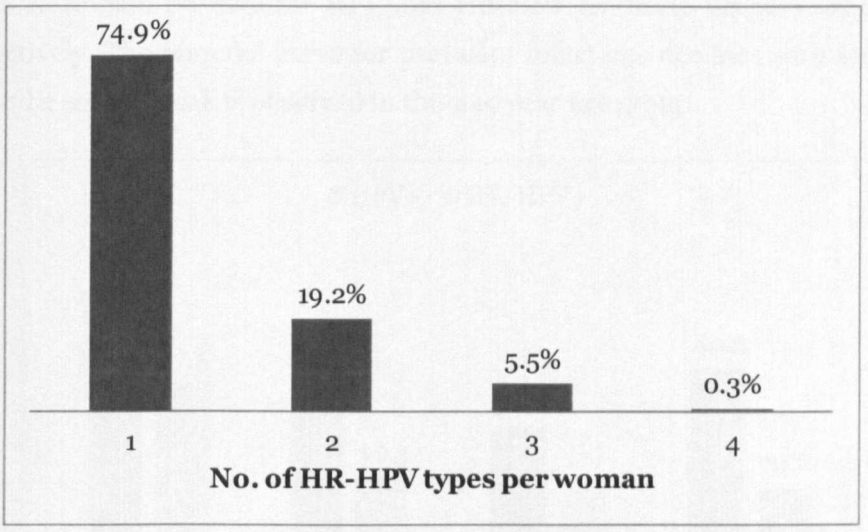


Figure 4.4 Proportion (%) of 613 women infected with prevalent single or multiple high-risk human papillomavirus (HR-HPV) types

4.3.1 Age-specific distribution

Figure 4.5 shows the age-specific distribution of women with prevalent HR-HPV infections according to positivity for any HPV and HR-HPV. The age-specific distribution of women with prevalent infections presents a bimodal curve. The curve is skewed to the left with a peak HR-HPV prevalence of 56.2% and 37.5% of women positive for HPV and HR-HPV in the 18-28 years age group, respectively. The bimodal curve for prevalent infections declines with increasing age and a second peak is observed in the ≥49 year age group.

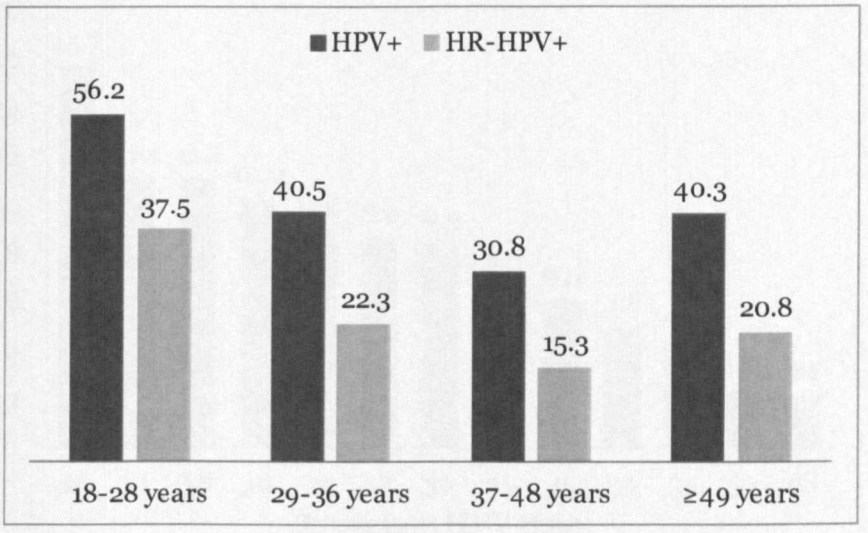


Figure 4.5 Age-specific distribution (%) of women with prevalent human papillomavirus (HPV) and high-risk human papillomavirus (HR-HPV) infections.

4.3.2 Type distribution of prevalent HR-HPV infections

Figure 4.6 shows the type-distribution of 805 prevalent HR-HPV types identified at baseline enrolment. The most prevalent type was HPV-16 (15.7%), followed by HPV-51, 58, 52, and 31. HPV-18 ranked as the sixth most prevalent HR-HPV type in this population.

HPV-16/18 represented 24.2% of the prevalent HR-HPV types and 75.8% of the types were other HR-HPV (not HPV-16/18).

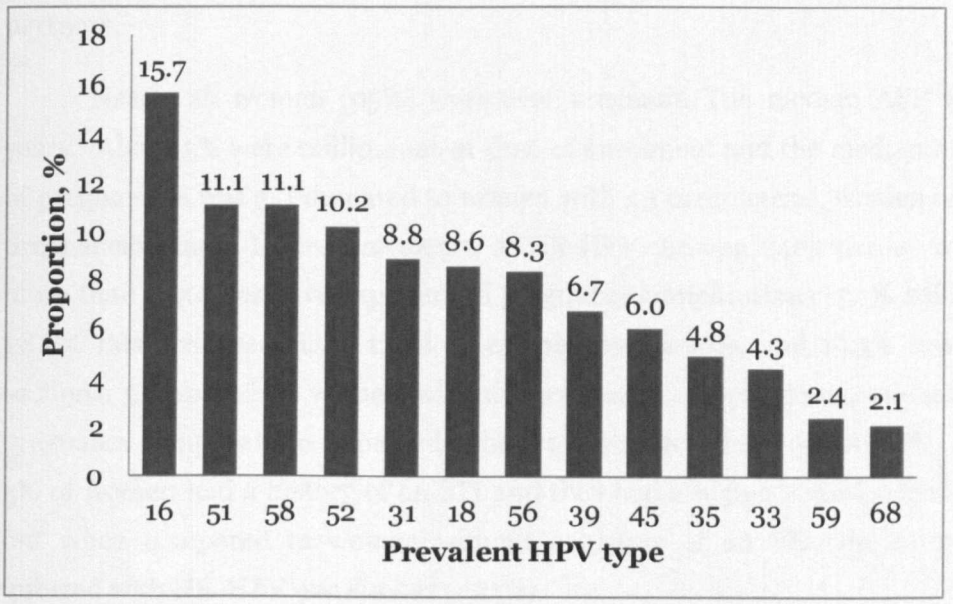


Figure 4.6 Type-distribution of 805 prevalent high-risk human papillomavirus (HR-HPV) types

4.3.3 Sexual and reproductive health characteristics at baseline

Table 4.1 shows SRH characteristics of 2537 sexually active women in the active cohort by HPV detection status at enrolment. Median age at first menarche and AFSI was 13 years and 17 years, respectively. Of the sexually active women, about 2% first had sex before starting menarche. Compared to women with AFSI ≥21 years, women AFSI ≤20 had a higher prevalence of HR-HPV (19% vs. 25%, respectively). The median number of lifetime number of sexually partners was two and they had higher prevalence of HR-HPV than those with one and ≥3 partners.

Nearly all women (95%) were ever pregnant. The median AFP was 19 years. About 5% were nulliparous at time of enrolment and the median number of pregnancies was 3. Compared to women with ≤3 pregnancies, women with >3 pregnancies had a higher prevalence of HR-HPV. Among 2405 parous women, more than a quarter have experienced pregnancy complications: 7.7% stillbirths, 28.8% miscarriages, 16.9% tubal or ectopic pregnancies, and 15.3% caesarean sections. Compared to women with no pregnancy complications, women with pregnancy complications appeared to have a lower prevalence of HR-HPV. About 5% of women had a history of an STI and they had a higher prevalence of HPV, but when compared to women without a history of an STI, the proportion infected with HR-HPV was similar (~25%).

Table 4.1. Select sexual and reproductive health characteristics of sexually active women (n=2537) in the Guanacaste cohort according to human papillomavirus (HPV) status at enrolment

Subject characteristics	Total no. of women	Total no. of HPV+ women	%	Total no. of HR-HPV+ women	%
Age at enrolment					
18-28 years	674	379	56.2	253	37.5
29-36 years	615	249	40.5	137	22.3
37-48 years	662	204	30.8	101	15.3
≥49 years	586	236	40.3	122	20.8
Age at first menarche		1068		613	
≥15 years	506	202	39.9	114	22.5
14 years	480	188	39.2	97	20.2
13 years	620	266	42.9	151	24.4
≤12 years	929	410	44.1	250	26.9
Age at first sexual intercourse					
≥21 years	407	141	34.6	80	19.7
18-20 years	687	306	44.5	171	24.9
16-17 years	719	317	44.1	179	24.9
≤15 years	724	304	42.0	183	25.3
Time of first sexual intercourse from first menarche*					
>0 years	2333	980	42.0	564	24.2
0 years	151	65	43.0	35	23.2
<0 years	53	23	43.4	14	26.4
Lifetime number of sexual partners*					
1	1110	396	35.7	229	20.6
2	504	248	49.2	153	30.4
≥3	923	424	45.9	231	25.0
Pregnancy‡					
Never	132	77	58.3	53	40.2
Ever	2405	991	41.2	560	23.3
Age at first pregnancy‡					
≥21 years	722	278	38.5	148	20.5
18-20 years	850	366	43.1	213	25.1
16-17 years	558	227	40.7	137	24.6
≤15 years	274	120	43.8	62	22.6
Missing					
Number of pregnancies‡					
0	132	77	58.3	53	40.2
1-3	1156	525	45.4	315	12.8
4-5	498	180	36.1	97	19.5
≥7	751	286	38.1	148	19.7
Live Births					
0	158	91	57.6	54	34.2
1-2	1283	566	44.1	346	27.0
3-5	458	169	36.9	85	18.6
≥6	638	242	37.9	128	20.1

No. of stillbirths					
Never	2221	911	41.0	523	23.5
Ever	184	80	43.5	37	20.1
No. of miscarriages					
0	1712	708	41.4	422	24.6
1	437	181	41.4	87	19.9
2+	256	102	39.8	51	19.9
No. of tubal or ectopic pregnancies					
Never	2129	932	43.8	537	25.2
Ever	407	136	33.4	76	18.7
Caesarean section					
Never	2018	842	41.7	488	24.2
Ever	367	138	37.6	71	19.3
History of STI*					
No	2419	1011	41.8	582	24.1
Yes	117	56	47.9	30	25.6

*HR-HPV, high-risk human papillomavirus infection; STI, sexually transmitted infection

4.3.4 Risk of 2-year follow-up persistence of prevalent HR-HPV infections according to patient characteristics

Table 4.2 shows the risk of 2-year persistence of prevalent HR-HPV infections according to patient characteristics. For the specific SRH factors of interest, there was no statistically significant association with AFSI and 2-year persistence; whereas, there was an increasing risk of 1-year and 2-year persistence with decreasing AFP among women infected with prevalent HPV-16/18. Compared to women with AFP ≥21 years, the odds ratio of prevalent HPV 16/18 persistence at 2-years was 1.32 (95% CI: 0.65-2.69) for AFP 18-20 years, 1.54 (95% CI: 0.70-3.38) for AFP 16-17 years, and 2.03 (95% CI: 0.76-5.43) for AFP ≤15 years.

As compared to women with one lifetime sexual partner, women with two lifetime sexual partners had a nearly 3-fold increase risk of 2-year persistence (OR=2.96; 95% CI: 1.26-6.97).

Women positive for any HR-HPV and ever had a history of one miscarriage was 1.8 times likely to have 1-year persistence but this association ceased at 2-years. Interestingly, an inverse association with 2-year persistence was found among women positive with any HR-HPV with a history of tubal or ectopic pregnancy (OR=0.54; 95% CI: 0.33-0.89). This association was more pronounced among those infected with other HR-HPV (non-HPV-16/18) (OR=0.49; 95% CI: 0.26-0.92).

Table 4.2 Risk of 2-year follow-up persistence of prevalent high-risk human papillomavirus (HR-HPV) infection according to patient characteristics

	HR-HPV					HPV-16/18					Other HR-HPV (not HPV-16/18)				
Subject characteristics	No. of prevalent HR-HPV infections at baseline	No. of HR-HPV infections persist/no persist at 1-year	Age-adjusted OR (95% CI)	No. of HR-HPV infections persist/no persist at 2-year	Age-adjusted OR (95% CI)	No. of prevalent HR-HPV infections at baseline	No. of HR-HPV infections persist/no persist at 1-year	Age-adjusted OR (95% CI)	No. of HR-HPV infections persist/no persist at 2-year	Age-adjusted OR (95% CI)	No. of prevalent HR-HPV infections at baseline	No. of HR-HPV infections persist/no persist at 1-year	Age-adjusted OR (95% CI)	No. of HR-HPV infections persist/no persist at 2-year	Age-adjusted OR (95% CI)
Age at first menarche															
15+ years	145	45/66	1.00	28/14	1.00	41	20/19	1.00	14/3	1.00	104	25/47	1.00	14/11	1.00
14 years	127	29/62	0.80 (0.44-1.46)	17/9	0.85 (0.51-1.41)	29	11/14	0.78 (0.28-2.17)	5/4	0.63 (0.26-1.53)	98	18/48	0.85 (0.40-1.82)	12/5	1.03 (0.55-1.96)
13 years	199	40/99	0.71 (0.41-1.22)	16/16	0.66 (0.41-1.06)	45	17/23	0.78 (0.31-1.96)	8/3	0.77 (0.35-1.70)	154	23/76	0.70 (0.35-1.39)	8/13	0.63 (0.35-1.14)
≤12 years	333	89/152	1.14 (0.70-1.86)	33/31	0.99 (0.65-1.51)	80	29/39	0.77 (0.34-1.77)	12/5	0.74 (0.36-1.53)	253	60/113	1.47 (0.79-2.75)	21/26	1.23 (0.73-2.09)
Age at first sexual intercourse															
21+ years	103	28/49	1.00	17/9	1.00	27	9/14	1.00	7/1	1.00	76	19/35	1.00	10/8	1.00
18-20 years	221	58/102	1.09 (0.64-1.94)	25/16	0.98 (0.60-1.60)	53	22/26	1.39 (0.50-3.89)	10/4	1.10 (0.45-2.71)	168	36/76	0.99 (0.49-2.00)	12/12	0.97 (0.53-1.77)
16-17 years	235	51/122	0.84 (0.47-1.51)	24/22	0.75 (0.46-1.23)	53	20/27	1.24 (0.44-3.52)	9/6	0.90 (0.6-2.23)	182	31/95	0.74 (0.36-1.51)	15/16	0.71 (0.39-1.31)
≤15 years	246	66/106	1.19 (0.67-2.11)	28/23	1.01 (0.62-1.63)	62	26/28	1.50 (0.54-4.15)	13/4	1.26 (0.52-3.09)	184	40/78	1.07 (0.53-2.15)	15/19	0.91 (0.50-1.65)
Missing															
Time of first sexual intercourse from first menarche															
<0 yrs	740	182/352	1.00	79/62	1.00	179	68/89	1.00	32/814	1.00	561	114/263	1.00	47/48	1.00
0 yrs	46	16/23	1.09 (0.55-2.17)	11/7	1.08 (0.61-1.92)	14	7/6	-	5/1	-	32	9/17	0.98 (0.41-2.35)	6/6	0.95 (0.47-1.96)
≥1 yrs	19	5/4	1.86 (0.49-7.17)	4/1	2.13 (0.69-6.61)	2	2/0		2/0		17	3/4	1.33 (0.28-6.27)	2/1	1.52 (0.41-5.54)
Lifetime number of sexual partners															
1	294	64/105	1.00	28/24	1.00	72	23/34	1.00	10/6	1.00	222	41/71	1.00	18/18	1.00
2	213	36/78	0.71 (0.43-1.19)	16/9	0.83 (0.53-1.31)	45	19/11	2.68 (1.06-6.77)	8/1	2.96 (1.26-6.97)	168	17/67	0.39 (0.20-0.77)	8/8	0.49 (0.28-0.88)
3+	298	72/132	0.84 (0.55-1.30)	33/26	0.89 (0.61-1.30)	78	25/31	1.17 (0.54-2.52)	14/6	1.22 (0.62-2.41)	220	47/101	0.74 (0.44-1.27)	19/20	0.79 (0.50-1.25)
Pregnancy															
Never	86	8/17	1.00	7/2	1.00	18	3/3	1.00	3/0	1.00	68	5/14	1.00	4/2	1.00
Ever	719	157/283	1.01 (0.42-2.45)	70/57	0.79 (0.38-1.64)	177	60/71	0.87 (0.16-4.59)	29/13	0.59 (0.13-2.62)	542	97/212	0.97 (0.33-2.86)	41/44	0.82 (0.34-1.98)
Age at first pregnancy															
21+ years	213	50/109	1.00	28/14	1.00	52	15/33	1.00	10/1	1.00	161	35/76	1.00	18/13	1.00
18-20 years	295	70/136	1.16 (0.74-1.82)	33/24	1.02 (0.69-1.51)	64	27/31	1.94 (0.86-4.35)	12/7	1.32 (0.65-2.69)	231	43/105	0.93 (0.54-1.61)	21/17	0.90 (0.56-1.45)
16-17 years	193	46/98	1.11 (0.67-1.82)	18/18	0.90 (0.58-1.40)	49	21/21	2.19 (0.90-5.32)	10/4	1.54 (0.70-3.38)	144	25/77	0.81 (0.43-1.51)	8/14	0.67 (0.39-1.15)
≤15 years	76	28/29	2.18 (1.17-4.09)	10/11	1.46 (0.85-2.52)	23	11/8	3.03 (1.00-9.17)	5/2	2.03 (0.76-5.43)	53	17/21	1.84 (0.85-3.98)	5/9	1.21 (0.62-2.36)
Number of pregnancies															
0	86	8/17	1.00	7/2	1.00	18	3/3	1.00	3/0	1.00	68	5/14	1.00	4/2	1.00
1-3	413	72/169	0.91 (0.37-2.22)	31/18	0.79 (0.37-1.65)	101	28/45	0.66 (0.12-3.60)	12/5	0.55 (0.11-2.50)	312	44/124	0.90 (0.30-2.70)	19/13	0.83 (0.34-2.02)
4-5	117	28/46	1.10 (0.40-3.03)	10/11	0.78 (0.33-1.82)	30	13/12	1.30 (0.20-8.30)	6/2	0.90 (0.17-4.78)	87	15/34	0.86 (0.24-3.02)	4/9	0.61 (0.21-1.73)
7+	189	57/68	1.35 (0.51-3.58)	29/28	0.82 (0.37-1.83)	46	19/14	1.34 (0.22-8.28)	11/6	0.60 (0.12-3.02)	143	38/54	1.28 (0.39-4.22)	18/22	0.91 (0.34-2.42)
Live Births															
0	88	9/20	1.00	8/2	1.00	18	3/3	1.00	3/0	1.00	70	6/17	1.00	5/2	1.00
1-2	449	83/175	1.02 (0.44-2.37)	34/24	0.81 (0.40-1.64)	110	31/48	0.72 (0.13-3.87)	13/6	0.57 (0.12-2.62)	339	52/127	0.97 (0.35-2.68)	21/18	0.81 (0.36-1.86)
3-5	108	27/44	1.02 (0.38-2.74)	12/10	0.76 (0.33-1.74)	26	13/10	1.65 (0.25-10.96)	7/2	1.14 (0.21-6.36)	82	14/34	0.66 (0.19-2.22)	5/8	0.51 (0.18-1.40)
6+	160	46/61	1.08 (0.41-2.80)	23/23	0.68 (0.31-1.50)	41	16/13	1.12 (0.18-7.14)	9/5	0.52 (0.10-2.71)	119	30/48	0.93 (0.29-2.93)	14/18	0.68 (0.26-1.75)
No. of stillbirths															
Never	678	173/330	1.00	77/61	1.00	162	64/79	1.00	30/13	1.00	516	109/251	1.00	47/48	1.00
Ever	41	14/17	1.20 (0.56-2.54)	9/5	1.16 (0.61-2.20)	15	7/8	0.85 (0.27-2.67)	6/1	0.97 (0.36-2.60)	26	7/9	1.36 (0.48-3.83)	3/4	1.08 (0.45-2.60)
No. of miscarriages															
0	543	126/275	1.00	57/42	1.00	132	50/68	1.00	26/8	1.00	411	76/207	1.00	31/34	1.00
1	107	40/43	1.82 (1.11-2.98)	16/15	1.41 (0.92-2.16)	28	15/10	1.86 (0.76-4.58)	6/5	1.14 (0.52-2.49)	79	25/33	1.78 (0.97-3.25)	10/10	1.55 (0.92-2.61)
2+	69	21/29	1.23 (0.65-2.35)	13/9	1.08 (0.63-1.86)	17	6/9	0.76 (0.24-2.39)	4/1	0.76 (0.28-2.06)	52	15/20	1.54 (0.70-3.38)	9/8	1.34 (0.70-2.56)
No. of tubal or ectopic pregnancies															
Never	706	182/325	1.00	87/59	1.00	170	68/82	1.00	36/12	1.00	536	114/243	1.00	51/47	1.00
Ever	99	21/54	0.58 (0.33-1.02)	7/11	0.54 (0.33-0.89)	25	9/13	0.89 (0.32-2.45)	3/3	0.65 (0.27-1.60)	74	12/41	0.48 (0.24-0.98)	4/8	0.49 (0.26-0.92)
Cesarean section															
Never	621	166/294	1.00	82/59	1.00	157	66/73	1.00	35/13	1.00	464	100/221	1.00	47/46	1.00
Ever	96	20/52	0.70 (0.41-1.18)	3/7	0.70 (0.41-1.18)	20	5/14	0.42 (0.14-1.23)	1/1	0.41 (0.15-1.15)	76	15/38	1.11 (0.57-2.18)	2/6	0.94 (0.50-1.75)
History of STI															
No	763	188/359	1.00	89/65	1.00	186	74/89	1.00	38/14	1.00	577	114/270	1.00	51/51	1.00
Yes	39	15/20	1.39 (0.69-2.82)	5/5	1.21 (0.64-2.26)	9	3/6	0.56 (0.13-2.41)	1/1	0.44 (0.12-1.61)	30	12/14	2.07 (0.91-4.69)	4/4	1.74 (0.84-3.59)

-, no convergence

OR, odds ratio; CI, confidence interval; STI, sexually transmitted infection

4.4 Discussion

The Guanacaste Cohort remains the largest natural history cohort to have been conducted in a less developed country where investigations fully evaluated the role of HPV DNA and the role of risk factors. This provided a dataset that would allow full evaluation of SRH factors and risk of HPV persistence, which would complement the investigations carried out in the IARC multi-centric case-control studies described in *Study 1* (**Chapter 3**). The results from this *Study 2* support the role of decreasing age at first pregnancy with increasing risks of 2-year HPV persistence among women infected with prevalent HPV-16/18. Risks were increased by two-fold among women with AFP ≤15 years compared with AFP ≥21 years. It was not surprising that a positive association with 2-year persistence was found with increasing lifetime number of sexual partners as it has been found to be associated with risk HPV acquisition and invasive cervical cancer.

Other prospective natural history studies evaluating the risk of HPV persistence have been carried out in young women from populations in developed countries where the risk of cervical cancer is low (Brown *et al*, 2005; Ho *et al*, 1998; Maucort-Boulch *et al*, 2010; Moscicki *et al*, 2001; Winer *et al*, 2005; Woodman *et al*, 2001) and SRH factors significantly differ. Previous studies did not differentiate between risks of prevalent and incident infections or considered whether there were differences by type-specific HPV infections (HPV-16/18 vs. other HR-HPV). However, similar to this study, one prospective study conducted in Colombia, a high-risk area for cervical cancer, did not identify any associations neither between AFSI, parity and risk of HR-HPV persistence over a median duration of 9-year follow-up (Munoz *et al*, 2009).

4.4.1 Age at first pregnancy

The majority of women (79%) in this cohort who initiated first coitus were also pregnant within the same year. This observation was similar to that observed in *Study 1* and reflects the close temporal sequence of events of early AFSI and AFP occurring in developing countries. No association was found between AFSI and HPV persistence. In contrast, *Study 1* identified similar increased risk associations between invasive cervical cancers with both AFSI and AFP, which

made it difficult to disentangle any possible independent associations (**Chapter 3**). These findings in *Study* 2 suggest that AFP may play a more relevant role in cervical carcinogenesis than AFSI and highlight the potential biological susceptibility of the cervix during adolescence and pregnancy when exposed to increased levels of hormonal changes (Singer & Monaghan, 2000). As discussed previously, augmented levels of exposure to oestrogens in the presence of HPV may increase the probability of persistence, resulting in neoplastic changes (Elson *et al*, 2000; Hwang *et al*, 2009; Shai *et al*, 2007). This phenomenon is primarily dependent on the first pregnancy rather than subsequent pregnancies (Singer & Monaghan, 2000) which may partially explain the lack of association with multiparity.

Increased risks of invasive cervical cancer have been identified with high parity (International Collaboration of Epidemiological Studies of Cervical Cancer, 2006; Munoz *et al*, 2002) and long-term use of oral contraceptives (Moreno *et al*, 2002) suggesting that continuous endogenous oestrogen levels (pregnancy) and exogenous oestrogen exposure (oral contraceptive use) may modulate immune response to favour HPV persistence rather than clearance (Giannini *et al*, 1998; Giannini *et al*, 2002; Jacobs *et al*, 2003; Marzi *et al*, 1996). However, parity was not associated with HPV persistence in this study. It has been hypothesised that short-intervals between pregnancies might sustain these elevated levels of oestrogens that may influence HPV persistence (Mukherjee *et al*, 1994; Munoz *et al*, 2002). The cohort did not have detailed data to measure the time between the first and subsequent pregnancies to clarify whether this could explain the lack of association with persistence.

4.4.2 Pregnancy complications

The associations between HPV persistence and cervical trauma experienced during pregnancy remains unclear. Similar to previous findings in the pooled case-control studies of invasive cervical cancer, women who reported caesarean deliveries appeared to show a protective effect although not statistically significant (Munoz *et al*, 2002). Pregnancy miscarriage has not been found to be associated with invasive cervical cancer (Munoz *et al*, 2002), however, there appears to be a weak positive association with HPV persistence for women with a history of miscarriages/abortions. A plausible role of HPV in pre-term births (Zuo

et al, 2011) and spontaneous abortions (Hermonat *et al*, 1997) has been suggested. Similar to other hormonal hypotheses, these associations may be explained by the impaired immune response during pregnancy when there is an increased production of oestrogen that causes up-regulation of HPV gene expression.

Interestingly, there was an inverse association between tubal/ectopic pregnancy and HPV persistence. This finding could be due to the effects of random chance. However, risks of ectopic pregnancy have been linked to luteal phase defect which could reduce levels of mid-luteal follicle-stimulating hormone (FSH), luteinising hormone (LH), estradiol (E2) and progesterone (Guillaume *et al*, 1995). The mechanical barrier of an ectopic pregnancy, like that of tubal ligation (Li & Thomas, 2000), could alter blood supply to the ovary which reduces the gonadotrophin signal to the ovary, altering hormone levels and therefore disrupting ovarian stimulation. The reduction of oestrogen levels could interfere with the efficiency of HPV to facilitate metaplastic transformation in the cervix that would result in cervical neoplasia, thus showing a protective effect. Furthermore, data from a WHO collaborative study of Neoplasia and Steroid Contraceptives hospital-based case-control study collected in eight countries show that tubal ligation does not increase the risk of cervical cancer (Li & Thomas, 2000). This study suggested that tubal sterilisation may offer an alternative management strategy to hysterectomy for treatment of cervical precancerous lesions and secondary prevention of cervical cancer; however, further research is warranted.

4.4.3 Prevalent HR-HPV infections

Given the possible differences in natural histories for prevalent and newly detected or "incident" HR-HPV infection, the analysis concentrated on evaluating the risk of prevalent infections. Prevalent HPV infections identified at enrolment may represent longer duration of infections since one point prevalence estimate does not inform when the infection was acquired. Among young women, it could be speculated that these infections were newly acquired if time since first sexual intercourse and study enrolment was short. For older women, it is difficult to disentangle what these prevalent HPV infections represent when taking into account their full sexual history. These infections could be newly acquired

infections, persistent infections, or latent infections that have re-activated. It is also possible that these prevalent and newly detected infections are persisting at undetectable low-levels and may have a longer duration of persistence than observed, which has been shown particularly for HPV-16 (Weaver *et al*, 2011). It could be hypothesized that possible HPV reactivation of these infections may occur at a later stage of the natural history, which may explain why our observed associations were not consistent with the established association between parity and invasive cervical cancer.

4.4.4 Type-specific HR-HPV infection

Interestingly, the associations of AFP and lifetime number of sexual partners were mainly observed among women with HPV-16/18 and not other HR-HPV infections. This further supports the differences in types-specific risks of HPV persistence and of natural histories. It has been shown in a study in the US that the 10-year cumulative risks for ≥CIN-3 were 17.6% among women positive for HPV-16, 13.6% for HPV-18 and only 3% for women with other HR-HPV (not HPV-16/18) (Khan *et al*, 2005). These results have recently been corroborated in a study conducted in Taiwan that showed 16-year cumulative risks for cervical cancer among women positive for HPV-16, HPV-58 (without HPV-16) or other HR-HPV (not HPV-16/58) were 13.5%, 10.3% and 4%, respectively (Chen *et al*, 2011). This study also found 2-year persistence to be a biomarker of chronic or long-term infection that was predictive of an increased risk of subsequent cancer development. The increased type-specific risks of HPV-16/18 suggest that different cervical screening modalities could be considered for those specifically infected (Cuzick *et al*, 2008). For example, partial typing for HPV-16/18 may provide risk stratification management for clinicians to manage separately those HPV-16/18 positive from those who are other HR-HPV-positive (not HPV-16/18) and HPV-negative.

4.4.5 Methods of estimating HPV persistence

Measuring HPV persistence is not straightforward. Assumptions were made to accommodate incomplete data produced in natural history studies where HPV infections may be missing at certain time points. For example, a patient may have skipped their study visit for unknown reasons but the type-specific HPV infection may still be present at the conclusion of the study but this patient was

censored. This may have contributed to our weakened associations with follow-up time as shown for AFP. Also, to cope with intermittent missing HPV results, an assumption was made about a woman who is HPV positive when a study visit is missed following her baseline positive result and she returns with a positive HPV result at the subsequent study visit (as described in section 4.2.5.2). These assumptions cannot exclude the potential contribution of bias when estimating the duration of HPV persistence (Mitchell *et al*, 2011).

4.4.6 Strengths and limitations of the study

Strengths of this study include its population-based design, the long-term follow-up period of women (median duration 7 years), the detailed information collected on SRH factors and the full assessment of HPV DNA to adequately evaluate the persistence of prevalent HR-HPV infections. A limitation of this study is the older age range recruited in the cohort (≥18 years), which didn't allow us to evaluate young women who began AFSI and AFP at an early age (≤15 years) and follow their early pregnancies closely to understand the role of hormonal changes on HPV persistence.

4.5 Conclusions

In summary, this study identified early AFP on imparting a different risk of HPV persistence depending on the prevalence of HPV-16/18. Findings provide evidence of the role of early AFP in HPV persistence, a critical step in cervical carcinogenesis that would lead to progression of clinically relevant cervical neoplasia. Further assessment of whether AFP is associated with progression to cervical pre-cancer would complete its contribution to the spectrum of the natural history of HPV from acquisition, to persistence, and to progression of cervical pre-cancer and cancer. The protective effect of tubal /ectopic pregnancies against HPV persistence provides increasing culminating evidence of the role of female reproductive hormones involved in immune response to HPV infection and the cervical carcinogenic process. Additional research is needed to elucidate the role of oestrogens on HPV which could potentially be translated into treatment strategies for management of cervical neoplasia (i.e. control hormone levels to prevent HPV infection sequelae).

Cover sheet for each 'research' paper included in a research thesis

NB: A section of Chapter 5 has already been published:

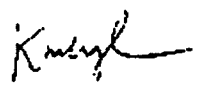
Louie KS, Castellsague X, de Sanjose S, Herrero R, Meijer CJ, Shah K, Munoz N, Bosch FX, for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Smoking and passive smoking in cervical cancer risk: pooled analysis of couples from the IARC multi-centric case control studies. Cancer Epidemiology, Biomarkers & Prevention 2011; 20 (7)1379-1390.

1. For a 'research paper' already published
    a. Where was the work published?
    Cancer Epidemiology, Biomarkers & Prevention
    b. When was the work published? **2011**
        i. If the work was published prior to registration for your research degree, give a brief rationale for its inclusion. **N/A**
    c. Was the work subject to academic peer review? **Yes**
    d. Have you retained the copyright for the work?

    No, refer to Appendix 10 for permission from copyright holder.
2. For a 'research paper' prepared for publication but not yet published
    a. Where is the work intended to be published? **N/A**
    b. List the paper's authors in the intended authorship order **N/A**
    c. Stage of publication **N/A**

3. For multi-authored work, give full details of role in the research included in the paper and in the preparation of the paper.

    I, Karly S Louie, was the primary author who designed and planned the study with SS, XC, FXB; conducted the statistical analysis; interpreted the results; and wrote the manuscript.



Candidate's signature

Senior author, F Xavier Bosch, signature to confirm role as stated in (3)

5 Study 3. Male role in the aetiology of cervical cancer

5.1 Introduction

Behavioural risk factors of the man have important implications in the consequent female partner risk of human papillomavirus (HPV) acquisition and of invasive cervical cancer (ICC). As HPVs involved in cervical carcinogenesis are sexually transmitted, it is central to understand patterns of sexual behaviour in HPV transmission including the behaviours of both men and women. It has been debated that a woman's risk for cervical cancer will depend more on the full sexual history of the male partner than on her own behaviour (Skegg *et al*, 1982). This is particularly relevant in societies where women tend to be virgins at marriage and monogamous thereafter. Epidemiological studies have tried to characterise the male role and the consequent female partner risk of HPV acquisition and of cervical cancer (Bleeker *et al*, 2005; Castellsague *et al*, 2003; Hernandez *et al*, 2008; Partridge & Koutsky, 2006). While a number of studies mostly involving monogamous women have observed an association between the number of sexual partners of the husband and his wife's risk for cervical cancer (Brinton *et al*, 1989; Buckley *et al*, 1981; Pridan & Lilienfeld, 1971), other studies have not (Bosch *et al*, 1996; Kjaer *et al*, 1991; Munoz *et al*, 1996; Thomas *et al*, 2001). Other inconclusive associations with history of contact with sex workers have been identified (Bosch *et al*, 1996; Kjaer *et al*, 1991; Munoz *et al*, 1996; Thomas *et al*, 2001).

Besides sexual behaviour, other male factors, such as tobacco smoking has been less explored despite it being a well-established risk factor for cervical pre-cancer and cancer (Munoz *et al*, 2006; Plummer *et al*, 2003). Several reviews have summarised the epidemiological and biological association of passive smoking on the risk of cervical cancer (IARC, 2004; National Cancer Institute, 1999; U.S. Dept. of Health and Human Services & National Center for Chronic Disease Prevention and Health Promotion, 2006), however, the evidence has been suggestive rather than sufficient to implicate the role of passive smoking in the aetiology of cervical cancer among lifetime non-smoking women. Among the studies identified, the recognised limitations include small sample sizes of non-

smoker controls and cases of cervical cancer, lack of specific information on HPV and sexual behaviour and most studies obtained spousal history of smoking through questioning of the women rather than the men. Furthermore, most of the studies involved cervical intraepithelial neoplasia grade 3 (CIN-3)/carcinoma in situ (CIS) rather than invasive disease, which is a relevant distinction since evidence suggests that smoking acts in the stages of progression from CIS to invasive cancer (Plummer *et al*, 2003).

To evaluate the male role in the aetiology of cervical cancer, and specifically the risk related to passive smoking in the context of couples, we performed a pooled analysis of five case-control studies involving ICC and two case-control studies involving CIS, of couples in which husbands or stable partners of ICC and CIS case and control women participated. The studies were conducted in three continents, mainly in developing countries, and were coordinated by the International Agency for Research on Cancer (IARC) in Lyon, France and the Catalan Institute of Oncology (ICO) in Barcelona, Spain. The IARC/ICO series of case-control studies remain the largest dataset of sexual couples on etiological investigations of ICC that fully addresses the role of HPV DNA and of independent cofactors. Some of the associations with risk factors (i.e. penile HPV infection and male circumcision) have been assessed in subsets of the participants in this analysis (Bosch *et al*, 1996; Castellsague *et al*, 2002; Castellsague *et al*, 1997; Franceschi *et al*, 2002; Munoz *et al*, 1996; Plummer *et al*, 2003). For this study, we characterize in-depth male factors, the role of passive smoking in the context of couples with the full dataset on HPV and their associations with invasive cervical cancer.

5.2 Methods

The IARC/ICO case-control program included a series of studies on ICC and CIS from eleven countries with a broad range of cervical cancer incidence rates. Among these, seven studies conducted in five countries, enrolled husbands or stable partners of women with CIS or cervical cancer and control women were pooled for these analyses. Methods of each study and primary results related to women have been published previously. Countries included Brazil (Eluf-Neto *et al*, 1994) and Colombia (Bosch *et al*, 1996; Munoz *et al*, 1993; Munoz *et al*, 1996), the Philippines (Ngelangel *et al*, 1998), Thailand (Chichareon *et al*, 1998) and

Spain (Bosch *et al*, 1996; Bosch *et al*, 1992). Briefly, women with histologically confirmed incident cervical CIS, invasive squamous cell carcinoma, adenocarcinoma or adenosquamous-cell carcinoma were recruited from reference hospitals before treatment. Control women were recruited from the general population in two of the studies of ICC in Spain and Colombia and from the same hospitals as the cases for the other studies. Control women were frequency-matched to case patients by five-year age groups.

Current husbands or stable partners (herein referred to as husbands) of enrolled women were defined as men who reported having had regular sexual intercourse with the women for at least six months, irrespective of whether or not they were married or lived together.

Informed consent was obtained from both men and women who agreed to participate.

5.2.1 Study questionnaire

A standardised questionnaire was administered to participants by a trained interviewer that included questions about socio-demographic factors, sexual behaviour, hygienic practices, and history of sexually transmitted infections (STIs). For specific questions on smoking habits, subjects were first asked to classify themselves as life-time never smoker, ex-smoker (defined as a former smoker who stopped smoking at least 1 year prior to the interview) or current smoker. Ever smokers were also asked at what age they started smoking regularly, the duration and how many cigarettes per day they smoked. Additional questions were asked on the type of tobacco (blond, black, or other) and type of filter (filter, no filter, or both) used. Ex-smokers were asked the age at which they stopped smoking.

5.2.2 Penile and cervical HPV DNA sampling

Two samples of exfoliated cells were obtained from the penis: one from the distal urethra with the use of a very thin, wet, cotton-tipped swab and one from the external surface of the glans and coronal sulcus with the use of a standard-sized wet, cotton-tipped swab.

Two samples of cervical exfoliated cells were collected with wooden spatulae and endocervical brushes. After preparation of one Papanicolaou smear,

the remaining cells were eluted in saline, centrifuged and frozen at -70 °C until shipment to the central laboratory for HPV DNA testing. A tumor-biopsy sample was obtained from cases and frozen. Cytology and histology diagnosis were reviewed and confirmed by a panel of expert pathologists that agreed on a diagnosis by consensus or majority.

5.2.3 Detection of HPV DNA

Detailed descriptions of the polymerase-chain-reaction (PCR) assays used in these studies have been described elsewhere. HPV DNA was detected by PCR amplification of a small fragment of the *L1* gene using MY09 and MY11 consensus primers for the studies in Spain and Colombia (Hildesheim *et al*, 1994) and GP5+/6+ general primer system for the other studies (de Roda Husman *et al*, 1995; Jacobs *et al*, 1995; Walboomers *et al*, 1992b). Beta-globin primers were used to amplify the beta-globin gene to assess the quality of the DNA in the specimen. HPV DNA in PCR products was analysed with the use of a cocktail of HPV-specific probes and genotyped by hybridization with type-specific probes for 33 HPV types in the case of cervical samples and for at least 6 HPV types (6, 11, 16, 18, 31, and 33) in the case of the penile samples. Samples that tested positive for HPV DNA but did not hybridise with any of the type-specific probes were labeled as HPV X.

5.2.4 Ethical approval

All relevant ethical approvals was obtained from IARC, the Lead Investigator from the IARC multi-centric case control studies on cervical cancer and LSHTM as described in **Chapter 3.2.5** (Appendix 6 and 7).

5.2.5 Statistical analyses

5.2.5.1 Univariate and multivariate analyses

To evaluate the association between male risk factors, smoking habits and risk of CIS or ICC, we first used age- and country-adjusted univariate logistic regression analyses to determine the effects of each of the following potential male factors using an alpha-level of 0.05: age, education, sexual history (age at first sexual intercourse, lifetime number of sexual partners, history of contact with sex workers, history of STIs, and history of anal sex), hygienic practices (i.e. pay attention to uncover penis and to wash the region, able to fully uncover

spontaneously or by pulling the penis from the skin prepuce, and wash before and after sexual intercourse), male circumcision status, and history of smoking (non-smoker, current smoker, or ex-smoker, lifetime pack-years, and use of tobacco and filter type); and to control for potential confounding of male characteristics and risk of cervical cancer. Final models were adjusted for male factors that contributed a ≥10% change to any of the estimated odds ratios (OR) and 95% confidence intervals (CI). Factors identified as being significant in univariate logistic regression models were further selected in multivariate models. Only significant multivariate models are presented.

Lifetime number of sexual partners (a significant risk factor of exposure to HPV) was identified to be heterogeneous across study countries and an interaction term combining lifetime number of sexual partners and country were included in the fully adjusted multivariate models (refer to Section 5.3.4).

5.2.5.2 Potential confounders of the woman

To control for additional potential confounding by characteristics of the women, female risk factors (education, age at first sexual intercourse, lifetime number of sexual partners, history of pap smear 12 months prior to study enrolment, use of oral contraceptives, parity, and smoking) for cervical cancer were fitted into the final multivariate models a) for the CIS adjusted models if they contributed to any change to the OR estimates for male characteristics; and b) for the final ICC adjusted models as they are well-established risk factors known to be associated with ICC. However, when we adjusted the OR estimates with all female risk factors in the CIS model, the estimates did not significantly differ (data not shown).

5.2.5.3 CIS vs. ICC model

A statistically significant interaction between some male risk factors (e.g. age at first sexual intercourse, lifetime number of sexual partners, history of sexual intercourse with a sex worker, and HPV-positivity status) and case status (i.e. ICC versus CIS) justified the use of two separate models for each disease stage. This is in agreement with our current understanding of the natural history of CIS, as it has been estimated that about 31% of CIS cases will develop cancer within 30 years, leaving a proportion of CIS cases that will not advance to invasive disease (McCredie *et al*, 2008). Thus, some of the risk factors associated

with CIS incidence may differ from those associated with progression from CIS to ICC. However, for male circumcision we present the pooled analysis of CIS/ICC as well as the stratified models of CIS and ICC since we did not find heterogeneity with this risk factor and disease status as compared to the other male risk factors as described above. This is consistent with what was previously published (Castellsague *et al*, 2002).

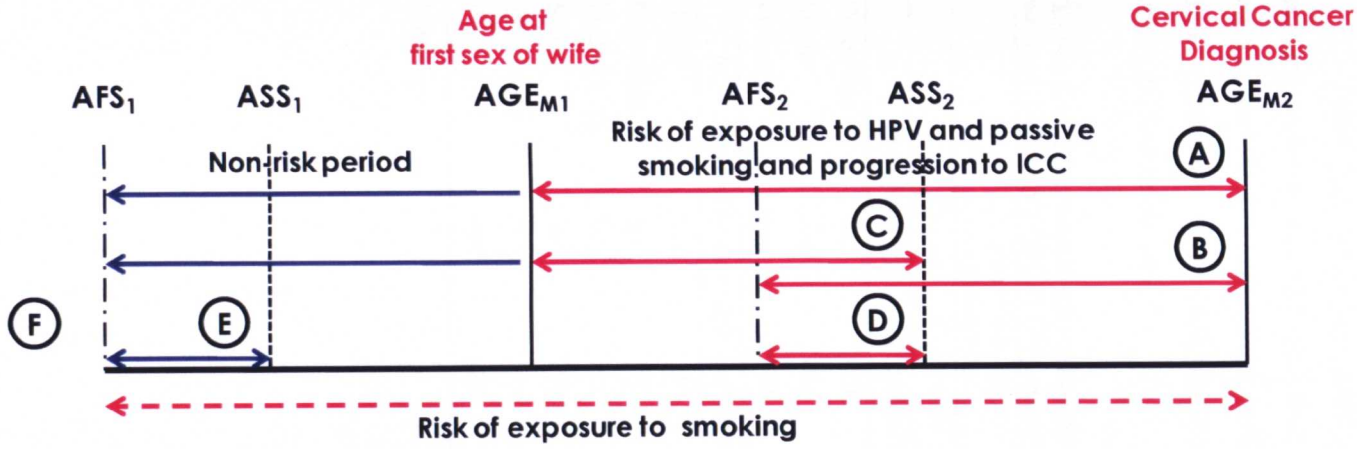
Furthermore, to better clarify the relationship between male characteristics and cervical cancer in their female partners, we removed the potential effect of previous male partners the woman may have had by calculating and comparing two different statistical models for CIS and ICC: one included all study couples and the second model included only couples with monogamous women.

5.2.5.4 Passive smoking classification

Besides the two models of all study couples and only couples with monogamous women, a third model was constructed for couples with lifetime non-smoking monogamous women when evaluating the role of passive smoking in the couples context. The third model was restricted to 765 couples with lifetime non-smoking monogamous women. In addition, the men's smoking history was further reclassified according to the risk period for which the woman would have been exposed to HPV infection (a necessary factor in cervical carcinogenesis), passive smoking and risk of progression to cervical cancer (Figure 5.1). Ninety male ex-smokers (n=44 cases and n=43 controls) of couples with monogamous women were reclassified as non-smokers, and the duration of exposure to passive smoke and smoking pack-years were recalculated (Table 5.1.).

Table 5.1. Reclassification of male smoking status according to period of risk of exposure as defined in Figure 1

	Couples with with monogamous women (n=981)		**Couples with non-smoking monogamous women (n=765)**	
	Reported smoking status	**Reclassified smoking status**	**Reported smoking status**	**Reclassified smoking status**
	Cases/Controls	Cases/Controls	Cases/Controls	Cases/Controls
Non-smoker	98/148	142/194	71/126	112/167
Ex-smoker	100/113	56/67	80/94	39/54
Current Smoker	293/229	293/229	207/186	207/186



AFS_1 and **AFS_2**, age at first smoke; **ASS_1** and **ASS_2**, age at stop smoking; **AGE_{M1}**, age of husband at time of age at first sexual intercourse of the wife; **AGE_{M2}**, age of husband at time of cervical cancer diagnosis of the wife.

Figure 1. Model of risk of exposure to HPV and passive smoking and progression to invasive cervical cancer (ICC) among non-smoking monogamous women according to the husband's history of smoking. Period of risk is defined between age at first sexual intercourse of the wife and cervical cancer diagnosis.

Duration of exposure to passive smoking is defined as: **A)** Current smoker=(AGE_{M2}– AFP) if AFS_1; **B)** Current smoker=(AGE_{M2}– AFS_2); **C)** Ex-smoker=(ASS_2 - AGE_{M1}) if $AFSI_1$; **D)** Ex-smoker=(ASS_2– AFS_2). Women are not at risk for HPV and passive smoking if **E)** Ex-smoker=(ASS_1– AFS_1) or their husband is a **F)** Non-smoker.

Smoking status of the husband is classified as follows: Current smokers=**A) + B)** , Ex-smokers=**C) + D)**, and Non-smokers=**E) + F)**

5.3 Results

5.3.1 Patient characteristics

Table 5.2 describes selected characteristics of the male and female subjects. Of the 291 CIS and 692 ICC cases and 936 control women, 59.8%, 70.9%, and 81.2%, were monogamous, respectively. In general, husbands were older than their wives, and husbands and wives of CIS cases and controls were younger than those of ICC.

Table 5.2. Characteristics of both corresponding husbands of (a) women and without CIN-3/CIS (b) invasive cervical cancer

	Total no. of husbands		Age of husbands*		Age of wives*	
	Cases	**Controls**	**Cases**	**Controls**	**Cases**	**Controls**
a. Cervical intraepithelial neoplasia grade 3/ Carcinoma in situ						
Colombia	127	164	40	41.5	36	35
Spain	164	184	38	37	34	35
Pooled	291	348	38	39	34	35
b. Invasive cervical cancer						
Brazil	72	76	52	53.5	46	48
Colombia	91	89	47	52	43	44
Philippines	155	111	46	46	46	44
Spain	146	139	50	50	50	52
Thailand	228	173	46	46	46	47
Pooled	692	588	50	50	45	46

*Median

5.3.2 Penile HPV prevalence

Table 5.3 shows penile HPV prevalence among husbands of cases and controls of CIS and ICC by history of smoking and country. Penile HPV detection was doubled in husbands of cases than controls of CIS and was higher among cases than controls of ex-smokers (2.4% vs. 0%) and current smokers (13.9% vs. 3.8%). Similar penile HPV detection was found in husbands of cases and controls of ICC (17.6% vs. 16.2%), which was also similar among cases and controls of ex-smokers and current smokers. However, penile HPV was more prevalent among husbands of cases than controls of ICC (9.7% vs. 6.6%, respectively).

Table 5.3. Penile HPV prevalence among husbands of cases and controls of CIS and ICC by history of smoking and country

	HPV Tested*		HPV-positive		HPV-positive among those tested					
					Non-smokers		Ex-smokers		Current Smokers	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
Invasive Cervical Cancer	444	346	78 (17.6)	56 (16.2)	16 (3.6)	18 (5.2)	19 (4.3)	15 (4.3)	43 (9.7)	23 (6.6)
Brazil	53	56	19 (35.8)	22 (39.3)	4 (7.5)	5 (8.9)	7 (13.2)	8 (14.3)	8 (15.1)	9 (16.1)
Colombia	49	48	16 (32.7)	14 (29.2)	6 (12.2)	10 (20.8)	2 (4.1)	1 (2.1)	8 (16.3)	3 (6.3)
Philippines	149	106	9 (6.0)	5 (4.7)	2 (1.3)	1 (0.9)	0 (0.0)	2 (1.9)	7 (4.7)	2 (1.9)
Spain	84	62	10 (11.9)	2 (3.2)	0 (0.0)	0 (0.0)	3 (3.6)	1 (1.6)	7 (8.3)	1 (1.6)
Thailand	109	74	24 (22.0)	13 (17.6)	4 (3.7)	2 (2.7)	7 (6.4)	3 (4.1)	13 (11.9)	8 (10.8)
Carcinoma in situ	165	186	35 (21.2)	14 (7.5)	8 (4.8)	7 (3.8)	4 (2.4)	0 (0.0)	23 (13.9)	7 (3.8)
Spain	102	106	22 (21.6)	4 (3.8)	2 (2.0)	0 (0.0)	4 (3.9)	0 (0.0)	16 (15.7)	4 (3.8)
Colombia	63	80	13 (20.6)	10 (12.5)	6 (9.5)	7 (8.8)	0 (0.0)	0 (0.0)	7 (11.1)	3 (3.8)

* HPV testing of adequate specimens that were beta-globin positive

5.3.3 Male characteristics and their univariate associations with risk of CIS and ICC

Table 5.4 presents selected male characteristics and their univariate associations with risk of CIS and ICC, stratified by all couples and couples with monogamous women. In general, similar associations were observed in all couples and couples with monogamous women models, except for associations of CIS with education and smoking status. Hygienic practices (able to fully uncover spontaneously or by pulling the penis from the prepuce and washing before and after sexual intercourse) and history of anal sex were not associated with CIS or ICC in univariate analyses (data not shown). The following husband's risk factors were found to be associated with a woman's increased risk of CIS or ICC: early age at first sexual intercourse, history of sexual intercourse with a sex worker (ever and while with current wife), history of gonorrhoea, increasing number of STIs, being a current smoker and increasing lifetime smoking pack-years. Lack of education was associated with an increased risk of ICC but not CIS, whereas the absence of circumcision and being penile HPV-positive were associated with an increased risk of CIS but not ICC. An inverse relation between use of "black" tobacco type, as compared to "blond" tobacco type among smokers, and the risk of CIS was observed, as well some hygienic practices such as lack of attention to uncover the penis to wash the region and the risk of ICC.

Table 5.4. Male characteristics and their univariate association with a woman's risk of cervical intraepithelial neoplasia grade 3 (CIN-3)/carcinoma in situ (CIS) or invasive cervical cancer (ICC)

	CIN-3/CIS				ICC			
	All couples		Husbands with monogamous women		All couples		Husbands with monogamous women	
	Cases/ Controls	Odds ratio (95% CI)[a]	Cases/ Controls	Odds ratio (95% CI)[a]	Cases/ Controls	Odds ratio (95% CI)[a]	Cases/ Controls	Odds ratio (95% CI)[a]
	No.		No.		No.		No.	
Education								
≥Secondary	120/157	1.00	61/123	1.00	226/236	1.00	165/197	1.00
Primary School	148/167	1.21 (0.86-1.68)	100/129	**1.58 (1.04-2.38)**	374/299	**1.62 (1.24-2.12)**	260/250	**1.52 (1.12-2.07)**
No School	21/23	1.36 (0.70-2.64)	12/17	1.49 (0.65-3.41)	92/53	**2.81 (1.83-4.33)**	66/44	**2.71 (1.66-4.42)**
Age at first sexual intercourse								
≥21	31/78	1.00	17/68	1.00	175/190	1.00	132/173	1.00
17-20	105/129	**2.36 (1.43-3.89)**	68/103	**3.02 (1.62-5.64)**	317/25	**1.49 (1.14-1.96)**	222/213	**1.51 (1.12-2.04)**
≤16	154/141	**4.48 (2.58-7.81)**	89/98	**5.79 (2.91-11.55)**	199/143	**1.89 (1.36-2.64)**	136/104	**2.14 (1.47-3.12)**
History of sexual intercourse with a sex worker								
Never	81/131	1.00	43/109	1.00	216/236	1.00	155/213	1.00
Ever	210/217	**1.72 (1.21-2.45)**	131/160	**2.22 (1.42-3.45)**	473/349	**1.58 (1.25-2.01)**	333/277	**1.78 (1.36-2.34)**
History of sexual intercourse with a sex worker while with current wife								
Never	81/131	1.00	43/109	1.00	216/236	1.00	155/213	1.00
Never sexual intercourse while with wife	113/142	1.41 (0.96-2.08)	63/101	**1.68 (1.03-2.74)**	197/187	1.28 (0.96-1.71)	115/142	1.23 (0.87-1.72)
Ever sexual intercourse while with wife	97/75	**2.32 (1.52-3.56)**	68/59	**3.21 (1.90-5.39)**	245/162	**1.87 (1.42-2.46)**	218/135	**2.29 (1.69-3.11)**

History of STIs								
Never	153/208	1.00	89/168	1.00	393/394	1.00	284/347	1.00
Syphilis only	4/4	1.37 (0.33-5.62)	3/2	2.71 (0.44-16.59)	12/13	0.96 (0.42-2.14)	9/10	1.19 (0.47-2.99)
Gonorrhoea only	48/40	**1.82 (1.12-2.95)**	23/27	1.81 (0.95-3.43)	154/77	**1.96 (1.43-2.69)**	105/57	**2.26 (1.57-3.27)**
Herpes only	2/7	0.43 (0.09-2.13)	2/5	0.85 (0.16-4.54)	5/3	1.59 (0.37-6.76)	4/3	1.55 (0.34-7.08)
Condyloma only	7/5	1.88 (0.58-6.06)	4/3	2.47 (0.54-11.36)	12/6	2.07 (0.76-5.62)	9/5	2.39 (0.78-7.30)
Other STIs only	32/44	1.06 (0.64-1.77)	24/34	1.44 (0.79-2.61)	51/28	**2.01 (1.23-3.28)**	35/19	**2.52 (1.39-4.55)**
≥2 STIs	45/40	**1.83 (1.10-3.04)**	29/30	**2.12 (1.14-3.94)**	65/67	1.07 (0.72-1.58)	45/50	1.21 (0.76-1.92)
Number of STIs								
Never	153/208	1.00	89/168	1.00	393/394	1.00	2/347	1.00
1	93/100	1.37 (0.95-1.96)	56/71	**1.63 (1.04-2.56)**	234/127	**1.87 (1.44-2.42)**	162/94	**2.18 (1.61-2.96)**
≥2	45/40	**1.81 (1.09-3.00)**	29/30	**2.13 (1.15-3.97)**	65/67	1.04 (0.70-1.55)	45/50	1.20 (0.75-1.90)
Circumcision								
Yes	15/36	1.00	8/26	1.00	179/139	1.00	138/130	1.00
No	271/311	**2.33 (1.23-4.43)**	163/242	**2.29 (1.00-5.24)**	511/449	1.15 (0.76-1.73)	352/361	1.33 (0.84-2.10)
Pay attentinon to uncover your penis and to wash the region								
Yes	220/256	1.00	130/191	1.00	621/507	1.00	438/471	1.00
No	71/92	0.79 (0.53-1.18)	44/78	0.75 (0.46-1.21)	62/80	**0.60 (0.39-0.91)**	49/73	**0.61 (0.38-0.97)**
Time since smoking cessation								
Non-smoker	83/137	1.00	56/105	1.00	149/183	1.00	98/148	1.00
Exsmoker >11 years	11/8	2.34 (0.90-6.10)	5/7	1.35 (0.41-4.45)	45/50	1.21 (0.76-1.93)	32/47	1.08 (0.64-1.84)
Exsmoker ≤10 years	24/29	1.41 (0.76-2.60)	7/20	1.67 (0.27-1.69)	83/79	1.31 (0.89-1.92)	68/66	**1.56 (1.01-2.40)**
Current smoker	171/172	**1.67 (1.15-2.43)**	104/136	1.48 (0.95-2.31)	414/274	**1.85 (1.41-2.43)**	293/229	**1.94 (1.41-2.66)**
Use of tobacco type by smokers								
Blond	37/25	1.00	20/19	1.00	299/222	1.00	216/179	1.00
Black	68/84	**0.46 (0.24-0.90)**	41/67	**0.42 (0.19-0.97)**	80/62	1.13 (0.56-2.28)	59/56	0.89 (0.41-1.91)
Both	82/84	0.60 (0.33-1.11)	47/63	0.59 (0.27-1.28)	74/42	1.49 (0.72-3.07)	55/35	1.39 (0.62-3.11)
Others	4/2	1.10 (0.18-6.74)	3/1	1.82 (0.16-20.50)	69/54	0.98 (0.63-1.51)	49/51	0.82 (0.50-1.34)

Use of smoking filter type by smokers								
Filter	89/84	1.00	56/66	1.00	277/205	1.00	205/165	1.00
No filter	15/19	0.80 (0.37-1.73)	7/15	0.51 (0.19-1.42)	42/42	0.86 (0.51-1.45)	25/36	0.63 (0.35-1.15)
Both	94/99	0.91 (0.58-1.43)	50/76	0.72 (0.41-1.25)	154/99	1.32 (0.89-1.97)	114/87	1.15 (0.74-1.78)
Others	4/2	1.86 (0.32-10.69)	3/1	3.09 (0.30-31.96)	69/54	0.98 (0.63-1.53)	49/51	0.79 (0.48-1.30)
Smoking lifetime pack-years [a]								
Non-smoker	83/137	1.00	56/105	1.00	149/183	1.00	98/148	1.00
Low no. of pack-years	96/112	1.41 (0.94-2.10)	49/89	1.05 (0.64-1.71)	272/202	**1.58 (1.18-2.11)**	196/174	**1.65 (1.18-2.30)**
Medium/High no. of pack-years	106/95	**2.00 (1.30-3.06)**	65/72	**1.79 (1.08-2.97)**	263/198	**1.75 (1.30-2.36)**	190/164	**1.82 (1.29-2.57)**
HPV status								
Negative	130/172	1.00	77/140	1.00	366/290	1.00	266/253	1.00
Positive	35/14	**3.25 (1.67-6.32)**	18/9	**3.68 (1.57-8.62)**	78/56	1.22 (0.82-1.81)	50/41	1.27 (0.80-2.03)
No HPV result/ Inadequate	126/162	1.04 (0.75-1.44)	79/120	1.21 (0.81-1.81)	248/242	0.83 (0.64-1.08)	175/197	0.91 (0.67-1.22)

STIs, sexually transmitted infections; CI, confidence intervals; Bolded odds ratios (95% CI) were statistically signifant associations ($p<0.05$).

[a] Adjusted for age and study country

[b] ***CIN-3/CIS model:*** smoking lifetime pack-years for the all husbands model is defined as low (36.5-6022.5 pack-years) and medium/high (6132-56611.5 pack-years); and for the husbands with monogamous women model, low (36.5-6716 pack-years) and medium/high (6825.5-38963.8 pack-years); ***Invasive cervical cancer:*** smoking lifetime pack-years for the all husbands model is defined as: low (36.5-7300 pack-years) and medium/high (7354.8-43435 pack-years); and for the husbands with monogamous women model, low (36.5-7665 pack-years) and medium/high (7829.3-43435 pack-years)

5.3.4 Lifetime number of sexual partners

Table 5.5 shows the varying association between lifetime number of sexual partners and risk of CIS or ICC by country. An increased risk of CIS was observed for both Colombia (statistically significant) and Spain with increasing lifetime number of sexual partners in the age-adjusted models of all couples and couples with monogamous women, but only Spain showed a significant increased risk (p-trend<0.001) in the fully adjusted multivariate model. An increased risk of ICC with increasing lifetime number of sexual partners was observed for the Philippines and Spain (p-trends<0.01); whereas, no statistically significant increased risk was observed for Brazil, Colombia, and Thailand in both univariate and multivariate models except for the univariate model for Thailand.

Table 5.5. Univariate and multivariate associations between lifetime number of sexual partners and risk of cervical intraepithelial neoplasia grade 3 (CIN-3)/ carcinoma in situ (CIS) or invasive cervical cancer (ICC) stratified by study country

		All couples		Couples with monogamous women [c]	
	Cases/ Controls	Age-adjusted	Mulitivariate	Age-adjusted	Multivariate
Lifetime no. of sexual partners	No.	Odds ratio (95% CI)		Odds ratio (95% CI)	
CIN-3/CIS[a]					
Colombia					
1-5	9/15	1.00	1.00	1.00	1.00
6-20	49/66	4.84 (2.84-8.26)	1.31 (0.51-3.37)	4.67 (2.50-8.75)	1.66 (0.50-5.48)
≥21	69/83	6.68 (3.77-12.95)	1.61 (0.62-4.18)	8.60 (4.14-17.89)	1.62 (0.47-5.64)
Spain					
1-5	45/124	1.00	1.00	1.00	1.00
6-20	65/36	1.29 (0.52-3.21)	3.52 (1.99-6.22)	1.55 (0.49-4.92)	3.74 (1.95-7.18)
≥21	54/22	1.56 (0.63-3.86)	5.33 (2.77-10.27)	1.56 (0.47-5.13)	7.29 (3.37-15.76)
p-trend			<0.001		<0.001
ICC[b]					
Brazil					
1-5	18/20	1.00	1.00	1.00	1.00
6-20	26/17	1.63 (0.64-4.16)	1.22 (0.38-3.93)	1.84 (0.54-6.28)	0.99 (0.15-6.48)
≥21	27/36	0.80 (0.35-1.82)	0.76 (0.23-2.59)	1.07 (0.38-2.99)	1.21 (0.19-7.74)
Colombia					
1-5	7/7	1.00	1.00	1.00	1.00
6-20	34/25	1.43 (0.44-4.71)	2.34 (0.49-11.17)	2.29 (0.47-11.03)	7.67 (0.75-78.91)
≥21	50/57	0.95 (0.31-2.94)	1.05 (0.23-4.89)	1.13 (0.24-5.34)	3.11 (0.26-36.49)

Philippines					
1-5	69/69	1.00	1.00	1.00	1.00
6-20	46/31	1.60 (0.91-2.80)	1.61 (0.81-3.23)	1.65 (0.89-3.06)	1.84 (0.89-3.82)
≥21	35/10	3.50 (1.60-7.65)	4.99 (1.75-14.20)	3.65 (1.64-8.12)	5.21 (1.80-15.08)
Spain			0.003		0.002
1-5	41/77	1.00	1.00	1.00	1.00
6-20	51/35	2.72 (1.53-4.85)	1.93 (0.86-4.36)	2.55 (1.36-4.76)	1.73 (0.72-4.20)
≥21	52/27	3.62 (1.98-6.62)	3.43 (1.43-8.21)	3.71 (1.94-7.09)	3.94 (1.48-10.48)
p-trend			0.006		0.007
Thailand					
1-5	89/87	1.00	1.00	1.00	1.00
6-20	70/45	1.54 (0.95-2.50)	1.37 (0.66-2.84)	2.12 (1.21-3.71)	1.43 (0.61-3.36)
≥21	60/36	1.60 (0.96-2.68)	1.49 (0.65-2.84)	1.92 (1.05-3.50)	1.84 (0.73-4.65)

a CIS multivariate model for all couples was adjusted for age of the husband and wife, circumcision status, HPV status, age at first sexual intercourse of the wife, wife's lifetime number of sexual partners; and the model for couples with monogamous women was adjusted for age of the husband and wife, age at first sexual intercourse of the wife, and HPV status

b ICC multivariate model was adjusted for age of the husband and wife, level of education of the husband and wife, lifetime number of sexual partners of the husband and wife, husband's history of STIs, husband's time since last smoke, age at first sexual intercourse of the wife , lifetime smoking pack-years of the wife , oral contraceptive use, parity, pap smear history 12 months prior to study enrollment.

c Not adjusted for wife's lifetime number of sexual partners

5.3.5 Multivariate associations of male characteristics and risk of CIN-3/CIS

Table 5.6 shows the association between significant male characteristics and cervical CIS in multivariate analyses after adjusting for both male and female risk factors. Generally, similar associations were identified in the two analyses of all couples and only couples with monogamous women, therefore we will describe our findings herein forward according to the all couples model. An increased risk for cervical CIS was observed for women with men who were positive for penile HPV (odds ratio, OR=2.17; 95% confidence interval, CI: 1.06-4.44) and men who were uncircumcised (OR=2.67; 95% CI: 1.31-5.45).

Table 5.6. Multivariate associations between male characteristics and risk of CIS

	Odds ratio (95% CI)	
Male characteristics	**All couples [a]**	**Couples with monogamous women [b]**
Circumcision		
Yes	1.00	1.00
No	2.67 (1.31-5.45)	2.25 (0.92-5.53)
HPV result		
Negative	1.00	1.00
Positive	2.17 (1.06-4.44)	2.44 (0.97-6.12)
No HPV result/inadequate	1.07 (0.74-1.53)	1.35 (0.87-2.10)

[a] Adjusted for all variables in the table, age of the husband and wife, study country, age at first sexual intercourse of the wife, wife's lifetime number of sexual partners.
[b] Adjusted for all variables in table except for circumcision, age of the husband's wife and age at first sexual intercourse of the wife.

5.3.6 Multivariate associations of male characteristics and risk of ICC

Lack of education, having a history of gonorrhoea, and having had at least one STI were also associated with an increased risk of ICC (Table 5.7).

Table 5.7. Multivariate associations between male characteristics and risk of ICC

Male characteristics	All couples [a]	Couples with monogamous women [b]
	Odds ratio (95% CI)	**Odds ratio (95% CI)**
Education		
≥Secondary	1.00	1.00
Primary School	1.63 (1.18-2.26)	1.59 (1.09-2.32)
No School	2.82 (1.68-4.75)	2.74 (1.51-4.98)
p-trend	0.001	0.001
History of STIs [b]		
Never	1.00	1.00
Syphilis only	0.61 (0.23-1.62)	0.64 (0.21-1.97)
Gonorrhea only	1.49 (1.00-2.22)	1.72 (1.08-2.74)
Herpes only	0.75 (0.10-5.39)	0.64 (0.08-5.50)
Condyloma only	1.73 (0.59-5.05)	1.86 (0.56-6.18)
Other STI only	1.53 (0.87-2.69)	1.69 (0.85-3.35)
≥2 STIs	0.88 (0.54-1.45)	0.94 (0.52-1.70)
Number of STIs[c]		
Never	1.00	1.00
1	1.41 (1.01-1.96)	1.58 (1.07-2.32)
≥2	0.88 (0.54-1.45)	0.94 (0.52-1.70)

STI, sexually transmitted infection

[a] Adjusted for age of the husband and wife, centre, and all variables in the table, level of education of the wife (≥secondary level, primary level, no schooling), age at first sexual intercourse of the wife (≥21 years, 17-20 years, ≤16 years), lifetime smoking pack-years of the wife (non-smoker, low, medium, and high smoking lifetime-pack-years), oral contraceptive use (never, 1-4 years, ≥5 years), parity (nulliparous, 1-6, ≥7) pap smear history 12 months prior to study enrollment (never, ever), and lifetime number of sexual partners of the wife (1, ≥2). Number of STIs and smoking history of smoking were not included in the final model.

[b] Did not adjust for number of STIs

[b] Did not adjust for history of STIs

5.3.7 Male circumcision

To further clarify the different associations of circumcision and a woman's risk for CIS or ICC, we stratified the analysis according to the male's sexual behaviour, similarly to a previous analysis of the present data (Castellsague *et al*, 2002) in order to support the evidence that the elevated risk of CIS or ICC linked to the absence of circumcision would be greater among women whose male partners were at a higher risk for HPV exposure.

Table 5.8 shows the association between circumcision and risk of CIS or ICC stratified by sexual behaviour characteristics of the men. Overall, women with uncircumcised men were at an increased risk of CIS (OR=2.67; 95% CI: 1.31-5.45), but not ICC (OR=1.09; 95% CI: 0.67-1.79). The positive association between uncircumcised men and the risk of CIS was stronger among women whose partners had a history of sexual intercourse with a sex worker, initiated sexual intercourse at an early age (≤16 years vs. 21 years), and more weakly associated with increasing sexual behaviour index with a four-fold risk among those with a high risk index. Although these positive associations between uncircumcised men and the risk of ICC were similarly observed when stratified by sexual behaviour characteristics of the husband, the ORs did not reach statistical significance. In the overall combined analysis of CIS/ICC, there was a moderate increased risk of marginal statistical significance (OR=1.43; 95% CI: 0.98-2.09) among women with uncircumcised men. Consistent to the CIS model, in the combined analysis, we observed an increased risk of CIS/ICC linked to the absence of circumcision when stratified by sexual behaviour characteristics, but the magnitude was slightly smaller as compared to the risks reported for CIS.

Table 5.8. Risk of cervical intraepithelial neoplasia (CIN-3)/carcinoma in situ (CIS) or invasive cervical cancer (ICC) among female partners of uncircumcised men, as compared with those of circumcised men, according to selected sexual behaviour characteristics of men

	CIN-3/CIS[a]		ICC[b]		Pooled CIN-3, CIS, ICC[c]	
	No. of circumcised/ uncircumcised	Odds ratio (95% CI)	No. of circumcised/ uncircumcised	Odds ratio (95% CI)	No. of circumcised/ uncircumcised	Odds ratio (95% CI)
Circumcision	51/582	**2.67 (1.31-5.45)**	318/960	1.09 (0.67-1.79)	369/1542	1.43 (0.98-2.09)
History of sexual intercourse with a sex worker						
Never	21/189	2.17 (0.60-7.87)	153/299	0.86 (0.37-2.01)	174/488	0.91 (0.49-1.69)
Ever	30/393	**2.59 (1.07-6.25)**	165/655	1.23 (0.66-2.29)	195/1048	**1.72 (1.06-2.80)**
Age at first sexual intercourse						
≥21	23/84	3.39 (0.73-15.84)	117/247	0.91 (0.33-2.52)	140/331	1.24 (0.63-2.46)
17-20	17/215	1.24 (0.40-3.87)	148/422	0.81 (0.40-1.62)	165/637	1.03 (0.60-1.77)
≤16	11/283	**5.12 (1.22-21.54)**	53/289	4.11 (0.82-20.54)	64/572	**4.20 (1.50-11.76)**
Sexual behaviour risk index[d]						
Low	24/141	1.75 (0.53-5.75)	146/297	0.63 (0.27-1.48)	170/438	0.88 (0.49-1.59)
Intermediate	17/179	2.40 (0.79-7.29)	129/389	1.29 (0.64-2.61)	146/568	1.57 (0.90-2.72)
High	10/260	4.13 (0.94-18.08)	40/253	6.66 (0.60-74.10)	50/513	**5.06 (1.55-16.56)**
Unknown	0/2		3/21			

[a] CIS multivariate model was adjusted for age of the husband and wife, study country, lifetime number of sexual partners of the husband and wife, husband's HPV status, and age at first sexual intercourse of the wife.

[b] ICC model for age of the husband and wife, study country, level of education of the husband and wife , lifetime number of sexual partners of the husband and wife, history of STIs of the husband, age at first sexual intercourse of the wife, lifetime smoking pack-years of the wife , oral contraceptive use, parity, and pap smear history 12 months prior to study enrollment.

[c] CIS+ICC pooled model was adjusted for age of the male and female subjects, study country, level of eduction of the male, age at first sexual intercourse of the male and ffemale, frequency of genital washing after sex of the male, lifetime number of sexual partner of the male. This is the same model that was previously published in

Castellsague X, NEJM 2002.

[d] High-risk men were those who had six or more sexual partners and who had first had intercourse before the age of 16 years. Low-risk men were those who had five or fewer sexual partners and who were at least 17 years of age when they first had intercourse. The remaining men were classified as being at intermediate risk. In the CIS/CIS, ICC, and pooled CIN-3/CIS/ICC models, age at first sexual intercourse of the male and lifetime number of sexual partners were not adjusted for in the final model.

5.3.8 Passive smoking

Table 5.9 shows the association between selected male smoking characteristics and cervical CIS and ICC in multivariate analyses. Generally, similar associations were identified in the two analyses of all couples and only couples with monogamous women, therefore we will describe our findings herein forward according to the all couples model. No statistically significant increased risk of CIS was observed for women whose partners had a history of smoking. An increasing risk of ICC was observed with decreasing time since smoking cessation with current smokers having the highest risk (OR=1.61; 95% CI: 1.16-2.24), suggesting passive smoking as a potential risk factor for cervical cancer. No increased risk of ICC was observed for women with male partners who used a specific tobacco type or filter. An increased risk of ICC was observed for women with partners who smoked at least a low number of smoking pack-years (OR=1.62; 95% CI: 1.14-2.29).

Table 5.9. Multivariate associations between selected male smoking characteristics and risk of cervical intraepithelial neoplasia grade 3 (CIN-3)/ carcinoma in situ (CIS) or invasive cervical cancer (ICC)

	Odds ratio (95% CI)	
Male characteristics	**All couples**	**Couples with monogamous women**
CIN-3/CIS[a]		
Time since smoking cessation		
Non-smoker	1.00	1.00
Ever smoker	1.33 (0.89-1.99)	1.16 (0.71-1.88)
Exsmoker >11 years	1.51 (0.53-4.31)	0.84 (0.22-3.19)
Exsmoker ≤10 years	1.17 (0.58-2.34)	0.67 (0.24-1.87)
Current smoker	1.36 (0.88-2.07)	1.26 (0.76-2.09)
Use of tobacco type by smokers		
Blond	1.00	1.00
Black	0.49 (0.23-1.06)	0.33 (0.12-0.88)
Both	0.49 (0.24-0.99)	0.36 (0.14-0.91)
Others	0.89 (0.12-6.39)	1.03 (0.07-15.53)
Use of smoking filter type by smokers		
Filter	1.00	1.00
No filter	0.76 (0.29-1.96)	0.49 (0.14-1.68)
Both	0.78 (0.45-1.32)	0.61 (0.31-1.18)
Others	1.63 (0.25-10.73)	2.79 (0.21-36.93)
Smoking lifetime pack-years [c]		
Non-smoker	1.00	1.00
Low no. of pack-years	1.18 (0.75-1.85)	0.89 (0.51-1.57)
Medium/High no. of pack-years	1.52 (0.93-2.47)	1.45 (0.53-2.56)
Invasive cervical cancer [b]		
Time since smoking cessation		
Non-smoker	1.00	1.00
Ever smoker	**1.57 (1.15-2.15)**	**1.55 (1.07-2.23)**
Exsmoker >11 years	1.46 (0.84-2.52)	1.11 (0.60-2.07)
Exsmoker ≤10 years	1.50 (0.95-2.37)	1.59 (0.95-2.67)
Current smoker	**1.61 (1.16-2.24)**	**1.63 (1.11-2.40)**
p-trend	0.006	0.01
Use of tobacco type by smokers		
Blond	1.00	1.00
Black	0.91 (0.27-3.04)	0.63 (0.17-2.33)
Both	1.42 (0.40-5.10)	0.83 (0.21-3.31)
Others	0.85 (0.32-2.27)	0.66 (0.21-2.08)
Use of smoking filter type by smokers		
Filter	1.00	1.00
No filter	1.34 (0.55-3.27)	0.95 (0.33-2.69)
Both	1.41 (0.72-2.72)	1.31 (0.63-2.71)
Others	0.98 (0.38-2.55)	0.83 (0.27-2.54)

Smoking lifetime pack-years [d]		
Non-smoker	1.00	1.00
Low no. of pack-years	**1.62 (1.14-2.29)**	**1.64 (1.09-2.47)**
Medium/High no. of pack-years	**1.48 (1.03-2.12)**	1.39 (0.91-2.10)

CI, confidence interval; STIs, sexually transmitted infections

a CIS multivariate model was adjusted for age of the husband and wife, study country, interaction terms (husband's lifetime number of sexual partners*study country and circumcision status*study country), age at first sexual intercourse of the wife (≥21 years, 17-20 years, ≤16 years), wife's lifetime number of sexual partners (1, ≥2); and husbands with monogamous women model was adjusted for all variables in the table except for circumcision, interaction term (husband's lifetime number of sexual partners*study country), age of the husband's wife and age at first sexual intercourse of the wife.

b ICC model for all husbands and husbands with monogamous women were adjusted for age of the husband and wife, study country, interaction term (husband's lifetime number of sexual partners*study country), level of education of the husband and wife (≥secondary level, primary level, no schooling), history of sexually transmitted infections, age at first sexual intercourse of the wife (≥21 years, 17-20 years, ≤16 years), lifetime smoking pack-years of the wife (non-smoker, low, medium, and high smoking lifetime-pack-years), oral contraceptive use (never, 1-4 years, ≥5 years), parity (nulliparous, 1-6, ≥7) pap smear history 12 months prior to study enrollment (never, ever), and lifetime number of sexual partners of the wife (1, ≥2).

c CIN-3/CIS model: smoking lifetime pack-years for the all husbands model is defined as low (36.5-6022.5 pack-years) and medium/high (6132-56611.5 pack-years); and for the husbands with monogamous women model, low (36.5-6716 pack-years) and medium/high (6825.5-38963.8 pack-years)

d ICC model: smoking lifetime pack-years for the all husbands model is defined as: low (36.5-7300 pack-years) medium/high (7354.8-43435 pack-years); and for the husbands with monogamous women model, low (36.5-7665 pack-years) medium/high (7829.3-43435 pack-years)

Table 5.10 shows the association between passive and active smoking history and risk of ICC after reclassifying smoking status of the husband according to Figure 1. As compared to the active smoking model, we did not observe an association between male smoking habits and risk of cervical cancer among couples with lifetime non-smoking monogamous women.

Table 5.10. Association between passive and active smoking history and risk of invasive cervical cancer after reclassification of smoking status of the husband

	Passive Smoking[a]		Active smoking[b]	
	Couples with non-smoking monogamous women		Couples with monogamous women	
	Cases/ Controls	Odds ratio (95% CI)[c]	Cases/ Controls	Odds ratio (95% CI)[c,d]
Smoking status				
Non-smoker	112/167	1.00	357/407	1.00
Ever	246/240	1.28 (0.88-1.85)	134/84	**1.77 (1.23-2.56)**
Ex-smoker	39/54	1.01 (0.56-1.83)	38/31	1.48 (0.83-2.65)
Current smoker	207/186	1.34 (0.91-1.96)	96/53	**1.94 (1.26-2.98)**
Duration of exposure to smoking				
0 years	112/167	1.00	357/407	1.00
1-20 years	101/108	1.51 (0.93-2.45)	52/35	1.58 (0.91-2.73)
≥21 years	137/124	1.13 (0.73-1.75)	43/30	1.56 (0.88-2.79)
No. of cigarettes per day				
Non-smoker	112/167	1.00	357/407	1.00
1-10 cigarettes/day	96/84	1.51 (0.95-2.39)	73/46	**1.63 (1.07-2.46)**
≥11 cigarettes/day	144/152	1.10 (0.73-1.66)	24/17	1.30 (0.91-1.84)
Smoking lifetime pack-years[e]				
Non-smoker	112/167	1.00	357/407	1.00
Low	77/81	1.44 (0.88-2.36)	29/24	0.56 (0.12-2.56)
Medium	72/78	1.12 (0.68-1.83)	35/18	4.14 (0.96-17.8)
High	84/70	1.25 (0.76-2.05)	31/21	0.55 (0.14-2.25)

CI, confidence interval

[a] For the passive smoking models, characteristics of the husband's smoking history were classified according to the wife's risk period of exposure to HPV and passive smoking as outlined in Figure 1.

[b] For the active smoking models, the risk of cervical cancer is based upon the woman's history of smoking

[c] Models were adjusted for age of the husband and wife, study country, level of education of the husband and wife, lifetime number of sexual partners of the husband, history of sexually transmitted infections, age at first sexual intercourse of the wife, oral contraceptive use, parity, and pap smear history 12 months prior to study enrollment.

[d] Husband's history of smoking (smoking status, duration of smoking, no. of cigarettes per day and smoking lifetime pack-years) were adjusted for in the final model accordingly to the woman's smoking habits.

[e] Husband's moking lifetime pack-years for the passive smoking model is defined as: low (36.5-3832.5 pack-years) medium (3942-7884 pack-years) and high (7938.75-67890 pack-years); and the women's smoking lifetime pack-years for the active smoking model is defined as: low (18.25-930.75 pack-years), medium (1022-3558.75 pack-years), and high (3577-19710 pack-years).

Although passive smoking was not independently associated with risk of ICC, there was an increased OR from 1.23 to 2.26 when women were exposed to passive smoking alone or to both active and passive smoking. The interaction term was however not statistically significant (p=0.77) (Table 5.11).

Table 5.11. Risk of cervical cancer according to the husband's and wive's smoking status among couples with monogamous women

	Cases/Controls	Odds ratio (95% CI)
Both non-smokers	112/167	1.00
Female non-smoker/Male ever-smoker	245/240	1.23 (0.85-1.77)
Female ever-smoker/Male non-smoker	30/27	1.63 (0.83-3.22)
Both ever-smokers	104/57	2.26 (1.40-3.64)
p-trend		0.001

5.4 Discussion

This study systematically reviewed the contribution of male factors in relation to cervical cancer risk. We found lifetime number of sexual partners to be the most consistent predictor of a woman's risk for CIS and ICC. This is not surprising as increasing lifetime number of sexual partners is a proxy measure of an increased probability of being exposed to HPV. Being positive for penile HPV and the absence of circumcision were significantly associated with an increased risk of CIS, whereas, lack of education and history of gonorrhoea were other male-related risk factors associated with an increased risk of ICC in the partner. In addition, a two-fold increased risk was found among couples with both ever smoking men and women. The difference in risk factors identified may reflect different stages of the natural history of cervical carcinogenesis with risk factors for CIS reflecting risk of exposure to HPV and risk factors for ICC reflecting risk of progressing to invasive disease.

Overall, since more than three-quarters of couples were with monogamous women, it is likely our results are a conservative evaluation of the male's role in cervical carcinogenesis. However, this conservative approach allowed us to explore the male risk factors in depth and to limit any potential residual confounding that may exist if women were not monogamous. In addition, our results may also represent societies in which women report lifetime monogamy and multiple partnerships are more common among men, which is a pattern that is generally seen in developing countries rather than in developed countries.

5.4.1 Risk factors of CIS

Although the absence of circumcision did not appear to be an independent male risk factor for ICC, we cannot exclude its contribution to the risk of cervical cancer by acting at the early stages of the oncogenic process, namely HPV acquisition. The strong positive association with CIS may likely reflect the early events of exposure and acquisition since prospective cohort studies have suggested that the time lag between infection to high-grade precancerous lesions could be within a few months of incident infection in prospective studies and estimated up to 7-15 years in cross-sectional studies (Moscicki *et al*, 2006). Once a woman has been exposed to infection and progressed to CIS, it is unlikely that circumcision status of the partner (which remains unchanged) at that stage would

affect a women's potential to progress to invasive cervical disease, suggesting other exogenous cofactors are involved in the disease process, which are discussed later.

Recent evidence suggests that circumcision may decrease the acquisition of high-risk (HR) oncogenic HPV and multiple HR-HPV infections (Gray *et al*, 2010). As seen in our data, the highest risk of CIS was among women with uncircumcised men who had high-risk sexual behaviour. In addition, two studies have shown a reduced clearance of penile HPV infection in uncircumcised men (Gray *et al*, 2010; Hernandez *et al*, 2010), increasing the probability of harbouring penile HPV infection and exposing their female partners; thus, explaining the high HPV prevalence observed among uncircumcised men compared to circumcised men (8.3% vs. 2%, respectively) in our study. The biological mechanisms by which male circumcision plays an important role in modulating HPV risk is likely multi-factorial and hypothesized to include post-circumcision anatomical, immunological, and microbiological changes. One recent study suggests that circumcision could decrease the amount of bacterial microenviroment that may otherwise exist under the foreskin, mediate genital mucosal inflammation and coinfections of STIs; therefore, the loss of the foreskin inner mucosa may help reduce the risk of HPV acquisition in circumcised men (Price *et al*, 2010). These results collectively support conclusions that male circumcision may be protective against HPV infections. The World Health Organization (WHO) has already recommended male circumcision as an important intervention to reduce the risk of HIV acquisition in heterosexual men, and this could in effect provide additional prevention against transmission of HPV (Averbach *et al*, 2010; Smith *et al*, 2010; WHO, 2007b) particularly when some data now suggest that HPV infections may also increase the likelihood of HIV acquisition (Averbach *et al*, 2010; Smith *et al*, 2010).

5.4.2 Risk factors for ICC

The consistent finding of low education of the woman and of the male partner as a risk factor for ICC (Franceschi *et al*, 2009), may be explained by similar patterns of mixing with partners of similar socioeconomic (SES) (Burchell *et al*, 2006) standing, as the majority of wives were with husbands who had obtained either the same or lower education level; for example, 96% of men with

primary level schooling were with wives who had equal or less schooling. The woman's level of education and early age at first sexual intercourse strongly attenuated a substantial fraction of the husband's education gradient on cervical cancer risk in our study (data not shown), which could explain this excess risk of ICC (Franceschi *et al*, 2009; Louie *et al*, 2009a).

In this study, information on time of diagnosis of *N. gonorrhoea* was not available, so the temporal sequence of infection with HPV or *N. gonorrhoea* could not be established. Data suggest that there is a lack of innate and adaptive immune response to induce a protective response when exposed to *N. gonorrhoea* infection, suggesting that co-infection with HPV could impair subsequent immune responses, favouring persistence rather than clearance of HPV (Sheung *et al*, 2008; Song *et al*, 2008; Sparling, 2008). Therefore, concomitant infection with HPV could potentially be responsible for persistent infection and cervical carcinogenesis, and could be further complicated by high recurrence of gonorrhoea. Consequently, this would also increase the probability of transmission of both HPV and *N. gonorrhoea* in their sexual partners. Although misreporting of self-history of gonorrhoea cannot be excluded, among those who reported gonorrhoea and had an adequate HPV test result, 19.9% were positive for penile HPV infection. In this study, 39 ICC couples both self-reported history of gonorrhoea, of whom 61.5% (n=24) were couples with monogamous women. Conversely, if women with persistent HPV infection were subsequently infected with *N. gonorrhoea,* it could be speculated that the association in this study identifies *N. gonorrhoea* as a risk factor that modulates the oncogenic potential of persistent infection to progress to invasive disease. Studies are needed in prospective cohorts to better understand the mechanisms of *N. gonorrhoea* to modulate the natural history of HPV infection.

5.4.3 Penile HPV detection

The interpretation of the lack of association between penile HPV prevalence, male behaviour and a women's risk of ICC are difficult to elucidate from our data. Firstly, we cannot rule out the contribution of penile HPV to a woman's risk of ICC as detection of HPV at study enrolment does not necessarily represent the time-point of exposure as the current understanding of the natural history of HPV in men shows that HPV is more readily transmitted from men to

women than from women to men, and these infections are less likely to persist among men with approximately 75% likely to clear infection at one year (Giuliano *et al*, 2008). In addition, we cannot exclude the possibility of reverse causality since HPV-infected husbands could clear HPV, and be re-infected by their wives who have cervical cancer and have been replicating HPV prior to the onset of cancer. Among 116 ICC case husbands who reported no history of sex with a sex worker or a casual partner while with their wife, 6 were HPV-positive (of whom 5 women reported lifetime monogamy) making it impossible to know who the source of HPV exposure came from if there was no underreporting. Secondly, detection of penile HPV DNA (17%) in our study was lower than recently reported prevalence estimates in men and this may result from incomplete sampling of the male genitalia as it has been suggested that for optimal HPV detection, sampling should include multiple anatomic sites of the penile shaft, glans penis/coronal sulcus, scrotum, anus, or perianus with optimal detection found in the penile shaft (Nielson *et al*, 2007). Although we did not sample the scrotum nor the penile shaft, we believe our samples from the coronal sulcus and glans penis adequately assessed HPV status as these two sites are in direct contact with the cervix.

5.4.4 Passive Smoking

This study showed no independent association of passive smoking and risk of cervical cancer in the absence of active smoking. In the first two models of all couples and couples with monogamous women, the lack of association with CIS and the significant association with ICC suggests that passive cigarette smoking could potentially acts as a late carcinogen in the transition from persistent infection/pre-invasive lesions to invasion. These findings are not new and are consistent with previous findings (IARC, 2004; Tay & Tay, 2004; Trimble *et al*, 2005; U.S. Dept. of Health and Human Services & National Center for Chronic Disease Prevention and Health Promotion, 2006). However, when we considered the possibility of misclassification bias in our third model of couples with lifetime non-smoking monogamous women and reclassified the men's smoking status according to the risk period for which the woman would be exposed to both HPV infection and passive smoking, no independent association could be found. The greatest risk estimate was more than two-fold for couples who were both ever-smokers.

5.4.4.1 Misclassification of smoking status

The contradicting results as shown in the different models highlight the distortion of estimates probably resulting from misclassification of smoking status. This suggests that a model considering only the time period of exposure to HPV and passive smoking should be used to determine susceptibility to carcinogenesis. The timing of exposure to tobacco smoke relative to cervical cancer development is important in defining exposure. Since we had detailed information on smoking and sexual history we were able to define and calculate exposure based on a series of responses. The strict definitions of exposure to tobacco smoke in our analyses showed associations with risk of cervical cancer that were obscured by using simpler definitions. Non-smoking monogamous women with men classified as ex-smokers who have quit smoking prior to initiating a sexual relationship may not be as susceptible to passive smoking. In addition, the men's lifetime duration of smoking does not necessarily include the whole period of the couple's relationship if he stops smoking during the relationship or he starts and stops smoking during the relationship. Although the possibility of misclassification of the women's smoking status cannot be excluded, we do not believe inclusion of non-smokers who were actually true smokers would cause substantial bias since female smoking prevalence in these study countries is low (WHO, 2009b). In epidemiologic studies of cervical cancer etiology, the definitions of exposure should reflect a model of risk to HPV infection and cervical carcinogenesis.

5.4.4.2 Strength of evidence for passive smoking

This study strengthens the current evidence for several reasons. First, this study has the largest dataset of couples with non-smoking women to measure passive smoking. Since our study obtained direct information from interviews with both the husband and wife, our results are considered reliable as previous studies have found good agreement in responses concerning spousal smoking status to range from 90 to 100 percent (U.S. Dept. of Health and Human Services & National Center for Chronic Disease Prevention and Health Promotion, 2006) and previous cotinine studies of never-smokers have validated the use of spousal history as a marker of exposure to tobacco smoke and people who live with smokers tend to mix with smokers outside the home (IARC, 2004). In contrast, previous studies had small sample sizes with small numbers of non-smokers.

Secondly, previous studies lacked adequate information on HPV and sexual behaviour indicators to control for potential confounding, and we were able to control for both male and female risk factors. Thirdly, as we currently understand the natural history of cervical cancer, not all pre-cancerous lesions will progress to invasive cervical cancer (McCredie *et al*, 2008), we were able to evaluate the effect of passive smoking by stage of disease (pre-invasive vs. invasive).

Previous studies did not evaluate the combined effects of different exposure of active and passive smoke (non-smokers, female ever-smoker/male non-smoker, and female non-smoker/male ever-smoker). Although the other combinations showed an increased risk, only the combination of ever-smoking couples showed a statistically significant increased risk. The lack of an independent association with passive smoking does not necessarily discount its contribution to ICC risk. This may suggest that the direct effect of active smoking outweighs the indirect carcinogenic effects passive smoking may have. One of the limitations of epidemiological studies using questionnaire data is its decrease in sensitivity or power of a study to show a positive association when the effect may only be moderately related to passive smoking (U.S. Dept. of Health and Human Services & National Center for Chronic Disease Prevention and Health Promotion, 2006). In addition, lifetime number of sexual partners of the men largely attenuated the observed effect of passive smoking on ICC risk.

5.4.4.3 Biological plausibility of passive smoking

A biological mechanism by which active and passive smoking could influence cervical carcinogenesis is not clearly understood. However, tobacco smoke contains known carcinogens such as polycyclic aromatic hydrocarbons that could potentially have a direct transformation effect on the cervix or could cause immunosuppression, allowing HPV infections to persist and progress to cancer (IARC, 2004). Detectable levels of nicotine and cotinine, a measurement of smoke exposure, have been found in cervical mucus as well as DNA adduct levels in the cervical epithelium of non-smokers, supporting the evidence that these chemicals can reach distant sites such as the cervix (National Cancer Institute, 1999). Another hypothesis includes mutagenic semen due to smoking is plausible and direct cervical contact with semen of smoking partners may represent another source of exposure (U.S. Dept. of Health and Human Services & National Center for Chronic Disease Prevention and Health Promotion, 2006). This study lacked data measurement levels of cotinine/nicotine in the cervix, therefore, additional studies are needed to obtain these data to complement our epidemiological findings.

Penile HPV detection was more prevalent among current smokers compared to ex-smokers and non-smokers which is consistent to previous findings (Vardas *et al*, 2011). This suggests that smokers may be more likely to have persistent infections compared to non-smokers, making them more likely to expose their wives to HPV infection. However, the interpretation of penile HPV detection at study enrolment is not straightforward (as discussed in Section 5.4.3). Other studies have not found smoking to be associated with penile HPV acquisition nor persistence (Kjaer *et al*, 2005; Lu *et al*, 2009).

5.4.4.4 Social implications of passive smoking

There are 1 billion active smokers worldwide and one third of adults are regularly exposed to passive smoke with the burden of tobacco-related disease, disability and death being the highest in developing regions. Moreover, the rate of increase in cigarette consumption in developing countries is ten times that of industrialised countries (WHO, 2010). This burden is likely to increase in the coming decades if current trends persist with more than 90% of the world's population not protected by comprehensive smoke-free policies and there is low

compliance (2%) in countries where there are comprehensive smoke-free laws (WHO, 2009b). Globally, there is an increasing trend of females aged 13-15 smoking in recent years (WHO, 2010), which needs to be considered along with reported median age at first sexual intercourse to occur for most women is 15- 19 years (Wellings *et al*, 2006) when assessing risk of ICC. The data presented here support that in addition to female tobacco smoking as an established cofactor for cervical carcinogenesis, there is a potential role of passive smoke on ICC, which suggest that the estimated burden of tobacco-related diseases may increase and magnify the need for effective tobacco control, notably in developing countries.

5.5 Conclusions

In conclusion, male circumcision could be an effective prevention measure against CIS, particularly in developing countries, where HPV vaccines are predominantly unavailable and cervical screening is largely ineffective or non-existent. The burden of tobacco-related diseases is projected to increase dramatically in the developing world with increasing rates of exposure to active and passive smoking. If more sufficient evidence confirms the causation of passive smoke on cervical cancer, the estimated burden of tobacco-related diseases will only increase, magnifying the need for both effective tobacco and cervical cancer control.

6 Overall discussion and implications

6.1 Introduction

This thesis presents a set of robust analyses to evaluate the associations of sexual and reproductive health (SRH) risk factors and risk of cervical cancer in developing countries. The culmination of work involved the use of the best available data possible to investigate the natural history of human papillomavirus (HPV) and invasive cervical cancer (ICC) with a range of different statistical method approaches to fully characterise and address the inconclusive SRH associations with ICC risk. The IARC/ICO series of case-control studies is the largest set of aetiological investigations that fully addresses the role of HPV DNA and of the independent established cofactors with a standardised protocol (*Study 1 and 3)*. In addition, these investigations were conducted in developing countries where ICC burden is the highest and data are often limited. Similarly, the Guanacaste Study was the largest population-based longitudinal cohort study of HPV and cervical neoplasia in the world (*Study 2)*. The intense effort to systematically evaluate and follow-up 10,000 women over a period of 7-years provided a richness of data that would allow time-dependent assessment of factors to be linked to different stages of cervical carcinogenesis, specifically, HPV persistence. In aggregate, these two datasets provide an opportunity to fully evaluate SRH factors throughout the natural history of HPV from stages of acquisition of infection, to persistence, cervical neoplasia and cervical cancer.

Each chapter for *Study 1* (**Chapter 3**), *Study 2* (**Chapter 4**), *and Study 3* (**Chapter 5**) has included its own individual discussion. This chapter will highlight key results and present a general discussion about the findings in this thesis. The implications of this research for primary and secondary cervical cancer prevention will also be discussed as well as provide some foresight into future research directions.

6.2 Did the thesis address the hypothesis?

This thesis aimed to evaluate the associations of SRH factors and risk of cervical cancer. One of the main hypotheses was that early age at first sexual intercourse (AFSI) and first pregnancy (AFP) are associated with cervical cancer, placing an HPV-positive woman at higher susceptibility for HPV persistence and greater risk of cervical cancer development. This research confirmed AFSI and

AFP as independent risk factors for cervical cancer, irrespective of other established cofactors (*Study 1*). However, any independent effects of these two events could not be distinguished. There is a possible additional increase in risk when early initiation of first sex (≤15 years) is followed by a pregnancy. The mechanisms of these associations may include the immature cervix and increased levels of exposure to hormonal (oestrogens) levels during adolescence and pregnancy that may facilitate HPV persistence. In order to confirm these findings in a cohort study to complete another stage in the natural history picture of HPV from acquisition to persistence, it was hypothesised that these SRH factors would be confirmed in a longitudinal analysis.

Research presented in *Study 2* showed that there was an increasing risk of 2-year persistence with decreasing age at first pregnancy among prevalent HPV-16/18 infections. The highest risk was found among women with AFP ≤15 years. These results provide additional evidence of the contributing role of early AFP and its affect on cervical cancer risk. Findings also support the different natural histories that may exist for type-specific HPV infections. HPV-16 is the most prevalent type worldwide and presents the greatest magnitude of risk of progression to cervical cancer if persistent. Interestingly, ectopic pregnancies also appeared to show a protective effect against HPV persistence. It is possible that luteal phase deficiency is causing these ectopic pregnancies which impairs ovarian stimulation and disrupts the efficiency of HPV to persist and cause cervical metaplastic changes and neoplasia. This highlights the increasing evidence of the role of female sex hormones in cervical carcinogenesis.

Another hypothesis of this thesis was that a woman's risk of cervical cancer depended less on her sexual behaviour than on that of her husband or male partner. Women who initiate sexual intercourse during adolescence tend to have older male sexual partners and men report more lifetime number of sexual partners than women who are predominantly monogamous. This research identified the age gap difference (≥8 years) between couples was an independent predictor of risk of ICC, particularly among girls with AFSI ≤16 years (**Chapter 3**). Sexual behaviour patterns suggest that men are more likely to be the source of STI transmission in marital relationships, which is particularly relevant in developing countries where most women are virgins at marriage and remain

lifetime monogamous. Although there is evidence of rapid change in sexual behaviour (i.e. pre-marital sex), these changes are more notably in developed countries (Wellings *et al*, 2006).

Increasing lifetime number of sexual partners of the male partner was a predictor of CIS and ICC risk depending on country, which is characterised by the varying sexual behaviour patterns in the population. This finding was not surprising as increasing lifetime number of sexual partners is a proxy of increase probability of being exposed to HPV. Being HPV DNA positive in penile specimens and the absence of circumcision were significantly associated with an increased risk of CIS; whereas lack of education, having a history of gonorrhoea were other male-related risk factors associated with ICC. Besides the husband's sexual behaviour, other lifestyle behaviour, in the context of couples, identified a two-fold increased risk of ICC among couples with both ever smoking men and women. Passive smoking was not identified as an independent risk factor cervical cancer; however, the combined effects of active and passive smoking suggest its potential adverse role in cervical carcinogenesis. The difference in male factor profiles for risk of CIS and ICC may reflect different steps of the natural history of cervical carcinogenesis with risk factors for CIS reflecting risk of exposure to HPV and risk factors for ICC reflecting risk of progressing to invasive disease.

6.3 Natural history of HPV and cervical cancer

There is now a dearth of research demonstrating clear aetiological associations between SRH factors and risk of cervical cancer. As a few expert HPV etiologists (Schiffman *et al*, 2011) suggest, "We do not need to question the sexual correlates of HPV infection" since it is well-established that infection with HPV is common and sexually transmitted. Furthermore, "depending on regional social/sexual practices, low education and age at first sexual intercourse can plausibly be linked to higher risk of acquisition. Apart from sociologic analyses, as etiologists, we do not need to question the sexual correlates of HPV infection." The research in this thesis did not question the plausible link of these SRH factors with HPV infection. Rather this research tried to clarify how the role of these factors contributed throughout the natural history of HPV.

This thesis confirmed the aetiological association of SRH factors with cervical cancer in large pooled analyses that provide more conclusive agreement

than previously understood. These specific pooled analyses with a common protocol has also allowed us to study the role of each AFSI and AFP with finer detail than previously possible and compared to other pooled analyses with differing protocols and data collected (International Collaboration of Epidemiological Studies of Cervical Cancer, 2009). A unique aspect of the research presented was the separation of risk factors associated with critical transition states in the natural history of HPV and cervical cancer - persistence, pre-cancer and invasive disease. A summary of factors consistently reported to play a role at different stages in the natural history of HPV and cervical neoplasia has been presented previously (Moscicki *et al*, 2006). Additional SRH factors identified in this thesis are highlighted in Figure 6.1.

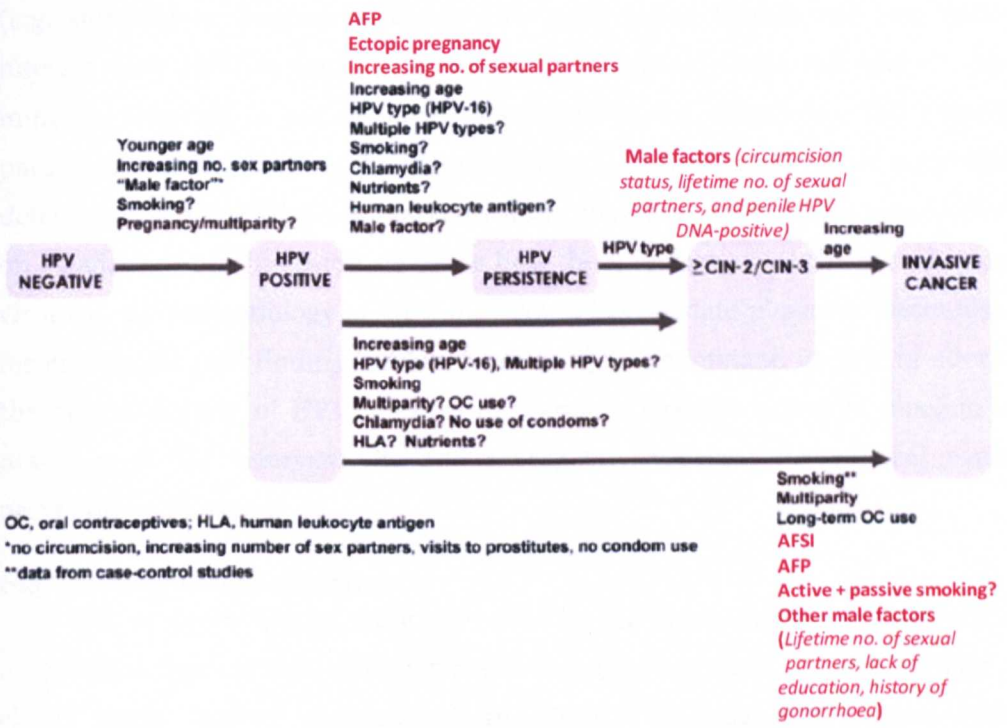


Figure 6.1 Overview of factors reported to play a role at different stages of the natural history of HPV and cervical neoplsia and additional sexual and reproductive health factors (red) identified in this thesis. Age at first pregnancy (AFP) and increasing lifetime number of sexual partners were associated with 2-year HPV persistence; and ectopic pregnancy showed a protective effect from HPV persistence. Male-related factors, absence of male circumcision, increasing lifetime number of sexual partners, and penile HPV-positivity were associated with carcinoma in situ. Age at first sexual intercourse (AFSI) and AFP were associated with invasive cervical cancer (ICC). Combined effects of active and passive smoking suggest an increased risk of ICC. Other male-related factors, lifetime no. of sexual partners, lack of education and history of gonorrhoea, were also associated with ICC. Adapted from (Moscicki *et al*, 2006).

No new studies regarding sexual correlates with HPV infection may be needed but ongoing pooled analyses such as the ones presented in this thesis will continue to provide relevant information and elucidate other remaining questions of the natural history. These research results have raised biological hypotheses from these epidemiological findings as well as support the increasing role of female sex hormones in the role of cervical carcinogenesis. Recent publications on time since first sexual intercourse (Plummer *et al*, 2011) and the contribution of endogenous sex steroids (Rinaldi *et al*, 2011) on the risk of cervical cancer, underscores that there are still remaining questions about the role of AFSI and AFP but they are in the process of being clarified.

Moreover, additional questions remain about the molecular mechanisms (e.g. steroidal hormones) by which SRH behavioural factors and host factors interact with HPV to cause progression to invasive disease and why the host immune response is not effective in controlling the infection. The research paradigm of understanding the natural history is shifting. Rather than addressing determinants of HPV infection and cervical cancer, researchers are now working on providing scientific evidence about how these factors affect the basic biology, virology, and immunology of HPV in order i) to elucidate plausible mechanisms for epidemiological findings and vice versa; ii) to understand its role in altering the natural history of HPV; and iii) to therefore feed the potential pipeline for new targets for interventions and therapeutic vaccines for cervical cancer prevention and control.

6.4 Methodological issues

This research was partially motivated by the inconsistent results identified in previous studies and different methodological approaches were applied to clarify these varying associations. In *Study 1* **(Chapter 3)**, three HPV-adjustment models (HPV unadjusted, HPV-adjusted, and restricted to HPV DNA positive cases and controls) were constructed to estimate the impact of these different strategies on associations between SRH factors and cervical cancer risk. Comparison of these methods have been applied previously on evaluating associations between parity, oral contraceptive use and smoking and ICC risk (Castellsague & Munoz, 2003). Similarly, regardless of the adjustment method used, the same conclusion regarding the direction of association and statistically significant association were found. Of the three adjustment methods, because

HPV is a necessary cause for cervical cancer, it was originally argued that the HPV-restricted model is a better strategy than the other two methods as it was a strict approach to getting rid of residual confounding. The other strategies would in effect underestimate the magnitude of the associations.

However, as a counter argument, adjusting for HPV status is not necessary as the adjustments do not contribute to removing any confounding effect. Nearly all cases of ICC are positive for HPV DNA (Bosch *et al*, 2002). Cases that are HPV-negative are believed to be associated with the limitations of HPV detection methodologies. Adjusting for HPV when it is assumed to be ubiquitous in all cases of ICC is unnecessary. In addition, restriction to only HPV-positive cases and controls is not appropriate as detection of HPV at one time-point for controls does not necessarily represent the same time–point in which a case was exposed and acquired infection.

In *Study 3* **(Chapter 5)**, to better clarify the relationship between passive smoking characteristics and cervical cancer in their female partners, three different models were computed to include all couples, couples with monogamous women and a third model included couples with lifetime non-smoking monogamous women. In the third model, we were able to remove the potential effect of previous male partners and the effect of the woman's active smoking behaviour. Furthermore, the men's smoking history was reclassified to reflect the risk period for which the woman would have been exposed to HPV infection, passive smoking and risk of progression to cervical cancer. Ninety male ex-smokers of couples with monogamous women were reclassified and the duration of exposure to passive smoke and smoking pack-years were recalculated. The contradicting results in previous studies and as shown in the different models compared in this study highlight the distortion of estimates probably resulting from misclassification of smoking status. Since the objective was to explore associations between cofactors among HPV-exposed women, a model considering only the time period of exposure to HPV and passive smoking should be used to determine the susceptibility to cervical carcinogenesis.

6.5 Public health implications

Well-characterisation of SRH factors and other cofactors in the natural history of HPV and cervical cancer has led to the development of primary and

secondary cervical cancer prevention tools. Stakeholders working in cervical cancer prevention need to integrate our understanding of these SRH behaviour patterns in monitoring and implementing effective prevention programmes. It is also important to understand how these SRH behaviour factors fit into the broader social and cultural context and contribute to the burden of cervical cancer. Much of the work that lies ahead is utilising what is understood about cervical cancer and operationalising the tools that exist for cervical cancer control.

6.5.1 Monitoring sexual behaviour and HPV prevalence in general populations

Aetiological studies on sexual behaviour may not be needed, but ongoing surveys on sexual behaviour trends and the prevalence of HPV are needed to assess interventions, particularly in the era of HPV vaccination. Since HPV infection often occurs soon after first sexual intercourse, AFSI is a reasonable proxy measure of exposure. Similar to UNAIDS using AFSI as one of the indicators for highlighting prevention priorities for HIV, it could be similarly used for prevention of HPV and cervical cancer. The WHO/ICO Information Centre on HPV and Cervical Cancer has aggregated available data to summarise the burden of HPV infection and cervical cancer and IARC has conducted a large number of population-based HPV prevalence surveys carried out mainly in less developed countries with unscreened populations covering four continents. These data will be useful in providing baseline data from the pre-vaccination era. Additional HPV surveillance studies should be carried out in the post-vaccination era to measure the long-term impact of vaccination on the burden of cervical cancer in order for each country to consider the most cost-effective prevention strategy (i.e. vaccination alone, vaccination plus cervical screening albeit at different screening intervals, or cervical screening alone). Repeated measurements over time of these SRH indicators and of HPV prevalence will inform policy makers on progress of reducing the burden of cervical cancer.

From another perspective, the global variation of sexual behaviour (Wellings *et al*, 2006) and HPV prevalence (Bruni *et al*, 2010) has been used to explain not only the heterogeneity of cervical cancer rates between developed vs. developing countries but also heterogeneity within developing regions (Louie *et al*, 2011). It has been shown that the global variability among adolescents first

initiating sexual activity is influenced by many factors that include economic inequalities (Nahmias & Nahmias, 2011). Patterns of early AFSI and AFP (Louie *et al*, 2009a) and low education (de Sanjose *et al*, 1997; Franceschi *et al*, 2009) highlight the existing social inequalities that may also need to be addressed when planning prevention programmes.

6.5.2 Primary prevention of cervical cancer

The availability of prophylactic HPV vaccines gives new promises for a primary prevention strategy, particularly in developing countries where lack of infrastructure and resources have been able to provide none or ineffective cervical screening programmes. The risks associated with early AFSI and AFP, two highly prevalent events in developing countries highlight the urgency to provide access to vaccines for girls prior to sexual debut. There is additional evidence that first sexual intercourse for very young girls in developing countries is forced, and in a vulnerable sub-population of adolescent girls sexual coercion can be as high as 40% (Brown *et al*, 2001), placing young women at great physical vulnerability. Moreover, it is likely that the partners of these adolescents who report sexual coercion are sexually experienced and at high risk for HPV infection.

The bivalent and quadrivalent vaccines have shown ≥95% efficacy in preventing vaccine HPV types and related cervical neoplasia (Schiller *et al*, 2008); and more recently, clinical trials have shown that the HPV quadrivalent vaccine to be 85% efficacious in preventing HPV persistent infections in boys aged 16-26 (Giuliano *et al*, 2011). Inclusion of boys into routine immunisation programmes is under high debate. At the individual-level, vaccination of boys would offer herd immunity at the population level as well as reduce their own risk of HPV-related morbidities, such as genital warts and other related neoplasias at the anus (Palefsky *et al*, 2011) and possibly also the penis. There is still a question about the magnitude of herd immunity that would be conferred to girls if boys were also vaccinated. The cost-effectiveness of including males and the additional reduction of morbidity and mortality gained as a result of vaccinating both females and males remain unclear.

The opportunity of these vaccines to have an effective impact in developing countries, where the burden is the highest, will not be realised until they become affordable and integrated within the framework of national immunisation

programmes (Kane *et al*, 2006). WHO recommends vaccination of girls aged 9-13 years through national immunisation programmes where cervical cancer is considered a public health priority and where vaccine introduction is feasible and finance can be secured, sustained and be considered cost-effective (WHO, 2009a).

The GAVI Alliance, a public-private organisation dedicated to increase children's access to vaccines in developing countries, recently committed to supporting the introduction of HPV vaccines as well as received a commitment from the quadrivalent HPV vaccine manufacturing company, Merck & Co Inc., to offer the HPV vaccine at US$5 per dose. GAVI can offer the vaccine at a subsidised price to the world's poorest countries, increasing the likelihood of reaching the girls most at risk for HPV.

6.5.3 Secondary prevention of cervical cancer

Cytology screening programmes have been successful in curbing the incidence of ICC in developed countries but they have largely failed in most developing countries due to other competing health priorities and lack of resources including health infrastructures (trained cytotechnologists, laboratories, and services for diagnosis and treatment), human and financial resources (Denny *et al*, 2006; Sankaranarayanan *et al*, 2001).

VIA (visual inspection with acetic acid) or VILI (visual inspection with Lugo's iodine) are less laboratory dependent strategies and have been advocated as screening alternatives in developing countries. However, visual inspection methods are highly subjective and require good provider training and sustained quality assurance in order to achieve substantial gains in prevention of cervical cancer in routine settings (Cuzick *et al*, 2008).

A more objective and reproducible screening test is testing for HPV DNA that has shown to be more sensitive than cervical cytology to detect high-grade lesions (Dillner *et al*, 2008). A rapid HPV DNA test that can provide a patient with a result within the same visit would be the most effective approach to impact low resource settings where loss to follow-up is often the primary factor for an ineffective screening programme. A number of inexpensive rapid HPV DNA screening technologies have been under development and evaluation but it is unclear when these tests will become commercially available (Gravitt *et al*, 2008).

Results from *Study 2* (**Chapter 4**) provided additional evidence of the different type-specific risk of HPV persistence and of natural histories. These results corroborate with other studies that identified HPV-16/18 infections to be at greater risk for HPV persistence and for cancer development (Chen *et al*, 2011; Khan *et al*, 2005). Different screening technologies that discriminate for HPV-16/18 and other HR-HPV are under evaluation and may offer a greater positive predictive value of risk for cervical neoplasia; have the potential to increase specificity; and provide an objective output (Gravitt *et al*, 2008). A number of technologies suggested for screening management may include HPV genotyping, measuring HPV-16 viral load, and p16 enzyme linked immunosorbent assay. However, many of these technologies are currently highly complex, expensive, and require high quality control laboratories to perform these tests; and therefore, not appropriate for low-resource settings unless these tests become inexpensive, were able to provide results at the same time as the screening visit and easy to perform.

6.5.4 Other cervical cancer prevention measures

In settings where cervical cancer control with primary (vaccination) and secondary (screening) prevention measures is not possible, male circumcision could offer an alternative prevention strategy to reducing a woman's risk of cervical cancer. *Study 3* (**Chapter 5**) showed that the absence of circumcision increased a woman's risk of CIS. A recent systematic review and meta-analysis showed that circumcised men are at substantially lower risk of prevalent HPV infections than uncircumcised men (Larke *et al*, 2011). Also, data from randomised-controlled clinical trials have shown that a substantial reduction in risk among female partners becoming infected with HR-HPV was found among couples with circumcised men (Wawer *et al*, 2011). Circumcision services are being expanded in sub-Saharan Africa as an HIV prevention strategy and may represent an additional opportunity to reduce HPV as well as HIV in men (Larke *et al*, 2011). Approximately 30% of the world's male population ≥15 years are circumcised (WHO, 2007a). Circumcision could be another intervention to reduce HPV infection in men and reduce a woman's risk of HPV acquisition. Additional research is needed to evaluate strategies to integrate male circumcision services in the broader context of STI prevention and cervical cancer control.

6.6 Conclusions

In summary, cervical cancer ranks as the second most common cancer among women in the developing world. The problem disproportionately affects women most vulnerable to early age at first sexual intercourse and first pregnancy and high parity. The investigations presented in this thesis show that AFSI and AFP are not only linked to age at first HPV exposure but also linked to the natural history of cervical carcinogenesis. The role of men contributing to a woman's risk cannot be underestimated. The uncertainties of the role of these SRH factors have been clarified through this body of work that evaluated different assumptions on methodologies that may have previously provided inconsistent results. The evidence presented can conclude with confidence that these sexual and reproductive health behaviour and lifestyle factors (e.g. active and passive smoking) can plausibly drive the biological mechanisms of HPV and the carcinogenic pathway. The general steps of this pathway are understood but there are still fine details (e.g. role of oestrogens) that need to be revealed and these investigations are underway.

7 Dissemination of research findings

7.1 Conference presentations

7.1.1 The literature review has been presented as a poster presentation
Louie KS, de Sanjose S, Mayaud P. The under prioritised burden of cervical cancer in sub-Saharan Africa. Programs and Abstracts of the 25th International Papillomavirus Conference and Clinical Workshop; May 8-14, 2009; Malmo, Sweden. Abstract P-30.33.

7.1.2 Study 1 results have been presented as a poster presentation
Louie KS, de Sanjose, Diaz M, Castellsagué X, Herrero R, Meijer CJL, Shah K, Franceschi S, Muñoz N, Bosch FX. Sexual debut and pregnancy are risk factors for cervical cancer. Programs and Abstracts of the 25th International Papillomavirus Conference and Clinical Workshop; May 8-14, 2009; Malmo, Sweden. Abstract P-30.09.

7.1.3 Study 3 results have been presented as poster presentation
Louie KS, Castellsague X, de Sanjose S, Herrero R, Meijer CJ, Shah K, Munoz N, Bosch FX, for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Smoking and passive smoking in cervical cancer risk. Programs and Abstracts of the 27th International Papillomavirus Conference and Clinical Workshop; September 17-22, 2011; Berlin, Germany

7.1.4 Extended research
Findings from this thesis research have initiated further research into the subject of sexual and reproductive risk factors and risk of cervical cancer for which I am involved. Results have been presented as poster presentations at the following conferences.

Louie KS, Pineda S, Castanon A, Almonte M. Sexual behaviour factors and cervical cancer rates in Latin America. Programs and Abstracts of the 27th International Papillomavirus Conference and Clinical Workshop; September 17-22, 2011; Berlin, Germany (Oral Poster Presentation)

Ferrer E, **Louie KS**, Bruni L, Diaz M, Albero G, Muñoz J, de Sanjose S, Bosch FX, Castellsague X. Systematic review: cervical cancer awareness in sub-Saharan África. Programs and Abstracts of the 25th International Papillomavirus Conference and Clinical Workshop; May 8-14, 2009; Malmo, Sweden. Abstract P-22.27.

Louie KS, Albero G, Muñoz J, Bruni L, Ferrer E, Diaz M, Castellsagué, Bosch FX, de Sanjose S. HIV, fertility and cervical cancer rates in Africa. Programs and Abstracts of the 24th International Papillomavirus Conference and Clinical Workshop; November 3-9, 2007; Beijing China. Abstract PS8-21.

7.2 Peer-reviewed publications

7.2.1 Literature review

Louie KS, de Sanjose, Mayaud P. The burden of human papillomavirus (HPV) infection and cervical cancer in sub-Saharan Africa: prevention opportunities and challenges. Tropical Medicine & International Health 2009; 14 (10): 1287-1302.

7.2.2 Study 1

Louie KS, de Sanjose, Diaz M, Castellsagué, Herrero R, Meijer CJL, Shah K, Franceschi S, Muñoz N, Bosch F, for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Early age at first sexual intercourse and early pregnancy are risk factors for cervical cancer in developing countries. British Journal of Cancer 2009; 100 (7):1191-7.

7.2.3 Study 3

Louie KS, Castellsague X, de Sanjose S, Herrero R, Meijer CJ, Shah K, Munoz N, Bosch FX, for the International Agency for Research on Cancer Multicenter Cervical Cancer Study Group. Smoking and passive smoking in cervical cancer risk: pooled analysis of couples from the IARC multi-centric case control studies. Cancer Epidemiology, Biomarkers & Prevention 2011; 20(7):1379-1390.

7.3 Other publications and reports

The results from this PhD thesis project have been summarised or partially republished in other publications and reports to disseminate information to relevant stakeholders working in cervical cancer prevention, particularly in developing countries.

7.3.1 Literature review

Louie K, Didelot, MN, Damay A, Nagot N, Mayaud P, Segondy M. Papillomavirus humains (HPV) et cancers associés: aspects épidémiologiques (In French). Revue Francophone des Laboratoires 2008; 38(45): 27-34.

7.3.2 Study 1

Louie KS, de Sanjose S, Mayaud P. *Cervical cancer prevention in Africa.* Africa Health (2009); 32 (1): 27-29.

Louie KS. *Preventing Cervical Cancer in sub-Saharan Africa.* Programme for Research & Capacity Building in Sexual & Reproductive Health & HIV in Developing Countries; Fact Sheet, October 2009. Access at: http://www.srh-hiv.org/

Louie KS and Mayaud P. *Feature: Challenges in introducing vaccination for human papillomavirus (HPV) infection to prevent cervical cancer in sub-Saharan Africa.* IDS Health and Development Information; Health Reporter, October 2009.

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9 Appendices

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Appendix 1. Publication arising from the *Literature Review* conducted for this thesis

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Review

Epidemiology and prevention of human papillomavirus and cervical cancer in sub-Saharan Africa: a comprehensive review

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Summary

OBJECTIVES To identify the gaps of knowledge and highlight the challenges and opportunities for controlling cervical cancer in sub-Saharan Africa (SSA).

METHODS A comprehensive review of peer-reviewed literature to summarize the epidemiological data on human papillomavirus (HPV) and invasive cervical cancer (ICC) by HIV status, to review feasible and effective cervical screening strategies, and to identify barriers in the introduction of HPV vaccination in SSA.

RESULTS ICC incidence in SSA is one of the highest in the world with an age-standardized incidence rate of 31.0 per 100 000 women. The prevalence of HPV16/18, the two vaccine preventable-types, among women with ICC, does not appear to differ by HIV status on a small case series. However, there are limited data on the role of HIV in the natural history of HPV infection in SSA. Cervical screening coverage ranges from 2.0% to 20.2% in urban areas and 0.4% to 14.0% in rural areas. There are few large scale initiatives to introduce population-based screening using cytology, visual inspection or HPV testing. Only one vaccine safety and immunogenicity study is being conducted in Senegal and Tanzania. Few data are available on vaccine acceptability, health systems preparedness and vaccine cost-effectiveness and long-term impact.

CONCLUSIONS Additional data are needed to strengthen ICC as a public health priority to introduce, implement and sustain effective cervical cancer control in Africa.

keywords human papillomavirus, cervical cancer, HIV, sub-Saharan Africa, cervical screening, HPV vaccine

Introduction

Cancer of the cervix uteri is the most common cancer among women in sub-Saharan Africa (SSA). The magnitude of the problem has been under-recognized and under-prioritized compared to competing health priorities such as HIV/AIDS, tuberculosis and malaria. This is due to lack of epidemiological data and poor awareness, lack of human and financial resources, non-existent cancer service policies and lack of political will to address the complex problem (Denny *et al.* 2006; Parkin *et al.* 2008).

Organized screening and early treatment programmes have been effective in preventing cervical cancer in industrialized countries but they are costly and difficult to implement in resource-constrained settings. Despite our understanding of the causal relationship of the human papillomavirus (HPV) and cervical cancer (Bosch *et al.* 2002) and the availability of effective HPV vaccines to prevent infection and disease (Schiller *et al.* 2008), the opportunity of these vaccines to have an effective impact in SSA will not materialize until they become affordable and integrated within the framework of national immunization programmes (Kane *et al.* 2006). Until then, cervical cancer prevention will rely on secondary prevention measures.

This review aims to summarize the current epidemiology of HPV and cervical cancer and the complexity of implementing prevention in sub-Saharan Africa; to identify gaps of knowledge and to highlight the challenges and opportunities for controlling cervical cancer in the region.

Methods

We conducted a comprehensive review of peer-reviewed literature in databases of the World Health Organization

and Institut Català d'Oncologia (WHO/ICO) Information Centre on HPV and Cervical Cancer (http://www.who.int/hpvcentre), the International Agency for Research on Cancer (IARC) Screening Group (http://www.screening.iarc.fr), PubMed/MEDLINE, and reports from the World Health Organization (WHO). The following medical subject heading (MESH) and text words were used alone or in combination: 'HPV', 'cervical cancer', 'cervical screening', 'HPV vaccination' and 'sub-Saharan Africa'. This review summarizes the evidence from recent systematic reviews, meta-analyses, narrative reviews (non-systematic reviews), epidemiological studies, modelling and related analyses and practice guidelines.

Results

Cervical cancer in sub-Saharan Africa

An estimated number of 70 722 new cases of invasive cervical cancer (ICC) occur annually in sub-Saharan Africa and it is responsible for one-quarter of all female cancers (Parkin *et al.* 2008). ICC incidence in sub-Saharan Africa is one of the highest in the world with an estimated overall age-standardized incidence rate (ASR) of 31 per 100 000 women and varies by region with 42.7 in East Africa, 38.2 in Southern Africa, 28 in Central Africa and 29.3 in Western Africa (Figure 1) (Ferlay *et al.* 2004; Parkin & Bray 2006). In contrast, the ASR is 12.1 in Northern Africa and 11.9 in Europe (Parkin *et al.* 2008). However, better cancer incidence data are needed to characterize the burden of disease. At present, only a few population-based cancer registries exist in Africa, covering 11% of the population, and fewer produce high quality incidence data (Parkin *et al.* 2008). In the period 1998–2002, only two cancer registries in the region, Kyadondo County in Uganda and Harare in Zimbabwe, produced high quality data and reported ASR of 45.8 and 47.3 per 100 000, respectively (Curado *et al.* 2007).

It remains unclear whether the HIV epidemic (Figure 1) has affected the incidence of ICC in sub-Saharan Africa as incidence rates appear to have remained unchanged between the 1960s and 1990s as seen in Nigeria and South Africa or increasing in Bulawayo, Zimbabwe and Kampala, Uganda (Parkin *et al.* 2008). Furthermore, it is unknown whether more effective AIDS control interventions, such as better access to antiretroviral treatment (ART) will reduce the incidence of ICC because of immune reconstitution or increase the incidence as a result of longer life expectancy and potential risk for disease progression. It is projected that, irrespective of changing risk, population growth and ageing, the likely future burden of cervical cancer in sub-Saharan Africa will rise to about 118 000 new cases in 2025, which is a 67% increase from 2002 (Ferlay *et al.* 2004).

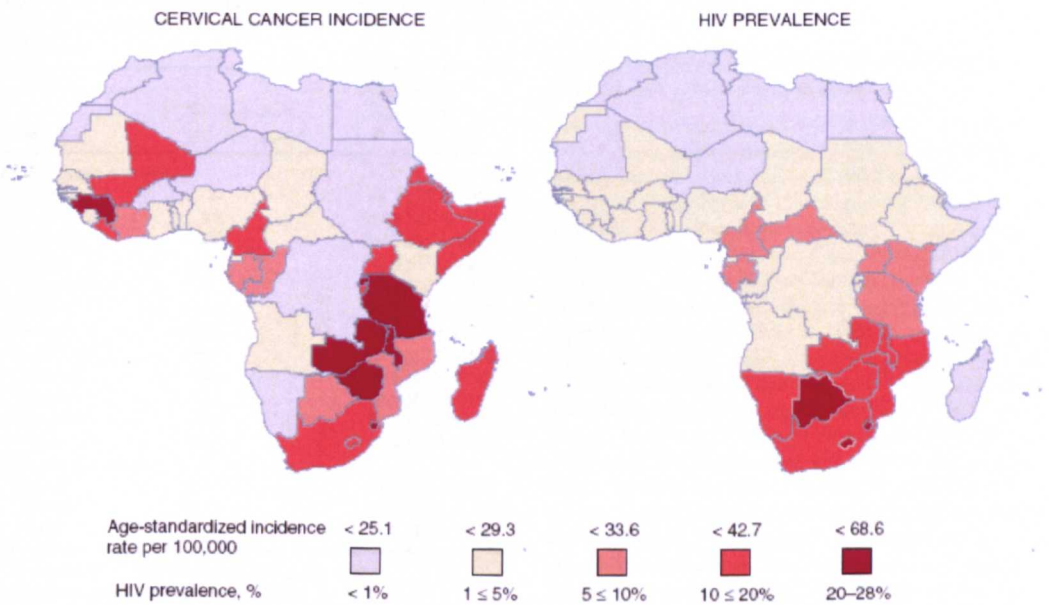

Figure 1 Age-standardised (world) incidence rates of cervical cancer (Ferlay J et al. 2002) and HIV prevalence (UNAIDS, 2008) in Africa.

Epidemiology of HPV infection and ICC

Among the 15 high-risk (HR) oncogenic HPV genotypes that have been identified, HPV16 and 18, the two vaccine-preventable types, show a greater risk of progression to pre-cancerous lesions than other HR types (Khan *et al.* 2005; Schiffman *et al.* 2005). In designing effective prevention strategies, HPV type-specific data are therefore essential to estimating the impact of HPV vaccines and cervical cancer screening.

Burden of HPV prevalence and type-distribution in the general population

Cross-sectional studies have shown that the overall prevalence of any HPV type in the general populations of sub-Saharan Africa for women with normal cytology is 21.8% (HPV Information Centre 2009). The prevalence of HPV types 16 and 18 among ICC cases ranges from 43.7% in Senegal to 90.2% in Ethiopia (Table 1) (HPV Information Centre 2009). The overall combined estimate of HPV16/18 prevalence among ICC cases in sub-Saharan Africa is 69.2%, which is consistent with the worldwide estimate of 70% (HPV Information Centre 2009).

Figure 2 shows the five most common HR HPV types among women with normal cytology, squamous intraepithelial lesions (SIL) and ICC in the general population of sub-Saharan Africa from a meta-analysis (HPV Information Centre 2009). The five most frequent HPV types among women with ICC according to ranking order are HPV 16, 18, 45, 33 and 35, a distribution which does not differ significantly from the worldwide distribution (HPV 16, 18, 33, 45 and 31) (HPV Information Centre 2009). An ongoing international survey using a centralized HPV testing and pathology laboratory to evaluate a collection of 10 000 archived cervical cancer tissue samples worldwide confirmed a similar distribution for the sub-Saharan Africa region although this only included samples from three countries, Mozambique, Nigeria and Uganda (de Sanjose *et al.* 2009).

While HPV prevalence and type-distribution have been aggregated and summarized for the vast region of sub-Saharan Africa, there are, however, large gaps in this epidemiological picture with virtually no data available for Central African countries, and a paucity of country-specific data in some sub-regions. These data would ideally be required to better understand factors influencing local epidemiology of ICC and to predict the local impact of vaccines.

Table 1 HPV16/18 prevalence in cases of invasive cervical cancer (ICC) from published studies in sub-Saharan Africa, according to HIV status

Country	No. of women with ICC tested	HPV16/18 Prevalence, % (95% CI*)
HIV-negative women[a]		
Benin	6	66.7 (28.9–100.0)
Guinea	18	44.4 (21.5–67.4)
Ethiopia	163	90.2 (85.6–94.8)
Kenya	261	60.9 (55.0–66-8)
Mozambique	302	79.1 (74.6–83.7)
Mali	123	54.5 (45.7–63.3)
Senegal	71	43.7 (32.1–55.2)
South Africa	307	62.9 (57.5–68.3)
Tanzania	102	72.6 (63.9–81.2)
Uganda	154	74.0 (67.1–81.0)
Zimbabwe	98	79.6 (71.6–87.6)
Total	1605	69.2 (66.9–71.4)
HIV-positive women		
Kenya[b]	51	68.6 (55.9–81.4)
Zambia[c]	28	53.6 (35.1–72.0)
Total	79	63.3 (52.7–73.9)

[a] Estimates from HPV Information Centre, 2009.
[b] Estimates from De Vuyst *et al.*, 2008.
[c] Estimates from Sahasrabuddhe *et al.*, 2007.
*CI, confidence interval.

Sexual and reproductive behaviour and risk of HPV infection and cervical carcinogenesis

Early age at first sexual intercourse (AFSI) and early pregnancy have been identified as risk factors for ICC in developing countries (Louie *et al.* 2009). AFSI is an important determinant of exposure to HPV infection and pregnancy as a determinant of risk for ICC, particularly in those who are highly parous (Louie *et al.* 2009). This is highly relevant in the African context, in which young women initiate these two events at an early age and experience high parity. Data on selected sexual and reproductive health factors are shown in Table 2 as proxy measures that could be used to inform on cervical cancer prevention strategies (i.e. appropriate age for HPV vaccination). Median AFSI for girls ranges from 15 to 20 years and total fertility ranges from 1.9 to 6.9 in sub-Saharan Africa. Condom use is not often reported by young African women except for non-barrier methods of contraception (Cleland *et al.* 2006). If unprotected sex is practiced, uncircumcized men with a history of multiple sexual partners, in particular, may increase the risk of cervical cancer in their female partners (Castellsague *et al.* 2002).

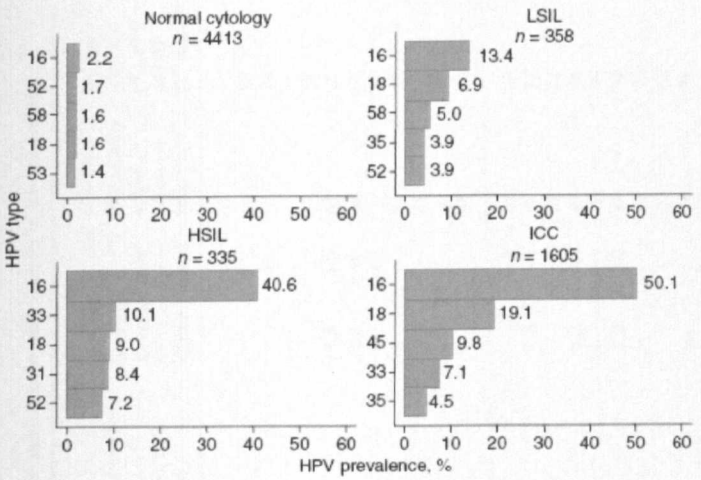

Figure 2 The five most frequent high-risk HPV types among women in the general population with normal cytology, low-grade intraepithelial lesions (LSIL), high-grade intraepithelial lesions (HSIL), and invasive cervical cancer (ICC) in sub-Saharan Africa [HPV Information Centre, 2009].

The role of HIV infection in the development of cervical cancer

With the diverse HIV epidemic in sub-Saharan Africa, we must consider how it may affect HPV-related cervical disease and other genital neoplasms. This may greatly vary by region, since the estimated number of women living with HIV ranges from <1000 cases in Comoros to 3.2 million in South Africa (Table 2) (UNAIDS 2008).

Epidemiological studies, mostly conducted in developed countries, have shown that HIV-infected women are at higher risk of being infected with HR HPV (Sun *et al.* 1997; Ahdieh *et al.* 2000; Jamieson *et al.* 2002), and are at a higher risk for persistence and associated cervical disease progression than HIV-uninfected women (Mayaud *et al.* 2001; Didelot-Rousseau *et al.* 2006; Moscicki *et al.* 2006; Palefsky *et al.* 2006; Blossom *et al.* 2007). This suggests that CD4$^+$ T-lymphocyte counts may play a role in the natural history of HPV infection. However, HPV persistence and SIL have been observed in young HIV-positive women with normal CD4$^+$ counts, which indicates that immune factors other than CD4$^+$ counts may play a role in disease progression. Clinical studies have shown conflicting results with some favouring HPV clearance and others persistence (Moscicki *et al.* 2006). In the African setting, high prevalence of sexually transmitted infections and other communicable diseases may influence immune status in cervical carcinogenesis, but few or no data are available to clarify these associations.

Both HIV and HPV infections have viral factors, and thrive on host factors that impair the immune system, which can cause considerable comorbidity and mortality. HIV-related immune alteration appears to increase the risk of cervical disease progression (Strickler *et al.* 2003; Hawes *et al.* 2006). However, after antiretroviral therapy (ART), the immune system is able to recover and better able to control HIV-related opportunistic infections and cancers, such as human herpes virus-8 (HHV-8) and associated Kaposi's sarcoma (Ahdieh-Grant *et al.* 2004; Hessol *et al.* 2004; Clifford *et al.* 2006). It has been hypothesized that ART has the potential to restore the immune response against HPV, reduce HPV persistence, and should therefore be able to reduce the occurrence of pre-cancerous lesions and favour regression. Some studies have shown a beneficial effect of ART in European (Heard *et al.* 1998) and US cohorts (Ahdieh-Grant *et al.* 2004). However, other studies have not found the same benefits of ART on regression of lesions (Orlando *et al.* 1999; Lillo *et al.* 2001; Moore *et al.* 2002; Schuman *et al.* 2003; Clifford *et al.* 2005). In a pooled analysis of data from six European centres where women had access to ART and one community-based South African cohort of HIV-positive women with no access to ART, similar hazard ratios for developing high-grade cervical disease were observed in the European centres and in South Africa (Kitchener *et al.* 2007). Among these conflicting data, a consensus on the effects of ART, and on how to best manage and prevent cervical cancer in HIV-positive women, remains to be found.

The rapid extension of access to antiretroviral programmes in sub-Saharan Africa, with its more intensive and organized follow-up offers the opportunity to assess the effects of ART on cervical disease. To inform possible screening strategies, HPV infection should be evaluated at various stages of HIV disease, such as in women who do

Table 2 Potential target HPV vaccine populations and selected sexual and reproductive health, HIV, education and immunisation indicators in sub-Saharan Africa

	Total population, Women (thousands)[a]	Women Aged 10-14 (thousands)[a]	Women Aged 15-24 (thousands)[a]	Age at first sexual intercourse[b]		Total Fertility[b]	Estimated number of women 15+ years living with HIV (thousands)[d]	Cervical Screening Coverage, %[c,e]		Net primary school enrolment ratio (%)[c, f]	Vaccine coverage (%) 2007 of DTP (3rd dose)[g]
				Female	Male			Urban	Rural		
Central Africa											
Angola	8426	1088	1670	–	–	6.9	110	–	–	–	83
Cameroon	8928	1107	1862	15.5	18.8	5.2	300	–	–	–	82
Central African Republic	2089	248	416	15.5	17.5	5.2	91	–	–	–	54
Chad	5045	629	982	15.5	18.5	6.6	110	9.5	4.0	49	20
Congo	1713	210	356	–	–	4.7	43	20.2	14.0	58	80
DRC Congo	29828	3853	5786	–	–	7.3	–	–	–	–	87
Equator. Guinea	307	38	56	–	–	5.6	6	–	–	83	33
Gabon	687	86	138	15.5	17.5	4.3	27	–	–	88	38
Sao Tome and Principe	77	10	18	–	–	4.7	–	–	–	95	97
West Africa											
Benin	3912	480	346	17.5	17.5	5.8	37	–	–	69	67
Burkina Faso	6896	861	636	17.5	20.5	6.2	61	7.8	5.1	39	99
Cape Verde	250	32	25	–	–	4.2	–	–	–	89	81
Cote d'Ivoire	9402	1168	890	15.5	18.5	4.7	250	6.9	3.1	49	76
The Gambia	770	91	65	–	–	6.0	5	–	–	72	90
Ghana	10812	1292	1025	17.5	19.5	4.6	150	3.2	2.2	64	94
Guinea	4565	561	406	15.5	17.5	5.6	48	–	–	63	75
Guinea-Bissau	743	86	61	–	–	6.8	9	–	–	37	63
Liberia	1680	205	152	–	–	5.2	19	–	–	58	88
Mali	5992	758	567	15.5	19.5	6.9	56	7.6	3.4	52	68
Mauritania	1474	174	139	–	–	4.6	4	4.5	1.0	79	75
Niger	6548	822	571	–	20.5	7.1	17	–	–	36	39
Nigeria	70381	8535	6632	15.5	20.5	5.7	1400	–	–	59	54
Senegal	5681	730	543	15.5	20.5	5.2	38	6.8	6.9	68	94
Sierra Leone	2626	303	248	–	–	6.6	30	–	–	–	64
Togo	3028	371	292	15.5	–	5.4	69	–	–	72	88
East Africa											
Burundi	3776	487	857	–	–	5.6	53	–	–	55	74
Comoros	307	34	67	18.5	–	5.1	<0.1	7.7	5.6	50	75
Djibouti	403	50	85	–	–	4.2	9	–	–	31	88
Eritrea	2279	271	493	–	–	5.2	21			45	97
Ethiopia	37544	4882	7315	15.5	18.5	5.7	530	1.6	0.4	64	73
Kenya	17934	2197	3951	17.5	16.5	5.0	–	4.0	2.6	76	81
Madagascar	8841	1124	1665	16.5	–	5.4	3	–	–	93	82

Table 2 (*Continued*)

	Total population, Women (thousands)[a]	Women Aged 10-14 (thousands)[a]	Women Aged 15-24 (thousands)[a]	Age at first sexual intercourse[b]		Total Fertility[c]	Estimated number of women 15+ years living with HIV (thousands)[d]	Cervical Screening Coverage, %[e]		Net primary school enrolment ratio (%)[f]	Vaccine coverage (%) 2007 of DTP (3rd dose)[g]
				Female	Male			Urban	Rural		
Malawi	6883	882	1307	16.5	17.5	6.1	490	3.7	2.5	95	87
Mauritius	631	54	98	–	–	1.9	4	–	–	96	97
Mozambique	10758	1262	2032	15.5	18.5	5.6	810	–	–	73	72
Reunion	401	34	65	–	–	2.4	–	–	–	–	97
Rwanda	4651	546	1100	20.5	18.5	5.9	78	–	–	75	99
Somalia	4215	493	765	–	–	5.7	7	–	–	–	39
Tanzania	14348	1936	2896	16.5	18.5	6.8	760	–	–	–	64
Uganda	19601	2421	3932	16.5	17.5	5.7	480	–	–	97	83
Zambia	5893	738	1182	16.5	16.5	5.9	560	5.7	1.8	93	80
Zimbabwe	6426	865	1568	18.5	–	3.9	680	10.8	5.2	82	62
Southern Africa											
Botswana	924	108	211	–	–	3.3	170	–	–	86	97
Lesotho	1058	128	238	–	–	4.1	150	–	–	77	83
Namibia	1021	127	212	18.5	18.5	4.2	110	17.8	5.8	79	86
South Africa	24413	2481	4907	17.5	–	2.9	3200	17.3	9.6	88	97
Swaziland	578	79	132	–	–	4.4	100	2.0	1.9	77	95
Sub-Saharan Africa	461810	55320 (12.0%)[h]	94121 (20.4%)[h]	–	–	–	12000	–	–	–	–

[a]Population in 2005. World population prospects: the 2008 revision. New York, Population Division, Department ot Economic and Social Attairs, United Nations Secretariat, 2009.
[b]Median age at first sexual intercourse [Wellings et al., 2006].
[c]World fertility patterns 2007. New York, Population Division, Department of Economic and Social Affairs, United Nations Secretariat, 2008.
[d]2008 Report on the global AIDS epidemic, UNAIDS/WHO, July 2008. [UNAIDS, 2008].
[e]Proportion of females aged 18-69 years who self-reported a pap smear test in the last 3 years [WHO, 2002].
[f]Net primary school enrolment is the number of boys and girls of primary-school-age that are enrolled in primary education, expressed as a percentage of the total population in that age group. It shows the extent of participation in primary education of children beloing to the official age group corresponding to primary education in the given country. UNESCO Institute for Statistics Data Centre [online database]. Montreal, UNESCO Institute for Statistics, 2007 (http://stats.uis.unesco.org, accessed 28 Jan 2009).
[g]WHO/UNICEF coverage estimates for 1980-2007, as of August 2008. http://www.who.int/inmiunization_monitormg/routine/immunization_coverage/en/index4html.
[h]Percentage of Total population in sub-Saharan Africa.

not yet require ART, in women initiating ART, or in women with long-term use of ART. This will not only help shed light on the effects of different levels of HIV immunosuppression on the temporal development of pre-neoplastic changes, but it will also help clarify the potential sustainable gains in preventing cervical cancer with anti-retroviral therapy.

HPV prevalence and genotype-distribution in HIV positive women

Few studies have reported on the prevalence of HPV among HIV-positive women in SSA. Among the available data, Figure 3 shows the five most common HR-HPV types among HIV-positive women with normal cytology and SIL from a meta-analysis (HPV Information Centre 2009) and two ICC studies in Kenya and the Zambia (Sahasrabuddhe *et al.* 2007; De Vuyst *et al.* 2008). Few studies have included ICC cases with HPV type-specific data according to HIV serostatus in the region (Table 1). One study in Kenya (n = 204) found no significant differences in HPV 16/18 prevalence distribution according to HIV serostatus (De Vuyst *et al.* 2008). HIV-positive women with ICC have more multiple infections than in HIV-negative women, which complicates the HPV type distribution comparison by HIV status (De Vuyst *et al.* 2008). About 50% of HIV-positive women with HPV16 and/or 18 ICC cases are co-infected with another HPV type, so it is unclear whether the other HR types would be responsible for invasive disease in the absence of HPV16 or 18 (De Vuyst *et al.* 2008). These data suggest that current HPV vaccines containing HPV16/18 have the potential to prevent perhaps fewer ICC cases among HIV-positive women. It would be important to understand the potential differences between HIV-positives and negatives with additional immune correlated data among HIV-positives, such as $CD4^+$ count.

Cervical cancer screening

Cytology screening programmes have been successful in curbing the incidence of ICC in developed countries (Sankaranarayanan *et al.* 2001), but they have largely failed in most developing countries due to competing health priorities and lack of resources including health infrastructure (trained cytotechnologists, laboratories and services for diagnosis and treatment), human and financial resources (Denny *et al.* 2006). According to population-based surveys conducted by WHO in 2001 and 2002, cervical screening coverage was at best 20.2% of urban and 14.0% of rural areas in the Congo, and at worst, 1.6% of urban and 0.4% of rural areas in Ethiopia (WHO 2002) (Table 2). South Africa is the only country in sub-Saharan Africa to have established a national cytology-based cervical screening programme in 2001. However, coverage remains poor and the impact on ICC rates is unknown (Kawonga & Fonn 2008).

VIA (visual inspection with acetic acid) or VILI (visual inspection with Lugol's iodine) are less laboratory dependent strategies and have been advocated as screening alternatives in developing countries (Denny *et al.* 2006). In various evaluation studies, VIA has shown to have a

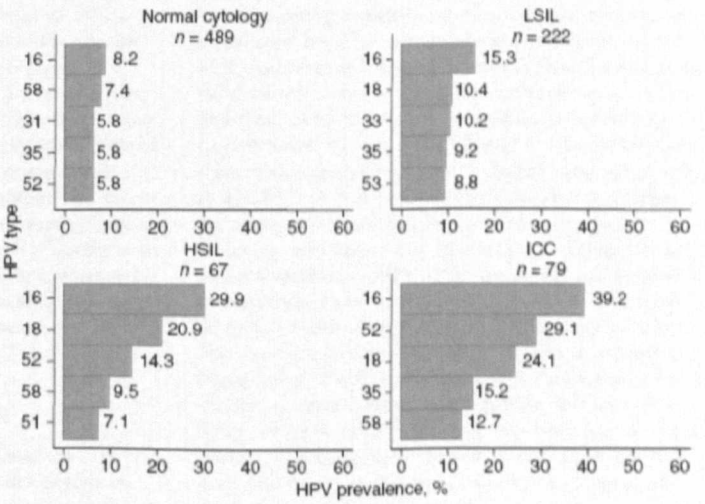

Figure 3 The five most frequent high-risk HPV types among HIV-positive women with normal cytology, low-grade intraepithelial lesions (LSIL), high-grade intraepithelial lesions (HSIL), and invasive cervical cancer (ICC) in sub-Saharan Africa [Clifford et al., 2006; Sahasrabuddhe et al., 2007 de Vuyst et al., 2008).

sensitivity and specificity of 60–94% and 74–94%, respectively, to detect high-grade lesions in Africa; and VILI has shown to have a sensitivity and specificity of 90–97% and 73–91%, respectively (Sankaranarayanan *et al.* 2001). The specificity of VIA is however lower among HIV-positive women, which may be attributed to high rates of coinfections in the lower genetal tract (Denny *et al.* 2002). A number of completed and ongoing screening activities using VIA and VILI have been evaluated as different strategies in the region (Table 3) (IARC Screening Group 2009). Among 20 countries reporting screening activities, 11 countries have ongoing programmes. Of the 49 projects that have been initiated, 27 projects are ongoing, but only six projects were funded by the Ministry of Health in the country. Most screening programmes in sub-Saharan Africa have been initiated as research or pilot projects (88%), and only a limited number of countries ($n = 6$) have received financial support or have the local political support to scale up national screening programmes.

While visual inspection methods appear effective for primary screening, they are still prone to subjectivity, requiring good provider training and sustained quality assurance in order to achieve substantial gains in prevention of cervical cancer in routine settings (Sankaranarayanan *et al.* 2007; Muwonge *et al.* 2009).

A more objective and reproducible screening test is testing for HPV DNA that has shown to be more sensitive than cervical cytology in detecting high-grade lesions (ACOG Practice Bulletin 2005; Dillner *et al.* 2008). A screening trial using HPV testing for 6553 unscreened women (35–65 years) in South Africa showed an 80% reduction of CIN2+ by 36 months of follow-up among HIV-infected women, which was similar to the reduction among HIV-uninfected women (Kuhn *et al.* 2009). In addition, a cluster-randomized trial of about 132 000 women (30–59 years) in rural India showed that a single round of HPV DNA testing significantly reduced the rate of advanced stages of cervical cancers and associated deaths compared to VIA, cytology and no screening after 8 years of follow-up (Sankaranarayanan *et al.* 2009). These studies suggest that HPV testing is an appropriate primary screening approach in low-resource settings to reduce cases of high-grade lesions, advanced stages of ICC and mortality in HIV-infected and uninfected women.

Rapid HPV DNA testing

The limitations of HPV DNA testing include the cost (i.e. US$20–30 per test), infrastructure, and time needed to obtain a result. However CareHPV (Qiagen Gaithersburg Inc., MD, USA) has been developed as a simple, rapid and operational HPV test for low-resource settings that can produce results within 3 h (Qiao *et al.* 2008). The compact, portable and battery-operated technology has stable conditions, and the test can be conducted by workers with minimal training. Data from China showed that, compared to VIA, CareHPV has a higher sensitivity (90% *vs.* 41%) and a reasonably comparable specificity (84% *vs.* 94%) to detect high-grade lesions. Moreover, a modelling analysis found that CareHPV has the potential to reduce the incidence of cervical cancer by 56% in China if given just three times over a woman's lifetime and effective treatment is available (Levin *et al.* 2008), suggesting its potential impact in reducing the burden of ICC in comparable settings. Regulatory approval is anticipated in developing countries in the near future, and this test will be provided at a low-cost. CareHPV represents a promising alternative screening test, however, its performance and diagnostic value to detect pre-cancerous lesions need to be evaluated in African settings.

Effective cervical screening programmes

Apart from affordable, acceptable and effective screening tools, the performance of a screening programme is dependent on multiple factors, such as information and education for women and communities in order to obtain their participation into screening programmes. After screening, acceptable and accessible referral services for diagnosis and effective treatment, and good follow-up are required for a successful programme (Gravitt *et al.* 2008). At present, this may involve a three-visit strategy with initial screening evaluation at the first visit, performing colposcopy for those who screen positive at the second visit, and treating biopsy-confirmed cervical lesions at the third visit. In order to avoid loss-to-follow-up, a 'screen-and-treat' approach should be considered, where referral/treatment is offered immediately to screen-positives cases with a reduced number of clinical visits (Blumenthal *et al.* 2007). A cost-effective model for five developing countries which included South Africa showed that screening with one-visit or two-visits in a lifetime using visual inspection or HPV DNA test coupled with immediate cryotherapy for screened positives for women aged 35 years or older has the potential to reduce the lifetime risk of cancer by 25–35% compared with no screening (Goldie *et al.* 2005). In sub-Saharan Africa, where minimal cervical screening services are available, this once in a lifetime screen-and-treat strategy may have an important impact in reducing the incidence of ICC and needs to be evaluated locally to determine whether it is logistically feasible, acceptable and safe.

Table 3 Completed or ongoing cervical screening activities in sub-Saharan Africa

Country	Date	Project/Program Title	Type of Programme	Source of Funding	Current Status
Angola	2002–2004	Comparative evaluation of early detection of cervical cancer precursors by VIA and with VILI and establishment of a cervical cancer prevention training centre	Research	IARC (Screening services continue with local funds to date in 2008)	Active
Benin	2008–2009	Screening of cervical intra epithelial lesions in women living with HIV; Case-control study about pap-smear done in Parakou hospital between 2008 and 2009.	Research associated with HIV project	Unknown	Completed
Botswana	2005–2009	Botswana National Cervical Cytology (Cancer) Screening Programme	MoH	None	Active
Burkina Faso	2000–2002	Comparative evaluation of early detection of cervical cancer precursors by VIA and VILI	Research	IARC	Completed
	2003–2004	Human papillomavirus genotype distribution and cervical squamous intraepithelial lesions among high-risk women with and without HIV-1 infection	Research	Montpellier University Hospital	Completed
	2007–2012	Prevention of cervical cancer in Burkina Faso (Ouagadougou and Bobo Dioulasso)	Pilot project	Societe des obstetriciens et gyncecologues du Canada (SOGC); Agence canadienne de developpement international (ACDI)	Approved/ Not yet active
Congo, Republic of	2000–2004	Comparative evaluation of early detection of cervical cancer precursors by VIA and VILI	Research	IARC	Completed
	2004–2008	Screening and treatment of cervical precancerous lesions	Research	Local funds	Active
Ethiopia	2006–2008	Comparison of PAP smear, VI A/VILI for cervical cancer screening	MoH	WHO, UNFPA, UNICEF	Active
Ghana	2001–2003	Safety, Acceptability, and Feasibility of a Single Visit Approach to Cervical Cancer Prevention – A Demonstration Project	Pilot project	JHPIEGO; Bill and Melinda Gates Foundation	Completed
Guinea	2000–2003	Comparative evaluation of early detection of cervical cancer precursors by VIA and VILI	Research	IARC (Screening services continue with local funds to date in 2008)	Completed
	2003–2004	Organized cervical cancer screening program using visual inspection methods in the region of Khorira	Demonstration project	IARC (Screening services continue with local funds to date in 2008)	Completed
	2004–2005	Organized cervical cancer screening program using VIA/VILI in Conakry	Demonstration project	IARC (Screening services continue with local funds to date in 2008)	Active
Kenya	2000–2004	Western Kenya Cervical Cancer Prevention Project (WKCCPP)	Unknown	PATH	Completed

Table 3 (*Continued*)

Country	Date	Project/Program Title	Type of Programme	Source of Funding	Current Status
Madagascar	2006–2008	Prevention of cervical cancer through screening using VIA and treatment with cryotherapy – A demonstration project in Madagascar	Pilot project	WHO (Headquarters)	Active
Mali	2001–2003	Comparative evaluation of early detection of cervical cancer precursors by VIA and VILI	Research	IARC	Completed
	2004–2007	Organized cervical cancer screening program using VIA/VILI in the region of Bamako	Demonstration project	IARC	Completed
Malawi	2000–2002	Cervical Cancer Screening & Early Treatment (CCSET) Pilot Project	Pilot Project	DFID (Project Hope) Bill and Melinda Gates Foundation (JHPIEGO)	Completed
	2004–2007	Cervical cancer prevention programme (CECAP)	MoH	USAID (2004–2005), Bill and Melinda Gates (2005–2006), USAID (2007)	Completed
	2006–2008	Prevention of cervical cancer through screening using VIA and treatment with cryotherapy in Lilongwe	Pilot project	WHO (Headquarters)	Active
Niger	2000–2004	Comparative evaluation of early detection of cervical cancer precursors by VIA and VILI	Research	IARC	Completed
	2004–2008	Outreach clinics/campaigns	Outreach clinics/campaigns	SEM le president de la republique (programme special)/ UNFPA	Active
Nigeria	2006–2008	Prevention of cervical cancer through screening using VIA and treatment with cryotherapy – A demonstration project in Sgamu	Pilot project	WHO (Headquarters)	Active
	2006–2008	Lagos cervical cancer screening project		Institute for Women's health	Active
	2006–2010	Operation "Stop cervical cancer in Nigeria"	Research	NCI/NIH/FED-EX/T. BOONE PICKINS/EXXONMOBIL FOUNDATION/	Active
	2008–2009	Edo State Cervical Cancer Control programme	MoH	Edo State Government	Active
South Africa	1996–1999	Khayelitsha cervical cancer screening project (KCCSP)		Engender Health / University of Cape Town	Completed
	2000–2003	Randomised control trial of screening and treating women based on D VI and HPV testing (KCCSP)		University of Cape Town / Columbia University (New York) / Engender Health	Completed
	2003–2009	The Cervical Health Implementation Project (CHIP)		University of Cape Town / Columbia University (New York) / Engender Health	Active

Table 3 (*Continued*)

Country	Date	Project/Program Title	Type of Programme	Source of Funding	Current Status
	2007–2009	Increasing Access to Cervical Cancer Prevention in South Africa's North West Province	Pilot Project	JHPIEGO / Northwest Province (NWP) health department	Active
	2007–2010	Application and cost-effectiveness of new technologies such as liquid based cytology and computer-assisted cervical screening to women at high risk for the development of cervical cancer.	Cervical cancer prevention training actions	National Health Laboratory Service, Johannesburg	Active
Senegal	2009–2010	Cervical cancer prevention by single-visit approach in Dakar	Pilot project	Seeking Funds	Not yet active
Tanzania	2002–2008	Comparative evaluation of VIA and VILI to test cervical cancer in the early detection and prevention of cervical neoplasia and establishment of a cervical cancer prevention training centre in Tanzania	Research	IARC / Ocean Road Cancer Institute (ORCI) / International Network of Cancer Treatment and Research (INCTR) (Screening services continue with local funds to date in 2008)	Active
	2006–2008	Prevention of cervical cancer through screening using VIA and treatment with cryotherapy – A demonstration project in Moshi, Kilimanjaro region	Pilot Project	WHO (Headquarters)	Active
	2006–2008	Prevention of cervical cancer through screening using VIA and treatment with cryotherapy – A demonstration project in Peramiho	Pilot Project	WHO (Headquarters)	Active
	2006–2008	Outreach clinics/campaigns in 5 regions (Mwanga, Morogoro, Muheza, Mwanza and Bagamoyo)	Outreach clinics/ campaigns	Ocean Road	Active
Uganda	2006–2008	Prevention of cervical cancer through screening using VIA and treatment with Cryotherapy – A demonstration project in Masaka	Pilot project	WHO (Headquarters)	Active
	2006–2009	UCL – Uganda Women's Health Initiative (Kampala project)		Institute for Women's Health, University College, London	Active
	2007	Kisoro district hospital		Albert Einstein University (USA) MRC/UVRI Entebbe	Active
	2007–2010	Nsambya Hospital		CDC/PEPFA (USA) MRC	Active
	2008 2008	Project Mildmay Center Nsyamba hospital/Gulu hospital			Active
	2008–2011	VIA (Mbarara Regional referral hospital)	Cervical cancer prevention training actions	PATH	Active
	2008–2012	Kisenyi/Mulago		Canadian group	Active
	2008–2012	Mulago Hospital – Obstetrics & Gynecological Department		Ministry of Health and Makerere	Active

Table 3 (*Continued*)

Country	Date	Project/Program Title	Type of Programme	Source of Funding	Current Status
	2008–2012	START-UP Project: Uganda (Mulago hospital)	MoH	PATH	Active
Zambia	2006–2008	Prevention of cervical cancer through screening using VIA and treatment with cryotherapy in Lusaka	Demonstration project	WHO (Headquarters)	Active
	2006–2008	CIDRZ project at University Teaching Hospital, Lusaka	MoH	CIDRZ	Active
Zimbabwe	1996–1997	VIA as a cervical cancer test: accuracy validated using latent class analysis.	Research	USAID / JHPIEGO	Completed

Data aggregated from [IARC Screening Group, 2009]. Accessed 23 April 2009. *VIA, visual inspection with acetic acid; VILI, visual inspection with Lugol's iodine

Prospects of HPV vaccination: opportunities and challenges

Prophylactic HPV vaccines give new promises for a primary prevention strategy for HPV infection and cervical cancer. The vaccines have shown high safety, efficacy and immunogenicity for both the quadrivalent HPV 16/18/6/11 vaccine (Gardasil®, Merck & Co., Inc.) and the bivalent HPV 16/18 vaccine (Cervarix™, GlaxoSmithKline Biologicals) (Schiller *et al.* 2008). A number of countries in sub-Saharan Africa have licensed the HPV vaccines (Table 4). However, implementation plans are lagging and will depend largely on the affordability of the vaccines, and a clear cost-benefit ratio. We further discuss some of the challenges that will be met prior to introduction of the HPV vaccines in sub-Saharan Africa.

Vaccine efficacy, safety and immunization schedules

HPV vaccines have shown 95% efficacy in preventing vaccine HPV types and related precancerous lesions for up to 7 years in large international cohorts of women, although noticeably, excluding Africa (Schiller *et al.* 2008). However, safety and immunogenicity bridging studies are currently being conducted in young HIV-negative women in Senegal and Tanzania (ClinicalTrial.gov

Table 4 HPV vaccines licensure in sub-Saharan Africa, as of March 2009

Quadrivalent HPV 6/11/16/18 Vaccine (Gardasil® Merck, SA) (18)	Bivalent HPV 16/18 Vaccine (Cervarix®, GSK) (10)
Botswana	Congo
Burkina Faso	Cote d'Ivoire
Cameroon	Gabon
Central African Republic	Ghana
Chad	Kenya
Congo	Namibia
Cote d'Ivoire	Nigeria
Democratic Republic of Congo	Senegal
Equatorial Guinea	South Africa
Ethiopia	Uganda
Gabon	
Kenya	
Malawi	
Mauritania	
Mauritius	
South Africa	
Togo	
Uganda	

*Source: HPV Information Centre, 2009.

NCT00481767). In addition, these studies will investigate the effect of the vaccines in populations whose immunological system may be challenged by multiple co-infections such as malaria and helminths, which may possibly modify immune response (Lehtinen *et al.* 2006). Until recently, there were no data on HPV vaccination among those infected with HIV. Clinical trials are underway in HIV positive women in South Africa (ClinicalTrial.gov NCT00586339) and results are eagerly awaited from this endemic region. In addition, initial results from a quadrivalent vaccine trial of 126 perinatally infected HIV-infected children (7–12 years) in North America have shown that the vaccine is generally safe and nearly 100% seroconverted although antibody titres for HPV 16 and 18 were lower than in healthy children (Moscicki *et al.* 2009).

In order to evaluate the potential reductions in costs, a multi-centre study involving about 16 000 girls aged 10–18 years to evaluate the effectiveness and safety of two- *vs.* three-doses HPV vaccine in preventing cervical cancer is underway (Sankaranarayanan *et al.* 2008) and trials of giving HPV vaccines to babies as part of the standard infant immunization schedule are being discussed (Garland *et al.* 2008). Results from these studies may facilitate a wider rollout of HPV vaccine in sub-Saharan Africa.

Vaccine delivery challenges

The adoption of the vaccines into a national immunization programme will only be realized when the HPV vaccines drop in price and they become part of the essential WHO Expanded Program of Immunization (EPI) vaccines (Kane *et al.* 2006). EPI programmes have been successful in improving access and delivery of vaccines to children and achieving high coverage worldwide. In 2007, vaccination coverage of the target population for DTP for the third dose was as low as 33% in Equatorial Guinea to 99% in Burkina Faso (Table 2). Infrastructure of trained staff, cold chain and logistics, clinics and outreach services and information systems are already established to deliver these infant vaccines. However, even if the vaccine is incorporated onto the EPI list, there still remains the challenge of delivering an 'adolescent' vaccine since no programme exists targeting this population. In order to be highly efficacious, vaccines should be delivered prior to sexual initiation when risk of exposure to HPV increases.

Current vaccines target pre-adolescent and young women, and developing a school-based programme with this target population will be challenging, as few continue their secondary school education beyond primary school. Net school enrolment in primary school ranges from as low as 31% in Dijibouti to 97% in Tanzania (Table 2). Even if the vaccine were to be considered at the primary school level, those who need the vaccine the most may be less likely to attend school (Kane *et al.* 2006) as sexual activity among adolescents in out-of-school females is common (Brown *et al.* 2001). However, measles campaigns in Africa using schools as vaccination sites to bring non-school attendees to the vaccination site has been reported and is a strategy that could be explored for HPV vaccines (Kane *et al.* 2006).

Local communities will need to first identify factors that influence vaccine acceptability and uptake among target populations and healthcare providers, and then decide the most appropriate way for vaccine deployment. For example, PATH, an international NGO which focuses on reproductive health research, has conducted formative research in Uganda to evaluate the psychosocial aspects of vaccine acceptability and to understand health systems that are required to guide effective vaccine delivery strategies, communication and advocacy (PATH 2009). The results from this research are being used to explore two possible vaccination implementation strategies: (i) 'Child Days Plus' is a semi-annual event that aims to vaccinate children as part of an integrated health service programme; and (ii) school-based vaccination programme. Findings from the demonstration project are expected in 2010.

Vaccine costs and cost-effectiveness

At US$360 for the three-dose HPV vaccine, it is the most expensive vaccine in history. This is difficult to accept as 80% of cases of cervical cancer occur in developing countries (Parkin & Bray 2006), where health budgets are very limited. In order to overcome cost barriers, work is being done to facilitate innovative financial mechanisms to accelerate the introduction of vaccines, such as advanced market commitments, which provides an assured price subsidy for developing country purchase of a future vaccine meeting predefined standards and will provide industry with assurances of earning a reasonable return on their investment. Another solution would be public-private partnerships, which will allow both the public and private sectors to share the risks and costs of developing, scaling-up and introducing priority vaccines (Batson *et al.* 2006). In 2008, the GAVI Alliance a public-private organization dedicated to increasing children's access to vaccines in developing countries has included the HPV vaccine amongst its priority of new vaccines to be considered for introduction. If subsidized by the GAVI Alliance, the vaccine could reach over 80% of the countries in sub-Saharan Africa.

An economic analysis evaluating the impact of vaccination in GAVI eligible countries showed that with 70% coverage of pre-adolescents, HPV vaccines have the potential to reduce the lifetime risk of cervical cancer by 31–60% in the region (Goldie *et al.* 2008). Hypothetically, if the cost to vaccinate a girl was US$2 per dose, the vaccine would be cost-effective compared to no vaccination in all sub-Saharan African countries and it would be cost-saving and less than international dollars I$100 per disability-adjusted years of life (DALYs) averted in a few countries, e.g. Comoros, Guinea, Lesotho, Zimbabwe, Rwanda, Eritrea, Uganda and Djibouti. However, each country will need to conduct its own economic analyses to evaluate their budgets for introduction of the HPV vaccine among other new childhood vaccines (i.e. rotavirus, pneumococccal, meningococcal meningitis) being considered. It is still unknown whether one lifetime cervical screening and treatment may be more cost-effective than HPV vaccination in preventing cervical cancer deaths in each of these sub-Saharan African countries. Further evaluation of a number of strategies considering vaccination and screening only or in combination will also be needed to inform the most appropriate cost-effectiveness prevention strategy.

Summary of gaps in knowledge

In order to strengthen the efforts of cervical cancer prevention in sub-Saharan Africa, we highlight the following gaps in knowledge:

Epidemiology:

- Better cancer registry data to assess the burden of cervical cancer;
- Basic epidemiological data of HPV prevalence and genotype-distribution among women in the general population according to HIV serostatus to evaluate the potential impact of HPV vaccines and cervical cancer screening strategies;
- Longitudinal data on HIV infected women to evaluate the impact of ART on HPV infection and cervical disease;
- Long-term impact of HPV vaccines in preventing actual cases of cervical cancer.

Cervical screening:

- Evaluation of VIA, VILI in conjunction with HPV testing (e.g. with CareHPV) as screening tools in a 'screen-and-treat' approach;
- Evaluation of screening strategies in HIV-positive populations, including those taking ART.

HPV vaccine delivery:

- Vaccine trials in infants to evaluate the potential inclusion as part of an EPI standard immunization schedule;
- Vaccine deployment considerations: (i) community acceptability; (ii) health system capacity and channels of vaccine delivery; (iii) vaccination strategies, including appropriate age and sex, and catch-up strategies, (iv) health economics and impact modelling;
- Cost-effectiveness studies of vaccine and/or cervical screening strategies;
- Comparative analyses of cervical screening and/or vaccination strategies on disease impact.

Conclusions

The burden of cervical cancer is potentially large in sub-Saharan Africa and there is an urgency to make it a public health priority. Sub-Saharan Africa is vastly heterogeneous and each country will need to decide on a realistically feasible cervical cancer prevention and control strategy. Existing data should be used to initiate plans and projects to better understand the current situation and programmes will need to take into consideration the impact of HIV co-infection to adequately address both health needs. The availability of low-cost cervical screening technologies of HPV testing and visual inspection methods and HPV vaccines represent tools that provide realistic opportunities for cervical cancer prevention in sub-Saharan Africa. In order to successfully introduce, implement and sustain a prevention programme, good data from across multi-disciplines dedicated to the prevention of cervical cancer are needed.

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Appendix 2. Publication arising from *Study 1* in this thesis

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Early age at first sexual intercourse and early pregnancy are risk factors for cervical cancer in developing countries

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Early age at first sexual intercourse (AFSI) has long been associated with an increased risk of invasive cervical carcinoma (ICC). Age at first pregnancy (AFP) and ICC have been investigated less, although AFSI and AFP are strongly interrelated in most developing countries. A pooled analysis of case–control studies on ICC from eight developing countries with 1864 cases and 1719 controls investigated the roles of AFSI, AFP, and ICC risk. Age at first sexual intercourse, AFP and age at first marriage (AFM) were highly interrelated and had similar ICC risk estimates. Compared with women with AFSI ⩾21 years, the odds ratio (OR) of ICC was 1.80 (95% CI: 1.50–2.39) among women with AFSI 17–20 years and 2.31 (95% CI: 1.85–2.87) for AFSI ⩽16 years (*P*-trend <0.001). No statistical interaction was detected between AFSI and any established risk factors for ICC. The ICC risk was 2.4-fold among those who reported AFSI and AFP at ⩽16 years compared with those with AFSI and AFP at ⩾21 years. These data confirm AFSI and AFB as risk factors for ICC in eight developing countries, but any independent effects of these two events could not be distinguished.
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Early age at first sexual intercourse (AFSI) has been associated with an increased risk of high-risk human papillomavirus (HPV) infection, a sexually transmitted infection (STIS), that in susceptible women is responsible for virtually all cases of invasive cervical cancer (ICC) (Bosch *et al*, 2002). As sexual behaviour determines exposure to HPV, AFSI is of particular interest as it has been associated with riskier sexual behaviour, such as having unprotected sex, having multiple sexual partners, as well as a woman's partner having multiple partners. It has also been speculated that the increased risk of HPV is because of a biological predisposition of the immature cervix during adolescence that may be more susceptible to persistent HPV infections and therefore have a greater risk of cancer development (Kjaer *et al*, 1998). A number of studies have identified an increased risk of high-grade lesions and/or cervical cancer with early AFSI, whereas others have not (IARC, 2007). However, many of these studies were conducted before HPV assessment was feasible, and therefore, the association remains inconclusive. Age at first marriage (AFM) is often used as a proxy measure for AFSI, and those who engage in early sexual intercourse may also consequently become pregnant at an early age. Besides early AFSI, early childbearing has also been linked as a risk factor for cervical carcinogenesis and attributed to the cervical trauma experienced during early age at first pregnancy (AFP), or subsequently, by high-parity births (IARC, 2007). The interpretation of the mechanisms by which these sexual and reproductive events occurring early in life might affect ICC risk three or more decades later is not straightforward. The objective of this study is to further characterise and provide robust estimates of the risk of cervical cancer and its association with AFSI, interrelated characteristics such as AFP and AFM in a series of studies that fully considered the association of HPV with cervical cancer.

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MATERIALS AND METHODS

The programme of HPV and cervical cancer studies has been coordinated by the International Agency for Research on Cancer (IARC) in Lyon, France and the Institut Català d'Oncologia (ICO) in Barcelona, Spain. They included a series of case-control studies on ICC from eight developing countries with a broad range of rates of incidence of cervical cancer that were pooled for analysis. Regions covered include Morocco (Chaouki *et al*, 1998) and Algeria (Hammouda *et al*, 2005) in Africa; the Philippines (Ngelangel *et al*, 1998), Thailand (Chichareon *et al*, 1998) and

Madras in Asia; and Brazil (Eluf-Neto *et al*, 1994), Colombia (Munoz *et al*, 1993), Paraguay (Rolon *et al*, 2000) and Peru (Eluf-Neto *et al*, 1994) in South America. Although Spain (Munoz *et al*, 1993) was part of the series of case–control studies, the sexual and reproductive behaviour of this population was heterogeneous to the other countries (late AFSI and low parity) and the study site was therefore excluded from this analysis.

The methods of each study have been described elsewhere. Briefly, women with histologically confirmed incident invasive squamous cell carcinoma (SCC), adenocarcinoma or adenosquamous-cell carcinoma were recruited from reference hospitals before treatment. Written informed consent was obtained from those who agreed to participate. Hospital-based controls were frequency-matched to case patients by 5-year age groups.

A standardised questionnaire was administered to the participants by a trained interviewer, which included questions about sociodemographic factors, sexual and reproductive behaviour, smoking habits, pap screening history, hygienic practices, and history of sexually transmitted diseases.

Two samples of cervical exfoliated cells were collected with wooden spatulae and endocervical brushes. After preparation of one Papanicolaou smear, the remaining cells were eluded in saline, centrifuged and frozen at −70°C until shipment to the central laboratory for HPV DNA testing. A tumor-biopsy sample was obtained from cases and frozen. Cytology and histology diagnoses were reviewed and confirmed by a panel of expert pathologists that agreed on a diagnosis by consensus or majority.

Detailed descriptions of the polymerase-chain-reaction (PCR) assays used in these studies have been described elsewhere. HPV DNA detection was detected by PCR amplification of a small fragment of the *L1* gene using MY09 and MY11 consensus primers for the study in Colombia (Hildesheim *et al*, 1994) and the GP5+/6+ general primer system for the other studies (Walboomers *et al*, 1992; Jacobs *et al*, 1995; Roda Husman *et al*, 1995). β-Globin primers were used to amplify the *β*-globin gene to assess the quality of the DNA in the specimen. HPV DNA in PCR products was analysed using a cocktail of HPV-specific probes and genotyped by hybridisation with type-specific probes for 33 HPV types. Samples that tested positive for HPV DNA but did not hybridise with any of the type-specific probes were labelled as HPV X.

Statistical analysis

Unconditional logistic regression was used to estimate odds ratios (ORs) and 95% confidence intervals (95% CI). To assess the association of AFSI with the risk of ICC, three different statistical models to adjust for HPV DNA detection were computed and compared: (1) one model included all patients and controls, and it was not adjusted for HPV DNA status, (2) a second model included all patients and controls, and included a variable to adjust for HPV DNA status, and (3) a third model was restricted to HPV–DNA-positive cases and controls. To control for potential confounding, final models were adjusted for age (<40, ⩾40), country, lifetime number of sexual partners (1,>1), parity (0, 1–4, ⩾5), and educational level (never, primary, secondary or higher). Each variable included in the adjustment models was assessed for interaction with AFSI. Test for trend was carried out when appropriate, using the log-likelihood-ratio test. Only subjects who reported ever having been married and/or ever having had children were included in the analyses of AFM and AFP.

We evaluated other potential confounding factors such as smoking (never, ever), oral contraceptive use (never, 1–4 years, ⩾5 years), history of pap smears excluding those in the 12 months before enrolment (never, ever), having had first sexual intercourse before menarche and the timing of first sexual intercourse relative to age at menarche (data not shown), but they were not adjusted for in the final analysis as they did not contribute any change to the OR estimates for AFSI in the adjusted models.

RESULTS

Table 1 describes some characteristics of the 1864 ICC cases and 1719 corresponding controls that entered the final analysis. Ninety-five percent of case patients and 17% of controls tested positive for HPV DNA. The majority of cases (92%) had SCC. Case patients were older than controls with a median age of 49 *vs* 48, respectively. Median AFSI was earlier in case patients (17 years) compared with controls (19 years), and this was found to be consistent in each country.

Table 2 shows the risk of ICC by AFSI according to the three different adjustment models. An increased risk of ICC was consistently observed with decreasing AFSI (*P*-trend <0.001). Compared with AFSI ⩾21 years, the OR of ICC was 1.80 (95% CI: 1.50–2.16) for AFSI 17–20 years, and 2.31 (95% CI: 1.85–2.87) for AFSI ⩽16 years, after adjusting for age, centre, lifetime number of partners, parity, and education level in the HPV-unadjusted model. According to the different model adjustments, women reporting AFSI ⩽16 years of age had a 2.3–2.5-fold risk of ICC and 1.8–2.1-fold risk for AFSI 17–20 years of age (Table 2). Given the consistent association of AFSI and the risk of ICC across the different models, HPV-unadjusted models were used for the remainder of the results.

We calculated the risk of ICC for each country study, and, in general, each study showed an increasing risk of ICC with decreasing AFSI (data not shown). There was no evidence of heterogeneity with respect to study country ($P = 0.58$).

Table 1 Characteristics of cases of invasive cervix carcinoma and controls

	HPV tested		HPV positive				Age[a]		Age at sexual debut[a]	
	Cases	Controls	Cases	%	Controls	%	Cases	Controls	Cases	Controls
Country	1864	1719	1769	94.9	285	16.6	49	48	17	19
Algeria	142	145	132	93.0	18	12.4	53.5	52	16	18
Morocco	188	176	182	96.8	38	21.6	49	40	16	18
Madras (India)	187	184	180	96.3	51	27.7	48	46.5	17	18
Philippines	364	380	349	95.9	35	9.2	47.5	47	19	21
Thailand	378	259	363	96.0	41	15.8	49.5	50	18	20
Brazil	187	190	181	96.8	32	16.8	51	52	18	19
Colombia	110	124	87	79.1	21	16.9	46	45.5	17	18
Paraguay	112	86	109	97.3	18	20.9	48.5	45.5	16	19
Peru	196	175	186	94.9	31	17.7	48	48	16	18

Abbreviation: HPV = human papillomavirus. [a]Median.

Table 2 Effect of different strategies of multivariate model adjustments on the association between age at first sexual intercourse and risk of ICC (from IARC case–control studies)

Sexual debut	Cases *n* (%)	Controls *n* (%)	Age and centre adjusted Odds ratio (95% CI)	HPV unadjusted[a] Odds ratio (95% CI)	HPV adjusted[a] Odds ratio (95% CI)	HPV-positive only[a] Odds ratio (95% CI)
⩾21 years	341 (16.9)	656 (35.4)	1.00	1.00	1.00	1.00
17–20 years	813 (40.2)	667 (36.0)	2.44 (1.07–2.87)	1.80 (1.50–2.16)	1.78 (1.32–2.39)	2.10 (1.49–2.97)
⩽16 years	710 (35.1)	396 (21.4)	4.09 (3.38–4.94)	2.31 (1.85–2.87)	2.09 (1.48–2.96)	2.48 (1.65–3.73)
P-trend				<0.001	<0.001	<0.001

Abbreviations: HPV = human papillomavirus; ICC = invasive cervical carcinoma; IARC = International Agency for Research on Cancer; CI = confidence interval. [a]Adjusted for age, study country, lifetime number of partners (1, ⩾2), parity (0, 1–4, ⩾5), and education (never, primary, secondary).

We stratified the analysis according to the established risk factors for ICC and the positive association of ICC with decreasing AFSI remained at each level of exposure for each of these characteristics (Table 3). Similar associations were observed for AFP. No interaction was observed between any of the examined risk factors and AFSI. Although not statistically significant, the risk linked to AFSI seemed to be stronger among parous women compared with nulliparous women.

Age at first pregnancy and AFM were both directly correlated with AFSI in these populations ($P<0.001$). Approximately, 92% of women reported AFSI to be the same as AFM. One-quarter of women reported AFP to be the same as AFSI. Cumulatively, 62.4% of women reported giving birth within the first year of AFSI. Among women with AFSI ⩽16 years, 52.4% were pregnant within the first year of sexual intercourse. Figure 1 shows the high correlation between AFSI and AFP, and the similar decreasing risk of ICC with increasing age of AFSI/AFP. Given the high correlation between the two variables, we did not adjust for AFSI in the AFP final model and vice versa.

We further evaluated the combined effect of AFP and AFSI on the risk of cervical cancer (Table 4). An increased risk emerged in subsequent strata of decreasing AFP with decreasing AFSI. Given this combined effect, we assessed the latency period (AFP–AFSI) between these two events to clarify whether it affected the cervical cancer risk. Although there was no statistical difference across strata, the data suggested that within each AFSI strata, women with a latency period for a subsequent pregnancy of < 2 years may be at a slight increased risk compared with women with a larger time gap (data not shown).

DISCUSSION

The IARC/ICO series of case–control studies remain the largest set of aetiological investigations on ICC that fully addresses the role of HPV DNA and of the independent established cofactors. This is probably also the largest dataset reporting on ICC in the developing world in which early AFSI, AFP and high parity are prevalent phenomenons. The results show that early AFSI and early AFP are risk factors for cervical cancer, irrespective of other known risk factors for the disease. The data presented show a possible additional increase in risk when the early event of first sexual intercourse is shortly followed by a pregnancy.

The mechanism by which the early experience of first sexual intercourse and first pregnancy could influence the risk of cervical carcinogenesis may be explained by the steroid hormonal influence on HPV infection and on the host's immune response to HPV during pre-adolescence and adolescence. The transformation zone of the cervical epithelium has been recognised as the site in which HPV infection tends to cause cancer, and the susceptibility of this area is believed to be related to its denudation of the stratified epithelium, thus facilitating exposure of the basal layer to HPV with minimal trauma. Biological immaturity during adolescence has also been proposed as an additional susceptibility factor (Moscicki *et al*, 1989; Elson *et al*, 2000; Singer and Monaghan, 2000). During adolescence and pregnancy, the cervix is exposed to augmented levels of hormonal changes (Singer and Monaghan, 2000), in which oestrogen stimulation facilitates acidification of the vaginal cavity, a determinant of squamous metaplasia when the endocervical epithelial everts (Elson *et al*, 2000). When this oestrogen-stimulated metaplastic transformation occurs in the presence of HPV, the probability of cell transformation increases, resulting in neoplastic changes (Elson *et al*, 2000; Shai *et al*, 2007, 2008; Hwang *et al*, 2009). This phenomenon is dependent primarily on parity, and is more likely to occur during the first pregnancy rather than subsequent pregnancies (Singer and Monaghan, 2000). Although it has been postulated that these metaplastic changes are also influenced by the trauma and repair experienced during delivery, no increased risk for cervical carcinoma was observed in this same dataset when traumatic partition was evaluated (Munoz *et al*, 2002).

Increased risks of cervical carcinoma have been identified in women with long-term use of hormonal steroids (Moreno *et al*, 2002) and those who are highly parous (Munoz *et al*, 2002). In addition, HPV-16 transgenic mouse models have shown that those treated with longer durations of oestrogen were more likely to develop larger tumours and have a significantly higher number of tumours than those treated with a shorter duration (Elson *et al*, 2000; Brake and Lambert, 2005), supporting the human observations of a susceptible cervix to carcinogenic progression by continuous exogenous oestrogen exposure or increased endogenous oestrogen levels. If indeed oestrogen is needed for cervical carcinogenesis, close follow-up of young women and of their early pregnancies may be relevant to further understanding the role of steroids in the acquisition and persistence of HPV infections.

The influence of oestrogens on immune response may offer another explanatory effect (Mitrani-Rosenbaum *et al*, 1989; Arbeit *et al*, 1996), particularly during the follicular phase of the ovarian cycle and pregnancy, when levels of oestrogens are increased up to 3–8-fold the normal levels (Duncan *et al*, 1994; Marzi *et al*, 1996; Jabbour *et al*, 2008). The higher density of oestrogen receptors and their expression in the transformation zone may synergise with the effects of HPV oncoproteins, decreasing levels of cytotoxic cytokines that may down-regulate the cervical cell-mediated immune response, which favour persistent HPV infections instead of clearance (Marzi *et al*, 1996; Giannini *et al*, 1998; 2002; Jacobs *et al*, 2003). Additional research is needed to further understand the interaction between oestrogens and the regulation of immunomodulators, which may contribute to anti-tumour immunity.

The varying results between studies regarding the roles of AFSI and AFP may reflect the true differences between the study populations. In our study, the similar increased risks shown for AFSI and AFP may, in general, reflect the fact that in most developing countries women initiate these events at an early age,

Table 3 Age at first sexual intercourse and risk of cervical cancer according to various characteristics

	Number of cases/controls	Odds ratio (95% CI)[a]	Odds ratio (95% CI)[b]	P-trend
Parity				
Nulliparous				
⩾21 years	14/40	1.00	1.00	
17–20 years	7/14	1.88 (0.53–6.66)	1.61 (0.40–6.57)	
⩽16 years	7/6	3.60 (0.83–15.52)	1.50 (0.17–13.55)	0.56
Ever parous				
⩾21 years	327/614	1.00	1.00	
17–20 years	804/645	2.47 (2.07–2.94)	1.97 (1.63–2.36)	
⩽16 years	703/387	4.10 (3.36–4.99)	2.59 (2.08–3.21)	<0.001
P-heterogeneity between AFSI and ever parous = 0.64				
Parity (1–4 births)				
⩾21 years	171/399	1.00	1.00	
17–20 years	273/287	2.58 (1.98–3.35)	1.99 (1.51–2.62)	
⩽16 years	169/115	4.72 (3.41–6.54)	2.71 (1.89–3.87)	<0.001
Parity (⩾5 births)				
⩾21 years	156/215	1.00	1.00	
17–20 years	531/358	2.01 (1.57–2.59)	1.71 (1.32–2.23)	
⩽16 years	534/272	2.88 (2.19–3.78)	2.08 (1.55–2.78)	<0.001
P-heterogeneity between AFSI and parous groups (nulliparous, 1–4 births, and ⩾5 births) = 0.90				
Oral contraceptive use				
Never				
⩾21 years	218/400	1.00	1.00	
17–20 years	453/364	2.33 (1.87–2.90)	1.77 (1.40–2.25)	
⩽16 years	376/184	4.28 (3.29–5.55)	2.45 (1.82–3.29)	<0.001
1–4 years				
⩾21 years	64/162	1.00	1.00	
17–20 years	117/114	2.64 (1.76–3.94)	1.67 (1.07–2.61)	
⩽16 years	111/89	3.94 (2.53–6.13)	1.95 (1.15–3.30)	0.01
⩾5 years				
⩾21 years	36/52	1.00	1.00	
17–20 years	124/72	3.26 (1.85–5.72)	2.46 (1.36–4.46)	
⩽16 years	96/49	4.48 (2.39–8.40)	2.80 (1.38–5.65)	0.006
Smoking				
Never				
⩾21 years	272/569	1.00	1.00	
17–20 years	587/552	2.34 (1.93–2.83)	1.68 (1.36–2.06)	
⩽16 years	550/348	3.76 (3.03–4.67)	2.06 (1.61–2.64)	<0.001
Ever				
⩾21 years	67/85	1.00	1.00	
17–20 years	221/113	2.73 (1.81–4.13)	2.32 (1.50–3.60)	
⩽16 years	152/43	5.63 (3.42–9.27)	3.62 (2.10–6.26)	<0.001
Lifetime number of sexual partners				
Monogamous				
⩾21 years	270/569	1.00	1.00	
17–20 years	529/499	2.33 (1.92–2.83)	1.80 (1.46–2.22)	
⩽16 years	349/214	3.89 (3.05–4.96)	2.38 (1.83–3.11)	<0.001
Partners >1				
⩾21 years	69/74	1.00	1.00	
17–20 years	280/151	2.03 (1.37–3.01)	1.74 (1.15–2.63)	
⩽16 years	352/156	2.75 (1.84–4.11)	2.14 (1.39–3.28)	0.001
P-heterogeneity = 0.36				
Education				
Never go to school				
⩾21 years	54/57	1.00	1.00	
17–20 years	255/155	1.68 (1.09–2.60)	1.46 (0.93–2.30)	
⩽16 years	402/173	2.41 (1.55–3.75)	2.09 (1.31–3.35)	0.001
Primary school				
⩾21 years	164/238	1.00	1.00	
17–20 years	404/311	1.99 (1.54–2.56)	1.62 (1.24–2.12)	
⩽16 years	236/167	2.51 (1.87–3.39)	1.71 (1.24–2.36)	0.001
Secondary school				
⩾21 years	118/359	1.00	1.00	
17–20 years	150/198	2.68 (1.95–3.68)	2.29 (1.65–3.20)	
⩽16 years	72/55	4.79 (3.07–7.47)	3.36 (2.07–5.47)	<0.001
P-heterogeneity = 0.08				

Table 3 (*Continued*)

	Number of cases/controls	Odds ratio (95% CI)[a]	Odds ratio (95% CI)[b]	*P*-trend
Ever have a pap smear 12 months before study enrolment				
Never				
⩾21 years	183/320	1.00	1.00	
17–20 years	434/371	2.20 (1.74–2.79)	2.29 (1.65–3.20)	
⩽16 years	416/205	3.65 (2.79–4.78)	3.36 (2.07–5.47)	<0.001
Ever				
⩾21 years	158/336	1.00	1.00	
17–20 years	379/296	2.65 (2.02–3.46)	1.92 (1.44–2.57)	
⩽16 years	294/191	4.49 (3.32–6.06)	2.30 (1.63–3.24)	<0.001

Abbreviations: CI = confidence interval; AFSI = age at first sexual intercourse. [a]Adjusted for age and study centre. [b]Adjusted for age, study country, lifetime number of partners (1, ⩾2), parity (0, 1–4, ⩾5), and education (never, primary, secondary).

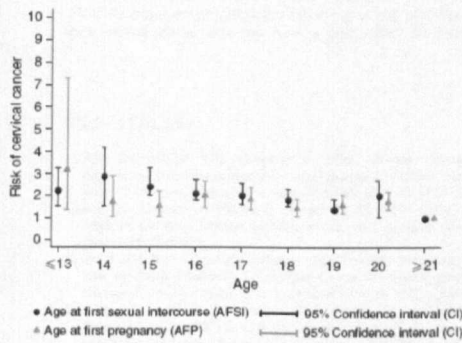

Figure 1 Age at first sexual intercourse and age at first pregnancy are highly correlated with the risk of invasive cervical cancer (*P*-trend<0.001). Models were adjusted for age, centre, lifetime number of sexual partners (1, >1), parity (0, 1–4, ⩾5), and education (never, primary, secondary).

Table 4 Interaction between age at first pregnancy and age at first sexual intercourse in the risk of cervical cancer

	Age at first pregnancy		
Age at sexual debut	**⩾21**	**17–20**	**⩽16**
⩾21 years	1.00		
17–20 years	1.58 (1.22–2.03)	1.93 (1.58–2.36)	
⩽16 years	2.17 (1.35–3.47)	2.28 (1.74–2.99)	2.36 (1.82–3.07)

and experience high parity, making their effects difficult to distinguish from one another. In contrast, results of studies in more developed countries where there is a longer latency period between sexual initiation and AFP, as in Spain, the US (Brinton *et al*, 1987) or Italy (Parazzini *et al*, 1989) tend to show an increased risk with early AFSI but not with AFP as first pregnancies tend to occur much later. It is interesting that, in countries like the UK, where the rates of teenage pregnancies are high, women with AFSI of ⩽17 years had a 2–3-fold increased risk for cervical cancer compared with those with AFSI ⩾20 years (Green *et al*, 2003). Consistently, women with an early AFP of 15–19 years had a two-fold increased risk for cervical cancer compared with those with AFP ⩾25 years (Green *et al*, 2003). These observations merit further exploration but, in aggregate, tend to indicate a significant increase in risk of neoplastic disease when early AFSI occurs (surrogate of early HPV exposure and a period of increased cervical susceptibility) and is followed closely by an early pregnancy (surrogate of early exposure to high oestrogen levels).

Irrespective of their lifetime number of sexual partners, women have a similar increased risk of ICC with early AFSI as shown by the 2.4-fold risk among monogamous women with AFSI ⩽16 years as compared with the 2.2-fold risk among women with >1 lifetime number of sexual partners. It has long been suggested that a cervical cancer risk will also depend on the sexual history of the woman's male partner in addition to her own behaviour (Skegg *et al*, 1982). This is particularly relevant in societies where most women are virgins at marriage and monogamous thereafter, where the incidence of cervical cancer for a population may vary depending on the behaviour of the male partner. Of our study women, 70% were monogamous. In several studies among monogamous women, the risk of cervical cancer was reported to be two to eight times for women with husbands who had multiple partners (Pridan and Lilienfeld, 1971; Buckley *et al*, 1981; Brinton *et al*, 1989). The sexual history of the male partner was not evaluated in this analysis; however, promiscuity, history of other STIS, and lack of male circumcision are factors that have been associated with the male role in cervical carcinogenesis (Castellsague *et al*, 2003).

In interpreting our results, we must emphasise the difficulty in fully disentangling a woman's sexual and reproductive profile in relation to her cancer risk (Schroder *et al*, 2003). We cannot exclude misclassification bias if AFSI and the number of sexual partners were inaccurately reported, leading to some residual confounding, However, the presence of established risk factors for ICC, use of oral contraceptives, smoking, and pap smear history did not seem to significantly affect the strength of the association between AFSI, AFP, and risk of ICC.

We examined the different stratified methodologies (unadjusted, HPV-adjusted, and HPV-positive restricted) used to evaluate the association between AFSI and risk of ICC traditionally employed in the literature. This was done to exclude any spurious association related to statistical adjustment and to clarify inconsistent findings of the association found in earlier studies. Although in strict terms restriction of analyses to HPV-positive cases and controls seemed preferable, the consistency of the results across the three different methods provides convincing evidence of the risk associated with AFSI. Furthermore, these results indicate that for the evaluation of other risk factors, adjusting for HPV status is not necessary as the adjustments do not contribute to remove any confounding effect.

Sexual practices in the world indicate that very early intercourse might be occurring in adolescents with 44, 45 and 52% of girls between the ages of 13–19 years reporting being sexually experienced in Argentina, Botswana and Nigeria, respectively

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(Brown *et al*, 2001). In several case studies among young females, first sexual intercourse has been reported as forced in 5–15% of cases, and in some extreme cases worldwide, the estimates range from 21% among out-of-school adolescents in Botswana, 20% among secondary schools in Peru, and 41% among young urban females attending night schools in Peru. Among 15–30% of sexually active girls aged 15–19 years report forced first sexual intercourse (Brown *et al*, 2001). It is likely that the partners of these adolescents who report sexual coercion are adult males who are sexually experienced (Wellings *et al*, 2006) and at high risk of HPV exposure. Globally, these exposures might affect a high proportion of very young girls in areas of human strife, thus adding child sexual abuse to the burden of a lifetime increased risk of genital cancer.

Our study shows that women who initiate first sexual intercourse and experience their first pregnancy at a young age are at an increased risk of cervical cancer. The importance of HPV-vaccination programmes targeting young adolescents before first sexual intercourse can have a great effect in decreasing the incidence of cervical cancer; additional efforts are required in family planning and sexual education adapted to the extremely variable sociocultural contexts in the world.

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Conflict of interest

No conflict of interest is declared in relation to this manuscript.

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Appendix 3. Publication arising from *Study 3* in this thesis

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Cancer Epidemiology, Biomarkers & Prevention

Research Article

Smoking and Passive Smoking in Cervical Cancer Risk: Pooled Analysis of Couples from the IARC Multicentric Case–Control Studies

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Abstract

Background: The independent role of tobacco smoking in invasive cervical cancer (ICC) has been established. We evaluated the potential impact of passive smoking (PS).

Methods: A pooled analysis of 1,919 couples enrolled in one of seven case–control studies involving cervical carcinoma *in situ* (CIS) or ICC was investigated. Information on smoking and sexual behavior was collected from interviews. Specimens were taken from the cervix and penis for human papillomavirus (HPV) DNA testing. Three PS risk models were constructed with all couples, couples with monogamous women, and couples with lifetime nonsmoking monogamous women. For the third model, the analysis considered potential misclassification of smoking status and was restricted to the risk period for which the woman was exposed to both HPV, a necessary cause of ICC, and PS. Multivariable unconditional logistic regression was used to estimate associations between CIS or ICC and PS.

Results: An increased risk was found among couples with both ever smoking men and women (OR = 2.26; 95% CI: 1.40–3.64). No statistically increased risk of CIS was found with PS in the models analyzed. Similar significant increased risks of ICC with PS was found among all couples (OR = 1.57; 95% CI: 1.15–2.15) and couples with monogamous women (OR = 1.55; 95% CI: 1.07–2.23) but not among lifetime nonsmoking monogamous women married to ever smoking men.

Conclusion: PS could not be detected as an independent risk factor of ICC in the absence of active smoking.

Impact: The combined effects of exposure to active and PS suggest its potential adverse role in cervical carcinogenesis. *Cancer Epidemiol Biomarkers Prev; 20(7); 1379–90. ©2011 AACR.*

Introduction

Men play an important role in the transmission of human papillomavirus (HPV), the etiologic factor for invasive cervical cancer (ICC). As HPVs involved in cervical carcinogenesis are sexually transmitted, it is central to understand patterns of sexual behavior in HPV transmission including the behaviors of both men and women. It has been debated that the cervical cancer risk for a woman will depend more on the full sexual

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Note: Supplementary data for this article are available at Cancer Epidemiology, Biomarkers & Prevention Online (http://cebp.aacrjournals.org/).

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history of the male partner than on her own behavior (1). This is particularly relevant in societies where women tend to be virgins at marriage and monogamous thereafter. Epidemiologic studies have tried to characterize the male role and the consequent female partner risk of HPV acquisition and of cervical cancer (2–5). Although a number of studies mostly involving monogamous women have observed an association between the number of sexual partners of the husband and his wife's risk for cervical cancer (6–8), other studies have not (9–12). Other inconclusive associations with prostitution have been identified (9–12).

Besides sexual behavior, other male factors, such as tobacco smoking has been less explored despite it being a well-established risk factor for cervical precancer and cancer (13, 14). Several reviews have summarized the epidemiologic and biological association of passive smoking (PS) on the risk of cervical cancer (15–17), however, the evidence has been suggestive rather than sufficient to implicate the role of PS in the etiology of cervical cancer among lifetime nonsmoking women. Among the studies identified, the recognized limitations include small sample sizes of nonsmoker controls and cases of cervical cancer, lack of specific information on HPV and sexual behavior and most studies obtained spousal history of smoking through questioning of the women rather than the men. Furthermore, most of the studies involved cervical intraepithelial neoplasia grade 3 (CIN-3)/carcionoma *in situ* (CIS) rather than invasive disease, which is a relevant distinction since evidence suggests that smoking acts in the stages of progression from CIS to invasive cancer (14).

To evaluate the male role in the etiology of cervical cancer, specifically the risk related to PS, we carried out a pooled analysis of five case–control studies involving ICC and two case–control studies involving CIS, of couples in which husbands or stable partners of ICC and CIS case and control women participated. The studies were conducted in three continents, mainly in developing countries, and were coordinated by the International Agency for Research on Cancer (IARC) in Lyon, France, and the Catalan Institute of Oncology (ICO) in Barcelona, Spain. The IARC/ICO series of case–control studies remain the largest dataset of sexual couples on etiologic investigations of ICC that fully addresses the role of HPV DNA and of independent cofactors. Some of the associations with risk factors (i.e., penile HPV infection and male circumcision) have been assessed in subsets of the subjects in this analysis (9, 11, 14, 18–20). For this study, we characterize in depth the role of PS with the full dataset on HPV and risk factors of the men and their associations with ICC.

Materials and Methods

The IARC/ICO case–control program included a series of studies on ICC and CIS from eleven countries with a broad range of cervical cancer incidence rates. Among these, seven studies conducted in five countries, enrolled husbands or stable partners of women with CIS or cervical cancer and control women were pooled for these analyses. Methods of each study and primary results related to women have been published previously. Countries included Brazil (21) and Colombia (9, 11, 22), Philippines (23), Thailand (24), and Spain (9, 25). Briefly, women with histologically confirmed incident cervical CIS, invasive squamous cell carcinoma, adenocarcinoma, or adenosquamous-cell carcinoma were recruited from reference hospitals before treatment. Control women were recruited from the general population in two of the studies of ICC in Spain and Colombia and from the same hospitals as the cases for the other studies. Control women were frequency matched to case patients by 5-year age groups.

Current husbands or stable partners (herein referred to as husbands) of enrolled women were defined as men who reported having had regular sexual intercourse with the women for at least 6 months, irrespective of whether or not they were married or lived together.

Informed consent was obtained from both men and women who agreed to participate.

Questionnaire

A standardized questionnaire was administered to participants by a trained interviewer that included questions about socio-demographic factors, sexual behavior, hygienic practices, and history of sexually transmitted infections (STI). For specific questions on smoking habits, subjects were first asked to classify themselves as lifetime never smoker, ex-smoker (defined as a former smoker who stopped smoking at least one year prior to the interview) or current smoker. Ever smokers were also asked at what age they started smoking regularly, the duration and how many cigarettes per day they smoked. Additional questions were asked on the type of tobacco (blond, black, or other) and type of filter (filter, no filter, or both) used. Ex-smokers were asked the age at which they stopped smoking.

Penile and cervical HPV DNA sampling

Two samples of exfoliated cells were obtained from the penis: one from the distal urethra with the use of a very thin, wet, cotton-tipped swab, and one from the external surface of the glans and coronal sulcus with the use of a standard-sized wet, cotton-tipped swab.

Two samples of cervical exfoliated cells were collected with wooden spatulae and endocervical brushes. After preparation of one Papanicolaou smear, the remaining cells were eluted in saline, centrifuged and frozen at −70°C until shipment to the central laboratory for HPV DNA testing. A tumor-biopsy sample was obtained from cases and frozen at −70°C. Cytology and histology diagnosis were reviewed and confirmed by a panel of expert pathologists that agreed on a diagnosis by consensus or majority.

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Detection of HPV DNA

Detailed descriptions of the PCR assays used in these studies have been described elsewhere. HPV DNA was detected by PCR amplification of a small fragment of the *L1* gene by using MY09 and MY11 consensus primers for the studies in Spain and Colombia (26) and $GP5^+/6^+$ general primer system for the other studies (27–29). β-Globin primers were used to amplify the β-globin gene to assess the quality of the DNA in the specimen. HPV DNA in PCR products was analyzed with the use of a cocktail of HPV-specific probes and genotyped by hybridization with type-specific probes for 33 HPV types in the case of cervical samples and for at least 6 HPV types (6, 11, 16, 18, 31, and 33) in the case of the penile samples. *Samples that tested positive for HPV DNA but did not hybridize with any of the type-specific probes were* labeled as HPV X.

Statistical analyses

To evaluate the association between smoking habits, and risk of CIS or ICC, we first used age- and country-adjusted univariate logistic regression analyses to determine the effects of each of the following potential male factors by using an α-level of 0.05: age, history of smoking (nonsmoker, current smoker, or ex-smoker, lifetime pack-years, and use of tobacco and filter type), education, sexual history (age at first sexual intercourse, lifetime number of sexual partners, history of contact with sex workers, history of STIs, and history of anal sex), hygienic practices (i.e., pay attention to uncover penis and to wash the region, able to fully uncover spontaneously or by pulling the penis from the skin prepuce, and wash before and after sexual intercourse), male circumcision status, and to control for potential confounding of PS characteristics and risk of cervical cancer, final models were adjusted for male factors that contributed change to any of the estimated OR and 95% CI. To control for additional potential confounding by characteristics of the women, female risk factors (education, age at first sexual intercourse, lifetime number of sexual partners, history of pap smear 12 months prior to study enrolment, use of oral contraceptives, parity, and smoking) for cervical cancer were fitted into the final multivariate models (i) for the CIS adjusted models if they contributed to any change to the OR estimates for male characteristics; and (ii) for the final ICC adjusted models as they are well-established risk factors known to be associated with ICC. However, when we adjusted the OR estimates with all female risk factors in the CIS model, the estimates did not significantly differ (data not shown). We identified lifetime number of sexual partners (a significant risk factor of exposure to HPV) to be heterogeneous across study countries (Supplementary Table S1) and an interaction term combining lifetime number of sexual partners and country were included in the fully adjusted multivariate models.

In addition, we found a statistically significant interaction between some male risk factors (e.g., age at first sexual intercourse, lifetime number of sexual partners, history of sexual intercourse with a sex worker, and HPV-positivity status) and case status (i.e., ICC vs. CIS), which justified the use of 2 separate models for each disease stage. This is in agreement with our current understanding of the natural history of CIS, as it has been estimated that about 31% of CIS cases will develop cancer within 30 years, leaving a proportion of CIS cases that will not advance to invasive disease (30). Thus, some of the risk factors associated with CIS incidence may differ from those associated with progression from CIS to ICC.

Furthermore, to better clarify the relationship between PS characteristics and cervical cancer in their female partners, we removed the potential effect of *previous male partners the* woman may have had by *calculating and comparing 3 different statistical models* for CIS and ICC: one included *all study couples, the* second model included only couples with monogamous women, and the third model included couples with lifetime nonsmoking monogamous women. For the third model restricted to 765 couples with lifetime nonsmoking monogamous women, we further reclassified the husband's smoking history according to the risk period for which the woman would have been exposed to HPV infection (a necessary factor in cervical carcinogenesis), PS and risk of progression to cervical cancer (Fig. 1). Ninety male ex-smokers ($n = 44$ cases and $n = 43$ controls) of couples with monogamous women were reclassified as nonsmokers, and the duration of exposure to passive smoke and smoking pack-years were recalculated (Supplementary Table S2).

Results

Patient characteristics

Table 1 describes selected characteristics of the male and female subjects. Of the 291 CIS and 692 ICC cases and 936 control women, 59.8%, 70.9%, and 81.2%, were monogamous, respectively. In general, husbands were older than their wives, and husbands and wives of CIS cases and controls were younger than those of ICC.

Table 2 shows penile HPV prevalence among husbands of cases and controls of CIS and ICC by history of smoking and country. Penile HPV detection was doubled in husbands of cases than controls of CIS and was higher among cases than controls of ex-smokers (2.4% vs. 0%) and current smokers (13.9% vs. 3.8%). Similar penile HPV detection was found in husbands of cases and controls of ICC (17.6% vs. 16.2%), which was also similar among cases and controls of ex-smokers and current smokers. However, penile HPV was more prevalent among husbands of cases than controls of ICC (9.7% vs. 6.6%), respectively.

Table 3 presents selected male risk factors and their univariate associations with risk of CIS and ICC, stratified by all couples and couples with monogamous women. In

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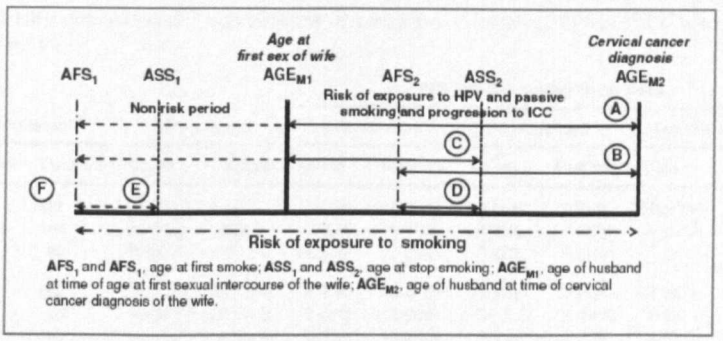

Figure 1. Model of risk of exposure to HPV and PS and progression to ICC among nonsmoking monogamous women according to the husband's history of husband. Period of risk is defined between age at first sexual intercourse of the wife and cervical cancer diagnosis. Duration of exposure to PS is defined as: (A) current smoker = (AGE_{M2}–AFP) if AFS_1; (B) current smoker = (AGE_{M2}–AFS_2); (C) ex-smoker = (ASS_2–AGE_{M1}) if $AFSI_1$; (D) ex-smoker = (ASS_2–AFS_2). Women are not at risk for HPV and PS if (E) ex-smoker = (ASS_1–AFS_1) or their husband is a (F) nonsmoker. Smoking status of the husband is classified as follows: current smokers = A + B; ex-smokers = C + D; and nonsmokers = E + F.

general, similar associations were observed in all couples and couples with monogamous women models, except for associations of CIS with education and smoking status. Hygienic practices (able to fully uncover spontaneously or by pulling the penis from the prepuce and washing before and after sexual intercourse) and history of anal sex were not associated with CIS or ICC in univariate analyses (data not shown). The following risk factors of husbands were found to be associated with an increased risk of CIS or ICC early age at first sexual intercourse, history of sexual intercourse with a sex worker (ever and while with current wife), history of gonorrhea, increasing number of STIs, being a current smoker and increasing lifetime smoking pack-years. Lack of education was associated with an increased risk of ICC but not CIS, whereas being uncircumcised and HPV positive were associated with an increased risk of CIS but not ICC. An inverse relation between use of "black" tobacco type, as compared with "blond" tobacco type among smokers, and the risk of CIS was observed, as well as some hygienic practices such as lack of attention to uncover the penis to wash the region and the risk of ICC.

Table 4 shows the association between selected male smoking characteristics and cervical CIS and ICC in multivariate analyses. Generally, similar associations were identified in the 2 analyses of all couples and only couples with monogamous women, therefore, we will describe our findings herein forward according to the all couples model. No statistically significant increased risk

Table 1. Characteristics of both corresponding husbands of women with and without (a) CIN-3/CIS or (b) ICC

	Total no. of husbands		Age of husbands[a]		Age of wives[a]	
	Cases	Controls	Cases	Controls	Cases	Controls
a. CIN-3/CIS						
Colombia	127	164	40	41.5	36	35
Spain	164	184	38	37	34	35
Pooled	291	348	38	39	34	35
b. ICC						
Brazil	72	76	52	53.5	46	48
Colombia	91	89	47	52	43	44
Philippines	155	111	46	46	46	44
Spain	146	139	50	50	50	52
Thailand	228	173	46	46	46	47
Pooled	692	588	50	50	45	46

[a]Median.

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Table 2. Penile HPV prevalence among husbands of cases and controls of CIS and ICC by history of smoking and country

	HPV tested[a]		HPV positive		HPV positive among those tested					
					Nonsmokers		Ex-smokers		Current smokers	
	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls	Cases	Controls
CIS	165	186	35 (21.2)	14 (7.5)	8 (4.8)	7 (3.8)	4 (2.4)	0 (0.0)	23 (13.9)	7 (3.8)
Spain	102	106	22 (21.6)	4 (3.8)	2 (2.0)	0 (0.0)	4 (3.9)	0 (0.0)	16 (15.7)	4 (3.8)
Colombia	63	80	13 (20.6)	10 (12.5)	6 (9.5)	7 (8.8)	0 (0.0)	0 (0.0)	7 (11.1)	3 (3.8)
ICC	444	346	78 (17.6)	56 (16.2)	16 (3.6)	18 (5.2)	19 (4.3)	15 (4.3)	43 (9.7)	23 (6.6)
Brazil	53	56	19 (35.8)	22 (39.3)	4 (7.5)	5 (8.9)	7 (13.2)	8 (14.3)	8 (15.1)	9 (16.1)
Colombia	49	48	16 (32.7)	14 (29.2)	6 (12.2)	10 (20.8)	2 (4.1)	1 (2.1)	8 (16.3)	3 (6.3)
Philippines	149	106	9 (6.0)	5 (4.7)	2 (1.3)	1 (0.9)	0 (0.0)	2 (1.9)	7 (4.7)	2 (1.9)
Spain	84	62	10 (11.9)	2 (3.2)	0 (0.0)	0 (0.0)	3 (3.6)	1 (1.6)	7 (8.3)	1 (1.6)
Thailand	109	74	24 (22.0)	13 (17.6)	4 (3.7)	2 (2.7)	7 (6.4)	3 (4.1)	13 (11.9)	8 (10.8)

[a]HPV testing of adequate specimens that were β-globin positive.

of CIS was observed for women whose partners had a history of smoking. An increasing risk of ICC was observed with decreasing time since smoking cessation with current smokers having the highest risk (OR = 1.61; 95% CI: 1.16–2.24), suggesting PS as a potential risk factor for cervical cancer. No increased risk of ICC was observed for women with male partners who used a specific tobacco type or filter. An increased risk of ICC was observed for women with partners who smoked at least a low number of smoking pack-years (OR = 1.62; 95% CI: 1.14–2.29).

Table 5 shows the association between passive and active smoking history and risk of ICC after reclassifying smoking status of men according to Figure 1. As compared with the active smoking model, we did not observe an association between male smoking habits and risk of cervical cancer among couples with lifetime nonsmoking monogamous women. Although PS was not independently associated with risk of ICC, there was an increased OR from 1.23 to 2.26 when women were exposed to PS alone or to both active and PS. The interaction term was, however, not statistically significant ($P = 0.77$; Table 6).

Discussion

This study shows no independent association of PS and risk of cervical cancer in the absence of active smoking. In the first 2 models of all couples and couples with monogamous women, the lack of association with CIS and the significant association with ICC suggests that passive cigarette smoking could potentially acts as a late carcinogen in the transition from persistent infection/preinvasive lesions to invasion. These findings are not new and are consistent with previous findings (15–17, 31–33). However, when we considered the possibility of misclassification bias in our third model of couples with lifetime nonsmoking monogamous women and reclassified the smoking status of men according to the risk period for which the woman would be exposed to both HPV infection and PS, no independent association could be found. The greatest risk estimate was more than 2-fold for couples who were both ever smokers.

The contradicting results as shown in the different models highlight the distortion of estimates probably resulting from misclassification of smoking status. This suggests that a model considering only the time period of exposure to HPV and PS should be used to determine susceptibility to carcinogenesis. The timing of exposure to tobacco smoke relative to cervical cancer development is important in defining exposure. Because we had detailed information on smoking and sexual history, we were able to define and calculate exposure based on a series of responses. The strict definitions of exposure to tobacco smoke in our analyses showed associations with risk of cervical cancer that were obscured by using simpler definitions. Nonsmoking monogamous women with men classified as ex-smokers who have quit smoking before initiating a sexual relationship may not be as susceptible to PS. In addition, the man's lifetime duration of smoking does not necessarily include the whole period of the couple's if he stops smoking during the relationship or he starts and stops smoking during the relationship. Although the possibility of misclassification of the woman's smoking status cannot be excluded, we do not believe inclusion of nonsmokers who were actually true smokers would cause substantial bias because female smoking prevalence in these study countries is low (34). In epidemiologic studies of cervical cancer etiology, the definitions of exposure should reflect a model of risk to HPV infection and cervical carcinogenesis.

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Table 3. Male characteristics and their univariate association with a risk of woman of CIN-3/CIS or ICC

	CIN-3/CIS				ICC			
	All couples		Husbands with monogamous women		All couples		Husbands with monogamous women	
	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]
	No.		No.		No.		No.	
Education								
≥Secondary	120/157	1.00	61/123	1.00	226/236	1.00	165/197	1.00
Primary school	148/167	1.21 (0.86–1.68)	100/129	**1.58 (1.04–2.36)**	374/299	**1.62 (1.24–2.12)**	260/250	**1.52 (1.12–2.07)**
No school	21/23	1.36 (0.70–2.64)	12/17	1.49 (0.65–3.41)	92/53	**2.81 (1.83–4.33)**	66/44	**2.71 (1.66–4.42)**
Age at first sexual intercourse								
≥21	31/78	1.00	17/68	1.00	175/190	1.00	132/173	1.00
17–20	105/129	**2.36 (1.43–3.89)**	68/103	**3.02 (1.62–5.64)**	317/25	**1.49 (1.14–1.96)**	222/213	**1.51 (1.12–2.04)**
≤16	154/141	**4.48 (2.58–7.81)**	89/98	**5.79 (2.91–11.55)**	199/143	**1.89 (1.36–2.64)**	136/104	**2.14 (1.47–3.12)**
History of sexual intercourse with a sex worker								
Never	81/131	1.00	43/109	1.00	216/236	1.00	155/213	1.00
Ever	210/217	**1.72 (1.21–2.45)**	131/160	**2.22 (1.42–3.45)**	473/349	**1.58 (1.25–2.01)**	333/277	**1.78 (1.36–2.34)**
History of sexual intercourse with a sex worker while with current wife								
Never	81/131	1.00	43/109	1.00	216/236	1.00	155/213	1.00
Never sexual intercourse while with wife	113/142	1.41 (0.96–2.08)	63/101	**1.88 (1.03–2.74)**	197/187	1.28 (0.96–1.71)	115/142	1.23 (0.87–1.72)
Ever sexual intercourse while with wife	97/75	**2.32 (1.52–3.56)**	68/59	**3.21 (1.90–5.39)**	245/162	**1.87 (1.42–2.46)**	218/135	**2.29 (1.69–3.11)**
History of STIs								
Never	153/208	1.00	89/168	1.00	393/394	1.00	284/347	1.00
Syphilis only	4/4	1.37 (0.33–5.62)	3/2	2.71 (0.44–16.59)	12/13	0.96 (0.42–2.14)	9/10	1.19 (0.47–2.99)
Gonorrhea only	48/40	**1.82 (1.12–2.95)**	23/27	1.81 (0.95–3.43)	154/77	**1.96 (1.43–2.69)**	105/57	**2.26 (1.57–3.27)**
Herpes only	2/7	0.43 (0.09–2.13)	2/5	0.85 (0.16–4.54)	5/3	1.59 (0.37–6.76)	4/3	1.55 (0.34–7.08)
Condyloma only	7/5	1.88 (0.58–6.06)	4/3	2.47 (0.54–11.36)	12/6	2.07 (0.76–5.62)	9/5	2.39 (0.78–7.30)
Other venereal disease only	32/44	1.06 (0.64–1.77)	24/34	1.44 (0.79–2.61)	51/28	**2.01 (1.23–3.28)**	35/19	**2.52 (1.39–4.55)**
≥2 STIs	45/40	**1.83 (1.10–3.04)**	29/30	**2.12 (1.14–3.94)**	65/67	1.07 (0.72–1.58)	45/50	1.21 (0.76–1.92)
Number of STIs								
Never	153/208	1.00	89/168	1.00	393/394	1.00	2/347	1.00
1	93/100	1.37 (0.95–1.96)	56/71	**1.63 (1.04–2.56)**	234/127	**1.87 (1.44–2.42)**	162/94	**2.18 (1.61–2.96)**
≥2	45/40	**1.81 (1.09–3.00)**	29/30	**2.13 (1.15–3.97)**	65/67	1.04 (0.70–1.55)	45/50	1.20 (0.75–1.90)
Circumcision								
Yes	15/36	1.00	8/26	1.00	179/139	1.00	138/130	1.00
No	271/311	**2.33 (1.23–4.43)**	163/242	**2.29 (1.00–5.24)**	511/449	1.15 (0.76–1.73)	352/361	1.33 (0.84–2.10)

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Table 3. Male characteristics and their univariate association with a risk of woman of CIN-3/CIS or ICC (Cont'd)

	CIN-3/CIS				ICC			
	All couples		Husbands with monogamous women		All couples		Husbands with monogamous women	
	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]	Cases/controls	OR (95% CI)[a]
Pay attention to uncover your penis and to wash the region								
Yes	220/256	1.00	130/191	1.00	621/507	1.00	438/471	1.00
No	71/92	0.79 (0.53–1.18)	44/78	0.75 (0.46–1.21)	62/80	**0.60 (0.39–0.91)**	49/73	**0.61 (0.38–0.97)**
Time since smoking cessation								
Nonsmoker	83/137	1.00	56/105	1.00	149/183	1.00	98/148	1.00
Ex-smoker >11 years	11/8	2.34 (0.90–6.10)	5/7	1.35 (0.41–4.45)	45/50	1.21 (0.76–1.93)	32/47	1.08 (0.64–1.84)
Ex-smoker ≤10 years	24/29	1.41 (0.76–2.60)	7/20	1.67 (0.27–1.69)	83/79	1.31 (0.89–1.92)	68/66	**1.56 (1.01–2.40)**
Current smoker	171/172	**1.67 (1.15–2.43)**	104/136	1.48 (0.95–2.31)	414/274	**1.85 (1.41–2.43)**	293/229	**1.94 (1.41–2.66)**
Use of tobacco type by smokers								
Blond	37/25	1.00	20/19	1.00	299/222	1.00	216/179	1.00
Black	68/84	**0.46 (0.24–0.90)**	41/67	**0.42 (0.19–0.97)**	80/62	1.13 (0.56–2.28)	59/56	0.89 (0.41–1.91)
Both	82/84	0.60 (0.33–1.11)	47/63	0.59 (0.27–1.28)	74/42	1.49 (0.72–3.07)	55/35	1.39 (0.62–3.11)
Others	4/2	1.10 (0.18–6.74)	3/1	1.82 (0.16–20.50)	69/54	0.98 (0.63–1.51)	49/51	0.82 (0.50–1.34)
Use of smoking filter type by smokers								
Filter	89/84	1.00	56/66	1.00	277/205	1.00	205/165	1.00
No filter	15/19	0.80 (0.37–1.73)	7/15	0.51 (0.19–1.42)	42/42	0.86 (0.51–1.45)	25/36	0.63 (0.35–1.15)
Both	94/99	0.91 (0.58–1.43)	50/76	0.72 (0.41–1.25)	154/99	1.32 (0.89–1.97)	114/87	1.15 (0.74–1.78)
Others	4/2	1.86 (0.32–10.69)	3/1	3.09 (0.30–31.96)	69/54	0.98 (0.63–1.53)	49/51	0.79 (0.48–1.30)
Smoking lifetime pack-years[b]								
Nonsmoker	83/137	1.00	56/105	1.00	149/183	1.00	98/148	1.00
Low no. of pack-years	96/112	1.41 (0.94–2.10)	49/89	1.05 (0.64–1.71)	272/202	**1.58 (1.18–2.11)**	196/174	**1.65 (1.18–2.30)**
Medium/high no. of pack-years	106/95	**2.00 (1.30–3.06)**	65/72	**1.79 (1.08–2.97)**	263/198	**1.75 (1.30–2.36)**	190/164	**1.82 (1.29–2.57)**
HPV status								
Negative	130/172	1.00	77/140	1.00	366/290	1.00	266/253	1.00
Positive	35/14	**3.25 (1.67–6.32)**	18/9	**3.68 (1.57–8.62)**	78/56	1.22 (0.82–1.81)	50/41	1.27 (0.80–2.03)
No HPV result/inadequate	126/162	1.04 (0.75–1.44)	79/120	1.21 (0.81–1.81)	248/242	0.83 (0.64–1.08)	175/197	0.91 (0.67–1.22)

NOTE: Bolded ORs (95% CI) were statistically significant associations ($P < 0.05$).

[a]Adjusted for age and study country.

[b]CIN-3/CIS model: Smoking lifetime pack-years for the all husbands model is defined as low (36.5–6,022.5 pack-years) and medium/high (6,132–56,611.5 pack-years); and for the husbands with monogamous women model, low (36.5–6,716 pack-years) and medium/high (6,825.5–38,963.8 pack-years); ICC: Smoking lifetime pack-years for the all husbands model is defined as: low (36.5–7,300 pack-years) and medium/high (7,354.8–43,435 pack-years); and for the husbands with monogamous women model, low (36.5–7,665 pack-years) and medium/high (7,829.3–43,435 pack-years).

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Overall, more than three-quarters of couples were with monogamous women, allowing us to explore the male PS factors in depth and to limit any potential residual confounding that may exist if women were largely not monogamous. Our results may predominantly represent societies in which women report lifetime monogamy and multiple partnerships are more common among men, which is a pattern that is generally more common in developing countries rather than in developed countries (35).

This study strengthens the current evidence for several reasons. First, this study has the largest dataset of couples with nonsmoking women to measure PS. Because our study obtained direct information from interviews with both the husband and wife, our results are considered reliable as previous studies have found good agreement in responses concerning spousal smoking status to range from 90 to 100 percent (17) and previous cotinine studies of never smokers have validated the use of spousal history as a marker of exposure to tobacco smoke and people who live with smokers tend to mix with smokers outside the home (15). In contrast, previous studies had small sample sizes with small numbers of nonsmokers.

Table 4. Multivariate associations between selected male smoking characteristics and risk of CIN-3/CIS or ICC

Male smoking characteristics	OR (95% CI)	
	All couples	Couples with monogamous women
CIN-3/CIS[a]		
Time since smoking cessation		
Nonsmoker	1.00	1.00
Ever smoker	1.33 (0.89–1.99)	1.16 (0.71–1.88)
Ex-smoker >11 years	1.51 (0.53–4.31)	0.84 (0.22–3.19)
Ex-smoker ≤10 years	1.17 (0.58–2.34)	0.67 (0.24–1.87)
Current smoker	1.36 (0.88–2.07)	1.26 (0.76–2.09)
Use of tobacco type by smokers		
Blond	1.00	1.00
Black	0.49 (0.23–1.06)	0.33 (0.12–0.88)
Both	0.49 (0.24–0.99)	0.36 (0.14–0.91)
Others	0.89 (0.12–6.39)	1.03 (0.07–15.53)
Use of smoking filter type by smokers		
Filter	1.00	1.00
No filter	0.76 (0.29–1.96)	0.49 (0.14–1.68)
Both	0.78 (0.45–1.32)	0.61 (0.31–1.18)
Others	1.63 (0.25–10.73)	2.79 (0.21–36.93)
Smoking lifetime pack-years[c]		
Nonsmoker	1.00	1.00
Low no. of pack-years	1.18 (0.75–1.85)	0.89 (0.51–1.57)
Medium/high no. of pack-years	1.52 (0.93–2.47)	1.45 (0.53–2.56)
ICC[b]		
Time since smoking cessation		
Nonsmoker	1.00	1.00
Ever smoker	1.57 (1.15–2.15)	1.55 (1.07–2.23)
Ex-smoker >11 years	1.46 (0.84–2.52)	1.11 (0.60–2.07)
Ex-smoker ≤10 years	1.50 (0.95–2.37)	1.59 (0.95–2.67)
Current smoker	1.61 (1.16–2.24)	1.63 (1.11–2.40)
P trend	0.006	0.01
Use of tobacco type by smokers		
Blond	1.00	1.00
Black	0.91 (0.27–3.04)	0.63 (0.17–2.33)
Both	1.42 (0.40–5.10)	0.83 (0.21–3.31)
Others	0.85 (0.32–2.27)	0.66 (0.21–2.08)

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Table 4. Multivariate associations between selected male smoking characteristics and risk of CIN-3/CIS or ICC (Cont'd)

Male smoking characteristics	OR (95% CI)	
	All couples	Couples with monogamous women
Use of smoking filter type by smokers		
Filter	1.00	1.00
No filter	1.34 (0.55–3.27)	0.95 (0.33–2.69)
Both	1.41 (0.72–2.72)	1.31 (0.63–2.71)
Others	0.98 (0.38–2.55)	0.83 (0.27–2.54)
Smoking lifetime pack-years[d]		
Nonsmoker	1.00	1.00
Low no. of pack-years	1.62 (1.14–2.29)	1.64 (1.09–2.47)
Medium/high no. of pack-years	1.48 (1.03–2.12)	1.39 (0.91–2.10)

[a]CIS multivariate model was adjusted for age of the husband and wife, study country, interaction terms (lifetime number of sexual partners of husband × study country and circumcision status × study country), age at first sexual intercourse of the wife (≥21 years, 17–20 years, ≤16 years), lifetime number of sexual partners of wife (1, ≥2); and husbands with monogamous women model was adjusted for all variables in the table except for circumcision, interaction term (lifetime number of sexual partners of husband × study country), age of the wife and age at first sexual intercourse of the wife.

[b]ICC model for all husbands and husbands with monogamous women were adjusted for age of the husband and wife, study country, interaction term (lifetime number of sexual partners of husband × study country), level of education of the husband and wife (≥secondary level, primary level, no schooling), history of STIs, age at first sexual intercourse of the wife (≥21 years, 17–20 years, ≤16 years), lifetime smoking pack-years of the wife (nonsmoker, low, medium, and high smoking lifetime-pack-years), oral contraceptive use (never, 1–4 years, ≥5 years), parity (nulliparous, 1–6, ≥7), pap smear history 12 months prior to study enrollment (never, ever), and lifetime number of sexual partners of the wife (1, ≥2).

[c]CIN-3/CIS model: Smoking lifetime pack-years for all husbands model is defined as low (36.5–6,022.5 pack-years) and medium/high (6,132–56,611.5 pack-years); and for the husbands with monogamous women model, low (36.5–6,716 pack-years) and medium/high (6,825.5–38,963.8 pack-years).

[d]ICC model: Smoking lifetime pack-years for the all husbands model is defined as: low (36.5–7,300 pack-years) and medium/high (7,354.8–43,435 pack-years); and for the husbands with monogamous women model, low (36.5–7,665 pack-years) and medium/high (7,829.3–43,435 pack-years).

Second, previous studies lacked adequate information on HPV and sexual behavior indicators to control for potential confounding, and we were able to control for both male and female risk factors. Third, as we currently understand the natural history of cervical cancer, not all precancerous lesions will progress to ICC (30), so we were able to evaluate the effect of PS by stage of disease (preinvasive vs. invasive).

Previous studies did not evaluate the combined effects of different exposure of active and passive smoke (both nonsmokers, female ever smoker/male nonsmoker, and female nonsmoker/male ever smoker). Although the other combinations showed an increased risk, only the combination of ever-smoking couples showed a statistically significant increased risk. The lack of an independent association with PS does not necessarily discount its contribution to ICC risk. This may suggest that the direct effect of active smoking outweighs the indirect carcinogenic effects PS may have. One of the limitations of epidemiologic studies by using questionnaire data is its decrease in sensitivity or power of a study to show a positive association when the effect may only be moderately related to PS (17). Lifetime number of sexual partners of the men largely attenuated the observed effect of PS on ICC risk. Studies have suggested that we need to consider the contribution of occupational exposure to tobacco smoke in addition to spousal/household smoking as 76% of nonsmokers who report no exposure to tobacco smoke at home have reported exposure at work (17) and about 75% of women in our study worked outside the home, which could have lead to additional misclassification of exposure and underestimated the impact of PS. In addition, although the possibility of couples not cohabiting together could lead to an overestimated PS impact, we believe the contribution is minimal as only 1.6% of couples with monogamous women reported periods of separation. To fully evaluate the impact of PS, measurement of exposure needs to take into account all environmental exposures within the household and workplace.

A biological mechanism by which active and PS could influence cervical carcinogenesis is not clearly

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Table 5. Association between passive and active smoking history and risk of ICC

	Passive smoking[a]		Active smoking[b]	
	Couples with nonsmoking monogamous women		Couples with monogamous women	
Male smoking characteristics	Cases/controls	OR (95% CI)[c]	Cases/controls	OR (95% CI)[c,d]
Smoking status				
Nonsmoker	112/167	1.00	357/407	1.00
Ever	246/240	1.28 (0.88–1.85)	134/84	1.77 (1.23–2.56)
Ex-smoker	39/54	1.01 (0.56–1.83)	38/31	1.48 (0.83–2.65)
Current smoker	207/186	1.34 (0.91–1.96)	96/53	1.94 (1.26–2.98)
Duration of exposure to smoking				
0 years	112/167	1.00	357/407	1.00
1–20 years	101/108	1.51 (0.93–2.45)	52/35	1.58 (0.91–2.73)
≥21 years	137/124	1.13 (0.73–1.75)	43/30	1.56 (0.88–2.79)
No. of cigarettes per day				
Nonsmoker	112/167	1.00	357/407	1.00
1–10 cigarettes/day	96/84	1.51 (0.95–2.39)	73/46	1.63 (1.07–2.46)
≥11 cigarettes/day	144/152	1.10 (0.73–1.66)	24/17	1.30 (0.91–1.84)
Smoking lifetime pack-years[e]				
Nonsmoker	112/167	1.00	357/407	1.00
Low	77/81	1.44 (0.88–2.36)	29/24	0.56 (0.12–2.56)
Medium	72/78	1.12 (0.68–1.83)	35/18	4.14 (0.96–17.8)
High	84/70	1.25 (0.76–2.05)	31/21	0.55 (0.14–2.25)

[a]For the passive smoking models, characteristics of the husband's smoking history was classified according to the wife's risk period of exposure to HPV and passive smoking as outlined in Figure 1.

[b]For the active smoking models, the risk of cervical cancer is based upon the woman's history of smoking.

[c]Models were adjusted for age of the husband and wife, study country, level of education of the husband and wife, lifetime number of sexual partners of the husband, history of STIs, age at first sexual intercourse of the wife, oral contraceptive use, parity, and pap smear history 12 months prior to study enrollment.

[d]Male smoking characteristics (smoking status, duration of smoking, no. of cigarettes per day, and smoking lifetime pack-years) were adjusted in the final model accordingly to the woman's smoking habits.

[e]Husband's lifetime smoking pack-years for the passive smoking model is defined as: low (36.5–3,832.5 pack-years), medium (3,942–7,884 pack-years), and high (7,938.75–67,890 pack-years); and the smoking lifetime pack-years of women for the active smoking model is defined as: low (18.25–930.75 pack-years), medium (1,022–3558.75 pack-years), and high (3,577–19,710 pack-years).

understood. However, tobacco smoke contains known carcinogens such as polycyclic aromatic hydrocarbons that could potentially have a direct transformation effect on the cervix or could cause immunosuppression, allowing HPV infections to persist and progress to cancer (15). Detectable levels of nicotine and cotinine, a measurement of smoke exposure, have been found in cervical mucus and DNA adduct levels in the cervical epithelium of nonsmokers, supporting the evidence that these chemicals can reach distant sites such as the cervix (16). Another hypothesis includes mutagenic semen due to smoking is plausible and direct cervical contact with semen of smoking partners may represent another source of exposure (17). This study lacked data measurement levels of cotinine/nicotine in the cervix, therefore, additional studies are needed to obtain these data to complement our epidemiologic findings.

Penile HPV detection was more prevalent among current smokers compared with ex-smokers and nonsmokers which is consistent to previous findings (36). This suggests that smokers may be more likely to have persistent infections compared with nonsmokers, making them more likely to expose their wives to HPV infection. However, the interpretation of penile HPV detection at study enrolment is not straightforward as it does not necessarily represent the time-point of exposure as the current understanding of the natural history of HPV in men shows that HPV is more readily transmitted from men to women than from women to men, and these infections are less likely to persist among men with approximately 75% likely to clear infection at one year (37). Other studies have not found smoking to be associated with penile HPV acquisition nor persistence (38, 39). In addition, we cannot exclude the possibility of reverse causality since

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Table 6. Risk of cervical cancer according to the smoking status of husbands and wives among couples with monogamous women

	Cases/controls	OR[a] (95% CI)
Both nonsmokers	112/167	1.00
Female nonsmoker/male ever smoker	245/240	1.23 (0.85–1.77)
Female ever smoker/male nonsmoker	30/27	1.63 (0.83–3.22)
Both ever smokers	104/57	2.26 (1.40–3.64)
P trend		0.001

[a]Model was adjusted for age of the husband and wife, study country, level of education of the husband and wife, lifetime number of sexual partners of the husband, history of STIs, age at first sexual intercourse of the wife, oral contraceptive use, parity, and pap smear history 12 months.

HPV-infected husbands could clear HPV, and be reinfected by their wives who have cervical cancer and have been replicating HPV prior to the onset of cancer. Among 116 ICC case husbands who reported no history of sex with a sex worker or a casual partner while living with their wife, 6 were HPV positive (of whom 5 women reported lifetime monogamy), making it impossible to know who was the source of HPV exposure if there was no underreporting. Second, detection of penile HPV DNA (17%) in our study was lower than recently reported prevalence estimates in men and this may result from incomplete sampling of the male genitalia as it has been suggested that for optimal HPV detection, sampling should include multiple anatomic subsites (40).

In conclusion, there are 1 billion active smokers worldwide and one-third of adults are regularly exposed to passive smoke with the burden of tobacco-related disease, disability, and death being the highest in developing regions. Moreover, the rate of increase in cigarette consumption in developing countries is 10 times that of industrialized countries (41). This burden is likely to increase in the coming decades if current trends persist with more than 90% of the world's population not protected by comprehensive smoke-free policies and there is low compliance (2%) in countries where there are comprehensive smoke-free laws (34). Globally, there is an increasing trend of females aged 13 to 15 smoking in recent years (41), which needs to be considered along with reported median age at first sexual intercourse to occur for most women is 15 to 19 years (35) when assessing risk of ICC. The data presented here support that in addition to female tobacco smoking as an established cofactor for cervical carcinogenesis, there is a potential role of passive smoke on ICC, which suggest that the estimated burden of tobacco-related diseases may increase and magnify the need for effective tobacco control, notably in developing countries.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgment

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Appendix 4. Permission granted to include the *Chapter 2* Literature Review publication in this thesis

Karly Louie - RE: TMIH paper to be included in PhD thesis

From: "Emmans, Verity - Oxford" <vemmans@wiley.com>
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Appendix 5. Permission granted to include the *Chapter 3* Study 1 in this thesis

Karly Louie

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Appendix 6. Permission granted from Lead Investigator of IARC multi-centric case control studies of invasive cervical cancer

ICO
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16 August 2010

Karly S. Louie
Department of Clinical Research
Faculty of Infectious and Tropical Diseases
Keppel Street
London WC1E 7HT

Dear Ms. Karly Louie,

As one of the Principal Investigators of the International Agency for Research on Cancer (IACR) Multicenter Cervical Cancer (CC) Study Group, I grant you access to the series of case-control studies on CC from Algeria, Brazil, Colombia, India, Morocco, Paraguay, the Philippines, Spain, and Thailand, to complete collaborative data analyses between IARC, the Catalan Institute of Oncology (ICO) from Barcelona, Spain, and the London School of Hygiene and Tropical Medicine (LSHTM). The data may be used to complete your thesis:

Study 1: Early age at first sexual intercourse, marriage and first pregnancy and risk of cervical cancer

Objective: To characterise and provide robust estimates of the risk of CC and its associatioin with age at first sexual intercourse (AFSI), interrelated characteristics such as age at first pregnancy and age at first marriage in a pooled analysis of case-control studies on CC from eight developing countries (Algeria, Morocco, India, Philippines, Thailand, Brazil, Colombia, Paraguay and Peru)

Study 2: Male sexual behaviour determinants and risk of cervical cancer in developing countries

Objective: To characterize in depth the male role in the aetiology of cervical cancer in different geographical settings in a pooled analysis of five case-control studies (Brazil, Colombia, Philippines, Spain and Thailand) involving ICC and two case-control studies (Colombia and Spain) involving cervical carcinoma in situ (CIS), of couples in which husbands or stable partners of ICC and CIS case and control women participated.

Co-authorship from these analyses will be mutually agreed upon Investigators at IARC, ICO (Xavier Bosch) and LSHTM (Karly Louie).

Sincerely,

F Xavier Bosch, MD, MPH PhD
Chief of Cancer Epidemiology Research Program
Cancer Epidemiology Research Program
Catalan Institute of Oncology (ICO)
Barcelona, Spain
Tel. (34) 93 2607812
Email: x.bosch@iconcologia.net

Appendix 7. Ethical approval granted from the London School of Hygiene and Tropical Medicine for this research

LONDON SCHOOL OF HYGIENE & TROPICAL MEDICINE

ETHICS COMMITTEE

APPROVAL FORM
Application number: 5819

Name of Principal Investigator	**Karly Louie**
Faculty	**Infectious and Tropical Diseases**
Head of Faculty	**Professor Simon Croft**

Title: **Sexual and reproductive health risk factors and risk of cervical cancer in developing countries**

This application is approved by the Committee.

Chair of the Ethics Committee .....

Date .....22 September 2010.....

Approval is dependent on local ethical approval having been received.

Any subsequent changes to the application must be submitted to the Committee via an E2 amendment form.

Appendix 8. Ethical approval granted for the Costa Rican Natural History Study of HPV and Cervical Neoplasia from US NCI

4/28/10

CLINICAL RESEARCH PROTOCOL **CONTINUING** REVIEW APPLICATION	PROTOCOL NO. OH99-C-N030	PRINCIPAL INVESTIGATOR (NIH Employee Name, Inst/Br, Address, Telephone and email): Allan Hildesheim/Mark Schiffman, NCI/DCEG, EPS

PROTOCOL TITLE:
Costa Rican Natural History Study of HPV and Cervical Neoplasia

PROTOCOL STATUS:
- ☐ Renew -Recruitment of participants has not yet begun.
- ☐ Renew -Participants are currently being recruited or enrolled.
- ■ Renew -No longer recruiting or enrolling participants, subject follow-up only.
- ☐ Renew -Participants have completed study; study and data analyses ongoing.
- ☐ Renew -Clinical Hold/Recruitment or enrollment of participants suspended.
- ☐ Terminate -Study closed. Participants have completed study. Recruitment and data analysis complete.

SUMMARY OF PROTOCOL ENROLLMENT (Aggregate): Only when the NIH is the coordinating site, provide totals and enrollment table for other site.

NIH Site	Other Sites	Total	
12,000		12,000	Accrual ceiling by IRB
0		0	New subjects accrued since last CR
11,545		11,545	Aggregate total accrued

Are you currently recruiting healthy volunteers? ☒ No ☐ Yes
Will the protocol involve adults unable to give informed consent? ☒ No ☐ Yes

Have analyses by sex, racial/ethnic subgroups been conducted for Phase 3 Clinical Trials as required? ☐ No ☐ Yes (answer a and b) ☒ N/A
a. Have analyses been reported? ☐ No (explain in narrative) ☐ Yes
b. Have significant differences been found? ☐ No ☐ Yes

Have any non-NIH investigators or sites been added since the last review?
☒ No
☐ Yes (Identify the persons or sites and describe the collaboration in the summary report)

WITH THIS REVIEW, I AM REQUESTING A CHANGE TO THE FOLLOWING:
*Include Name, Inst/Branch, Telephone, Address, e-mail. Check box if an NIH Employee and initial line. Attach sheet if necessary.

PRINCIPAL INVESTIGATOR:
Delete: _____
Add*: ☐ _____

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MEDICAL ADVISORY INVESTIGATOR:
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RESEARCH CONTACT:
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ASSOCIATE INVESTIGATOR(S):
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IONIZING RADIATION USE (X-rays, e.g., CT; radioisotopes, e.g. PET, etc.) check all that apply:
- ☒ None
- ☐ Medically indicated
- ☐ Research indicated. Since the last review,
  - ☐ Research usage HAS NOT changed.
  - ☐ Research usage HAS changed. (Explain in summary report)

INVESTIGATIONAL NEW DRUG/DEVICE: ☒ None ☐ IND ☐ IDE
*If reporting more than one IND/IDE, list on attached sheet.
FDA No. _____
Name: _____
Sponsor: _____
Who is the manufacturer of the above entity? _____

Does the protocol involve a Tech Transfer Agreement? ☒ No ☐ Yes

Does the protocol involve a drug/device/product that may lead to you or the NIH receiving payment and/or royalties?
☒ No
☐ Yes (Append a statement of disclosure)

Have there been any amendments since the last review?
☒ No
☐ Yes (Describe briefly in the attached narrative.)

Have there been any changes in the informed consent process or documentation since the last review?
☒ No
☐ Yes (Describe in Summary report)

Have there been any changes in the subject population, recruitment or selection criteria since the last review?
☒ No
☐ Yes (Explain changes in the attached narrative.)

Have any unexpected complications or side effects been noted since the last review?
☒ No
☐ Yes (Identify and explain in the attached narrative.)

Have any subjects withdrawn from this study since the last IRB approval?
☒ No
☐ Yes (Discuss in the attached narrative.)

Has any information appeared in the literature, or evolved from this or similar research, that might affect the IRB's evaluation of the risk/benefit analysis of human subjects involved in this protocol?
☒ No
☐ Yes (Discuss in the attached narrative.)

Has the NIH IRP COI Guide been distributed to new NIH investigators?
☐ No ☐ Yes ☒ N/A

Has the NIH IRP COI Guide been distributed to new Non-NIH investigators?
☐ No ☐ Yes ☒ N/A

CONFLICTS OF INTEREST REVIEW?
Date submitted to IC DEC: 2/24/10 Date cleared by IC DEC: 3/5/10

SIGNATURE	Principal Investigator	Allan Hildesheim Print/Type Name	Date 25 Feb 10	Send to Accountable Investigator
RECOMMENDATION	Accountable Investigator	Mark Schiffman Print/Type Name	Date 25 Feb 2010	Send to Branch Chief, or CC Dept. Head of Accountable Investigator
Expedited APPROVALS	Br Chief/CC Dept. Head or Acct. Invest	Mark Greene Print/Type Name	Date 3/3/10	Send to Clinical Director
	Clinical Director	William Dd Print/Type Name	Date 4/14/10	Send to Chair, Institutional Review Board
	Chair, For Institutional Review Board	Nancy Potischman Print/Type Name	Date 4/14/10	Send to Office of Protocol Services, through IRB Protocol Coordinator
COMPLETION	Protocol Specialist	Date 4/22/10	Protocol & Consent Approved Effective	

Appendix 9. Letter of request for data transfer for the Costa Rican Natural History Study of HPV and Cervical Neoplasia for *Study2* analysis

ICO
Institut Català d'Oncologia

Av. Gran Via, s/n Km 2,7
08907 L'Hospitalet - Barcelona
Tels. 93 260 77 33 / 93 335 70 11
Fax 93 260 77 83
www.iconcologia.net

9 February 2010

Mark Schiffman, MD MPH
Hormonal and Reproductive Epidemiology Branch
National Cancer Institute, National Institutes of Health
6120 Executive Blvd., Room 706
Bethesda, MD 20892 USA

Dear Dr. Schiffman,

As the NCI Co-Principal Investigator of the Proyecto Epidemiologico Guanacaste on the Natural History of HPV and Cervical Cancer Study, I am requesting data to complete a collaborative data analysis between NCI, Catalan Institute of Oncology (ICO), and the London School of Hygiene and Tropical Medicine (LSHTM).

I will be using these data for a study entitled "Sexual and reproductive behaviour determinants of human papillomavirus persistence in a prospective cohort from Guanacaste, Costa Rica". The objective of this study is:

- To evaluate the association between sexual and reproductive behaviour factors (age at first sexual intercourse, age at first birth, and parity) and the prospective risk for type-specific HPV persistence at 2-years among women positive for prevalent and incident HPV infection.

HPV persistence will be evaluated according to viral co-factors of type-specific persistence of HPV DNA, HPV viral load, and HPV serology. Other confounding risk factors such as lifetime number of sexual partners, smoking history, use of oral contraceptives, history of pap smear, and etc. will be considered.

Specifically, I am requesting the following Guanacaste cohort data

- Enrollment questionnaire: Sections (A) demographic and residential history, (B) pregnancy history, (C) sexual history, (D) contraceptive history, (E) medical history, (F) smoking history, and (G) marital/income information
- Referral questionnaire: Sections (B) vaginal douching and (C) Sexual history
- Follow-up questionnaire 1: Sections (B) smoking history, (C) pregnancy information, (D) vaginal douching, (E) Sexual history, (F) Contraceptive History, (G) Medical History
- Follow-up questionnaire 2: Sections (B) smoking history, (C) pregnancy information, (D) vaginal douching, (E) Sexual history, (F) Contraceptive History, (G) Medical History
- Viral HPV data: HPV DNA, HPV viral load, and HPV serology

Co-authorship from this analysis will be mutually agreed upon between the Investigators at NCI (Mark Schiffman), Guanacaste (Ana Cecilia Rodriguez), ICO (Silvia de Sanjose and Xavier Bosch) and LSHTM (Karly Louie also affiliated with ICO).

Thank you in advance for your attention to this data request and we look forward to this collaboration.

Sincerely,

Karly Louie, MSc
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London School of Hygiene and Tropical Medicine
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Appendix 10. Risk of 1-year follow-up persistence of prevalent type-specific HR-HPV infection according to patient characteristics

Appendix 10.1 Risk of 1-year follow-up persistence of prevalent "alpha 7 species" type-specific HR-HPV infection according to patient characteristics

	HPV-18		HPV-39		HPV-45		HPV-59		HPV-68	
Subject characteristics	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)
Age at first menarche										
15+ years	19	1.00	10	1.00	12	1.00	3.00	1.00	4	1.00
14 years	11	0.59 (0.10-3.39)	6	0.59 (0.05-6.78)	8	0.77 (0.12-4.93)	6	-	7	-
13 years	25	0.94 (0.24-3.74)	19	1.83 (0.32-10.59)	20	1.32 (0.29-5.96)	8		4	
≤12 years	44	1.20 (0.35-4.20)	21	1.22 (0.21-7.20)	24	0.71 (0.15-3.43)	11		12	
Age at first sexual intercourse										
21+ years	16	1.00	7	1.00	12	1.00	10	1.00	3	1.00
18-20 years	25	1.19 (0.22-6.34)	19	1.12 (0.20-6.33)	20	0.77 (0.12-4.93)	5	-	6	-
16-17 years	26	1.40 (0.27-7.23)	12	0.28 (0.02-3.24)	16	1.32 (0.29-5.96)	5		10	
≤15 years	32	1.87 (0.38-9.22)	18	1.18 (0.20-6.81)	17	0.71 (0.15-3.43)	8		8	
Missing										
Time of first sexual intercourse from first menarche										
<0 yrs	88	1.00		1.00	58	1.00				
0 yrs	10	-	54	-	6	-	27	1.00	45	1.00
≥1 yrs	1		2		1			-		-
Lifetime nubmer of sexual partners										
1	43	1.00	18	1.00	28	1.00	15	1.00	10	8
2	21	1.36 (0.51-3.62)	21	0.41 (0.84-2.02)	14	0.35 (0.07-1.68)	4	-	9	5
3+	35	0.45 (0.14-1.51)	17	0.25 (0.05-1.24)	23	0.51 (0.15-1.76)	9		23	14

Pregnancy										
Never	13	1.00	7	1.00	10	1.00	6	1.00	3	1.00
Ever	86	1.39 (0.17-11.66)	49	-	55	-	22		24	-
Age at first pregnancy										
21+ years	27	1.00	14	1.00	14	1.00	8	1.00	6	1.00
18-20 years	24	1.53 (0.37-6.30)	16	1.41 (0.33-6.11)	24	0.80 (0.19-3.31)	4	-	9	-
16-17 years	24	2.29 (0.55-9.61)	13	0.89 (0.14-5.58)	12	0.90 (0.19-4.22)	7		4	
≤15 years	11	3.77 (0.80-17.68)	6	1.00 (0.10-9.95)	5	0.70 (0.08-6.54)	3		5	
Number of pregnancies										
0	13	1.00	7	1.00	10	1.00	6	1.00	3	1.00
1-3	48	0.57 (0.11-2.83)	23	-	32	0.22 (0.04-1.62)	16	-	16	-
4-5	14	1.09 (0.16-7.26)	13		8	0.29 (0.04-2.12)	3		1	
7+	24	1.51 (0.22-10.33)	13		15	0.23 (0.03-1.63)	3		7	
Live Births										
0	13	1.00	8	1.00	10	1.00	6	1.00	3	1.00
1-2	41	0.80 (0.16-3.90)	17	-	22	0.24 (0.04-1.48)	12	-	15	-
3-5	23	0.44 (0.07-2.77)	19		21	0.21 (0.03-1.38)	7		4	
6+	22	1.10 (0.16-7.49)	12		12	0.28 (0.04-2.03)	3		5	
Still Births										
Never	76	**1.00**	47	1.00	55	1.00	21	1.00	24	1.00
Ever	10	**4.98 (1.38-18.00)**	2	-	-	-	1	-	-	-
Miscarriages/abortions										
0	62	1.00	37	1.00	41	1.00	19	1.00	20	1.00
1	17	1.75 (0.58-5.28)	7	1.82 (0.44-7.53)	6	0.54 (0.06-4.46)	3	-	2	-
2+	7	0.55 (0.06-4.67)	5	0.97 (0.11-8.93)	8	3.08 (0.67-14.14)			2	
Tubal or ectopic pregnancy										
0	89	1.00	48	1.00	52	1.00	26	1.00	24	1.00
1+	10	-	8	-	13	-	2	-	3	-
Cesarean section										
No	74	1.00	44	1.00	46	1.00	19	1.00	22	1.00
Yes	12	0.36 (0.05-2.79)	4	-	9	0.65 (0.08-5.20)	3	-	2	-
STI										
No	94	1.00	53	1.00	61	1.00	28	1.00	24	1.00
Yes	5	0.92 (0.12-7.19)	3	-	4	1.22 (0.15-9.86)		-	3	-

-, no convergence

OR, odds ratio; CI, confidence interval; STI, sexually transmitted infection

*Age-adjusted odds ratio

Appendix 10.2 Risk of 1-year follow-up persistence of prevalent "alpha 9 species" type-specific HR-HPV infection according to patient characteristics

	HPV-16		HPV-31		HPV-33		HPV-35		HPV-52		HPV-58	
Subject characteristics	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)	No. of infections	OR (95% CI)
Age at first menarche												
15+ years	39	1.00	21	1.00	10	1.00	8	1.00	24	1.00	18	1.00
14 years	31	0.68 (0.28-1.67)	20	1.77 (0.52-5.98)	14	3.07 (0.47-19.98)	1	-	23	1.18 (0.25-5.59)	21	0.18 (0.02-1.52)
13 years	44	0.56 (0.24-1.28)	21	0.47 (0.11-2.07)	9	1.30 (0.17-9.67)	17		22	0.75 (0.16-3.50)	39	0.70 (0.21-2.33)
≤12 years	76	0.57 (0.27-1.23)	37	1.17 (0.37-3.65)	18	2.08 (0.35-12.19)	22		45	2.32 (0.69-7.77)	50	0.88 (0.30-2.57)
Age at first sexual intercourse												
21+ years	27	1.00	14	1.00	7	1.00	7	1.00	20	1.00	16	1.00
18-20 years	55	1.17 (0.46-3.02)	33	1.14 (0.34-3.88)	13	1.15 (0.10-13.15)	13	0.19 (0.02-1.88)	28	1.18 (0.25-5.59)	35	0.18 (0.02-1.52)
16-17 years	44	0.96 (0.36-2.55)	27	0.37 (0.08-1.71)	20	4.07 (0.47-35.50)	14	0.41 (0.06-2.54)	27	0.75 (0.16-3.50)	36	0.70 (0.21-2.33)
≤15 years	64	1.21 (0.47-3.12)	25	1.27 (0.38-4.30)	11	1.02 (0.09-11.78)	14	0.64 (0.12-3.40)	39	2.32 (0.69-7.77)	41	0.88 (0.30-2.57)
Missing												
Time of first sexual intercourse from first menarche												
<0 yrs	170	1.00	89	**1.00**	47	1.00	42	1.00	104	1.00	120	1.00
0 yrs	10	1.44 (0.51-4.05)	8	**3.50 (1.06-11.58)**	3	-	4	-	7	0.57 (0.07-4.52)	5	-
≥1 yrs	5	1.97 (0.39-10.06)	2	2.30 (0.27-19.77)	1		2		3	4.08 (0.77-21.62)	3	
Lifetime nubmer of sexual partners												
1	72	1.00	40	1.00	18	1.00	14	1.00	51	1.00	42	1.00
2	48	0.95 (0.44-2.05)	26	0.48 (0.15-1.53)	14	3.78 (0.71-20.17)	11	0.48 (0.05-4.71)	27	1.77 (0.62-5.04)	38	1.35 (0.51-3.59)
3+	70	1.48 (0.77-2.84)	33	0.83 (0.35-2.01)	19	1.90 (0.34-10.78)	23	1.56 (0.36-6.85)	36	1.16 (0.41-3.30)	48	0.92 (3.44-2.48)

Pregnancy												
Never	13	1.00	12	1.00	5	1.00	4	1.00	10	1.00	11	1.00
Ever	177	2.80 (0.71-11.04)	87	1.68 (0.20-14.26)	46	-	44	-	104	-	117	-
Age at first pregnancy												
21+ years	51	1.00	25	1.00	10	1.00	14	1.00	31	1.00	31	1.00
18-20 years	56	1.11 (0.51-2.41)	35	1.53 (0.37-6.30)	18	-	14	-	34	0.72 (0.22-2.30)	48	1.83 (0.62-5.37)
16-17 years	49	1.46 (0.64-3.34)	17	2.29 (0.55-9.61)	14		11		27	1.30 (0.41-4.09)	24	0.41 (0.08-2.19)
≤15 years	21	1.61 (0.59-4.41)	10	3.77 (0.80-17.68)	4		5		12	1.86 (0.49-7.00)	14	2.71 (0.74-9.89)
Pregnancy												
Never	13	1.00	12	1.00	5	1.00	4	1.00	10	1.00	11	1.00
Ever	177	2.80 (0.71-11.04)	87	1.68 (0.20-14.26)	46	-	44	-	104	-	117	-
Number of pregnancies												
0	13	1.00	12	1.00	5	1.00	4	1.00	10	1.00	11	1.00
1-3	108	0.91 (0.29-2.83)	46	0.44 (0.12-1.71)	25	-	17	0.09 (0.01-1.67)	64	-	79	1.97 (0.25-15.65)
4-5	31	1.12 (0.30-4.22)	16	0.25 (0.04-1.48)	10		10	0.58 (0.04-8.16)	12		17	1.36 (0.13-14.79)
7+	38	0.80 (0.21-3.02)	25	0.46 (0.10-2.19)	11		17	0.71 (0.05-9.97)	28		21	2.59 (0.26-26.01)
Live Births												
0	13	1.00	12	1.00	5	1.00	4	1.00	11	1.00	11	1.00
1-2	80	1.02 (0.32-3.21)	41	0.57 (0.15-2.22)	13	0.08 (0.01-0.99)	12	-	49	1.54 (0.19-12.82)	64	2.07 (0.26-16.63)
3-5	66	0.97 (0.28-3.29)	27	0.39 (0.09-1.68)	23	0.18 (0.02-1.387)	18		32	1.37 (0.15-12.59)	33	1.75 (0.20-15.71)
6+	31	0.67 (0.17-2.54)	19	0.19 (0.03-1.05)	10	0.19 (0.03-1.34)	4		22	1.86 (0.19-18.37)	20	2.70 (0.26-28.01)
Still Births												
Never	168	1.00	82	1.00	45	1.00	41	1.00	96	1.00	113	1.00
Ever	9	0.74 (0.21-2.62)	5	1.18 (0.25-5.54)	1	-	3	-	8	1.72 (0.46-6.46)	4	0.66 (0.08-5.26)
Miscarriages/abortions												
0	138	1.00	64	1.00	39	1.00	28	1.00	80	**1.00**	94	1.00
1	21	1.23 (0.57-2.69)	14	2.43 (0.91-6.51)	5	-	7	-	17	**3.02 (1.14-8.04)**	14	1.11 (0.35-3.50)
2+	18	0.97 (0.33-2.81)	9	1.63 (0.41-6.51)	2		9		7	1.63 (0.40-6.66)	9	0.96 (0.20-4.71)
Tubal or ectopic pregnancy												
0	168	1.00	85	1.00	46	1.00	41	1.00	103	1.00	115	1.00
1+	22	2.24 (0.23-21.53)	14	-	5	-	7	-	11	-	13	5.03 (0.53-47.81)
Cesarean section												
No	157	1.00	74	1.00	38	1.00	40	1.00	86	**1.00**	98	1.00
Yes	20	0.55 (0.19-1.61)	13	0.70 (0.16-3.10)	8	-	4	-	17	**3.49 (1.22-9.92)**	19	0.56 (0.12-2.47)
STI												
No	182	1.00	88	1.00	51	1.00	48	1.00	107	1.00	121	1.00
Yes	8	0.68 (0.15-3.00)	10	4.79 (1.62-14.13)	-	-		-	7	1.92 (0.41-8.86)	7	2.67 (0.74-9.70)

-, no convergence

OR, odds ratio; CI, confidence interval; STI, sexually transmitted infection

*Age-adjusted odds ratio

Appendix 10.3 Risk of 1-year follow-up persistence of prevalent HR-HPV infection (alpha species 5 and 6) according to patient characteristics

	Alpha 5 (HPV 51)		Alpha 6 (HPV 56)	
Subject characteristics	No. of infections	OR (95% CI)*	No. of infections	OR (95% CI)*
Age at first menarche				
15+ years	26	1.00	12	1.00
14 years	24	-	14	1.00 (0.27-3.77)
13 years	40		21	0.76 (0.22-2.59)
≤12 years	56		30	0.44 (0.12-1.64)
Age at first sexual intercourse				
21+ years	23	1.00	7	1.00
18-20 years	49	-	18	1.00 (0.27-3.78)
16-17 years	40		28	0.76 (0.22-2.59)
≤15 years	34		24	0.44 (0.12-1.64)
Missing				
Time of first sexual intercourse from first menarche				
<0 yrs	137	1.00	70	1.00
0 yrs	6	-	4	-
≥1 yrs	3		3	
Lifetime nubmer of sexual partners				
1	69	1.00	35	1.00
2	29	-	20	0.89 (0.28-2.84)
3+	48		22	0.89 (0.31-2.57)
Pregnancy				
Never	15	1.00	5	1.00
Ever	131	-	72	-
Age at first pregnancy				
21+ years	36	1.00	15	1.00
18-20 years	60	-	27	1.29 (0.36-4.59)
16-17 years	27		25	2.34 (0.66-8.28)
≤15 years	8		5	0.65 (0.07-6.14)
Number of pregnancies				
0	15	1.00	5	1.00
1-3	73	-	37	-
4-5	29		12	
7+	29		23	
Live Births				
0	15	1.00	5	1.00
1-2	59	-	29	-
3-5	48		23	
6+	24		20	
Still Births				
0	124	1.00	70	1.00
1	7	-	2	0.92 (0.19-4.41)
2+				
Miscarriages/abortions				
0	97	1.00	50	1.00
1	20	-	12	0.70 (0.18-2.66)
2+	14		10	1.58 (0.48-5.18)
Tubal or ectopic pregnancy				
0	122	1.00	69	1.00
1+	24	-	8	-
Cesarean section				
No	100	1.00	64	1.00
Yes	31	-	8	-
STI				
No	136	1.00	76	1.00
Yes	9	-		-

-, no convergence

OR, odds ratio; CI, confidence interval

*Age-adjusted odds ratio

Appendix 11. Permission granted to include the *Chapter 5* Study 3 in this thesis

Karly Louie

From: permissions [Permissions@aacr.org]
Sent: 28 August 2011 18:23
To: Karly Louie
Subject: FW: Louie / Request for permission to reproduce article in PhD thesis
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Dear Karly,

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Hope this helps, please let me know if I can further assist.

Kind regards,
Karola

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