



This thesis is the basis for a joint degree of Doctor of Philosophy (PhD) between Karolinska Institutet and Makerere University.

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Thesis for doctoral degree (Ph.D.) 2009

Early Cervical Lesions Detected by Visual Inspection: Viral Factors, Management and Follow-up

Twaha Serunjogi Mutyaba

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Makerere University



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Early cervical lesions detected by visual inspection: viral factors, management and follow-up

Academic Thesis

The public defence for the degree of Doctor of Philosophy at Karolinska Institutet and Makerere University will be held at Davies lecture theatre, Mulago, Kampala. Friday, 30th October 2009, 09:00 am

Akademisk Avhandling

som för avläggande av gemensam medicine doktorsexamen vid Karolinska Institutet och Makerere University offentligen försvaras på det engelska språket i Davies Föreläsningssal, Mulago, Kampala, fredagen den 30 oktober 2009, kl 09.00

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Kampala and Stockholm 2009

ABSTRACT

Currently 80% of cervical cancer cases worldwide arise in low income countries. In Uganda, the age standardized incidence rate of cervical cancer is estimated at 40.7 per 100,000 women. Public health policies, logistical, socio-political-cultural factors and other inequities limit the delivery of services for cervical cancer prevention. This thesis presents data on biological factors and service delivery issues that may affect cervical cancer control.

Article I: A qualitative study using focus group discussions (FGDs) explored factors that influence the usage of reproductive health care services with an emphasis on cervical cancer. Barriers identified included: a lack of knowledge and misconceptions about cervical cancer, cultural constructs about the illness, economic factors, patriarchal domestic gender power relations, alternative authoritative sources of reproductive health knowledge, and unfriendly health care services.

Article II: An open interventional study with 2 arms, to evaluate the efficacy of male partner involvement in reducing the loss to follow-up among women referred for colposcopy after a positive cervical cancer-screening test. A total of 5,094 women were screened in two family planning/postnatal clinics at Mulago Hospital, Kampala, Uganda. 824 screened positive and were referred for colposcopy, half were allocated to the intervention group and half to the control group. In the intervention group, information about the screening findings and a request to assist their partner in attending the next examination were sent to the male partners. In the control group, a standard service was provided, which did not include a letter to the male partner. Women in the intervention group were more than twice as likely to return for colposcopy (odds ratio, OR 2.8, 95% confidence interval, CI 1.9–3.9).

Article III: We estimated the prevalence of cervical abnormalities detectable by visual inspection and cervical lesions diagnosed by colposcopy according to Human Immunodeficiency Virus (HIV) serostatus and described the outcomes of cryotherapy treatment.

In a ‘see and treat’ cervical prevention strategy, trained nurses screened women for cervical cancer using visual inspection with acetic acid (VIA) and visual inspection with Lugol’s iodine (VILI). Women with abnormal visual inspection findings were referred for colposcopic evaluation and HIV testing. Women with premalignant cervical lesions detected at colposcopy were treated mainly by cryotherapy, and were evaluated for treatment outcome after 3 months by a second colposcopy.

The colposcopy diagnosed abnormal cervical lesions in 27% of women who returned.

HIV seropositivity was associated with a higher likelihood of cervical lesions especially inflammation (Risk Ratio, RR=1.7, 95% CI 1.2-2.4) and low grade squamous intraepithelial lesions (LGSIL) (RR= 2.6, 95% CI 1.0-6.7).

Article IV: From 625 women who underwent colposcopic evaluation, information on social demographic characteristics was collected. They were tested for HIV testing and HPV typing was performed using SPF10/LiPA. The overall prevalence of HPV infection was 39.4 %. The most common HPV types in decreasing order of frequency were: HPV16, HPV52, HPV35, and HPV18. An age of less than 40 years, low income status and infection with HIV were statistically significant risk factors for any HPV infection.

Conclusions: The work in this thesis identified potential barriers for the successful implementation of cervical cancer programs in Uganda, proposed some practical solutions that may improve cervical cancer screening uptake and added to the body of knowledge about the distribution of HPV types that may be relevant to the development of second generation HPV vaccines.

Key words: cervical cancer, viral factors, visual inspection, uptake, follow up, Uganda

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**Early Cervical Lesions Detected by Visual Inspection: Viral
Factors, Management and Follow-up**

Twaha Serunjogi Mutyaba



MAKERERE UNIVERSITY



Kampala and Stockholm 2009

In the long history of humankind (and animal kind, too) those who learned to collaborate and improvise most effectively have prevailed.

Charles Darwin

Knowledge of what is does not open the door directly to what should be.

Albert Einstein

Cover photos by Bo Lambert.

Front photo: Portrays a day in the life of a rural Ugandan woman.

Back photo: Upper Mulago, the location of the two family planning/postnatal clinics, where the cervical cancer screening was performed.

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ISBN 978-91-7409-584-5

PERSONAL NOTE

As an undergraduate medical student, my priority was to pass and become a doctor. There was no particular focus on any speciality, but even then, one could pick out the major problems in each discipline. What stood out in gynaecology even then, was the high number of cervical cancer patients, taking up one third of all the bed space on gynaecological wards.

During my residency training in Obstetrics and Gynaecology, I researched the knowledge, attitudes and practices on cervical cancer screening, among the medical workers of Mulago Hospital. The findings were not encouraging, as 80% of the nurses interviewed had never been screened (Mutya et al 2006).

I brought my mother to my colleague and friend, Dr Evelyn Nabunya, with whom we did the residency training, for screening (Pap smear). Mother refused, saying that there was no way she was ever going to expose herself to a young doctor even if she was female. In any case, she did not have any symptoms. To date, I have failed to get her screened.

These were eye-openers to me that it will take more than knowledge of causes and methods of cervical cancer prevention, to actually reduce the incidence of cervical cancer in Uganda.

I dedicate all this to you, maama Twaha.

ABSTRACT

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LIST OF PUBLICATIONS

This thesis is based on the following scientific articles, which are subsequently referred to by their Roman numerals.

- I Mutyaba T, Faxelid E, Mirembe F, Weiderpass E. Influences on uptake of reproductive health services in Nsangi community of Uganda and their implications for cervical cancer screening. *Reproductive Health* 2007, 4:4.
- II Mutyaba T, Mirembe F, Sandin S, Weiderpass E. Male partner involvement in reducing loss to follow-up after cervical cancer screening in Uganda. *International Journal of Gynecology and Obstetrics*. *In press*.
- III Mutyaba T, Mirembe F, Sandin S, Weiderpass E. HIV serostatus and the role of colposcopy in a 'see and treat' program for cervical cancer prevention in Uganda. *Submitted*.
- IV Mutyaba T, Mirembe F, Kleter B, Quint W, Doorn LJ, Sandin S, Weiderpass E. HPV and HIV infections among women referred for colposcopy after screening with visual inspection in Mulago Hospital, Uganda. *Submitted*.

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1 LIST OF ABBREVIATIONS

HPV	Human papillomavirus
HIV	Human immunodeficiency virus
VIA	Visual inspection with acetic acid
VILI	Visual inspection with Lugol's iodine
LGSIL	Low grade squamous intraepithelial lesion
HGSIL	High grade intraepithelial lesion
PCR	Polymerase chain reaction
SPF10	Short PCR fragment
LiPA	Line probe assay
IARC	International Agency for Research on Cancer
HSV-2	Herpes Simplex Virus type 2
DNA	Deoxyribonucleic acid
CDC	Centers for Disease Control
LCR	Long Control Region
DEIA	DNA enzyme immunoassay
CIN	Cervical intraepithelial neoplasia
SIL	Squamous intraepithelial lesion
ASCUS	Atypical squamous cells of undetermined significance
LEEP	Loop electrosurgical excision procedure
VLPs	Virus like particles
UBOS	Uganda Bureau of Statistics
MOH	Ministry of Health
FGDs	Focus group discussions
LC1	Local Council 1
DEIA	DNA Enzyme Immuno Assay
RFLP	Restriction fragment length polymorphism
SIDA	Swedish International Development Cooperation Agency

2 INTRODUCTION

2.1 BURDEN OF CERVICAL CANCER AND GLOBAL TRENDS

Cervical cancer is the second most common cancer among females worldwide, with an estimated 493,243 new patients per year (IARC 2005). Of all new cases of cervical cancer, 80% occur in low income countries (Pagliusi 2004; Parkin 2006; Castlellsagué 2007). In Africa, it is the most common malignancy of women and it is estimated that 78, 897 new cases occur per year with mortality estimated at 61,671 deaths per year (Parkin 2005).

Cervical cancer is the most common malignancy of women in Uganda with age the standardized incidence estimated at 36.3 per 100,000 (IARC 2005). The incidence of squamous cell carcinoma of the cervix has declined in high income countries over time, largely due to effective screening services using the Pap smear (Mahlck 1994; Elovainio 1997; Gustafsson 1997; Hakama 1997; Hemminki 2002; Parkin 2005; Curado 2007).

Such programs which use cytological screening are too expensive to organize in most low income countries (Bradley 2005; Tsu 2005). A situational analysis of cervical cancer screening services in 5 countries in eastern, central and southern Africa found negligible cervical cancer screening services at most health units (Chirenje 2001).

Table 1 summarizes the distribution of the global burden of cervical cancer worldwide.

Table 1. Cancers of the uterine cervix. Incident cases, deaths and 5-year prevalence in 18 world regions in 2002.

	Cervix cancer		
	Cases	Deaths	5-year prevalence
World	492 800	273 200	1 409 200
More developed countries	83 400	39 500	309 900
Less developed countries	409 400	233 700	1 099 300
Eastern Africa	33 900	27 100	57 200
Middle Africa	8200	6600	13 900
Northern Africa	8100	6500	14 000
Southern Africa	7600	4400	13 100
Western Africa	20 900	16 700	35 700
Caribbean	6300	3100	18 400
Central America	17 100	8100	49 300
South America	48 300	21 400	139 200
Northern America	14 600	5700	58 200
Eastern Asia	61 100	31 300	191 900
South-Eastern Asia	42 500	22 500	132 500
South Central Asia	157 700	86 700	446 100
Western Asia	4400	2100	13 700
Eastern Europe	30 800	17 100	107 700
Northern Europe	5600	2800	21 100
Southern Europe	10 600	4100	40 900
Western Europe	12 700	5600	49 200
Oceania	2000	800	6500

Adapted from Sankaranarayanan (2006).

2.2 ETIOLOGICAL FACTORS

Persistent infection with oncogenic human papillomaviruses (HPV) has been established as the necessary causal factor for cervical cancer (zur Hausen 1996; Walboomers 1999; Bosch 2002). Harald zur Hausen, the 2008 Nobel Prize laureate for Physiology or Medicine, postulated as early as the 1970s that HPV infections were the causative agents, but in the absence of laboratory testing and difficulty of culturing the viruses, could only prove it in the eighties (Gissmann 1980; Durst 1983). With the advent of better technologies to detect viral genome, other researchers confirmed his hypotheses (Schiffman 1993; Gravitt 2000; Ifitner 2003; Muñoz 2003). The main route of transmission of HPV is sexual intercourse (Bosch 2007), but a few cases of vertical transmission have been reported (Puranen 1996). Many women get exposed to HPV but very few develop cervical cancer (Bosch 2007). This implies that there are other co-factors at play in the carcinogenic process. Behavioural determinants include the number of sexual partners, the sexual behaviour of male partner, the age of sexual debut, high parity, smoking and the long term use of oral contraceptives (Bosch 2007). Co- infection with other sexually transmitted diseases such as Chlamydia Trachomatis and Herpes Simplex Virus type 2 (HSV-2) increase the risk (Daling 1996; Szarewski 1996; Ferrera 1997; Deacon 2000; Plummer 2003; Smith 2004; Vaccarella 2006; Bosch 2007; International Collaboration of Epidemiological Studies of Cervical Cancer. 2007; Sasieni 2007). However, the role of HSV-2 in cervical cancer onset is contentious, and it is argued that the studies which concluded that it had a causal role in cervical cancer onset did not control for the role of HPV (Lehtinen 2002). Immunosuppression, as that is caused by infection with HIV infection and organ transplantation, has been associated with multiple infections with HPV, persistence and progression from premalignant lesions to cancer (CDC 1993; Fleming 1999; Moscicki 2004; Harris 2005; Clifford 2006; Moodley 2006; Banura 2008; Denny 2008).

2.3 EPIDEMIOLOGY AND NATURAL HISTORY OF HPV

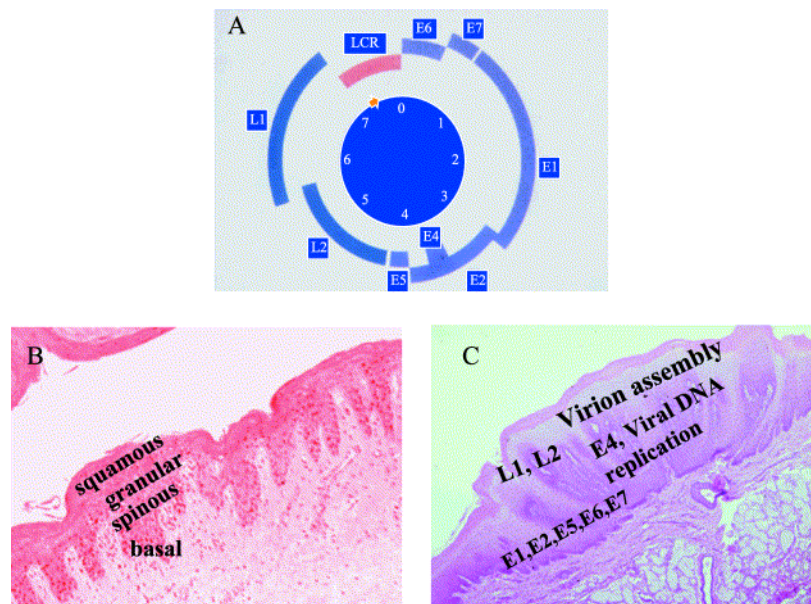
HPV is the most common sexually transmitted infection (Bosch 2007). A meta-analysis from 15 areas in 4 continents among women 15-74 years old, found overall HPV prevalence in 157,879 women with normal cervical cytology to be 10.4% (Bosch 2007). A global review of 346,160 women from 7 geographical regions reported prevalence ranging from 15 to 20% (Smith 2008).

Corresponding estimates for adult women by region were: Africa 22.1%, Central America and Mexico 20.4%, Northern America 11.3%, Europe 8.1% and Asia 8.0% (Bosch 2008). In all world regions, HPV prevalence peaked in women younger than 35 years of age, and decreasing in women of older age. In Africa, the Americas, and Europe, a clear second peak of HPV prevalence was observed in women aged 45 years or older. On the basis of these estimates, around 291 million women worldwide are carriers of HPV DNA, of whom 32% are infected with HPV16 or HPV18, or both (Bosch 2008). The HPV types most commonly detected are similar to those most commonly described in pre-neoplastic and cancer cases, although the relative contribution of HPV16 and HPV18 is substantially lower in cytologically normal women. Studies from Uganda have found prevalence ranging between 17% in adult women (Serwadda 1999; Blossom 2007; Asimwe 2008) to 74.6% among adolescents (Banura 2008).

Over 100 HPV genotypes have been identified. The HPV types are classified according to the nucleotide sequence of L1 gene coding for the major capsid protein. They are further grouped into genera if there is similarity of the L1 gene of less than 60% (de Villiers 2004). The HPV types which infect the genital mucosa belong to the genus alpha papillomaviruses (de Villiers 2004). Studies have shown that some related mucosal HPV types have oncogenic potential and have been designated as high risk. These include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, 73 and 82 which are categorized as carcinogenic, and HPV 26, 53 and 66 as probably carcinogenic (Schiffman 1993; Bosch 2002; Muñoz 2003; Munoz 2004; Smith 2008). Other HPV types cause no disease or cause benign lesions, with HPV 6 and 11 responsible for the majority of genital condylomata (Garland 2009).

Despite being a commonly transmitted virus, HPV infections are transient and the majority, over 90%, will be cleared within 3 years (Ho 1998; Bosch 2008). The majority of women infected with HPV do not develop cervical cancer (Nobbenhuis 1999; Castle 2005; Moscicki 2006). The replicative cycle of HPV is linked to the differentiation of HPV infected epithelium. HPV infect basal cells and the viral proteins override cell cycle arrest, with eventual production of new virions released in the squamous layers (O'Brien 2002), as shown in Figure 1.

Figure 1. The papillomavirus life cycle. Adapted from O'Brien (2002).



(A) Generic map of papillomavirus genome. The circular genome of approximately 7.8 kb is divided into 'early', 'late' and non-coding regions (LCR).
 (B) Histological section of normal epithelium, with the cellular layers indicated.
 (C) Epithelial papilloma. Expression of the early proteins E1, E2, E5, E6 and E7 starts in the basal and suprabasal layers; expression of E4 and viral DNA replication take place in the granular and spinous layers; production of the capsid proteins L1 and L2 takes place in the squamous layers; virions are assembled in the squamous layers and released with the keratin squames.

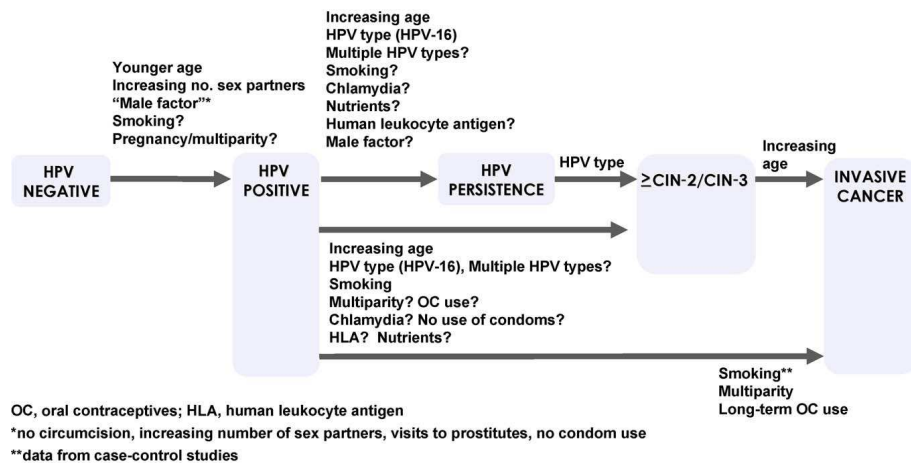
2.4 VIRAL EVASION OF THE HOST IMMUNE MECHANISMS

HPV is a double stranded DNA virus (Baker 1991; zur Hausen 1996; zur Hausen 2000). The genome is divided into 3 regions: the early region that encodes nonstructural viral regulatory proteins, the late region that encodes the 2 structural proteins (L1 & L2) and the long control region (LCR) that is non-coding (Stubenrauch 1999; O'Brien 2002). The Early region has the viral proteins E1, E2, E4, E5, E6 and E7 (Stubenrauch 1999; O'Brien 2002; Munger 2004). The major viral proteins in evasion of host immunity and immortalization of the cell are E6 and E7. E6 interferes with tumor suppressor protein p53 which regulates cell cycle arrest or apoptosis in case of DNA damage (Stubenrauch 1999; Munger 2004). E7 binds and

degrades retinoblastoma proteins (Rb), which are cell cycle regulators (Munger 2004). They control the transition from the G-phase to the S-phase of the cell cycle to allow for the repair of damaged DNA. By blocking these 2 tumour suppressor proteins, E6 and E7 transform the cell and immortalize it (zur Hausen 1996; Munger 2004). The L1 and L2 capsid proteins are expressed late in the infection (Ozbun 1997). L2 is responsible for presentation of the viral genome to the nucleus of the host cell after uncoating (Ozbun 1997). There is minimal viral antigenic exposure to the host immune system due to several reasons: the infection has no stage of viraemia, the initial viral proteins synthesized are nuclear and the virus does not induce cell death (Munger 2004). The pathological changes induced by the HPV oncoproteins are termed cervical intraepithelial neoplasia (CIN) (Kanodia 2007). The understanding of these mechanisms has informed the development of prophylactic vaccines targeting the L1 capsid protein. Research on the development of therapeutic vaccines targeting E6 and E7, the major oncogenes, is ongoing (Hung 2008).

2.5 THE EARLY PRE-MALIGNANT CERVICAL LESIONS

The diagram below shows the natural history of HPV and precancerous lesions and the role of different co-factors. Adapted from Moscicki (2006).



Several terminologies are used to refer to HPV-induced pathological changes in the cervical epithelium. In most of Europe, the Richart classification (Richart 1965; Richart 1973) uses cervical intraepithelial neoplasia (CIN). The Bethesda

classification uses squamous intraepithelial lesion (SIL) (Solomon 2002). Lesions are classified histologically in accordance to the degree of neoplastic progression, with a scale ranging from CIN1 to CIN3, or carcinoma in situ (CIS) by the Richart classification. The corresponding grades in the Bethesda classification are low grade SIL (LGSIL), for CIN1 and high grade SIL (HGSIL), for CIN2 and CIN3. Atypical cells of unknown significance (ASCUS) refer to unclear abnormal findings which may be due to neoplastic changes, inflammations or non infective processes (Stanley 2003; Steenbergen 2005).

Natural history studies of precancerous lesions of the cervix have reported regression of LGSIL in over 60% and progression to cancer of 1%, regression of CIN2 of 40% and progression to cancer of 5%, regression of CIN3 of 30% and progression to cancer of 12% (Castle 2009). The average modal time from infection with HPV to CIN3 has been estimated to be between 7 to 15 years and from CIN3 to invasive cancer averaging 10 years (Ostör 1993; Moscicki 2006; Tranbaloc 2008; Huh 2009).

2.6 PREVENTION OF CERVICAL CANCER

2.6.1 PAP SMEAR

First suggested in 1928 by Dr George Papanicolaou, with findings published in 1941 (Papanicolaou 1997), the Pap smear revolutionized cervical cancer prevention.

Taking advantage of the long period between CIN and invasive cancer and the ease of treatment of precancerous lesions, programs based on cytological screening have had a great impact on the reduction of cervical cancer incidence in mostly developed countries which can afford them. An example is the USA where in the past 60 years, the incidence of cervical cancer has been reduced by 75%, from 32.6 to 8.7 per 100,000 and mortality rates from cervical cancer decreased by 70%, from 9.3 to 2.5 per 100,000 (CDC 2005). Similar and sometimes better results were reported in Europe, especially the Nordic countries (Gustafsson 1997; Anttila 1999). Similar programs have proven too expensive to organize in most low income countries, and alternative feasible methods using visual screening have been suggested (Bradley 2005).

2.6.2 VISUAL INSPECTION METHODS

VIA and VILI

Visual inspection with acetic acid (VIA) involves naked-eye inspection of the cervix under bright light conditions at least 1 minute after the application of 3-5% diluted acetic acid (Blumenthal 2005). The test can be carried out by nurses or midwives (Megevand 1993; Sankaranarayanan 1998; University of Zimbabwe/ JHPIEGO project 1999).

A positive result is based on the appearance of well-defined, acetowhite areas in the transformation zone (Blumenthal 2005). This is the region on the cervix that undergoes metaplastic change from columnar epithelium to squamous epithelium. Reported sensitivity of VIA ranges from 52% to 79% and specificity from 49% to 88%, which are similar to those for cytology (Arbyn 2008). However, VIA is inefficient in detecting lesions located in the cervical canal of the uterus (Arbyn 2008).

Visual inspection with Lugol's iodine (VILI) uses Lugol's iodine solution applied to the cervix. It stains glycogen stored in cervical epithelial cells. Neoplastic and immature squamous metaplastic epithelium have less glycogen than the normal mature squamous epithelium and so do not turn mahogany brown. Instead they appear as mustard yellow changes, easily recognizable as the acetowhite changes associated with VIA. Sensitivity has been reported to vary from 78% to 98% and specificity from 73% to 91% (Sankaranarayanan 1998). Both VIA and VILI have an added advantage of giving immediate results (Arbyn 2008).

These two methods of visual inspection have been shown to be feasible as a primary means of screening for cervical cancer in low income settings and can be used by well trained nurses.

Because of organizational constraints and reported high rates of loss to follow up, strategies aiming at screening and treatment in the same visit ('see and treat') have been proposed. Effectiveness and acceptability have been evaluated in several studies and found to be good (Tsu 2005; Sankaranarayanan 2007; Luciani 2008; Kitpeerakoo 2009). Treatment of precancerous lesions is by either ablative methods, the most common being cryotherapy, or excisional methods, such as cone biopsy and loop electrosurgical excision procedure (LEEP). Unlike LEEP, cryotherapy does not avail a biopsy sample, and thus it is not possible to know if the whole lesion has been destroyed. Cryotherapy is easier to use and can be performed by nurses, making it

more applicable in low income settings (Sankaranarayan 2007). However, it may not be enough to treat all lesions detected, for example when the entire squamocolumnar junction cannot be visualized, when the lesion is too large for the cryotherapy probe to cover in one application, if lesion extends into the endocervical canal and when there is severe cervical atrophy (Gage 2009).

One disadvantage of 'see and treat' screening strategy is overtreatment which has been reported to range from 1.2 to 83.3% (Cárdenas-Turanzas 2005). This is likely to be worse in see and treat strategies without a colposcopy before treatment.

Colposcopy has been evaluated and could be an added asset in improving the effectiveness of 'see and treat' programs (Mitchell 1998; Benedet 2004). There is evidence that nurses can be trained effectively as colposcopists (Morris 1998; Todd 2002).

2.6.3 HPV DNA TESTING

Knowing that persistent high risk HPV infection is the necessary cause for cervical cancer, and the commercial availability of HPV detection tests, HPV testing has been suggested as a primary cervical cancer screening test. A recent study in India found a statistically significant reduction in mortality due to cervical cancer by a single round of HPV testing among women aged 30-59 years (Sankaranarayanan 2009). HPV DNA testing has also been suggested for triage of women with equivocal or low-grade cytological abnormalities, for follow-up of women with abnormal screening results who are negative at colposcopy or biopsy, and for prediction of the therapeutic outcome after treatment of cervical intraepithelial neoplasia (Scott 2002; Cuzick 2008). Primary HPV DNA-based screening with cytology triage with repeat HPV DNA testing of cytology-negative women has been reported to improve sensitivity by 35 % (Naucler 2009).

2.6.4 HPV PROPHYLACTIC VACCINATION

Since the identification of HPV 16 in cervical cancer biopsies (Durst 1983), the development of vaccines against the virus has happened in a relatively short time. The synthesis of virus-like particles (VLPs) which simulate the species-specific L1 protein of the virus capsid, has enabled scientists to produce type specific prophylactic vaccines against HPV (Kirnbauer 1992; Kirnbauer 1993).

For the largest effect on cervical cancer prevention, it was logical that the initial targets have been HPV 16 and 18, which are responsible for 70% of squamous cell cervical carcinomas, and HPV 6 and 11, which are responsible for most of condylomata (Bosch 2008). Currently, two HPV vaccines have been approved and are available on the market; a quadrivalent HPV6/11/ 16/18 vaccine (Gardasil®_Merck, Inc. Whitehouse Station, NJ), and a bivalent HPV16/18 vaccine (Cervarix® GlaxoSmithKline Biologicals, Rixensart, Belgium).

Numerous studies have reported on the safety, immunogenicity, efficacy (using development of CIN3 as the end point), and acceptability of the vaccines. Results have been overwhelmingly positive with efficacy close to 100 % (Harper 2004; FUTURE II Study Group 2007; Paavonen 2007; Harper 2009).

Because HPV vaccines are recent developments, some of the remaining questions concern how long the protection by vaccination lasts and issues of cross protection. After follow up of 6 year for Cervarix® and 5 years for Gardasil®, it was reported that the efficacy of preventing CIN remains 100% for both, and cross protection has been reported against HPV types 31 and 45 (Harper 2006).

With such promising results of the vaccines, there is a need for research on service delivery systems and issues of uptake to maximize the benefit from vaccination.

Indeed studies are ongoing and findings from Uganda indicated that school-based and community-based vaccination systems were highly acceptable (Katahoire 2008; Bingham 2009).

The availability of HPV vaccines will not negate the need for screening (Thiry 2009), and indeed it is suggested that there will be no immediate change in the screening policies even among vaccinated women, because of problems in identifying target populations, problems of compliance in having all three doses, and known factors which lower the vaccine efficacy over time, such as the number of sexual partners (Massad 2009). Screening guidelines are likely to remain the same, even among the vaccinated (Spitzer 2007; Apgar 2009).

2.7 DETERMINANTS OF UPTAKE AND LOSS TO FOLLOW UP IN CERVICAL CANCER PREVENTION

Using Pap smear programs, most high income countries have managed to reduce the incidence of cervical squamous cell carcinoma and therefore mortality from cervical cancer (Gustafsson 1997; Anttila 1999; CDC 2005; Curado 2007). The major burden

remains in the low income countries where 80% of new cervical cancer cases occur (Parkin 2005). However disparities of cervical cancer incidence exist within even the high income countries like the USA, due to socio-political and cultural issues (Tsu 2008; Fisher 2009) Inequities within income, gender, ethnicity, religion, education and geography underpin the unequal disease burden (Tsu 2008), since they affect the availability of services and usage.

Authoritative sources of knowledge influence whom women will believe regarding health issues (Davis-Floyd 1996; Justice 1999; Mutyaba 2007). Poverty is probably one of the strongest influences, and the issue of user fees in health units impacts negatively on access and utilization of health services (Nanda 2002; Bradley 2005). Gender influences the uptake of reproductive health services in two ways. At the household level, gender inequalities within a household influence who gets health care, and in many patriarchal societies, women are victims of this disparity (Markovic 2005; Basu 2006; Mutyaba 2007). Often, screening is within the services associated with management of sexually transmitted infections. However, women may shun these services, due to the associated stigma and fear of their male partners suspecting them of having sexually transmitted infections (Ahmed 2000; Manhart 2000; Adamson 2003; Bradley 2005; Tsu 2008). At the health service provision level, women may be uncomfortable being examined by a male health worker, and indeed in some communities, religious beliefs prohibit a woman to undress for any man other than her husband (Wood 1997; Thomas 2005; Oelke 2007; Azaiza 2008). Uganda has two health care models, the traditional system, which existed before the colonial times, and the western system, introduced along European models in the colonial times. People seek care depending on how they perceive their illness, the so called explanatory models. These determine people's understanding of the causes of illnesses, influence coping mechanisms, and influence discourse about health issues within different communities. More often than not, they are not consistent with those held by health professionals (Dein 2004; Shahid 2009). According to the health belief model, perceived barriers, susceptibility and perceived benefits act in moving people to action on issues of their health (Rosenstock 1988; Johnson 2008).

For effective cervical cancer prevention in low income settings, issues to consider should include the right policies and programs, proper logistical organization, the method of service delivery such as static versus mobile services, and the issue of user fees. Policy and program design should employ bottom-up as opposed to top-down approaches (Bradley 2005).

2.8 THE UGANDAN CONTEXT

Uganda is a low income country. The vast majority of the population (89%), is rural, and the major economic activity is subsistence agriculture (UBOS 2007). Selected demographic, health and economic indicators for Uganda from 1995 to 2007 are displayed in Table 2.

Table 2. Selected demographic, health and economic indicators for Uganda

Indicator	1995	2002	2007
Population (millions).	19.3	24.4	29.6
Population growth rate (%).	2.5	3.2	3.2
Total fertility rate (%).	6.9	6.9	6.7
HIV prevalence (%).	15	6.1	6.4
Maternal mortality ratio (per 100, 000 live births).	506	505	435
Contraceptive prevalence (%).	15	22.8	24.4
Unmet need for contraception (%).	29	35	41
Supervised deliveries (%).	38	38	42
Antenatal care, at least 1 visit.			94%
Antenatal care, 4 visits.			47%
Literacy - men (%).	-	-	76
Literacy - women (%).	-	-	63
Population below poverty line (<US \$ 1) (%).	35	38	31
Full immunization of children (%).	47	38	46
Government expenditure on health (as % of GDP).	2.1	2.2	-

Source: MOH (2006), UBOS (2007).

The uptake of reproductive health services in Uganda is low, as indicated by low contraceptive prevalence, supervised deliveries and antenatal attendance for the recommended four visits. A situational analysis done in six countries of East, Central and Southern Africa reported almost non-existent cervical cancer screening services

(Chirenje 2001). Another study done in Mulago Hospital, Kampala, Uganda, reported that 80% of the nurses interviewed had never had a cervical cancer screening test and only 14% of the final year medical students interviewed felt skilled enough to use a vaginal speculum (Mutya 2006).

3 AIMS AND OBJECTIVES OF THE STUDIES

General objective

The general objective of this thesis was to identify potential barriers for the successful implementation of cervical cancer prevention programs in Uganda, to identify some feasible solutions, and to contribute knowledge on the distribution of HPV types that may be relevant to the development of second generation HPV vaccines.

Specific objectives

Article I.

To better understand factors that influence usage of available reproductive health care services and how they would impact cervical cancer screening.

Article II.

To evaluate the efficacy of male partner involvement in reducing loss to follow-up among women referred for colposcopy after a positive cervical cancer screening using VIA/VILI.

Article III.

To determine the prevalence of cervical lesions diagnosed by colposcopy among women referred for colposcopy after screening positive by VIA/VILI.
To describe the influence of HIV serostatus on prevalence of lesions and outcome of treatment of premalignant lesions with cryotherapy.

Article IV.

To describe type specific prevalence of Human Papillomavirus (HPV) infection among women who returned for colposcopy after screening VIA/VILI.
To describe the association of HPV infection with HIV serostatus and HPV infection in cervical lesions as diagnosed by colposcopy.

4 SUBJECTS, MATERIALS AND METHODS

Table 3 summarizes the settings of each study carried out, the study designs and the participants involved.

Table 3. Showing study settings, study designs and participants.

<i>Article</i>	<i>Setting</i>	<i>Design</i>	<i>Participants</i>
I	Nsangi community, Uganda and Mulago Hospital, Kampala, Uganda	Qualitative study (FGDs)	Two groups of women and two groups of men from Nsangi community. Two groups of women attending Mulago family planning/postnatal clinic. Two groups of nurses/midwives from the family planning/postnatal clinics. Total number of participants for the FGDs = 82
Articles II, III and IV are based on data from 5,094 women, who were screened for cervical cancer by nurses in 2 family planning clinics between February 2007 and August 2008. Those screened positive were referred for colposcopy and treatment.			
II	Mulago Hospital, Kampala, Uganda	Interventional study, two arms	824 women who were referred for colposcopy: 415 in the interventional arm and 419 in the control arm
III & IV	Mulago Hospital, Kampala, Uganda	Cross sectional studies	625 women who returned for colposcopy.

Article I

Data collection was by focus group discussions (FGDs). The age range of participants was 28 to 63 years, with a mean of 45 years. The participants from Nsangi community were mainly subsistence farmers and the majority belonged to the Baganda ethnic group, the largest tribe representing about 20% of the population of Uganda. We contacted the Local Council (LC1) women secretary and the chairperson to inform the village about the study and plan the most convenient days, time and venue for the discussions. The male LC1 chairman helped in mobilizing the male participants and the woman LC1 secretary mobilized the women participants. We had no mixed groups with men and women because culturally among the Baganda and in many other tribes of Uganda, women are modest in the presence of men, and this would have affected the discussions negatively. We selected women who came to the postnatal clinic as representatives of the very few women (10%) who come for that service in Uganda. Nurses form the bulk of the Ugandan health work force and they are therefore familiar with the day-to-day problems of health service delivery. The nurse in charge of the postnatal/family-planning clinic invited women who sought family planning services and routine postnatal care to participate if they consented. Discussions in the community were held on Sundays when the participants had time and do not go to work in the agricultural fields. The interviews were held in a school classroom which was always free over the weekend and were moderated by the principal investigator and a female research assistant.

We conducted framework analysis through the following steps: familiarization, identifying a thematic framework, indexing, charting, mapping and interpretation. The taped discussions were transcribed within three days. We read and re-read the transcripts and observation notes to familiarize ourselves with the range of issues. We then developed a thematic framework from the a priori themes and emergent themes. This was then applied to the data to sort the data according to the themes.

Articles II, III and IV.

Articles II, III and IV are based on information derived from a 'see and treat' cervical cancer screening program. Between February 2007 and August 2008, a total of 5,094 women were screened by trained nurses using visual inspection with acetic acid (VIA) and Lugol's iodine (VILI). Screening was done at 2 family planning/postnatal clinics of Mulago Hospital, the national referral and teaching hospital of Uganda. It is the largest hospital in the country, and is located 2 km from the city center of Kampala. Group health education sessions were held at the clinics each morning

regarding the services available. The sessions provided information about the extent of the problem of cervical cancer, causes and risk factors, symptoms, and treatment options. Attendees were taught about prevention of the disease, and those eligible were offered the screening test. The nurses had been intensively trained on using VIA/VILI for screening. Lugol's iodine takes 3–5 days to disappear from the cervix and obscures the visualization of cervical vasculature and acetowhitening, which are essential for colposcopic evaluation. Therefore, women referred for colposcopy were told to return for the examination after at least 1 week, between Monday and Friday.

Article II

Women who screened positive at visual inspection were told the result and the implications, and assigned a date to return for colposcopy. They were informed that further management would depend on the colposcopic findings. Consenting women who screened positive following VIA/VILI and were living with a male partner in a stable relationship were eligible for the study. The intervention was a letter addressed to the male partner, in addition to the standard routine described above. The women were told about the contents of the letter, which they were asked to deliver to their male partner. The letter informed him that his partner had a condition that required further evaluation and requested that he offer her assistance in returning within the indicated period. The letter was written in English and Luganda, which is the major local language in the area where the study was conducted. Assignment into the 2 groups was according to the week in which women were screened, which approximated to group randomization. The main outcome measure was whether women returned for colposcopy within the study period. The probability of returning for colposcopy was compared between the study arms by fitting logistic regression models calculating the odds ratio (OR) and the associated 2-sided 95% confidence interval (CI). In supplementary logistic regression models, we adjusted for women's age, income status, education, distance from the clinic, male partner education, and male partner income status.

Article III

Participants were consenting women who returned for colposcopic evaluation having screened positive.

All women who returned for the colposcopic examination were tested for HIV after pre-test counseling. We used the Abbott Determine HIV-1/2 qualitative

immunochromatographic test (ABBOT JAPAN CO., LTD, Minato-Ku, Tokyo, Japan). Test results were validated using 2 other tests, the ChemBio HIV ½ Sta-Pak Dipstick (ChemBio Diagnostics Systems, Inc., 3661 Horseblock Road, Medford, NY 11763, USA), and the Trinity Biotech Uni-Gold HIV Test (Trinity Biotech Plc, Bray Co Wicklow, Ireland). The women had been informed about the HIV testing prior to their accepting to participate in the study. Post-test counseling was done and where necessary, women were directed to the relevant clinics that offer HIV care services. Blind as to the HIV status of the woman, the colposcopy was performed by the same investigator who graded the lesions using the Reid's colposcopic index, which considers 4 colposcopic signs: lesion margin, colour of acetowhitening, blood vessels, and iodine staining. It permits objective differentiation between squamous intraepithelial neoplasm (SIL) as low-grade cervical lesions (LGSIL) and high-grade lesions (HGSIL). Findings were recorded on a scale of increasing severity as: normal, inflammation, atypia/CIN/condylomata/wart/leukoplakia/HPV change, CIN2-3, and invasive carcinoma.

After taking a colposcopic guided biopsy, precancerous lesions were treated immediately using cryotherapy or loop electrosurgical excision procedure (LEEP), if the lesion extended over more than 75% of the transformation zone. The cryotherapy used nitrous oxide gas. All women who were treated were given a course of antibiotics, (Metronidazole and Doxycycline) for a week.

We did not perform biopsies from a cervix that appeared normal on colposcopy or where the colposcopist was convinced the diagnosis was inflammation. The women were told to return for re-evaluation after three months from the date of treatment. However, they could come back earlier if they experienced complications, such as bleeding or persistent vaginal discharge. Those who had other cervical conditions such as infections were appropriately treated, and those with cervical polyps or obvious cancer were admitted to the gynecological ward for appropriate management. Women who were treated for a premalignant cervical lesion had a colposcopic re-evaluation after 3 months, by the same colposcopist who performed the initial colposcopy to assess the outcome of treatment which was classified as: normal findings, persistent lesions, infection or inflammation, and cervical stenosis. The information was duly recorded on their data forms and women informed of the findings.

Article IV

Participants were consenting women who returned for colposcopic evaluation having screened positive for cervical abnormalities using visual inspection.

Collection of HPV samples was done by the gynecologist who carried out the colposcopic evaluation. After explaining the procedure to the woman, the vulva was inspected and a Grave's speculum inserted to expose the cervix. The cervical sample for HPV testing was collected using a cytobrush for the endocervical cells and an Ayre's spatula for the ectocervix. These were dipped and gently rotated in ThinPrep/PreservCyt transport medium contained in vials. The vial was sealed and labeled with the study identification number, name, and collection date and kept at room temperature. The samples were shipped to the DDL Diagnostic Laboratory (Voorburg, The Netherlands) for HPV testing.

Isolation of HPV DNA. Total DNA was isolated from 200 μ L of the suspension containing the cervical cells by use of the MagNA Pure LC instrument (Roche Diagnostics), using the Total DNA isolation kit (Roche Diagnostics). DNA was eluted in 100 μ L elution buffer, and 10 μ L was used for each polymerase chain reaction (PCR). Each run contained positive and negative controls to monitor the DNA isolation, PCR, HPV detection, and genotyping procedures.

PCR testing. The short PCR fragment (SPF) 10 primer set was used to amplify a broad spectrum of HPV genotypes. The primer set amplifies a fragment of 65 bp from the L1 region of HPV. Reverse primers contain a biotin label at the 5' end, enabling capture of the reverse strand onto streptavidin-coated microtiter plates. Captured amplimers were denatured by alkaline treatment, and the captured strand was detected by a defined cocktail of digoxigenin-labelled probes, which detects a broad spectrum of HPV genotypes. This method is called the HPV DNA enzyme immunoassay (DEIA), and it provides an optical density value. If the SPF10-DEIA yielded a borderline value (75% to 100% of the cut-off value), the SPF10 PCR was repeated and retested by DEIA. The same SPF10 amplimers can be used to identify the HPV genotype by reverse hybridization on a reverse-hybridization line probe assay (LiPA) that contains probes for 25 different HPV genotypes (SPF10 HPV LiPA, version 1; Labo Bio-medical Products; capable of detecting HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68 or 73, 70, and 74). Samples that were positive at SPF10 PCR primer but did not reveal any of the 25 aforementioned types were classified as positive for an unidentified HPV type (HPV X). The different HPV types were categorized as follows:

- High risk HPV types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66 and 68 or 73
- Low risk HPV types: 6, 11, 40, 43, 44, 54, 70, 74
- HPV 16 related types: 16, 31, 33, 35, 52, and 58
- HPV 18 related types: 18, 39, 45, 59, 68 or 73

4.1 ETHICAL ISSUES

All studies had clearance from the institutional review committees at Makerere University and Mulago Hospital. In addition we observed the following ethical issues during the conduct of the studies:

- Informed consent was obtained from the study participants.
- We involved the community leaders in the organization of the study in the community.
- We observed privacy in the screening and colposcopic examinations.
- All the women who declined to participate or were ineligible still received appropriate care.
- Participants had pre and post-test counseling for the HIV test and those who had a positive test were directed to the appropriate clinics which offer HIV care services.
- We offered no economic incentives to the women.

5 RESULTS

Article I

The following barriers were identified that might affect the uptake of cervical cancer prevention programs.

A lack of knowledge and misconceptions about cervical cancer: among the participants from the community, cervical cancer was not perceived as a major health problem and most women had never heard of it. The main reproductive health problems reported by the women were pregnancy related complications, diseases of the fallopian tubes, menstrual problems and unplanned pregnancy. Participants had misconceptions about cervical cancer: some attributed it to contraceptive usage and infertility. Although the nurses knew cervical cancer was a public health problem some had misconceptions about it too. One said it is caused by use of local herbs especially in pregnancy. Another said it is difficult to diagnose and therefore most women come to hospital with advanced disease.

Cultural constructs about cervical cancer (and related genital diseases) and alternative authoritative sources of knowledge: some discussants perceived cervical cancer as a "traditional" disease. Most women cited their paternal aunt, mother, elderly women and peers as the authoritative source of knowledge about reproductive health issues. Health workers were not perceived as authoritative sources of knowledge on reproductive health issues.

Economic factors and male partner influences: prioritization of available resources for other expenses such as school fees, informal charges and even bribery in the government units, discouraged women from seeking care from the health units. At the household level, women reported that the men control monetary resources. In the interviews with the men, it emerged that culturally, men in this ethnic group are not expected to get involved in reproductive health problems of their women, this indicated lack of dialogue at home regarding reproductive health issues.

Health services factors: government health units were reported by the women and men to be underequipped, understaffed and lacking medicines. The nurses were reported to be unfriendly. There was no privacy and waiting time in hospitals was reported to be too long. Nurses had justification for their behaviour citing low pay, and being overworked, and they reported a lack of essential equipment. The nurses did not feel appreciated by the authorities, despite the heavy workload.

Article II

In univariate analysis, the proportion lost to follow-up was higher among young (<30 years) and elderly women (>60 years), compared to women aged between 30–60 years (P=0.000, Table 1, Article II). Women with higher education and higher income were less likely to be lost to follow-up (P=0.005 and P=0.001, respectively). The percentage lost to follow-up was lower among women residing 10 km or less from the screening center (P=0.005) and among women with male partners of higher income status (P=0.007). A lower percentage was lost to follow-up among women residing in rural areas than among women residing in urban areas (P=0.029). Women in the intervention group were more likely to return for colposcopic evaluation than were women in the control group, with 16% and 34%, respectively, lost to follow-up (Table 2, Article II). The effect of the intervention remained statistically significant after adjustment for age, distance from the screening center, residence, education, income status and male partner's income status (OR 2.8; 95% CI, 1.9–3.9) (Table 3, Article II).

Article III

625 women who returned for the colposcopic evaluation were tested for HIV and had a colposcopic evaluation. 102 (16%) were HIV positive. At colposcopy, one hundred and seventy one (171) (27%) women had cervical lesions: 129 inflammation, 18 LGSIL, 14 HGSIL, 8 invasive cancer, and 2 inconclusive results. Thus, the false positive screening by visual inspection alone, calculated as the proportion of women with a positive visual inspection but a negative colposcopy, was 73% (454 out of 625).

There was no statistically significant association between age, age of sexual debut, parity and low or high grade SIL (Table 1, Article III). There was a highly significant statistical association between the risk of SIL and low education and low income status (Table 1, Article III).

There was a statistically significant association between HIV seropositivity and having a cervical lesion (P = 0.007). Bivariate analysis showed that HIV seropositivity was associated with a higher likelihood of inflammation (RR= 1.7, 95% CI: 1.2-2.4), LGSIL (RR= 2.6, 95% CI: 1.0-6.7), and any lesion (RR= 1.5, 95% CI: 1.1-2.0). There was no statistically significant association between HIV serostatus

and HGSIL (RR= 0.4, 95% CI: 0.1-3.0) (Table 2, Article III). All the 8 women who had invasive cancer were HIV negative.

The 32 women with SIL (18 LGSIL and 14 HGSIL) underwent treatment by cryotherapy (31 women) or LEEP (1 woman). After three months from the time of treatment, another colposcopic evaluation was performed. One woman had persistent LSIL, and one had inflammation. Both were HIV positive. Three women, all HIV negative, did not return after 3 months. All other 27 women had normal findings at the repeat colposcopy.

Article IV

The social demographic characteristics of the women are summarized in table I, Article IV. 102 (16%) women were HIV positive. Compared to older women, younger women (<40 years of age) were more likely to have any HPV (P < 0.001), low risk HPV (P < 0.001) and high risk HPV (P < 0.001). A low income status was associated with any HPV (P < 0.001), high risk HPV (P < 0.001). There was no statistically significant association between the age of sexual debut and HPV infection (Table 1, Article IV).

The overall prevalence of HPV infection was 39.4 % (246 out of 625) (95% CI 35-43). The 10 most common HPV types in decreasing order of frequency were: HPV16, HPV52, HPV35, HPV18, HPV33, HPV51, HPV66, HPV56, HPV6 and HPV31. High risk types were more prevalent at 26.6% than low risk types at 8.5%. The prevalence of unidentified types (HPVX) was 8.5%. The prevalence of HPV 16-related types and HPV 18-related types was 18.1% and 8.2% respectively. Together, HPV 16 and 18 types accounted for 8.8%. Types 6 and 11 accounted for 2.2%. Overall, 26 (4.2%) women had multiple infections. The prevalence of multiple HPV infection was higher (15.7%) among HIV positive women compared to HIV negative women (1.9%) (Table 2, Article IV).

Infection with HIV was a significant risk factor for any HPV infection (RR 2.2, 95% CI 1.8-2.6), low risk HPV (RR 3.4, 95% CI 1.8-2.6), high risk HPV (RR 2.8, 95% CI 2.2-5.6), HPV 16/18 (RR 2.1, 95% CI 1.2-3.6), HPV16 related infection (RR 3.0, 95% CI 2.2-4.2), HPV18 related (RR 3.4, 95% CI 2.0-6.0) (Table 3, Article IV).

By colposcopy, 35.4% of women with normal findings had an HPV infection (any type), and 22.9% had a high risk HPV infection. Among women with colposcopic diagnosis of inflammation, 53% had an HPV infection and 34.4% had a high risk HPV infection. Among women with LGSIL, 44% had an HPV infection and 38.9%

had a high risk HPV infection. Among women with HGSIL, 28.6% had an HPV infection and 21.4% a high risk HPV infection. Among women with invasive cancer, 88.9% had an HPV infection, all of them a high risk HPV infection (Table 5, Article IV).

6 DISCUSSION

6.1 METHODOLOGIES

Article I

The choice of method was determined by the study objectives, which could not be answered by quantitative methods. Recognition that health is a result of interplay between social, economic, political and environmental factors has driven the need for interpretative research to complement epidemiological methodologies (Baum 1995; Malterud 2001).

Uses of qualitative methods are broadly divided into 4 categories: contextual, to identify the form and nature of what exists, diagnostic, to explore reasons for or causes of what exists, evaluative, to appraise the effectiveness of what exists, and strategic, to identify new theories, policies and plans (Ritchie 1994).

Our inquiry was diagnostic to explain why the uptake of reproductive health is low as well as strategic, to identify ways of improving on the situation.

We chose FGDs over individual interviews as the data collection method for our objective. Advantages of FGDs include the generation of multiple perspectives on factors influencing motivations, synergetic discussion about the phenomenon of interest, ease of assembly, inexpensive and flexible in terms of format, types of questions and desired outcomes. They provide rich data through direct interaction between the researcher and participants. Group dynamics help to focus on most important issues and allow assessment of the extent to which there is a consistent and shared view (Carey 1994; Robinson 1999; Krueger 2000).

Trustworthiness

This could be likened to validity and veracity sought in epidemiological research, usually by minimizing bias and confounding. In qualitative research where the issue of the subjectivity of the researcher is often a concern, it is important that measures are taken to make the study findings believable, the truth value of the study findings (Dahlgren 2004). This is achieved in the process: planning the study, participant selection, conduct of the data collection and analysis. These reflect the rigor of the research.

We discuss the rigor and trustworthiness based on guidelines for reviewers of qualitative research reports as outlined by (Malterud 2001).

Relevance of aims: the research question was relevant to explore reasons for the low uptake of reproductive health in Uganda as reported in the Uganda Demographic and Health Survey (UBOS 2007).

Reflexivity: to minimize the influence of the researcher on the process, the focus groups involving the women in the community were facilitated by a female research assistant, who was a resident in the same community. The principal investigator kept a low profile and took notes during these sessions. Those with the men were moderated by the principal investigator who was male. The interviews with the nurses were facilitated by the principal investigator, a medical colleague known to them. Triangulation to enhance the consistency as well as get wider views about the issue of unfriendly health services was by a unique method. The taped discussions by the women and men in the community, especially those implicating the unfriendliness of health personnel as a factor keeping women away from government health units, were played back to the nurses to elicit reactions (confirmation/denial) and explanations for their behaviour.

Selection of participants and data collection: we selected adult women and men in the same community, at their convenience and acceptance to participate. Their age ranged from 28 to 63 years. They were mature enough to have experienced need for reproductive health care services and were mature enough to reflect on the issues raised using personal experiences. The interviews were conducted following a focus group guide that had been translated into the local language and tested. At the end of each session the notes were read back to the participants to agree whether what was written was what had transpired during the discussions.

Analysis and reporting: we had a priori themes which set the theoretical framework but were open to emergent themes. Audiotapes of the discussions were transcribed verbatim. Detailed notes of non-taped interviews, focus group observation notes, and field notes were also transcribed. We selected appropriate verbatim quotes to capture the themes identified.

Limitations

Because of limitations of time and costs, we did not address the constraints of health policy and financing which impact directly or indirectly on the uptake. Interviewing

policy makers and administrators would give some explanations as to why health units are understaffed and supply of drugs is erratic.

Article II

This was an interventional study. Intervention trials provide the strongest evidence with which to test hypotheses and randomization controls for known and unknown confounders (IARC/WHO 1999). In this study, participant assignment was done through a week of screening which approximated to group randomization, though the analysis was at the individual level. One limitation of this would have been the underestimation of the intracluster variation effect (Murray 2008). We tested the internal validity of the participant assignment into the two groups during the analysis in two ways: (1) by comparing the distribution of the demographic characteristics of the women in the two groups at the individual level, and (2) by comparing secondary outcomes such as distribution of cervical lesions within the two groups. We found no statistically significant difference.

We used intention to treat analysis as opposed to per-protocol analysis. Intention to treat analysis reflects the compliance or non-compliance when the intervention is introduced in practice (Passalacqua 2009). It also minimizes bias in case those who do not comply are different from those who do. It may however dilute the effect of the intervention (IARC/WHO 1999). The appropriate statistical methods were used in analysis. We fit logistic regression models calculating the odds ratio (OR) and the associated 2-sided 95% confidence interval (CI). In supplementary logistic regression models, we adjusted for confounding variables.

Article III

The validity of HIV testing: the HIV testing was performed using the Abbott Determine HIV-1/2 qualitative immunochromatographic test (ABBOT JAPAN CO., LTD, Minato-Ku, Tokyo, Japan). Positive tests were confirmed using The ChemBio HIV ½ Sta-Pak Dipstick (ChemBio Diagnostics Systems, Inc., 3661 Horseblock Road, Medford, Ny 11763, USA). If there was disagreement between the two, we used a third test: the Trinity Biotech Uni-Gold HIV Test (Trinity Biotech Plc, Bray Co Wicklow, Ireland), as a tie-breaker.

All colposcopies were performed by the same person, so there was no inter observer variation regarding colposcopy diagnosis.

We used the Wilcoxon rank sum test for the association of the different explanatory variables and the different cervical lesions. It is a non-parametric test with no normality assumptions.

Article IV

HPV testing methods are broadly categorized into non PCR-based tests and the PCR-based tests. Most signal amplification methods designed for the detection of HPV DNA are usually cocktails of probes for high risk HPV types, which use nucleic acid hybridization and are mainly used in the triage of women with inconclusive cytology results like ASCUS (Clavel 1998; Eder 2009). The most utilized is Hybrid Capture system, HCII (Digene).

Target amplification methods for identifying specific HPV sub-types mainly use consensus primer polymerase chain reaction (PCR) to amplify a broad spectrum of HPV types. Type specific primers are then used to detect a particular type (Brink 2007). After PCR, several methods can be used to detect the HPV types. Gel electrophoresis: in standard form it does not give typing information, but in other formats as used in restriction fragment length polymorphism (RFLP), it can give type information, though with limited sensitivity. Most methods use hybridization between PCR products and probes. The original hybridization assay was a Southern blot, in which amplicons are electrophoresed and then transferred to a membrane. Labeled probes are then added and hybridize to the amplicons (Kuypers 1993). Currently, the most frequently used amplicon detection methods are reverse hybridization, the most common one being the line probe assay (LiPA), as was used in this study. Captured amplicons were denatured by alkaline treatment, and the captured strand was detected by a defined cocktail of digoxigenin-labelled probes, which detect a broad spectrum of HPV genotypes. This method is called HPV DNA enzyme immunoassay (DEIA), and it provides an optical density value. If the SPF10-DEIA yielded a borderline value (75% to 100% of the cut-off value), the SPF10 PCR was repeated and retested by DEIA. The same SPF10 amplicons can be used to identify the HPV genotype by reverse hybridization on a reverse-hybridization line probe assay (LiPA) that contains probes for 25 different HPV genotypes (SPF10 HPV LiPA, version 1; Labo Bio-medical Products; capable of detecting HPV 6, 11, 16, 18, 31, 33, 34, 35, 39, 40, 42,

43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 66, 68or73, 70, and 74). Samples that were positive at the SPF10 PCR primer but did not reveal any of the 25 aforementioned types were classified as positive for HPV X. Sensitivity of the method is rated at 97.2% (Kleter 1999).

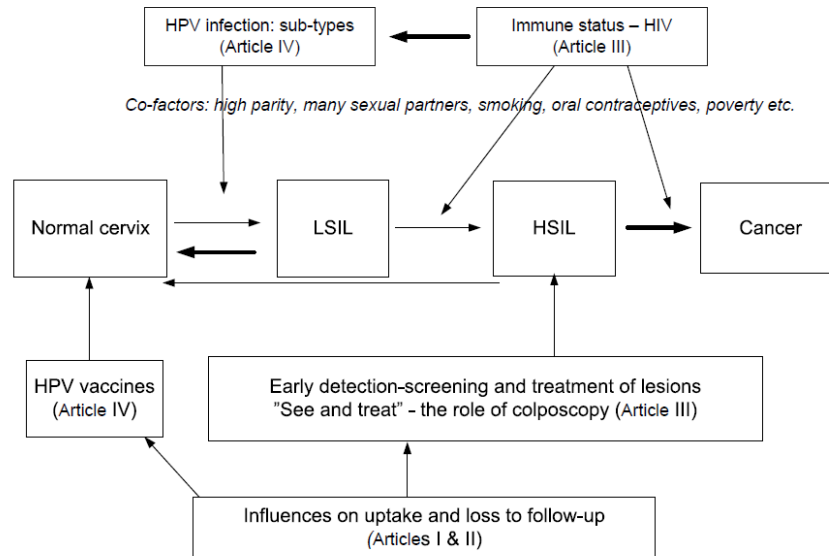
The specimens for HPV detection may be formalin fixed paraffin embedded tissue, Pap stained archival smears or liquid based sample. Liquid based samples are better at preserving the cell morphology and integrity of nucleic acids, and therefore giving less false negative HPV test results compared to formalin fixed paraffin embedded specimens (Ferraz 2004; Taha 2006).

Validity of methods used in the study: The collection of samples was done by an experienced gynecologist familiar with the techniques. The use of both Ayre's spatula and a cytobrush improved on the adequacy of the sample. The specimen were liquid based which ensured better preservation of nucleic acids, therefore minimizing false negative HPV testing. The method of HPV detection that was used is highly sensitive, with 97.2% sensitivity.

6.2 APPRAISAL OF FINDINGS OF THE STUDIES

Figure 2 conceptualizes the studies in this thesis.

Figure 2. Causal relationships and preventive measures for cervical cancer.



The figure depicts the causal pathway for cervical cancer. Above are the causes: HPV infection, the necessary cause, and immunological status which affects the clearance or persistence of HPV infection. In the background are the co factors: parity, smoking etc. Below are the preventive measures. Primary prevention, by HPV vaccination, and secondary prevention, by screening for, and treatment of premalignant lesions. The big arrows indicate major directions for example, most LSIL regress, and synergy between HIV and HPV.

Article I

Women perceived health services, especially in government units to be unfriendly. The quality of health service provision as an influence on its uptake has been reported in many studies, especially regarding the uptake of family planning services (Randrianasolo 2008; Agha 2009). The findings we reported were in agreement with these findings. What these studies do not report are the reasons for the unfriendly behaviour of the service providers which we reported in the study.

Our findings of gender influence at the household level were similar to reports from other studies (Markovic 2005; Tsu 2008; Thiel de Bocanegra 2009). However at the health unit level we found that the women did not mind the gender of the health service provider as reported in some other studies (Markovic 2005; Thiel de Bocanegra 2009).

Cultural constructs about cervical cancer and the existence of alternative authoritative sources of reproductive health knowledge may be explained by the evolution of health care in Uganda. The western model is a recent introduction after colonization. It is elitist, which is reflected in the language used in medical practice. The women reported that they leave the clinics without understanding their illness and cannot read the writings of the health workers. On the other hand, the alternative authoritative sources of knowledge may give explanatory models of illnesses more understandable to the women, since they are grounded in the local context (Baum 1995; Davis-Floyd 1996).

We reported a lack of knowledge about cervical cancer issues as another obstacle to uptake. Similar findings have been reported from other studies from the developing world (Bradley 2005; Anorlu 2008; Moodley J 2009).

Article II

We reported on an intervention of male involvement to reduce loss to follow-up of women who had a positive screening test. Most research reporting on the involvement of men in women's health issues is less about the effectiveness of the men's involvement, but more of evaluating programs and making suggestions about the ways services might change to involve more men (Sternberg 2004). Most of it is qualitative and reports an unwillingness of men to be involved (Flores 1995; Thiel de Bocanegra 2009). We quantified the effect of a targeted intervention and the findings indicated that male involvement reduced loss to follow-up. Since we used intention to treat analysis, we cannot ascertain whether the male partners actually received and

acted on the letter as requested. The intervention could have worked by a different mechanism, that is, by influencing the perception of the gravity of screening findings, and therefore influencing the decision to return, the health belief model (Rosenstock 1988). On the other hand, many studies have reported similar findings indicating male partner willingness to participate in women's health issues (Flores 1995; Schehl 1997; Mutyaba 2007). Their reticence to get involved may be due to cultural and economic constraints. In case only some of the women actually delivered the letter to their male partners and they acted on it, then we might have underestimated the effect.

Article III

The prevalence of HIV seropositivity was almost three times that of the general population (MOH 2006). This was not surprising. It was a population of women who had a positive cervical cancer screening test. The relationship between cervical cancer and HIV infection is well established (CDC 1993). HIV seropositivity was associated with a higher likelihood of having cervical lesions, a similar finding to what many studies have reported (Wasserheit 1992; Fleming 1999; Palefsky 2003; Danso 2006; Parham 2006).

At colposcopy, one hundred and seventy one (171: (27%) women had cervical lesions. This means that the false positive screening using visual inspection alone, calculated as the proportion of women with a positive visual inspection but a negative colposcopy, was 73% (454 out of 625). This would be even higher if one only considered SIL or cancer. Some 'see and treat' programs perform cryotherapy after a positive screen test using visual inspection. In this study, we treated after a positive colposcopy diagnosis of SIL. Overtreatment in 'see and treat' programs has been reported to range from 1.2 to 83.3% (Cárdenas-Turanzas 2005).

We were hampered by the small sample size of the women who underwent cryotherapy to measure the statistical difference on the treatment outcome between the HIV positive and negatives. Of the 31 who were treated and had a repeat colposcopy after 3 months, one woman had persistent LSIL, one had inflammation, and both were HIV positive. Three women, all HIV negative, did not return after 3 months. Reports from elsewhere have reported a higher recurrence rate of precancerous lesions after treatment among HIV positive compared to HIV negative, especially if the CD4 counts have been low (Vonau 2000; Danso 2006; Franceschi

2007). We did not measure CD4 counts of the women in this study which was another limitation

Article IV

Detection of specific genotypes of HPV is rationalized by the need to identify those at higher risk for CIN and subsequently cervical cancer, in order to monitor for a resolution of disease in follow-up after treatment, to describe geographical distribution of HPV that will enable the development of second generation vaccines and to monitor emergence of types that will be major causes after a reduction of HPV 16 and 18 incidence and prevalence following widespread vaccination (Eder, 2009). We studied a population that had screened positive in screening for cervical cancer. This selection impacted on the results reported. The overall prevalence of HPV infection was high at 39.4%. Comparative estimates for Africa range between 20-31% (de Sanjose 2007).

A study among young women aged 12-24 years in Kampala found higher prevalence of 74.6% (Banura 2008). This is mostly likely due to the difference in age structure of the study populations.

An increased risk of HPV among the HIV positive women was similar to other reported studies (Wasserheit 1992; Franceschi 2007). In this study we reported that in 66.7% of diagnoses of invasive cancer, types 16 or 18 were isolated. They are responsible for 70% of cervical cancers world wide (Bosch 1995; zur Hausen 2001; Harper 2009). Similar findings were reported by Odida et al (2008). Thus the same projected reductions will be expected for Uganda in the vaccinated population.

The four most prevalent types reported in Article IV were 16, 52, 35 and 18. Studying an adolescent population 12-24 years of age, Banura (2008) reported the most common prevalent HPV types to be: 52, 51, 18 and 16 in that order. In Zimbabwe, a study reported HPV types 58, 16, 70 and 18 to be the most frequent in a cohort of HIV negative women (Fukuchi 2009). Reported frequencies for Uganda are types 16, 18, 56 and 33 in cervical cancer, types 16, 52, 35 and 51 in high grade lesions and types 35, 16, 52 and 53 in low grade lesion(de Sanjose 2007) (Castellsagué 2007).

Although vaccination with bivalent vaccine against types 16 and 18 has been found to confer cross protection against types 31 and 45 of up to 40%, and other types (33, 52 and 58) of up to 25% (Brown 2009), the findings of different type specific distribution in our findings add support to the need for the development of second generation vaccines. Table 4 summarizes HPV studies from Uganda in the recent past

Table 4. Summary of recent HPV studies from Uganda.

Study	Population	HPV analysis	Results
Serwadda et al 1999	960 women aged 15–59yrs, self-collected vaginal Swabs	Signal amplification Hybrid Capture(II)	Overall HPV prevalence = 16.7%, HPV prevalence = 44.3% in HIV-positive and 10.2% in HIV negative women, HIV-1 prevalence = 17.8%.
Blossom et al 2007	106 women, STI clinic, Kampala	Target amplification Pure Kit (Roche) PCR/reverse blot assay	HPV prevalence = 46.2% Commonest high risk genotypes were 52, 58, and 16. HPV 16/18 = 18% HIV prevalence was 34.9%
Asimwe et al 2008	314 women, 18–49yrs, SW Uganda.	Signal amplification Hybrid Capture(II)	Prevalence high-risk HPV = 17.2% No HIV prevalence reported
Odida et al 2008	186 confirmed cervical carcinoma archival samples	Target amplification SPF10 HPV LiPA, version 1	114 out of 186 positive for HPV DNA. Specific HPV genotypes were identifiable in 109 cases: HPV 16, 18, 31, 35, 39, 44, 45, 51, 52 and 70. Single infections =96.3%, multiple infections =3.7%. HPV 16 or 18 = 80% of cases with single infection.
Banura et al 2008	1,275 sexually active women aged 12–24 years, Naguru teenage clinic.	Target amplification SPF10 HPV LiPA, version 1	Prevalence of HPV infection =74.6%, High-risk HPV types = 51.4%. Commonest: HPV 52, 51, 18 & 16. HIV + had higher prevalence HPV (87.8% vs 73.2%) and of multiple-types (64.6% vs. 37.3%), compared with HIV-.
Banura et al 2008	987 primiparous pregnant women aged <25 years	Target amplification SPF10 HPV LiPA, version 1	Prevalence of HPV = 60% and HIV = 7.3%. HPV 51 = 12.1%, HPV16 = 8.4% and HPV18 = 5.8%. New HPV infections detected in 42.9% of women between the 1st/2nd and 3rd trimesters, and 38.1% between pregnancy and delivery, but 50.4% and 71.8% of HPV infections, respectively, cleared,
Mutyaba et al 2009	625 women, 18–70 years, screened positive using VIA, Mulago	Target amplification SPF10 HPV LiPA, version 1	Overall prevalence HPV = 39.4 %, HIV = 16%. Commonest types HPV16, HPV52, HPV35, and HPV18. High risk types = 26.6%, low risk= 8.5%, unidentified types 8.5%. PV 16/18 = 8. Types 6/11 = 2.2%. Overall, 26 (4.2%) women had multiple infections with the prevalence higher (15.7%) among HIV positive women compared to HIV negative women (1.9%)

7 CONCLUSIONS

Article I.

The aim was to better understand factors that influence the usage of available reproductive health care services and how they would impact cervical cancer screening.

Conclusions: A lack of knowledge and misconceptions about cervical cancer, cultural factors and patriarchal domestic power relations, alternative authoritative knowledge sources, economic factors and unfriendly health services were barriers to the uptake of services.

Article II.

The aim was to evaluate the efficacy of male partner involvement in reducing loss to follow-up among women referred for colposcopy after a positive cervical cancer screening using VIA/VILI.

Conclusion: Male partner involvement in form of a letter reduced more than two fold, loss to follow-up among women referred for colposcopy.

Article III.

The aims were to determine the prevalence of cervical lesions diagnosed by colposcopy among women referred for colposcopy after screening positive by VIA/VILI and to describe the influence of HIV serostatus on the prevalence of lesions and outcome of treatment of premalignant lesions with cryotherapy.

Conclusions: The prevalence of cervical lesions diagnosed by colposcopy was 27%, implying a high false positive screening rate by visual inspection in this 'see and treat' program. The association between HIV seropositivity and the prevalence of premalignant cervical lesions was statistically significant. We could not conclude on the effect of HIV seropositivity on the treatment outcome because of the low number of women who were treated.

Article IV.

The aims were to describe the type specific prevalence of HPV infection, the association of HPV infection with HIV serostatus and HPV infection in cervical lesions

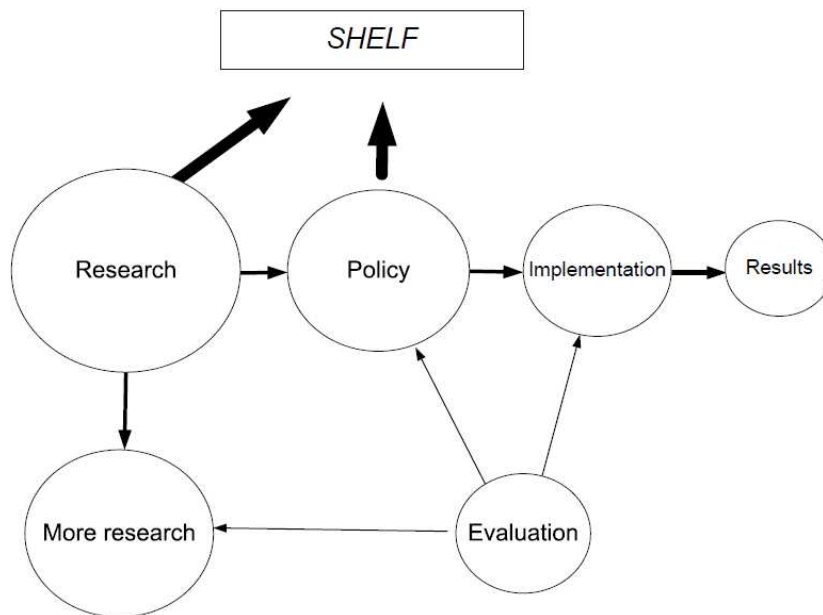
Conclusions: Prevalence of HPV infection was high at 39.4% with high risk types being more prevalent than low risk types. The most prevalent were: types 16, 52, 35

and 18. Being HIV positive was associated with higher risk of HPV infection. High risk HPV was identified in 90% of the women with invasive cancer.

8 IMPLICATIONS OF RESEARCH FINDINGS

Figure 3 depicts the research-policy nexus, what may happen to research findings.

Figure 3. Fate of research findings.



In the figure, large arrows indicate that most research will not feed into policy and many policies end up on the shelf. Uptake of research into policy and practice is not straightforward. Policy processes themselves are complex, multifactorial and non-linear. The successful integration of research into policy needs clear focus on current policy issues, political awareness and close engagement with policymakers, good communication, and seizing unexpected opportunities (Crew 2002).

For a cervical cancer prevention program to be effective, at least three criteria need to be fulfilled: high coverage of the population at risk, using an accurate screening test as part of high-quality services and ensuring that women with positive test results are properly managed (Bradley 2005).

The success of future cervical prevention in Uganda will depend on good vaccination coverage and screening of susceptible women. As shown in Table 2, the uptake of reproductive health services and completion of a vaccination schedule is low in Uganda.

8.1 IMPLICATIONS FOR POLICY AND IMPLEMENTATION

Article I. Policy and programme planners need to address the issues reported to improve uptake: provide information to the public about cervical cancer and its prevention, involve authoritative knowledge sources in the planning of prevention programs, involve men in women's reproductive health issues and improve quality of service delivery especially government health units.

Article II. A letter to the male partner reduced loss to follow-up. It is a simple practical intervention that can be introduced into practice at low cost.

Article III. Findings are relevant to the 'see and treat' programs which are recommended for the low income settings. Currently the pilot screening programs introduced in Uganda use cryotherapy after a positive VIA/VILI screen test. It is probable that there is a lot of over treatment.

Article IV. Aggregation of the data from this study with similar studies will contribute to the development of future second generation vaccines that will reduce the remaining 30% risk not covered by the current vaccines targeting types 16 and 18.

8.2 IMPLICATIONS FOR FURTHER RESEARCH

Article I. One of the themes identified was alternative authoritative knowledge sources on reproductive health issues. There is a need for further research to find out if this exists in the other tribes of Uganda, and to quantify it if it does.

The quality of service delivery, especially in government units, was reported to be very poor. We only reported on the reasons for the behaviour of the nurses. Further research is needed to explain why they are underequipped and understaffed, information that needs to be collected by interviewing policy planners and administrators.

Article II. We used a letter as the mode of male partner involvement. Further research can explore other means of involvement and test their effectiveness.

Article III. Due to low statistical power, we could not conclude on the effect of HIV seropositivity on the treatment outcome using cryotherapy. More studies with larger sample sizes and longer follow-up time will be needed to answer the question.

9 ACKNOWLEDGEMENTS

Many people contributed in different ways for this thesis to be. I may not remember all of you but I thank you all. I will mention some though.

Swedish International Development Cooperation Agency (SIDA), for making all this possible.

I am very grateful to my supervisors: Ass. Professor Elisabete Weiderpass, Karolinska Institutet, Department of Medical Epidemiology and Biostatistics and Ass. Professor Florence Mirembe, Makerere University College of Health Sciences, Department of Obstetrics and Gynaecology. Thank you for guiding me through all the stages: developing the concepts, full proposal, ethical approval, data collection, analysis and writing of the articles. You were there to ease the procurement issues. You went out your way to listen to my social issues when they arose, and for that, I am very grateful. Thank you for your unlimited patience and your belief in me.

My co-supervisor, Ass. Professor Elisabeth Faxelid, Karolinska Institutet, Department of Public Health Sciences Division of Global Health (IHCAR). Thank you first for your great contribution to the qualitative study. I learnt a lot from you up to the end. Thank you for your guidance in the writing of this thesis. I am very grateful

My tutor and friend, Professor Bo Lambert, thanks for all the support, both academic and social. I enjoyed the social more; sometimes the walks were too long and tiring. I could not tell you, because the sights were worth it.

Ass. Professor Stefan Peterson, thank you for being a good man, thank you for being practical, thank you for understanding the contextual differences between Sweden and Uganda, thank you for coordinating this collaboration. They could not have made a better choice.

Thank you Sven Sandin, for taking me over the statistical mountains. You make them look like anthills.

Thank you Paul Dickman, for the teaching of statistics to start with, and then administratively. Thanks for that big heart.

Sandra Eloranta and Therese Andersson, you never showed annoyance whenever I bumped into your offices with STATA problems. You made it look so easy. I wish I hope I get a chance one day to teach you something too, maybe how to perform a hysterectomy.

Prof. Sewankambo, Principal, MUCHS, Prof E K Rwakishaya, Director, SGS, Ass.Prof Katabira, Mr. Kijjambu and Dr Ibingira: thank you for all the effort you put into this program and demanding for results. You kept us going. Most of all, thanks for being so supportive.

Dr Sankaranarayan at IARC, Lyon, thank you for all your input and the opportunity you gave me to learn colposcopy, LEEP and programming cervical cancer screening in Barshi, India.

Sharon Grant at IARC, thanks for teaching me advanced literature search.

Richard Muwonge at IARC, thanks for your input in the early stages.

Bernhard Kleter, Wim Quint and Leen Jan van Doorn, thank you for performing the HPV analyses and for all your information on typing techniques.

Camilla Alqvist, thank you for the patience and foresight to know that I needed reminders to do those forms. Often I was behind the deadlines, but you understood.

Marie Dokken, thank you for being there to answer my questions and to help me with Swedish and administrative issues.

Gunilla Sonnebring, you never complained whenever I would dive into your office for help. Thank you for the immeasurable help in writing this thesis. Thank you for being so kind.

Pouran Almstedt, thanks for all the support, graphics and much more.

Margrethe Meo, thank you a million times for all you have done.

Luanne, thank you for all the corrections and advice.

Connie Nordlund and Gaston Nunez, thank you for all the help whenever I called on you.

Endre Kobold, you were the first I saw when I first entered Nobelsvag 12 A. Thank you for being a nice and easy person to deal with and for all the help.

Karin Sundström and Fatima Azerkan, you lent an ear, encouraged me, and offered all the help. Thank you, I wish you success too.

Elizabeth Katende, Jajja Beatrice Kibuuka, Shifa Nalufumu, Joyce Rwecungura, Gertrude Sentongo, Betty Gada, Lillian Namuli, Robinah Nanyonjo, and Fiona Chandiru. You are such a great team. I know I will work with you again in the future.

Maria Nakyewa thanks a million for always easing the financial complexities.

Colleagues: Josaphat Byamugisha, Dan Kaye, Lynn Atuyambe, Jolly Beyeza, Cecily Banura, Ateenyi Agaba, Michael Odida, Jackson Orem, Noeline Nakasujja, Janet Nakigudde, Romano Byaruhanga, and others. You all know, it is a long walk. Thanks for being there. Colleagues in the Department of Obstetrics and Gynaecology, thank you being so supportive.

Jane Nalunga, my friend, thank you for allowing me to use your facilities. Thank you for all you do for me.

Asia Kasambula, Charles Wasswa, Cottie, Ibra and Sadik in Stockholm, thank you for the companionship.

The women and men who participated in the studies. Your contribution is invaluable.

My family, all of you, thanks you for all the support and good wishes.

Adia and Shafique: my children, my loves, you are the reason I keep going.

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