HUMAN IMMUNODEFICIENCY VIRUS (HIV), HUMAN PAPILLOMAVIRUS (HPV) AND CERVICAL CANCER PREVENTION IN UGANDA

To my family, my wife Lydia, my daughters Edly and Victoria and my sons Enosh and Christian

Örebro Studies in Medicine 125

EDWARD KUMAKECH

HUMAN IMMUNODEFICIENCY VIRUS (HIV), HUMAN PAPILLOMAVIRUS (HPV) AND CERVICAL CANCER PREVENTION IN UGANDA:

PREVALENCE, RISK FACTORS, BENEFITS AND CHALLENGES OF POST-EXPOSURE PROPHYLAXIS, SCREENING INTEGRATION AND VACCINATION

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Abstract

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The aim of this thesis was to evaluate selected HIV, HPV and cervical cancer prevention services in Uganda. Four **sub studies I-IV** that employed quantitative and qualitative methods of data collection and analysis were used. The study participants included healthcare providers, policy makers, women, men, village health teams and 15-24 year old women in Uganda. The results from **sub study I** of the thesis showed that there was a high prevalence of occupational exposure to HIV among healthcare providers and majority of the exposure occurred through needle stick injuries (19.2%), of which 4.46% occurred with serologically confirmed HIV-infected blood. The high-risk group were nurses–midwives and students. The predisposing factors included lack of protective devices and recapping of needles. The use of post exposure prophylaxis for prevention of HIV sero-conversion after the occupational exposure was undermined by poor reporting of exposure, investigations and untimely access to the recommended antiretroviral drugs. From **sub study IV** among the 15-24 year old women, some moderate HIV prevalence (1.7%) was observed in addition to other sexually transmitted infections (STIs) in this case syphilis (1.2%) and HPV (33.7%). And they were associated with sexual risk behaviors. **Sub study IV** further revealed that of the HPV infections, 68.8% and 8.9% occurred with the high-risk HPV types and vaccine HPV-16/18 types respectively. The high-risk HPV infections were significantly less prevalent among the bivalent HPV-16/18-vaccinated group compared to non-vaccinated group [18.5% vs 28.1%, p 0.032, OR 95% CI 0.6(0.4-0.9)]. The difference between the two groups was more pronounced if vaccine HPV-16/18 only were compared [0.5% vs 5.6%, p 0.006, OR 95% CI 0.08(0.01-0.64)]. At type-specific level, significant difference was observed for HPV16 only. Bivalent HPV16/18 vaccination was not associated with increased prevalence of non-vaccine-types of HPV infections. Instead, other STIs (HIV/ syphilis) were associated with increased prevalence of HPV infections. The association of HIV infection with higher prevalence of HPV infections observed in sub **study IV** justifies the need for integration of HIV and cervical cancer prevention services. In response to that, in **sub studies II and III**, the Healthcare providers, policy makers and community members alike perceived integration of HIV and cervical cancer prevention particularly screening would be beneficial to all stakeholders but not without challenge.

Keywords: HIV, HPV, Cervical cancer, Prevention, Post-exposure prophylaxis, screening integration, vaccination, Uganda .

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Sammanfattning på svenska

Huvudmålsättningen för avhandlingsprojektet har varit att utvärdera förebyggande hälsovårdsinsatser för infektioner orsakade av HIV (humant immunbristvirus) och HPV (humant papillomvirus) och cervixcancer (livmoderhalscancer) i Uganda. Projektet har genomförts i fyra delarbeten där både kvalitativa och kvantitativa metoder använts. I studierna har deltagit frivilliga personer som bidragit med sin syn på hälsovården i Uganda, och i en del fall även med blod- och cervixprover. Dessa har inkluderat hälsoarbetare av olika kategorier, beslutsfattare, kvinnor och män som söker vård, byhälsoteam och unga kvinnor (15-24 år) i Uganda.

Resultaten från **delstudie I** visade att det fanns en hög förekomst av yrkesmässig exponering för HIV bland hälsoarbetarna. En majoritet av dessa expositioner hände genom stick på kanyler (19,2%), 4,5% var från konfirmerat HIV-infekterade individer. Högsta risken att drabbas löpte sjuksköterskor, barnmorskor och studenter. Predisponerande faktorer inkluderade brist på skyddsutrustning och när man tog av kanyler från sprutorna. Möjligheten att använda postexpositionsprofylax (PEP) mot HIV undergrävdes av bristande rapportering om stickincidenter och ojämn tillgång till de rekommenderade antivirala medlen.

Delstudie IV bekräftade förekomsten av HIV och andra sexuellt överförbara infektioner (STI) bland unga kvinnor (15-24 år) i den sydöstra regionen av Uganda. HIV-prevalensen var 1,7%, syfilis 1,2% medan 33,7% var infekterade med HPV. Dessa STI var bl a associerade med sexuellt riskbeteende såsom många partners. Vidare fann man att en majoritet av HPV-infektionerna orsakades av s k högrisktyper (68,8%), varav HPV-16/18 nära 9%. Dock var dessa högrisktyper av HPV signifikant lägre förekommande hos kvinnor som vaccinerats med det bivalenta HPV 16/18 – vaccinet ca fem år tidigare [18.5% hos vaccinerade vs 28.1% hos ovaccinerade, p 0.032, OR 95% CI 0.6(0.4-0.9)]. Skillnaden mellan grupperna var ännu starkare om man bara jämförde de i vaccinet ingående typerna HPV 16 och 18 [0.5% vs 5.6%, p 0.006, OR 95% CI 0.08(0.01-0.64)]. Av de enskilda HPV-typerna var det endast HPV 16 som hade statistiskt signifikant lägre nivå hos vaccinerade kvinnor. Det fanns

ingen association mellan vaccination och högre sexuellt riskbeteende eller förekomst av andra (icke-vaccin) HPV-typer. Däremot noterades ett samband mellan andra STI (HIV och syfilis) och högre förekomst av HPV. Detta är i linje med andra studier, regionalt och globalt, och understryker behovet av integration av hälsoinsatser för HIV och cervixcancer.

Detta studerades närmare i **delstudie II och III**, genom att inhämta synen på integrerad service från hälsoarbetare, beslutsfattare och allmänna medborgare, och den samlade uppfattningen var att det skulle vara bra med ökad integration. Detta kunde speciellt gälla screeningverksamhet. Praktiska aspekter som tid och reskostnader lyftes fram som viktiga faktorer för en ökad integrering. Speciellt gruppen redan HIV-infekterade kvinnor skulle vara värdefull att fånga upp i en integrerad serviceverksamhet. Vissa farhågor framfördes rörande risk för långa väntetider på hälsoenheterna, trötthet, stigmatiseringsproblematik rörande HIV samt den generella bristen på hälsovårdsarbetare i Uganda. En del samhällsmedborgare uttryckte oro för att de som drabbats av både HIV och cervixcancer skulle riskera att vara speciellt socialt utsatta.

Sammanfattningsvis, så är såväl exponering för HIV i arbetet (i sjuk och hälsvård) som HIV och HPV bland unga kvinnor vanligt förekommande i Uganda trots förekomsten av förebyggande hälsovård. Fördelarna med preventiva hälsoinsatser såsom PEP för HIV, screening för cervixcancer och HPV-vaccination riskerar att undermineras av diverse utmaningar i form av svag struktur av hälsosektorn och andra hälsoproblem. Fortsatta studier behövs för att belysa nyttan med PEP för hälsoarbetare och integrerad screening för HIV och HPV samt utvidgad HPV-vaccination.

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LIST OF ABBREVIATIONS

AGCUS:	Atypical glandular cells of undetermined significance
AIDS:	Acquired immunedeficiency syndrome
ART:	Antiretroviral therapy
ARVs:	Antiretroviral drugs
ASCUS:	Atypical squamous cells of undetermined significance
B cells:	B lymphocytes
bDNA:	Branched DNA
Bps:	Base pairs
cART:	Combination antiretroviral therapy
CC:	Cervical cancer
CCR5:	Chemokine receptor type 5
CD4:	CD4+ T lymphocytes
CD8:	CD8+ T lymphocytes
cDNA:	Complementary DNA
CI:	Confidence interval
CIN:	Cervical intraepithelial neoplasia
COC:	Combined oral contraceptives
CPE:	Cytopathic effect
CT:	Chlamydia trachomatis
CTL:	Cytotoxic T lymphocytes
CXCR4:	Chemokine receptor type 4
DB:	Dot plot hybridization
DBS:	Dried blood spot
DMPA:	Depot medroxyprogesterone acetate
DNA:	Deoxyribonucleic acid
E6-AP:	E6 associated protein
EIAs:	Enzyme-linked immunosorbent assays
ELISA:	Enzyme-linked immunosorbent assay
FDA:	US Food and Drug Administration
FGDs:	Focus group discussions
FP:	Family planning
Gp:	Glycoprotein
HAART:	Highly active antiretroviral therapy
HC:	Hybrid capture
HCPs:	Healthcare providers
HCWs:	Healthcare workers

HG:	High-grade
HIV:	Human immunodeficiency virus
HLA:	Human leukocyte antigen
HPV:	Human papillomavirus
HR:	High-risk
HSV:	Herpes simplex virus
IARC:	International Agency for Research on Cancer
ICC:	Invasive cervical cancer
IDIs:	Individual interviews
IEC:	Information education and communication materials
IFA:	Immunofluorescence assay
IL-1:	Interleukin-1
IL-6:	Interleukin-6
IRB:	Institutional review board
ISH:	In-situ hybridization
ISS:	Immunosuppressive syndrome
LCR:	Long control region
LEEP:	Loop electrosurgical excision procedure
LG:	Low-grade
LR:	Low-risk
LTR:	Long terminal repeat
MCH:	Maternal child health
MR:	Moderate-risk
mRNA:	messenger RNA
MTCT:	Mother to child transmission
NAT:	Nucleic acid tests
Non-vax:	Non-vaccine HPV-16/18 types.
ORFs:	Open reading frames
p:	Significance level
p53:	protein 53
PAF:	Proportion attributable fraction
PBMCs:	Peripheral blood mononuclear cells
PCR:	Polymerase chain reaction
PEP:	Post-exposure prophylaxis
PMs:	Policy makers
PMTCT:	Prevention of mother to child transmission
POC:	Point of Care

pRb:	protein Retinoblastoma
PrEP:	Pre-exposure prophylaxis
PV:	Papillomavirus
RDTs:	Rapid Diagnostic Tests
RH:	Reproductive health
RNA:	Ribonucleic acid
Rt-PCR:	Real-time polymerase chain reaction
RT-PCR:	Reverse transcriptase polymerase chain reaction
SIL:	Squamous intraepithelial lesion
SNP:	Single nucleotide polymorphism
SPSS:	Statistical package for social sciences
STH:	Southern transfer hybridization
STIs:	Sexually transmitted infections
SVA:	Single visit approach
T cells:	T lymphocytes
TLR:	Toll like receptors
Tm:	Melting temperature
TNF:	Tumor necrotic factor
TPHA:	Treponema pallidum heamoagglutination test
tRNA:	Transfer RNA
T-test:	Student t-test
URR:	Upstream regulatory region
VHTs:	Village health teams
VIA:	Visual inspection with 5% acetic acid
VILI:	Visual inspection with lugol iodine
VLPs:	Virus like particles
WHO:	World health organization
X^2:	Pearson's chi square test

ORIGINAL PAPERS

This thesis condenses the following four papers, which were numbered I-IV.

Paper I: **Kumakech E**, Achora S, Berggren V, Bajunirwe F. Occupational exposure to HIV: a conflict situation for health workers. Int Nurs Rev. 2011 Dec;58(4):454-62.

Paper II: **Kumakech E**, Andersson S, Wabinga H, Berggren V. Integration of HIV and cervical cancer screening perceptions of healthcare providers and policy makers in Uganda. BMC Public Health. 2014, 14:810.

Paper III: **Kumakech E**, Andersson S, Wabinga H, Berggren V. Integration of HIV and cervical cancer screening perceptions and preferences of communities in Uganda. BMC Women's Health 2015; 15(1):183.

Paper IV: **Kumakech E**, Berggren V, Wabinga H, Andersson S. Prevalence and risk factors for vaccine and non-vaccine types of Human Papillomavirus (HPV) infections among Bivalent HPV16/18 vaccinated and non-vaccinated young women in Uganda – 5 year follow up study. Submitted

OTHER ORIGINAL PAPERS

Mugisha E, LaMontagne DS, Katahoire AR, Murokora D, **Kumakech E,** Seruyange R, Tsu VD. Feasibility of delivering HPV vaccine to girls aged 10 to 15 years in Uganda. Afr Health Sci. 2015; 15(1):33-41.

Bansil P, Lim J, Byamugisha J, **Kumakech E,** Nakisige C, Jeronimo JA. Performance of Cervical Cancer Screening Techniques in HIV-Infected Women in Uganda. J Low Genit Tract Dis. 2014; 19(4).

LaMontagne DS, Mugisha E, Pan Y, **Kumakech E,** Ssemaganda A, Kemp TJ, Cover J, Pinto LA, Safaeian M, PhD. Immunogenicity of bivalent HPV vaccine among partially vaccinated young adolescent girls in Uganda. Vaccines (2014); 32(47):6303-11.

Paul P, Winkler JL, Bartolini RM, Penny ME, Huong TT, Nga le T, **Kumakech E,** Mugisha E, Jeronimo J. Screen-and-treat approach to cervical cancer prevention using visual inspection with acetic acid and cryotherapy: experiences, perceptions, and beliefs from demonstration projects in Peru, Uganda, and Vietnam. Oncologist. 2013; 18(12):1278-84.

LaMontagne DS, Barge S, Le NT, Mugisha E, Penny ME, Gandhi S, Janmohamed A, **Kumakech E,** Mosqueira NR, Nguyen NQ, Paul P, Tang Y, Minh TH, Uttekar BP, Jumaan AO. Human papillomavirus vaccine delivery strategies that achieved high coverage in low- and middle-income countries. Bull World Health Organ. 2011; 89(11):821-830B.

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INTRODUCTION

HIV/AIDS

Genome organization

HIV is a small of just over 9000 base pairs, roughly spherical, enveloped, positive-stranded RNA virus [1]. The envelope contains viral glycoproteins and is acquired by budding from the plasma membrane [2]. The envelope surrounds a capsid that contains two identical copies of the positive-strand RNA genome inside an electron-dense core [3].

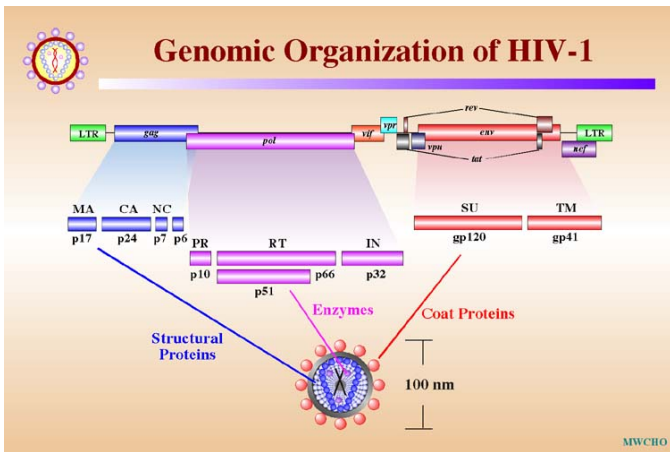

Figure 1. **HIV-1 genome and the frameshifting region.** The HIV-1 genome is shown with an expanded view of the *gag-pol* transframe region, which includes the elements important for ribosomal frameshifting. The frameshift signal is composed of the heptameric slippery site and an RNA stem-loop structure. *LTR*, long terminal repeat. Available at https://web.stanford.edu/group/virus/retro/2005gongishmail/HIV-1b.jpg

Inside the capsid core are also a few copies of retroviruses-typical RNA-dependent DNA polymerase (reverse transcriptase) and integrase enzymes and two cellular transfer RNAs (tRNA) [3]. The tRNAs are base paired to each copy of the genome to be used as a primer for the reverse transcriptase.

The genome consists of at least 3 major genes that encode polyproteins for the enzymatic and structural proteins of the virus: gag (group-specific antigen), pol (polymerase) and env (envelope) [1-4]. At each end of the genome are long terminal repeat (LTR) sequences. The LTR contains promoters, enhancers and other gene sequences for binding different cellular transcriptional factors [5]. HIV also encodes several regulatory proteins [5-6].

The capsid is composed of several proteins cleaved from a polypeptide encoded by the gag gene [7]. The viral enzymes are encoded by the pol gene, including the protease, reverse transcriptase and integrase [8]. These proteins are cleaved as part of the nucleocapsid assembly process. The viral glycoproteins are produced by proteolytic cleavage of the polyprotein encoded by the env gene [9]. In HIV, the glycoprotein is first produced as a precursor gp160 which is cleaved into the gp41 and gp120 [10]. These glycoproteins form lollipop-like spikes visible on the surface of the virion. The larger of the glycoproteins (gp120) is responsible for the tissue tropism of HIV [11-12] and is recognized by the neutralizing antibody [13]. The smaller subunit (gp41) forms the transmembrane component and promotes cell-to-cell fusion [14-15]. The gp120 of HIV is highly glycosylated [16-17], and its antigenicity can drift during the course of a chronic HIV infection [18]. Both of these factors impede immune clearance of the virus [19-20]. Detection of these glycoproteins is a useful marker of infection.

Classification

HIV belongs to the retroviruses family and Lentivirus subfamily [21]. Lentiviruses are slow disease onset viruses associated with neurological and immunosuppressive disease [21]. They are viruses with D-type cylindrical nucleocapsid core [21]. HIV has been subdivided into 2 types namely HIV-1 and HIV-2.

HIV-1 was zoonotic disease transmitted to man from chimpanzees in at least 4 groups M-P, corresponding to the Chimpanzee virus about 20 years ago [22]. As a result of its spread and evolution in man, HIV-1 group M got distributed worldwide. And therefore HIV-1 group M has been subdivided into subtypes A-K [23]. A higher prevalence of all subtypes is found in sub-Saharan Africa, of subtype B in the Americas (both

North and South) and Western Europe, of subtype C is southern Africa and India and of subtype A/E in Southeast Asia. HIV-1 group M subtype C is worldwide the most prevalent HIV-1. Other HIV-1 groups N and P are very rare standing at about 20 and 3 infected people respectively in Cameroon. HIV-1 group O is prevalent in 0.1-1% of sexually active people in Cameroon and neighboring countries of Gabon and Equatorial Guinea [24].

On the other hand, HIV-2 which also bears all the hallmarks of lentiviruses and has similar biology as HIV-1 has its origin in sooty mangabey monkeys in West Africa [25]. HIV-2 is also transmitted to humans in groups A-H, of which groups A and B are the most prevalent and perhaps the only pathogenic ones. The time of HIV-2 introduction to human is very close to that of HIV-1, about 20+ years ago. HIV-2 is less virulent than HIV-1 causing a chronic infection until signs of immunodeficiency and AIDS develops, and is associated with lower rates of mother to child transmission of about 2-7% [26]. The prevalence of HIV-2 infections in some communities in Africa can reach 10-16% but is limited to West Africa, Mozambique, Angola and Southwest India [27].

Lifecycle

HIV replication starts from the binding of the viral glycoprotein spikes (gp120) to specific cell surface receptor proteins [28]. The presence of virus receptors and co-receptors is the determinant of the tissue and host tropism of HIV [28].

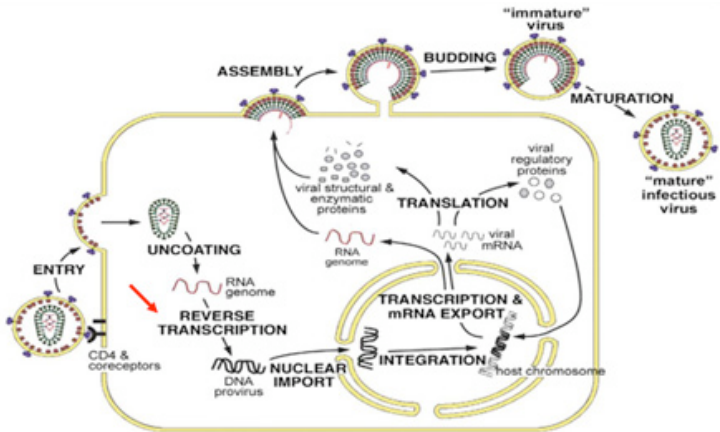

Figure 2: Lifecycle of HIV-1. Available at https://www.cbs.umn.edu/bmbb/contacts/ioulia-f-rouzina.

The gp120 of HIV interacts with a specific epitope of the CD4 surface molecule plus co-receptors such as CCR5 and CXCR4 expressed on T-helper lymphocytes and cells of the macrophage lineage (e.g. macrophages, dendritic cells, microglial cells) [29]. HIV enters the cells by receptor-mediated fusion of the envelope with the cellular plasma membrane [30] as opposed to endocytosis [31].

Once released into the cytoplasm, the reverse transcriptase uses the virion tRNA as a primer and synthesizes a complementary negative-strand DNA [32]. At the same time, the reverse transcriptase-associated RNase degrades the viral genomic tRNA template, and then synthesizes the positive-strand of the DNA [32-34]. During the synthesis of the virion DNA (provirus), sequences from each end of the genome (U3 and U5) are duplicated which juxtaposes the long terminal repeats (LTR) to both ends [33-34]. This process creates sequences that are required for integration of the viral genomic DNA into the host cellular genome and also enhancer and promoter sequences to regulate transcription. However, the reverse transcriptase is error prone. The error rate for HIV reverse transcriptase is approximately 5 per genome. This genetic instability from reverse transcriptase activity generates new strains of HIV during the course of an individual's disease [35], a property that may alter the pathogenesis of the virus and promote immune escape.

The reversed transcribed viral double-stranded DNA is then delivered to the nucleus and preferentially integrates into subset of transcriptionally active genes of the host chromosome with the aid of a viral-encoded, virion carried-integrase and host cell cofactors [36-37]. HIV produces a large amount of nonintegrated circular DNA (provirus) which is not transcribed efficiently but may contribute to the pathogenesis of the virus.

Once integrated the viral DNA is transcribed as a cellular gene by the host RNA polymerase II [38-39]. Transcription of the genome produces a full-length RNA, which is processed to produce several mRNA containing the gag, gag-pol, or env gene sequences [40]. The full-length transcripts of the genome can also be assembled into new virion.

As a cellular gene, HIV replication depends on the efficiency of its transcription. The efficiency of viral genome transcription and whether the virus remains latent depend on the ability of the cell to use the enhancers and promoter sequences encoded in the LTR region, the extent of methylation the DNA region, and the cells growth rate [41]. Stimulation of the cell by mitogens, certain lymphokines or infection with exogenous viruses (e.g. herpesviruses) produce transcription factors that also bind to the LTR and can activate transcription of the virus.

Replication of HIV is regulated further by other viral proteins [42-46]. The expression of HIV proteins is regulated by as many as six gene products. The nef protein represses expression of all the viral genes that may play a role in inducing latency. The tat is a trans-activator of transcription of viral and cellular genes. The rev regulates RNA splicing and promotion of export to cytoplasm. The nef, tat and rev genes produce proteins that create a network of regulatory factors that control their own synthesis and the synthesis of the virion's proteins. Vif protein helps to initiate replication. Vpu protein facilitates release of virus and vpr protein is a transactivator carried in virion.

HIV replication is also under cellular regulation, and activation of the T cell by a mitogen or antigen also activates the virus [47]. The viral glycoproteins are synthesized, glycosylated, and processed by the endoplasmic reticulum and golgi apparatus [48]. The glycoprotein is cleaved into a transmembrane and surface regions and associates to form dimers or trimers that migrate to the plasma membrane [48].

The gag and the gag-pol polyproteins are first synthesized as fusion product and then bind to two copies of viral progeny genome [49-50]. The association of two copies of the genome and cellular tRNA molecules with this aggregate triggers the release of the viral protease and cleavage of the gag polyproteins [49-50]. This action releases the reverse transcriptase plus its associated integrase and forms the virion core, which remains associated with the virion glycoprotein-modified plasma membrane [49-50].

The fully assembled virion buds (by exocytosis) from the plasma membrane and simultaneously acquires its envelope and is released from the cell as an infectious virion [51]. Alternatively, cell-to-cell spread of the HIV is further enhanced by HIV envelope glycoproteins ability to induce autophagy or apoptosis or cell-mediated cytotoxicity in the uninfected standby T cells in addition to form multinucleated giant cells, or syncytia [52-54]. Syncytia are fragile, and their lysis enhances the cytolytic activity of the virus and also viral spread. The virus may also remain latent (non-productive state) for long periods, but when activated in CD4 T cells or macrophages, productive replication ensues. This activation may occur after stimulation of the cell by an antigen or mitogen.

Pathogenesis and immunity

The major hallmarks of the pathogenesis and disease caused by HIV is persistent inflammation, progressive depletion of CD4 expressing T cells and macrophages and AIDS. CD4 T cells are the helper T lymphocyte cells that once activated by antigens presented to them by dendritic cells play roles in B cell activation for generation of specific or acquired immune response and also CD8 T lymphocyte cell activation for generation of specific cell mediated cytotoxicity (i.e. delayed type hypersensitivity reactions).

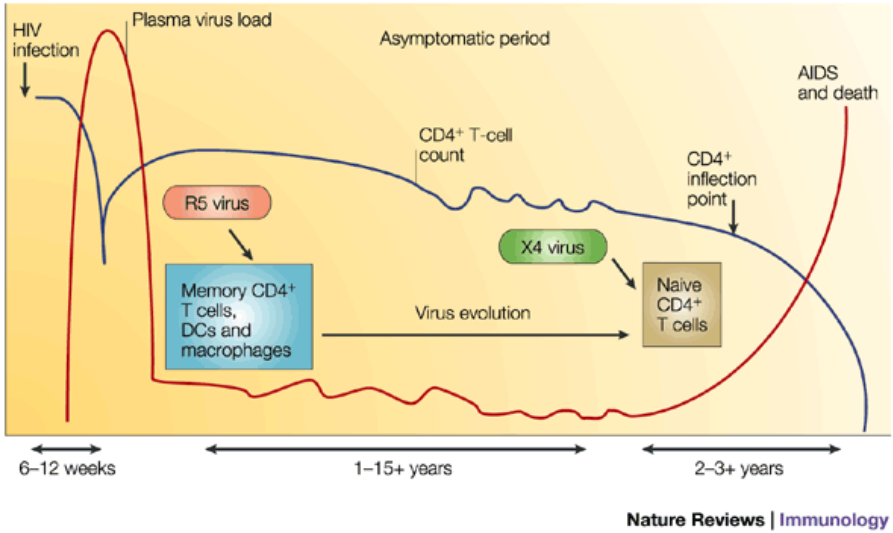

Figure 3: Schematic diagram of the course of HIV-1 infection. Source: Sarah L. Rowland-Jones. Timeline: *AIDS pathogenesis: what have two decades of HIV research taught us?*. *Nature Reviews Immunology 2003; 3(4): 343-348; doi:10.1038/nri1058*

HIV induces several programmed cell-death pathways that may kill the CD4 T cell or macrophages. These include apoptosis, autophagy, syncytia formation and lysis and necrosis-like cell death [55-59]. A lot of data indicate that HIV uses its envelope glycoprotein Env expressed on the surface of the infected cells to trigger autophagy in the uninfected standby T cells leading to their apoptosis [55]. Conversely, autophagy and apoptosis does not occur in the HIV infected cells, a probably viral survival mechanism [56]. The ability of HIV to kill the target cell correlates with the amount of CD4 expressed by the cell [57]. Another theory for the cytolytic activity of HIV is binding of HIV virions or the gp120 to CD4 molecules of uninfected standby T cells prevent its cell surface expression and immunological function and promote cell-to-cell fusion leading to syncytia formation and lysis [58]. There is also evidence that HIV infection not only triggers apoptosis in uninfected standby CD4 T cells but also mediate necroptosis (programmed cell death) in the infected producer CD4 T cells [59]. Unlike apoptosis, necroptosis mainly occurs in HIV-infected cells and spares the uninfected standby cells. Necroptosis occurs as an alternative programmed

cell death mechanisms in the absence of apoptosis machinery and also is partly involved in syncytia formation [59].

Macrophages are persistently infected with HIV. They may be spared the cytopathic effect of HIV gp120 because they express lesser amounts of CD4 than T cells [60]. Monocytes and macrophages are probably the major reservoirs and means of distribution of HIV [61]. Circulating macrophages, microglial cells of the brain, pulmonary alveolar macrophages, dendritic cells, and other cells of the monocyte-macrophage lineage can spread the virus and potentially contribute to HIV disease [62].

In addition to immunosuppressive disorders as a result of CD4 T cell depletion, HIV infection is also associated with neurological abnormalities [63]. Microglial cells from monocyte-macrophage lineage are the predominant cell type of the brain that gets infected with HIV, but neurons and glial cells may also be infected. HIV infected monocytes and microglial cells may release neurotoxic substances or chemotactic factors to promote inflammatory responses in the brain [64]. Direct cytopathic effects of the virus on neurons are also possible.

Following recognition of the HIV infection by the host immune system, innate immune response such as type 1 interferon, IL1, IL6, TNF alpha and inflammatory cytokines and adaptive humoral immune response such as neutralizing antibodies are generated against the gp120 protein, capsid proteins, regulatory proteins and viral nucleic acid [65]. HIV infection together with the innate immune response chronically active cells in the lymphoid tissue including T cells, B cells and myeloid lineage cells [66]. HIV also chronically activates the cellular components (T and B cells) of the adaptive immune system and this mediate antibody-dependent cellular cytotoxicity (ADCC) responses [67]. Part of the ADCC is the generation of CD8+ cytotoxic T cells against HIV-infected CD4+ cells. Other cell-mediated and cytokine responses may suppress the replication of HIV and promote latency following the initial acute phase of infection.

However, HIV especially HIV-1 has the ability to incapacitate the immune system for example not inducing innate immune response in monocyte derived dendritic cells, shield its immunogenic cDNA away from cytosolic innate immune sensors, remain latent in lymphocytes, and alter its antigenicity allows the virus to escape immune clearance and prevents resolu-

tion of the disease [66]. HIV establishes a latent and low-level chronic infection in every infected individual [67]. A slow progressive decrease in the levels of CD4 cells may precipitate immunodeficiency after long periods.

Activation of the CD4 T cells is one of the first steps in the initiation of adaptive immune response. Helper T cells secret lymphokines and gamma interferon (IFN-δ) required for activation of macrophages, other T cells, B cells and natural killer cells. When HIV kills CD4 T cells or makes them dysfunctional, antigen-specific immune responses (especially cellular immune responses) are incapacitated and humoral responses are uncontrolled [68]. HIV-associated depletion of the CD4 T cells responsible for adaptive immune response allows the outgrowth of many of the AIDS-associated opportunistic infections such as human papillomavirus (HPV) and cancers such as cervical cancer [69].

HIV Acquisition and Transmission

HIV is usually present in the blood, oropharyngeal tissues and semen and cervicovaginal fluids of the infected individuals [70-71] and therefore serves as potential sources of HIV transmission. Therefore, HIV can be transmitted from one person to another through non-sexual and sexual means involving contamination with the above potential sources of HIV.

Non-sexual transmission

Occupational exposure to HIV infected blood and or body fluids: Occupational exposure to HIV presents a low but potential source of HIV infection [72-73]. Prospective studies of healthcare workers (HCWs) have estimated the risk for HIV infection after an occupational exposure to an HIV-infected blood to be 0.3% after percutaneous exposure and 0.09% after mucous membrane exposure and without use of anti-retroviral drugs for postexposure prophylaxis (PEP) [74-77]. The risk is considerably higher in cases of deep injury, visible blood on the sharp device, a procedure that involves a needle placed in the patient's artery or vein, and a patient with advanced acquired immune deficiency syndrome (AIDS) [78]. The susceptible groups for occupational exposure to HIV are of course HCWs including nurses compared to other cadres of healthcare professionals [76].

The World Health Organization (WHO) estimates that 3 million percutaneous exposures occur annually among 35 million HCWs globally, corresponding to 1000 new HIV infections from occupational exposure with over 90% occurring in resource constrained countries [79]. This risk is probably highest in sub-Saharan Africa and Asia where incidence rates as high as nine exposures per health worker per year were reported [80-81].

The risk factors for occupational exposure to HIV among health workers are well documented and consistent across literature from Africa, Asia, Europe and America. The high-risk group or settings or circumstances for occupational exposure include being a trainee like intern/registrar doctor, a nurse–midwife and a surgeon; places like medical wards, intensive care units and operating theatres; and medical procedures like emergency surgery and Caesarean sections [82-86].

Literature is, however, scarce about the circumstances that predispose to occupational exposure to HIV, particularly those from the affected health worker's perspectives. The available literature indicates the incorrect use of needles, lack of safer needle holders and sharps disposal containers, continued recapping of needles after use, lack of training for health workers, long working hours of greater than 40 hours per week, failure to use gloves when handling needles and the belief among health workers that the risk of HIV sero-conversion from occupational exposure is low as the most important predisposing factors to needle stick injuries [87-90]. More so, a study conducted in Tanzania revealed insufficient measures to reduce the risk of HIV transmission, e.g. nonfunctional water taps, lack of plastic bags for disposal of medical wastes and shortage of gloves [80]. In Ethiopia, a study revealed the non-protective effect of work inexperience on occurrence of needle stick injuries [91]. However, none of the above studies examined the relationship between each of the above factors and the occurrence of occupational exposure to HIV in a cross-sectional survey design. Other factors missing in the literature are the role of health worker's level of training and concern about their personal safety during patient care on the use of safety devices and the occurrence of occupational exposure to HIV.

Intravenous drug use and sharing of syringes or needles: Sharing of contaminated syringe or needles is a common practice among intravenous drug users. HIV transmission occurs during the sharing of syringes or

needles for drug use. Globally, there are 16 million intravenous drug users of which 3 million are HIV infected (i.e. 18.8%) mostly through sharing of syringes or needles although intravenous drug users also engage in high-risk sexual behaviors which facilitate HIV transmission between them and different groups [92]. One systematic review concluded that the risk of HIV infections was significantly higher among intravenous drug users than among their counterparts who were not intravenous drug users [92].

Transfusion of blood, blood products and organ transplant: Before the introduction of blood and blood product screening, individuals receiving blood transfusions, organ transplants and hemophiliacs receiving clotting factors from pooled blood were at high risk of HIV infection. One systematic synthesis study has estimated the per-act risk of HIV transmission to be greatest for blood transfusion compared to other routes of HIV exposure such as vertical and sexual intercourse [93]. This is a possibility because HIV infection is prevalent among blood donors for example a study conducted in a hospital-based blood bank in Uganda in 1994 found 3.9% of the donated blood to be infected with HIV more so among donors with AB blood group [94].

Tattoo needles and contaminated inks: Tattoo needles and contaminated inks are another potential route of HIV transmission. It is also possible that tattooing and body piercing behaviors could be confounding other high-risk behaviors for HIV transmission. In fact, a study conducted among adolescent detainees found significant associations between tattooing and body piercing practices and alcohol, marijuana, antidepressants and sedative use which are known risk factors for HIV transmission [95]. However, HIV is not transmitted by casual contact, touching, hugging, kissing, coughing, sneezing, insects, water, food or utensils, toilets, swimming pools, or public baths.

Sexual transmission

HIV is predominantly a sexually transmitted infection (STI). The per-act and per-partner HIV transmission risks range from a low of 0.04% to a high of 40.4% depending on the whether it was oral intercourse, anal intercourse, vaginal intercourse, men sex with men or heterosexual intercourse [96-98].

Furthermore, the factors that facilitate sexual exposure to HIV include early age at sexual debut, multiple sexual partners, and condom non-use [99-103]. Studies among women and gay men respectively showed that early age at sexual initiation was associated with an increased risk of HIV infection [99-100].

Similarly, having multiple and concurrent sexual partners was associated with an increased risk of HIV infection [101-102]. Other sexually transmitted infections (STIs) particularly herpes simplex 2 (HSV) infection has also been associated with an increased risk of HIV infection [101, 103]. On the contrary, consistent and correct use of male condom protects against HIV in heterosexual sero-discordant couples by about 60-95% although the most recent information showed 80% protection [104].

Vertical transmission

HIV can also be transmitted vertically from mother to child. Before the introduction of combination antiretroviral therapy (cART), the risk of mother to child transmission of HIV ranged from 12-45% and was influenced by a variety of risk factors including advanced maternal HIV infection (high viral load, low CD4 count and AIDS diagnosis), prolonged rupture of membranes, first twin birth, prematurity or low birth weight, chorioamniosis, vaginal delivery, and maternal drug use such as opioids [105]. However, the use of cART has now reduced the risk of MTCT of HIV to as low as 1-2% in many countries [106]. And the principal determinants of the reduced risk are the maternal viral load and use of cART.

Co-factors for HIV infection

Gender: A systematic review has shown that HIV infections were more prevalent among women than men [107]. Several factors increase women's vulnerability to HIV infection including biological, behavioral, socio-economic, cultural and structural inequalities [108].

Age: The high risk groups for incident HIV infections include sexually active young people aged 20-24 years compared to other age group and women compared to men are disproportionally more affected [109].

Herpes Simplex 2 Co-infections: Genital herpes simplex 2 co-infection increases the likelihood of acquiring HIV infection as well as its shedding [110]. More so HSV 2 infection of B cells may induce HIV replication from latently infected cells [111].

Syphilis: Studies have shown a positive association between syphilis and HIV infections [112]. In fact, comorbid syphilis increases a person's susceptibility of acquiring and transmitting HIV by 2-5 folds [113].

Pregnancy, child birth and breast feeding: In the absence of ART, pregnancy is associated with a small but appreciable increase in the risk of acquistion and or progression of HIV infection at rates similar to high-risk cohorts [114]. In fact, pregnancy, child birth and breast feeding are prerequisites for vertical and perinatal transmission of HIV. MTCT risks are elevated among women with incident HIV infections during pregnancy [115].

Hormonal contraception: Previous data do not support an association between use of oral contraceptives and an increased risk of HIV infections [116]. For injectable contraceptives particularly depot medroxyprogesterone acetate (DMPA) however uncertainty persists with some studies showing small to moderately increased risk of HIV acquisition for all women using DMPA with a smaller increase in risk for women in the general population [116-117]. For the rest of the hormonal contraceptives including implants, most studies showed no significant increase in HIV risk [116-117].

Male circumcision: Randomized controlled trials have shown that adult male circumcision can reduce the risk of HIV acquisition by 60% in heterosexual men and may also provide indirect long-term benefit to women which may start after complete wound healing [118]. However, systematic reviews and meta-analysis revealed insufficient evidence that male circumcision provides direct protection against HIV or other STIs in women or men who have sex with men [119-121].

***Chlamydia trachomatis* (Lymphogranuloma Venereum):** Systematic reviews and meta-analysis have shown a strong positive association between *Chlamydia trachomatis* lymphogranuloma venereum and HIV infection

although it is not yet clear whether the association is due to biological or behavioral factors [122].

***Trichomonas vaginalis* and bacterial vaginosis:** Previous studies have shown a plausible association between vaginal infections such as *Trichomonas vaginalis* and bacterial vaginosis and HIV infection. A systematic review has indicated that HIV incidence were significantly higher among women with *Trichomonas vaginalis* and bacterial vaginosis compared to their counterparts without those infections [123].

Alcohol use: Studies conducted in sub Saharan Africa has shown that users of alcohol particularly heavy drinkers are more likely to be HIV positive than non-users [124]. Similarly, alcohol use also increases the risk of HIV acquisition [124]. The frequency or quantity of alcohol use was positively associated with HIV prevalence and male alcohol users were more affected than the female alcohol users [124]. It is very likely that use of alcohol indirectly influence HIV acquisition risk and prevalence through high risk sexual behaviors. In fact, many studies from Asia, Western and Sub Saharan African countries have associated alcohol use with diverse sexual risk behaviors [125-127] and also poor adherence and poor ART treatment outcome [128].

Preexisting *Human papillomavirus* (HPV) infections: Accumulating evidence including systemic reviews indicated that pre-existing genital HPV infection increases the risk of acquisition of HIV infection. A recent systematic review indicated that HIV incident infection was significantly associated with HR-HPV infection in five of six studies and with LR-HPV in two out of five studies [339]. A detailed discussion of the associations between HIV/AIDS, HPV and cervical cancer is presented on the later pages of this thesis under the integration of HIV, HPV and cervical cancer prevention justifications.

HIV/AIDS Disease Burden – Morbidity and Mortality: HIV/AIDS is a major cause of morbidity among adults globally and more so in developing countries. The prevalence of HIV among women aged 15–49 years in Uganda increased from 7.5% in 2005 to 8.3% in 2011 [129]. In terms of burden, between 2007 and 2013 the estimated number of people living with HIV in Uganda increased from 1.2 million to 1.5 million, and 56%

of the people living with AIDS were women aged 15 years and older [129].

HIV/AIDS prevention

Universal precaution in healthcare settings

To prevent occupational exposure to HIV in healthcare settings, universal precautions should be taken on blood and body fluids. Universal precaution means all patient's blood and body fluids should be assumed to be infectious for HIV and other blood-borne pathogens [130]. Protective wear (e.g. gloves, mask and gown) and other barriers should be used to prevent exposure to body fluids and blood products [130]. Contaminated surfaces should be disinfected with 10% household bleach, 70% ethanol or isopropanol, 2% glutaraldehyde, 4% formalaldehyde, or 6% hydrogen peroxide [130]. Laundry washing in hot water with detergent should be sufficient to inactivate the virus [130]. However, compliance and effectiveness of universal precaution measures against HIV and other blood borne pathogens remains to be demonstrated. A systematic review has showed that in many healthcare settings across the globe, healthcare workers' compliance to the recommended universal precaution measures is relatively poor [131]. In Uganda alike, no information was available on healthcare worker's compliance with universal precautions as a measure for HIV prevention.

Post-Exposure Prophylaxis (PEP)

Post-exposure prophylaxis (PEP) with anti-retroviral drugs can reduce the risk of HIV sero-conversion following occupational exposure to HIV. It has been demonstrated that anti-retroviral drugs can reduce by approximately 81% the risk for HIV infection after an occupational exposure [78]. Despite the effectiveness of PEP, reporting of exposure, uptake and adherence to PEP can be very poor among healthcare providers [132-136]. At the time of this thesis, in Uganda, ART programmes with the capacity to provide PEP services are available in many healthcare facilities but information were lacking on the factors influencing reporting of occupational exposures to HIV, uptake and adherence to PEP among healthcare workers.

HIV Testing/Screening

HIV testing/screening is an important HIV prevention strategy in that it serves the dual purpose of providing an entry point to HIV education/counseling and antiretroviral treatment (ART) programmes for those who test HIV-positive. HIV testing/screening methods commonly used in many countries including Uganda are serological methods (i.e. anti-HIV antibody testing and antigen testing), reverse transcriptase-polymerase chain reaction (RT-PCR) and T-lymphocytes cell count.

Serology

Enzyme linked immunosorbent assay (ELISA) or latex agglutination procedures are basic HIV screening tests. The ELISA test detects antibody to one or more HIV envelope proteins (e.g. gp120), is sensitive up to 100% but this poses a risk of false positive results (i.e. are less specific) [137-139]. Therefore, more specific tests such as Western blot and immunofluorescent assay (IFA) are subsequently used to confirm HIV seropositive results from ELISA.

The Western blot assay determines the presence of antibody to each of the viral antigens including the core protein (p24) and possesses up to 99.9% specificity [137-139]. False-positive HIV results with this algorithm of tests are extremely rare but may occur. In such situation, the test results are often corroborated with clinical or other laboratory information or repeated or supplemented. HIV antibody may develop slowly requiring 4 to 8 weeks in most patients but 6 months or more in as many as 5% of those infected. Unfortunately, the aforementioned serological tests (ELISA, Western blot) are unable to detect infections during this window period and give potentially false negative results. Also, serological tests such as ELISA may provide false-positive results to infants born to HIV-infected mothers who carry HIV antibodies vertically transmitted to them from their mother during intrauterine life but may not have the actual HIV infection (RNA) [138]. Nevertheless, serological tests provide a basic screening for HIV infection. Serological tests such as ELISA and Western Blot are available in Uganda but in research laboratories only [140].

Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR) Assays

HIV is detected by nucleic acid-based tests which quantify the viral RNA, transcripts of reverse transcriptase activity and it convey information about the viral load [141]. Viral load quantitation has become the major prognostic marker for disease prognosis and outcome of antiretroviral therapy in the treatment of HIV-infected individuals. The three major methodologies for viral load quantitation: the reverse transcriptase-polymerase chain reaction (RT-PCR; Amplicor HIV-1 Monitor Test, Roche Diagnostic Systems, Pleasanton, CA), the nucleic acid sequence-based amplification (NASBA; NucliSens HIV-1 QT Test, Organon Teknika, Bostel, The Netherlands); and a signal amplification methodology termed branched chain DNA (bDNA) technique (Quantiplex HIV-1 RNA test, Bayer Diagnostics, Emeryville, CA) [142]. Other commercially available nucleic acid-based tests for quantifying viral load include Cobas TaqMan, Abbott Real Time HIV-1, Versant HIV-1 RNA bDNA, Versant HIV-1 RNA kPCR and ExaVir Load. In terms of performance, a recent systematic review showed that all currently available HIV viral load assays are of sufficient sensitivity to detect plasma viral load of 1000 copies/mL as a threshold to initiate investigations on treatment adherence or possible treatment failure [143]. Sources of variability between viral load assays include differences in technology platform, plasma input volume, and ability to detect HIV-1 subtypes [143]. RT-PCR is currently the gold-standard for HIV diagnosis because it provides solution for HIV detection in the window period and infants with passive HIV antibodies but their clinical relevance is limited because of relatively poor reproducibility, especially for low copy numbers and unequal amplification dynamics for different serotypes, high cost and complicated laboratory standards. RT-PCR tests are available in Uganda and are being used for identification of HIV-1 infected infants and young children using dried blood spot (DBS) [144].

HIV core p24 antigen and p24 antigen-antibody assays

HIV may also be detected based on measuring the presence in serum or medium of viral core p24 antigen. The core p24 antigen can be detected in the lymphocytes of as many as 60% of patients with HIV infection and indicates that active viral replication is occurring. This antigen is detectable during the initial acute phase of HIV disease and then disappears as

the virus enters the late stage of infection, only to reappear years after infection when AIDS stage has been reached and widespread viral replication resumed. Core p24 antigen testing is sensitive and specific in diagnosing pediatric HIV infection, in predicting CD4+ T cell decline and clinical progression at early and late stage of infection, and may be suitable for antiretroviral treatment monitoring in both adults and children. Notably, core p24 antigen was measurable even in patients with stably suppressed viremia, and its concentrations were negatively correlated with the concentrations of CD4+ T cells and positively correlated with the concentrations of activated CD8+ T cell subsets. Systematic reviews have concluded that core p24 antigen is an excellent marker of HIV expression and disease activity and can be used in the same fields of application as HIV RNA is used [145]. The test is validated for both subtype B in the US and non-B subtypes in sub Saharan African country with good sensitivity and specificity profiles [146]. The core p24 antigen Perkin Elmer assay currently most often used has a sensitivity of 98.8% and a specificity of 100% (in infants 6 weeks of age) and is less costly and less complicated than the gold-standard PCR [147-148]. However, in clinical practice, plasma RNA determinations are in general most widely used [149].

T-lymphocyte cell count

Analysis of T-lymphocyte subsets (particularly CD4 and CD8) can provide an indication of an HIV infection but is mainly used for clinical follow up of disease progression in already known HIV infection. CD4 T-lymphocytes absolute count and the ratio of helper to inducer lymphocytes (CD4/CD8 ratio) are abnormally low in HIV-infected individuals. More so, the stage of AIDS disease is defined by the concentration of CD4 lymphocytes. World Health Organization (WHO) criterion for eligibility for ART is based on CD4 count levels. Currently, two T-lymphocyte phenotyping technologies are available on the market namely flow cytometer (single-platform technology, SPT) and flow cytometer/hematology analyzer (dual-platform technology, DPT). Systematic review of studies has shown a strong correlation between DPT and SPT platforms regarding their absolute and percentage CD4 count [150]. FACSCalibur, FACScount and PIMA CD4 T cell counting system are respective examples of flow cytometry-based DPT and SPT platforms in use in many low resource settings including Uganda. FACScount and PIMA are smaller point-of-care (POC) versions that generate both the absolute and percent CD4 plus or

minus CD8 or CD3 counts. Using the DPT FACSCalibur as the gold standard CD4 T cell count system, studies have shown that FACScount CD4 T cell count system provides reliable absolute and percentage CD4 count using capillary or venous blood and are suitable for monitoring adult and pediatric HIV infections in moderate – volume laboratories [151-152]. Similarly, the PIMA CD4 T cell count system also provide a reliable absolute or percentage CD4 count and can be used with capillary or venous whole blood but is suitable for screening eligible adult HIV patients for ART initiation in low-volume laboratories [151-153].

In Uganda, FACScount CD4 and PIMA CD4 count services have been established in all public hospitals and Primary Healthcare Centers throughout the country to support ART programmes in terms of ART initiation and monitoring of HIV-positive patients [153].

HIV Rapid Diagnostic Tests (HIV RDTs)

Standard HIV testing algorithms comprising of ELISA followed with confirmatory Western Blot test for ELISA positive cases require advanced laboratories and can take 1 week or longer and are therefore not scalable in low income countries. Innovative and simpler algorithms comprising of anti-HIV antibody Rapid Diagnostic Tests (RDTs) have been developed, validated and are currently in use in many low income countries including Uganda. The anti-HIV antibody RDTs currently available on the market includes Alere Determine® HIV-1/2 (Alere Medical Co. Ltd US), Statpak HIV-1/2 (Chembio Diagnostic Sys, US) and Uni-Gold™ Recombigen® HIV-1/2 (Trinity Biotech PLC, US). Alere Determine™ HIV-1/2 is an immunochromatographic rapid diagnostic test for the combined detection of anti-HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens. HIV 1/2 Stat-Pak® is an immunochromatographic rapid diagnostic test for the combined detection of anti-HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens. Uni-Gold™ HIV-1/2 also is an immunochromatographic rapid diagnostic test for the combined detection of HIV-1/2 antibodies in human serum/plasma and capillary/venous whole blood specimens. Blood sample (venipuncture whole blood, fingerprint whole blood or serum or plasma) may be used for the HIV test. WHO evaluation study has shown that sensitivity and specificity of Alere Determine HIV-1/2 RDT were 100% and 98.7% respectively, that for Stat-Pak HIV-1/2 were 99.5% sensitivity and

100% specificity and lastly for Uni-Gold™ HIV-1/2, the sensitivity was 99.8% and the specificity 99.9% compared to the reference assay results [154].

In Uganda, the Ministry of Health allows both serial and parallel HIV testing using the aforementioned HIV RDTs. In serial testing, a non-reactive result on Determine HIV-1/2 RDT ends the testing but a positive result is confirmed with Statpak HIV-1/2 RDT which ends the testing if positive result is obtained. However, in cases of discrepancy or tie between Determine HIV-1/2 RDT and Statpak HIV-1/2 RDT results, a third HIV RDT Uni-Gold test is performed to obtain the final diagnosis. An evaluation of this algorithm showed that if Stat-Pak was used as the first screening test for a serial algorithm, the sensitivity was 99.6% and specificity was 99.7% but if Determine was used as the first screening test for a serial algorithm, sensitivity was 97.3% and specificity was 99.9%. [155]. In Uganda, the main stay for provision of HIV voluntary counseling and testing (VCT) has been at health facilities. Home based VCT programmes were later initiated in the country by various healthcare players to improve on service coverage in a cost-effective way [156].

Oral Pre-Exposure Prophylaxis (PrEP)

Oral Pre-Exposure Prophylaxis (PrEP) is an HIV prevention approach that involves the use of oral antiretroviral drugs (ARVs) before sexual contact with an HIV-infected partner. It is recommended for use in recurrent high-risk sexual relations such as HIV discordant couples. In fact, two candidate antiretroviral drugs (tenofovir and emtricitabine) have been identified and phase 3 clinical trials conducted in Uganda and Kenya showed that use of tenofovir compared with no use was associated with an 85% relative risk reduction in HIV-1 acquisition and use of emtricitabine plus tenofovir was associated with an 93% relative risk reduction in HIV-1 acquisition [157]. When PrEP becomes available, injection drug users, men who have sex with men (MSM) at substantial risk for HIV, and HIV-negative partners within serodiscordant heterosexual couples are more likely to benefit from this new biomedical HIV prevention method. Regarding acceptability of oral PrEP for HIV prevention, existing research suggests that PrEP is reasonably acceptable to MSM in the US, but few men thought it was necessary [158].

Topical (vaginal or rectal) Microbicides Pre-Exposure Prophylaxis

Microbicides used as topical PrEP has the potential of preventing sexual transmission of HIV. However, clinical trials based on compounds such as nonoxynol-9 (N-9) known to inactivate the virus failed to prevent HIV transmission instead N-9 enhanced susceptibility to HIV infection [159]. Similarly, many other formulations of microbicides based on compounds that inhibit binding, fusion or entry of the virus into the host cell have also failed to show either safety or efficacy for prevention of HIV transmission [159]. Alike, microbicide formulations containing antiretroviral drug Tenofovir gel for vaginal or rectal use have also shown conflicting efficacy results, 39% level of protection compared to the placebo group in one trial but none in another trial [159-160].

Highly Active Antiretroviral Therapy (HAART) for PMTCT

Mother-to-child transmission (MTCT) also known as vertical transmission is the major route of HIV acquisition in children worldwide. MTCT of HIV occurs during pregnancy, labor and breastfeeding. Strategies to reduce MTCT include maternal and infant use of ARVs or HAART, caesarean section before onset of labor or rupture of membranes, and complete avoidance of breastfeeding. Where these interventions are available, the risk of MTCT of HIV has dropped to as low as 1-2%. In view of the fact that ARVs or HAART of HIV-positive mothers reduces maternal viral load thus minimizing the risk of MTCT of HIV, the WHO recommended treatment of all HIV-positive pregnant mothers with option B HAART comprising of Nevirapine (NVP) + Lamivudine (LMV)+ Zidovudine (AZT) at the fourth week of gestation followed by an intravenous NVP administration intrapartum and postpartum NVP syrup to the respective infants for six weeks to achieve rapid PMTCT. Pre-post studies have shown that option B HAART improves maternal CD4 count, reduces viral load and prevent MTCT of HIV in 90% of children born to HIV-positive mothers [161].

By 2010, the reported coverage of ART or HAART prophylaxis for PMTCT in sub Saharan Africa was 60% [162]. In Uganda, HIV testing and ART or HAART prophylaxis for PMTCT are targeted at all pregnant women and HIV-infected women respectively attending health facilities or from community outreaches. One study in Uganda has shown that HIV

infections in children could be reduced by 28% by increasing HIV testing capacity at health facilities to ensure 100% testing among women seeking antenatal care and providing ART or HAART prophylaxis to all eligible pregnant women would reduce MTCT of HIV by 18% [163]. The major challenges to elimination of vertical transmission of HIV has been and will remain low uptake, poor adherence and poor completion rates for PMTCT services including antenatal care attendance, HIV testing and ART prophylaxis.

Blood donor, blood product and transplant organ screening

Although a number of recent studies have confirmed that the residual risk of HIV infection from blood and blood components is very small, progressive donor screening measures are important in safeguarding blood supply from HIV. World Health Organization (WHO) established a goal of regional blood safety by 2012 through among others improved testing of donor blood as well as appropriate clinical use of blood [164]. This implied that blood, blood products and transplant organs should be tested for HIV and those found positive for HIV must not be used. The tests used for blood donor screening include the HIV antibody tests such as ELISA and western blot but because these serological tests cannot detect HIV infections during the window period (<12-14 days), the residual risks of transfusion-transmitted HIV may remain within this diagnostic window. Use of HIV nucleic acid amplification tests (NAT) for blood donor screening, the residual risk of transfusion transmitted HIV can be further decreased by up to 50% depending on the sensitivity of the NAT protocol and whether it is an individual or pooled blood donations that are screened [165]. However, in low income countries such as Uganda, NAT has not been introduced in the public health sector and so screening of blood donors for anti-HIV antibodies using either ELISA and Western blot or HIV RDTs continues to be used and this carries residual risk of transfusion transmitted HIV [166]. Another strategy for blood safety from HIV could be ensuring individuals anticipating the need for blood such as those awaiting elective surgery have donated blood beforehand.

Immunization and vaccination

Unlike many other viruses, natural infection with HIV does not result into protective immunity against re-infection or disease progression since re-

infections and progression to AIDS seems unavoidable. Trials of candidate HIV vaccines to protect against HIV infections are ongoing. Only one candidate vaccine has significantly reduced HIV-1 acquisition at a limited efficacy of 31%, and none have delayed disease progression in vaccinated individuals [167]. A desirable vaccine should elicit broad neutralizing antibodies to prevent or reduce acquisition of the virus by adults and transmission of the virus to infants by HIV-positive mothers and also should sufficiently stimulate effective cytotoxic T lymphocyte (CTL) response to block the disease progression in breakthrough infections.

Many approaches to HIV vaccine utilize viral envelope gp120 or its precursor gp160 as immunogen [168]. Specific epitopes and T-cell antigens are also being investigated [168]. However, the development of HIV vaccine is faced with several problems unique to the virus for example the antigenicity of the virus changes through mutation. The virus can be spread through syncytia and also remain latent in an individual, hiding from the antibody. HIV also infects and inactivates those CD4+ T cells required to initiate an immune response. The candidate vaccine should be able to elicit both humoral and cellular immune responses to the virus in order to prevent sexual transmission of the virus (i.e. initial protection) and such will be difficult to achieve with a single vaccine. Additionally, the efficacy of the vaccines must be tested in human trials and a proper regimen of vaccination developed to elicit protective immunity. Also evaluating the success of the vaccine in limiting the spread and morbidity of HIV infection will be difficult for example it is often difficult to differentiate vaccine-induced sero-positivity from HIV-infection-induced sero-positivity [169]. Long-term follow up will be required to monitor vaccine efficacy and serologic responses albeit may not be sufficient to indicate success.

HIV Screening Services and Delivery Mechanisms in Uganda

HIV screening is the organized testing of people at risk of HIV infection with the intention of early detection and linkage to treatment and care programmes of the HIV infected persons. In Uganda, the available HIV screening services delivery and access points include voluntary counseling and testing (VCT), STI clinics, antenatal care (ANC)/PMTCT clinics, HIV research participants enrolment/recruitment clinics, routine counseling and testing (RCT) or routine testing and counseling (RTC) options within clinical outpatient and inpatient departments of health facilities, blood donation and screening clinics and ART clinics.

Voluntary Counseling and Testing (VCT): Voluntary counseling and testing is an organized HIV screening programme whereby members of the general public are informed about risk of acquisition of HIV and those who perceive themselves at risk voluntarily present themselves for HIV counseling and testing. The HIV counseling is provided in 2 phases, at pretest and posttest. And during the pretest and posttest counseling sessions, information is given on risk factors for HIV infection, HIV prevention methods and the role of HIV testing as a preventive option. VCT programmes often have linkage to HIV treatment and care programmes (ART clinics) to which they refer the HIV positive cases for treatment and care. Therefore, VCT programmes target high-risk populations such as discordant couples, pregnant mothers, youths including adolescents, drug users, men sex with men (MSM), prisoners and widows and widowers with the objective of early detection and treatment of the HIV infected cases.

Many VCT programmes make use of the HIV RDTs because of the quick turnaround time for results and hence the possibility of delivering the pretest counseling, HIV testing and posttest counseling services in a single visit approach, eliminating the risk of loss to follow up of the positive cases.

In Uganda, various delivery mechanisms have been employed by HIV stakeholders to effectively deliver VCT services to high-risk populations. This includes community-based standalone VCT clinics, health facility-based standalone VCT clinics, VCT services integrated into antenatal care (ANC) and maternity services, VCT services integrated into community-based family planning clinics and VCT services integrated into community-based youth center programmes. Some non-governmental organizations (NGOs) and community-based organizations (CBOs) conduct mobile VCT services door-door and or from social gathering facilities such as churches, mosques, markets and schools.

STI clinics: Early diagnosis and treatment of sexually transmitted infections (STI) is another HIV prevention strategy in Uganda. In Uganda, syndromic STI management services are available in almost all health facilities countrywide. More so, in Uganda, VCT for HIV have been integrated into STI management programmes such that suspicious patients seeking STI treatment are opportunistically screened for HIV.

Routine HIV counseling and testing (RCT): Routine HIV counseling and testing (RCT) is another approach to counseling and testing for HIV in Uganda. In RCT approach, all outpatient and inpatient departments of health facilities approach patients seeking their services, counsel, obtain voluntary consent and perform HIV testing on those interested. RCT has been adopted by almost all health facilities in Uganda to improve access and utilization of HIV counseling and testing services.

Routine HIV testing and counseling (RTC): Routine testing and counseling for HIV (RTC) is another approach to HIV counseling and testing employed by selected health facilities in Uganda. In RTC approach, all patients are tested for HIV infection as part of comprehensive medical management and those found to be HIV positive are then counseled and informed about their test results if they are interested to know. Those who are not ready to receive their HIV results are given appointments for additional counseling sessions which can go on until they are ready for it. However, regardless of patient's consent, the HIV test result is used to inform the medical treatment/management of the patient for whichever disease they present with. The difference between RCT and RTC is that RCT is voluntary HIV counseling and testing whereas RTC is not VCT, the HIV test is done even before obtaining consent from the patient, it is at the stage of receiving the HIV results when the patient's consent is sought. Therefore RTC is not the typical VCT we know of.

Blood donor screening for HIV: It has become standard practice in Uganda to screen all blood donors for HIV. Blood banks at national and regional referral hospitals are the entities responsible for donor screening. Blood from HIV positive donors are not used to prevent transfusion transmitted infections (TTIs).

Antiretroviral therapy (ART) clinics: In Uganda, ART clinics have been established in almost all hospitals upto the level of primary healthcare centers countrywide. The primary role of ART clinics is treatment of eligible HIV infected patients with antiretroviral drugs (ARVs). However, ART clinics also provide VCT services mainly to family members, friends and relatives of their HIV infected patients and new clients.

HUMAN PAPILLOMAVIRUS (HPV) AND CERVICAL CANCER

Genome organization

Human Papillomavirus (HPV) is a circular, non-enveloped, double stranded DNA containing virus belonging to the Papillomavirus genus and *Papillomaviridae* family [170-171]. It is made up of about 8000 base pairs (bps) in length organized into only eight genes situated in specific regions called open reading frames (ORFs).

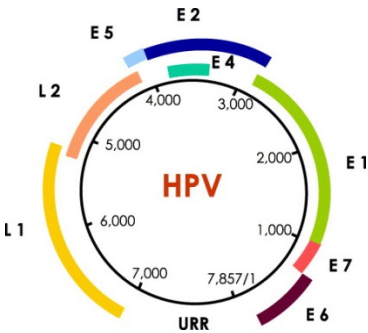

Figure 4: Schematic presentation of the HPV genome showing the arrangement of the early E or nonstructural genes, the capsid genes (L1 and L2) and the upstream regulatory region (URR).

The ORFs include early (E) and late (L) regions and the upstream regulatory region (URR) [172]. The E region comprises of six genes namely E1, E2, E4 and E5 required for viral replication and the E6 and E7 genes or oncogenes are required to maintain the cell-cycling state but also play a role in transformational changes [172-173]. The L region comprises of 2 genes namely L1 and L2 that encode the major and minor capsid proteins respectively, which encapsulate the HPV genome [172-173]. The URR contains gene sequence that control transcription but does not code for any protein [172-173].

Classification

Over 100 papillomavirus types have been isolated from mammals and humans and they together form the *Papillomaviridae* family. Classification allows for grouping and or subgrouping of the *Papillomaviridae* family

into genus, species, types, subtypes and variants. Papillomavirus (PV) classification is based on phylogenetic criteria of relatedness in the nucleotide sequence of its highly conserved L1 ORF gene [171, 174-176]. The process of classification involves comparing the L1 ORF gene nucleotide sequence of the newly isolated PV with a reference gene sequence, quantifying the magnitude of similarities or differences and graphically presenting the relationships in what is called phylogenetic trees. Starting from the center of the tree, the main branches also called *genera* share less than 60% of the L1 ORF gene nucleotide sequence and they are named by Greek letters such as alpha, Beta, Delta, Gamma etc [174-176]. Species or clades within a genus are a lower order of clusters of PV that share between 60% and 70% of the nucleotide sequence relatedness and considerable biological similarity [174-176]. PV types within a given species share between 71% and 89% of the LI ORF gene nucleotide sequence i.e. they differ by at least 10% and subtypes differ by 2% -10%. Finally, a PV variant within a given type differs by 1-2% in their LI ORF gene nucleotide sequence [174-176]. However, where there is greater intratypic variability in the non-coding URR, variants may differ by as much as 5% [174-176]. Subtypes are very rare and presently they were reported for a few HPV types such as 5, 8, 20, 34, 44, 54, 68 and 82. Each PV type is identified by a number based on the order of their discovery [174-176].

The phylogenetic criterion has managed to classify the Papillomaviridae family into 29 *genera* containing 189 papillomavirus types. Species within genera are also numbered in Arabic numerals such as 1, 2, 3 etc. For example, there are over 70 papillomavirus types belonging to the Alpha *genera* which are clustered into 15 *species* numbered 1- 15.

Papillomaviruses that infect humans also called Human Papillomavirus (HPV) incidentally are clustered in 5 genera namely the Alpha-, Beta-, Gamma-, Mu- and Nu- papillomaviruses [174-175] and they together contain more than 100 types. Of the over 100 HPV types that infect humans, about 40 types regularly or sporadically infect the cutaneous and mucosal epithelial surfaces of the skin, respiratory and genital tracts [177]. Incidentally all of them (the HPV types associated with both cutaneous and anogenital lesions such as warts and cancers) belong to the same genus, Alpha-papillomavirus [178]. Incidentally, all the HPV types that infect the cervix belong to the alpha genus and species 1, 3, 5, 6, 7, 8, 9, 10, 11, 13 and 15 which together contains over 40 HPV types [175].

HPV 16 and 18 the two commonest types associated with 70% of cervical cancer (CC) worldwide belong to Alpha 9 and Alpha 7 respectively [179]. Similarly, HPV6 and 11, the two commonest types associated with ano-genital warts (a form of cutaneous lesions) belong to the Alpha 10.

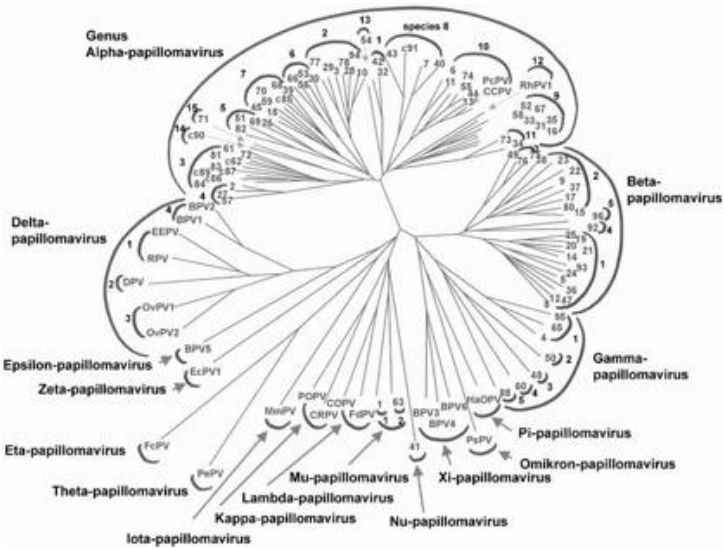

Figure 5A: Classification of papillomaviruses. Phylogenetic tree based on L1 ORF, including 118 different types from de Villiers et al 2004 [175].

Based on the potential to cause cancer (oncogenicity), HPV types from the 5 *genera* that infect humans are further classified into high-risk (HR) or carcinogenic group 1 and group 2A possibly carcinogenic , moderate-risk (MR) or probably carcinogenic group 2B and low-risk (LR) types or non-carcinogenic by the International Agency for Research on Cancer (IARC) [175, 180]. The HR-HPV types or the carcinogenic group 1 types include HPV16 and 18 with their genotypic relatives 31, 33, 35, 39, 45,51, 52, 56, 58 and 59 and carcinogenic group 2A type which is HPV 68 are the main types associated with cervical cancer globally. The MR HPV types or probably carcinogenic types include HPV 26, 30, 34, 53, 64, 66, 67, 69, 70, 73, 82 and 85 [181]. A recent systematic review has suggested the addition of HPV 26, 67, 68, 69, 73, and 82 to the Group 1 class since these genotypes has been shown to be more present in invasive cervical cancer (ICC) samples compared to in normal cytology [182]. The LR HPV

types or non-carcinogenic types include HPV 6, 11, 40, 42, 43, 44, 54, 61 and 62. The LR HPV 6 and 11 are associated with genital warts and are frequently associated with low grade squamous intraepithelial lesions [175, 180].

A

Genus + Species	Type	Invasive Cervical Cancer	IARC Category	Squamous Cell Carcinoma	Adeno Carcinoma	Tropism
Alpha 1	HPV32		3			mucosal
	HPV42					
Alpha 2	HPV3					cutaneous
	HPV10					
	HPV28		3			
	HPV29		3			
	HPV77		3			
	HPV94					
	HPV117					
	HPV125					
Alpha 3	HPV61	0.01	3			mucosal
	HPV62		3			
	HPV72		3			
	HPV81		3	0.4		
	HPV83		3	0.4		
	HPV84		3			
	HPV86		3			
	HPV87		3			
	HPV89		3			
	HPV102					
	HPV114					
Alpha 4	HPV2		3			cutaneous
	HPV27		3			
	HPV57		3			
Alpha 5	HPV26	0.37	2B	0.22		mucosal
	HPV51	1.25	1	0.75	0.54	
	HPV69	0.08	2B			
	HPV82	0.07	2B	0.26		
Alpha 6	HPV30	0.37	2B			
	HPV53	0.26	2B	0.04		
	HPV56	0.84	1	1.09		
	HPV66	0.08	2B	0.19		
Alpha 7	HPV18	10.28	1	11.27	37.3	
	HPV39	1.67	1	0.82	0.54	
	HPV45	5.68	1	5.21	5.95	
	HPV59	1.08	1	1.05	2.16	
	HPV68	1.04	2A	0.37		
	HPV70	0.11	2B			
	HPV85		2B			
	HPV97					
Alpha 8	HPV7		3		41.62	cutaneous (mucosal)
	HPV40		3		1.08	
	HPV43				0.54	
	HPV91	0.01	3		1.08	
Alpha 9	HPV16	61.35	1	54.38		mucosal
	HPV31	3.35	1	3.82	0.54	
	HPV33	3.83	1	2.06		
	HPV35	1.94	1	1.27		
	HPV52	2.71	1	2.25		
	HPV58	2.22	1	1.72		
	HPV67	0.31	2B			
Alpha 10	HPV6	0.11	3	0.07		mucosal
	HPV11	0.02	3	0.07		
	HPV13		3			
	HPV44	0.01	3			
	HPV74	0.01	3			
Alpha 11	HPV34	0.07				mucosal
	HPV73	0.52		0.49		
Alpha 12	HPV73					mucosal
Alpha 13	HPV54					
Alpha 14	HPV71					
	HPV90		3			
	HPV106		3			

B

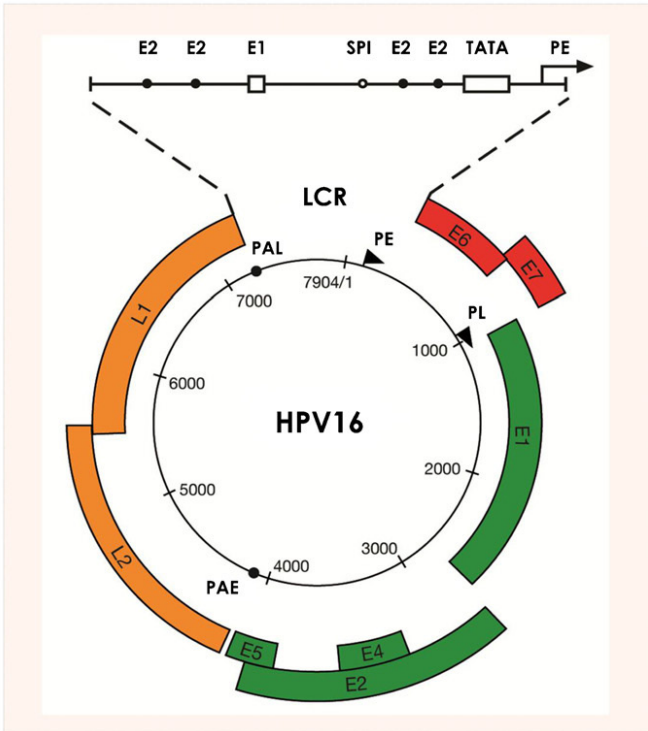

Figure 5B: Alpha Papillomavirus Disease Association and Genome Organisation. Source: Doorbar et al 2013 [Reference 172].

A. The high-risk Alpha types have been clearly linked with the development of squamous cell carcinoma (SCC) and adenocarcinoma (AC) of the cervix. IARC category 1 and 2A HPV types are classified (respectively) as carcinogenic and possibly-carcinogenic. Despite limited epidemiological data, the 2B classification is proposed for types that are probably carcinogenic because of their close phylogenetic relationship with the established carcinogenic types. HPV types in category 3 are considered non-carcinogenic. The remaining types have not yet been classified because of insufficient data. Types that are closely related evolutionarily (e.g., HPV16 and 31) can exhibit different degrees of cancer risk, which is thought to be related to different protein functions and patterns of gene expression. HPV16 is predominantly associated with SCC originating at the transformation zone and HPV18 with AC of the endocervix, but both can cause cancers at either type of tissue. Although cutaneous/mucosal classifications are not tight, the different Alpha species have tropism preferences which are indicated on the right.

B. The genome organisation of HPV16 is typical of the high-risk Alphapapillomaviruses (including HPV18), and comprises a long control region (LCR) and eight genes that are necessary for different stages of the virus life cycle. These genes encode a larger number of gene products as a result of mRNA splicing. The LCR contains binding sites for cellular transcription factors (e.g., SP1, AP1, Oct1), as well as for the viral E1 and E2 proteins that control viral replication and gene expression. HPV16 has two well-characterized promoter elements known as PE (early promoter; also referred to as p97) and PL (late promoter; also referred to as p670) that regulate the expression of differentially-spliced mRNAs during epithelial differentiation (position 97 and 670 in the HPV16 genome denote the 5_ cap site/RNA initiation site of viral transcripts). PAE and PAL indicate the positions of the early and late polyadenylation sites within the genome.

HPV Lifecycle

The lifecycle of HPV starts by entry into epithelial cells. HPV preferably colonizes mucocutaneous or cutaneous epithelia especially the proliferative basal epithelium. It has been suggested that HPV access the basal epithelium through naturally thin epithelial cells such as those in the transformation zone of the cervix in young women of reproductive age or through micro lesions or abrasions in the epithelium produced during sexual contact or other STIs [183]. Although the viral virulence factors, cellular receptors and molecular processes that facilitate initial attachment of the HPV virions to the basal cells are still unclear but there are studies that demonstrated that HPV virion gets adsorbed to Keratinocytes via membrane-associated heparan sulfate and integrin receptors and intermediate secreted into the extracellular matrix by the keratinocytes [184-186].

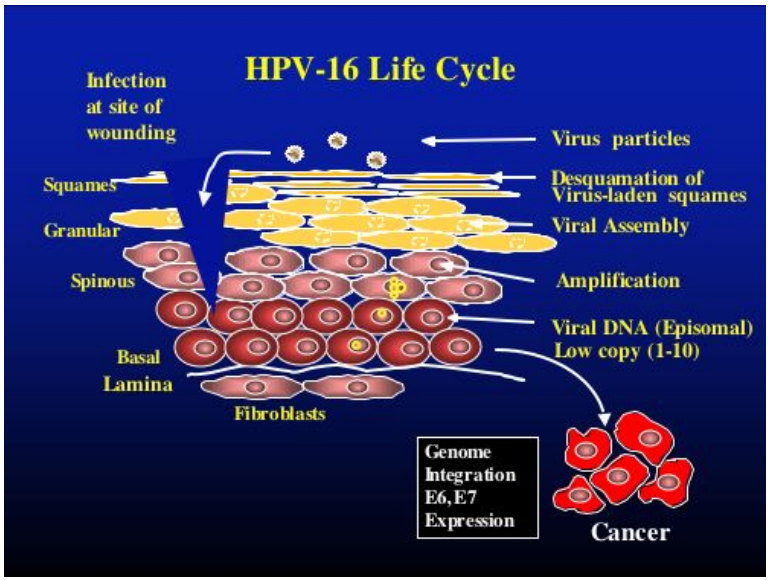

Figure 6: HPV lifecycle. Source: https://virologyspring2011.files.wordpress.com/2011/02/p_angeletti_hpv_lifecycle.jpg

After attachment, however, the HPV pseudovirion appears to be internalized into the basal epithelial cells by endocytosis [187-189]. In the cytosol, virus appears to reduce the PH environment of the cell resulting into disruption of intracapsomeric disulphide bonds in the endosomal membrane, uncoating and possible release and movement of the viral DNA to the nucleus of the basal cells along PH gradient [190-191]. In the nucleus, the viral DNA is integrated into the host cell chromosome close to fragile sites or cellular oncogenes [192-195]. Initially, low copies of about 1-10 virions are replicated and can persist in the basal epithelial cells in episomal form for varying periods of time. Although the actual pattern of viral gene expressions to facilitate integration, replication, assembly, maturation and release of the viral particles is not yet well understood, it seems the viral early genes E1 and E2 are expressed to maintain the viral DNA replication in episomal form [196-197] at the same time the E4 and E5 amplify the viral friendly environment [198] and E6 and E7 are minimally expressed just enough to enhance the proliferation of the infected cells and their lateral expansion [199]. As the basal keratinocytes continue to proliferate, the infected differentiating suprabasal cell's E6 and E7 gene

expressions are gradually upregulated to maintain the cells in proliferative state, blocking the exit of the daughter cells from the cell cycle [199]. However, as the daughter HPV infected cells matures and migrates towards the upper layers of the mucosa where cells reach the stage of terminal epithelial differentiation, E1, E2, E6 and probably E7 genes expressions are maximally upregulated, resulting in assembly, maturation and release of the viral particles [200].

The mature HPV virions are then released during the desquamation or sloughing of dead surface epithelial cells (koilocytes) and this serves as the mechanism of dissemination. The aforementioned HPV life cycle represent a complete productive cycle whereby progeny HPV virions are produced and released from the HPV infected cells. Alternatively, the HPV life cycle in the infected epithelial cells may enter into abortive cycle, which is responsible for severe and oncogenic outcome of the infection. In the abortive phase, differentiating suprabasal epithelial cells become neoplastic, there is increased epithelial thickening, cells appear immature, and are expressing E6 and E7 proteins, increased integration of the viral genome into the host cell's genome [201].

Carcinogenesis

Over the years it has been noted that it takes about 15-20 years between HPV infection and development of malignant transformation leading to cervical cancer. The critical event in the malignant transformation caused by HPV infection is the viral DNA integration into the host genome. HPV DNA integration into the host cell chromosomes causes genomic instability but ensures persistent replication and expression of the viral genes especially the oncogenes E6 and E7 in the basal and parabasal keratinocytes [202-205]. The viral oncongenes E6 and E7 proteins inhibit two cellular tumor suppressor genes namely p53 and Retinoblastoma (pRb) which are required for disruption of the cell-cycle progression and apoptosis in response to DNA damage respectively [206-207]. The oncogenic HPV E6 and E7 proteins are highly expressed in differentiating keratinocytes, where they inhibit p53 and retinoblastoma (pRb) proteins, two important transcriptional regulators. E6 inhibits p53, and E7 inhibits pRB. The net effect is that these important cancer controlling genes are shut-off, encouraging persistent replication of the transformed and unstable genome. Cell cycle without normal checks from p53 and pRb are proned to mutation

and it is the accumulated mutations that promote carcinogenesis [208-209].

HPV Immune Activation and Evasion

Cutaneous and genital HPV infections are very common but the immune responses of the majority of the individuals clear the infection without any overt clinical disease. The few who develop lesions, in most cases, are able to mount an effective delayed cell-mediated immune response (adaptive immunity) and the lesions regress. Failure to induce immune response against HPV exposure could be due to inefficient activation of innate immunity and ineffective priming of the adaptive immune cells, which facilitates viral persistence, a key feature of high-risk HPV infections. In persistent infection, however, antigen tolerance develops and host immune cells become compromised. HPV antigen-specific effector cells are poorly recruited to the infection focus and their activity may as well be down-regulated [210]. In such a tolerant and immunosuppressive state, HPV-infected neoplastic keratinocytes expressing high levels of E6 and E7 oncoproteins are not killed and may progress to high-grade lesions and cancer ensues. Also, the infectious cycle of HPV itself is a mechanism for immune evasion. Infections are localized without viraemia and very low levels of viral proteins are expressed but crucially HPV is not cytolytic. Virus replication and assembly occur in cells already destined for death by anoikis or "death by natural causes", there is no inflammation and no danger signal to alert the immune system. The interferon response for HPV infection, a key antiviral defense mechanism [211], is actively suppressed with the E6 and E7 proteins of the high risk HPVs inhibiting the interferon receptor signaling pathways and the activation of the interferon response genes [212]. The E7 proteins down regulate TLR9 [212] and overall HPV effectively evades the innate immune response delaying the activation of adaptive immunity.

Acquisition and Transmission

Non-sexual Transmission

Non-sexual transmission of genital HPV infection through modes like skin-to-skin contact, fingers and sex toys are rare occurrence but have been reported [213-214]. Similarly, vertical transmission of HPV infection

from mother to unborn child via the placenta during gestation or through direct exposure to genital and cervical HPV lesions during child birth are also rare occurrence but have been reported [215]. Non-sexual horizontal transmission of HPV infection from parents to children is also possible although rather rare. In preadolescent children, genital HPV infections are considered as sign of sexual abuse despite the possibilities of the aforementioned non-sexual horizontal transmission.

Sexual Transmission

Human papillomavirus (HPV) is predominantly a sexually transmitted infection (STI) and so share the same risk factors for STIs such as early age at sexual debut, multiple sexual partners and non-condom use [216-218].

Young age at sexual debut was associated with an increased risk of genital HPV infection [219]. A systematic review of several studies has also shown that early age at sexual initiation was associated with an increased risk of cervical cancer [220]. Similarly, the risk of genital HPV infection increases with decreasing interval between menarche and first sexual intercourse after controlling for other determinants of infection [221]. The risk of genital HPV infection reportedly increases with both the men's and women's number of lifetime sexual partners [222-224] although having only one partner was also associated with HPV infection [225].

Literature was conflicting about the association between consistent use of male condom and genital HPV infection. A systematic review of some studies reported that consistent use of male condoms does not protect from genital HPV infection [226] while others reported that consistent male condom use provide partial protection against HPV. For example, a prospective study of consistent use of male condom by couples [227] and a meta-analysis of 20 published studies [228] both concluded that the risk of genital HPV transmission and HPV-associated cervical lesions were reduced by consistent use of male condoms. However, due to the highly contagious or infectious nature of HPV, the protective effect of condoms is likely to be limited.

Literature was also conflicting about the association between male circumcision and genital HPV infection. Male circumcision was associated with a decreased prevalence of penile HPV infection but partially protec-

tive against new HPV acquisition in a prospective study [229]. Similarly, a meta-analysis has found that male circumcision was associated with decreased odds of genital HPV prevalence but partially protective (about 35%) against new genital HPV acquisition, genital HPV clearance, or genital warts in prospective design [230].

Co-factors of HPV infections

Several cofactors have been reported to directly or indirectly influence the risk of genital HPV infection and they include age, HIV/AIDS comorbidity, other STIs comorbidity, cigarette smoking, pregnancy state, use of hormonal contraception, use of vaginal lubricants and spermicides, and genetic predisposition.

Age: A systematic review has shown that across studies, the global age-specific HPV prevalence among females increases with an increasing age with a peak at the age of <25 years [231]. However, among males, peak HPV prevalence spanned a wide range of ages and was generally not concentrated in the younger age groups [232]. Generally, global age-specific HPV prevalence first peaks at younger ages of <25 years and in the Americas and Africa, the prevalence rebound at the age >45 years [233].

HIV/AIDS comorbidity: Systematic reviews and meta-analysis have consistently indicated the increased risk of acquisition and progression of HPV infections among HIV-positive women than their HIV-negative peers [234-235]. The exact mechanisms by which HIV/AIDS comorbidity increases the risk of HPV acquisition and progression is not well understood but it could be through reactivation of silent HPV infections [236]. It is also possible that the destruction of T helper cells (CD4 cells) and the resultant immunosuppressive state (ISS) from HIV infection provides immune unchecked environment for HPV infection and progression.

Other STIs comorbidity: Other STIs such as *Chlamydia trachomatis* (Ct) have been associated with an increased risk of genital HPV infection [237-238]. *Herpes simplex 2* infection has also been associated with an increased risk of HPV and cervical cancer [239-240]. For syphilis, a recent clinical study conducted in Mozambique also reported a positive association between syphilis infection and HPV infection [241]. It could be the

resultant inflammations and micro abrasions in the genital tract from other STIs that allows HPV direct access to the susceptible basal epithelium.

Cigarette smoking: A systematic review of previous studies has shown conflicting results regarding the association between cigarette smoking and risk of HPV infection with some studies reporting a positive association after controlling for sexual behaviors while others reporting an inverse association [242]. Cigarette smoking may be masking other risk factors although there was an evidence that tobacco-related carcinogens contained in cigarette smoke directly damages the genetic materials of genital cells increasing their susceptibility to HPV infection [243].

Pregnancy state: Previous studies were conflicting about the association between pregnancy state and HPV infections with some studies reporting a higher rate while other studies reporting the same rate of genital HPV infections among pregnant women compared to their non-pregnant counterparts [244-247]. In fact, a recent meta-analysis showed a significantly increased risk of HPV infection in pregnant women, especially for those aged <25 years [245]. Among pregnant women, HPV prevalence increases with gestational age [244, 247]. The increased risk of genital HPV infections in pregnant women could be due to several physiological hormonal changes during pregnancy such as increased secretions of uterine and ovarian hormones particularly progesterone that has the potential to undermine the host immune response to new or reactivation of quiescent HPV infections. Possibly, the anatomical change during pregnancy such as enlargement of the ectocervix where the transformation zone is situated exposes the susceptible basal epithelium to any viral particles in the genital fluid [248]. Another aspect could be the Th2 type of immune deviation during pregnancy which does not favor control of intracellular viral infections such as HPV [249].

Hormonal contraception: Previous studies were conflicting regarding the association between hormonal contraceptive use (both ever use and prolonged use for 5 or more years), genital HPV infections and cervical cancer [250-251]. A prospective study conducted in Thailand found that recent and long duration use of combined oral contraceptives (COC) for more than 6 years was associated with an increased risk of both any HPV and HR-HPV infections but no similar association was observed for recent or long duration use of progesterone-only contraceptives (depot medroxy-

progestrone acetate) when compared to never users [249]. Similarly, compared to never users, oral contraceptive use was associated with an increased risk of cervical cancer [250]. On the contrary, data from Latin America indicated that hormonal contraceptive use and duration of use were not independent risk factors for high-risk HPV infection or high-grade cervical cancer intraepithelial neoplasia [251].

Vaginal lubricants and spermicides: Use of vaginal spermicide containing Nonoxynol-9 (N-9) has on the contrary been associated with an increased risk of HPV infection (252]. This report about nonoxynol-9 containing vaginal spermicides concurred with studies in mice model that also found that N-9 greatly increased susceptibility to HPV infection but on contrary Carrageenan, a polysaccharide present in some vaginal lubricants prevented genital HPV infections even in the presence of N-9 [253]. However, the exact mechanism by which Carrageenan prevents genital HPV infection is still unknown.

Genetic predisposition: Race and geographical locations (which are genetic markers) have been associated with susceptibility and persistence of certain HPV types and variants. It has been demonstrated for example that variants of HPV16 and 18 persists longer in a host whose race and ancestral geographical location matches that of the variant i.e. European variants persists longer in white women and similarly African variants persist longer in African American women [254]. Other genetic markers such as polymorphism of the p53 genes (tumor suppressor gene responsible for cell cycle arrest) have also been associated with increased risks of certain HPV infections in Asian population [255] although no association was reported in the European population [256]. Similarly, Human Leucocyte Antigen (HLA) the gene responsible for foreign antigen presentation to the immune system polymorphism has also been associated with increased risk and progression of HPV infections [257-258] although the specific alleles varied between studies, supporting a genetic predisposition to cervical cancer. Last but not least, full and half sibling studies have shown familial aggregation of cervical cancer with higher risk among full siblings compared to half siblings [259-260]. In fact, one study conducted in Sweden has apportioned familiar risk for cervical cancer in full sibling into a heritable component accounting 64% and an environmental component accounting for 36% [259].

Pre-existing HPV infections: Studies have consistently reported that pre-existing HPV infection of specific types appears to increase the risk of acquisition of other HPV types [260-263]. One of studies found it was pre-existing HPV16 infection that was associated with an increased risk of acquisition of other HPV types over time [260-261] although one of the studies reported that any type of pre-existing HPV infection is associated with an increased risk of acquisition of any other type of HPV infection overtime [262]. However, it is still not clear whether HPV types influence each other's infectivity and the cellular and molecular mechanisms involved. Sexual co-transmission could be an alternative explanation where the vector sexual partner e.g. the man was carrying multiple HPV strains co-infections so that the victim is simultaneously exposed to multiple HPV strains in a single sexual act.

Simultaneous exposure to multiple HPV strains carrying vector appears to be insufficient to explain why pre-existing HPV infection is associated with an increased risk of acquisition of HPV of other types. One emerging theory seems to suggest that infection with the new HPV of other types occur in a non-independent manner [262]. This theory of non-independence of co-infections was supported by a report that indicated that the odds of acquiring concurrent infections with HPV31, 39 and 45 was increased 11-18 times in women with pre-existing HPV 18 infection than women without pre-existing HPV 18 type of infection [262]. Similarly, the odds of acquiring a new infection with HPV type 58 was increased by 5-7 times in women with pre-existing HPV 16 infections than those without pre-existing HPV of that type [262]. The exact mechanisms by which pre-existing types of HPV infections in a non-independent manner influences the likelihood of infection with other types of HPV is not yet well understood but could be through generation of cross-reacting or cross neutralizing antibodies that act across a certain phylogenetically related or unrelated HPV types and thus preventing their occurrence [263]. The generation of limited quantity of about 1% cross neutralizing antibodies against certain HPV types 16, 31, 33 45, 58 and 59 in addition to type-specific neutralizing antibodies by immunization of mice with L1 virus-like particles (VLP) has been demonstrated [264].

Another explanation for the non-independence of concurrent or sequential acquisition of different genital HPV types could be the endocytosis pathways they use to gain entry into the epithelial cells, whether they are

shared or type-specific. This theory is supported by a report that some HPV types use the same endocytosis pathway to gain entry into epithelial cells while others such as type 16, 31 and 58 though phylogenetically closely related use different endocytosis pathways to enter cells [266]. However, regarding the association between pre-existing HPV infection and persistence of any other HPV types, literature consistently reported that pre-existing HPV infection is not associated with persistence of any other HPV types [260-261, 266].

Burden of HPV infections and Type Distribution

The global prevalence of HPV DNA infections in women without cervical abnormalities stands in the range of 11-12% with higher rates in sub-Saharan Africa (24%), Eastern Europe (21%) and Latin America (16%) [267]. Worse affected regions were East Africa and the Caribbean where prevalence over 30% have been reported. The most common high-risk HPV type, HPV16, represented between 13% (in Africa) and 30% (in West and Central Asia) of all HPV types in women with normal cytology [268].

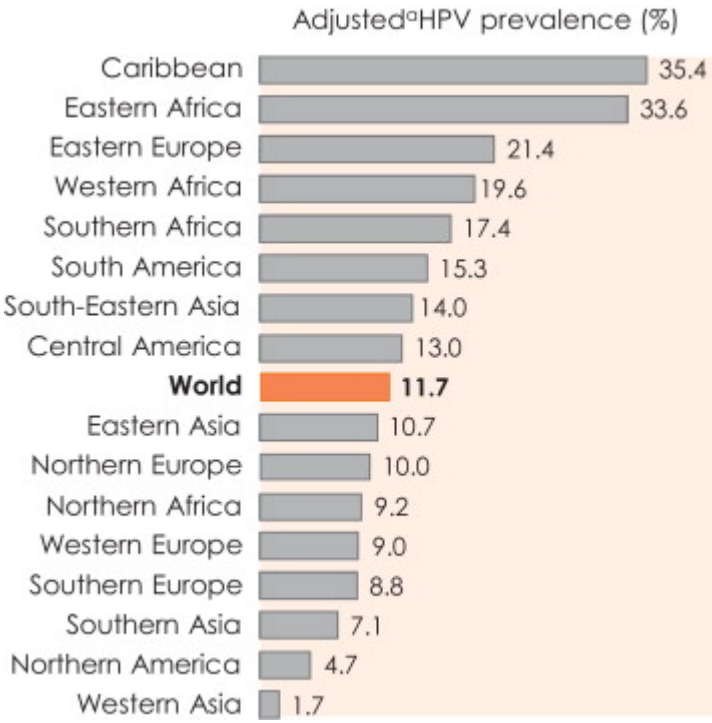

Figure 7: HPV prevalence among women with normal cytology: meta-analysis based on results from 1,016,719 women. Redrawn from Bruni L *et al.* 2010 (Reference 267).

There is a relationship between HPV prevalence and age seen globally, which shows peak prevalence in younger women (less than 25 years) with a monotonic decline at older ages [269]. Type-specific HPV prevalence data showed that the five most prevalent types worldwide were HPV16 (3.2%), HPV18 (1.4%), HPV52 (0.9%), HPV31 (0.8%) and HPV58 (0.7%) [269]. All other HPV types had a prevalence of 0.6% or less, including HPV45 (0.5%), as well as HPV6 (0.5%) and HPV11 (0.2%) (the two most prevalent types found in association with genital warts).

Globally, among women with cervical cytological abnormalities, HPV prevalence increases with the severity of the lesion reaching around 90% in women with cervical intraepithelial neoplasia grade 3 (CIN3) and invasive cervical cancer [268]. And globally, the type-specific prevalence of three commonest high-risk HPV types in women with abnormal cervical

cytology particularly invasive cervical cancer (i.e. HPV16, 18 and 45) were 63%, 16% and 5%, respectively, of women with cancer [268].

In Uganda, HPV DNA prevalence among women of unchecked cervical cytology stands in the range of 12.5-75.3% [270-271]. In terms of HPV seroprevalence, the range was 17-57% [270]. The six commonest HPV types among Ugandan women of unchecked cytology in order of decreasing prevalence were HPV 52, 58, 16, 45, 18 and 66 with highest reported type-specific prevalence of 14.2%, 7.5%, 7.5%, 5.1%, 3.5% and 3.0% respectively.

Among Ugandan women with invasive cervical cancer (ICC), HPV DNA prevalence ranges from 61.3-90.1% [270, 272-273]. In terms of HPV sero-prevalence, the range was 9-27% [270]. The 5 commonest HPV types among Ugandan women with ICC in the order of decreasing prevalence were HPV 16, 18, 45, 51 and 52 and their highest type-specific prevalence were 47.4%, 35%, 9.7%, 1.9% and 1.3% [270, 272-273].

Similarly in Uganda, young women aged <25 years harbors the highest HPV prevalence rates in the range of 60.0 - 75.3% [270-271]. Low-risk HPV types 6 and 11 prevalence among Ugandan young women aged <25 years stands in the range of 15.5-24.7% and 13.3-16.3% respectively [270-271]. Among Ugandan men aged 15-49 years, any HPV DNA prevalence stands in the range of 61.9-62.6% [270, 274].

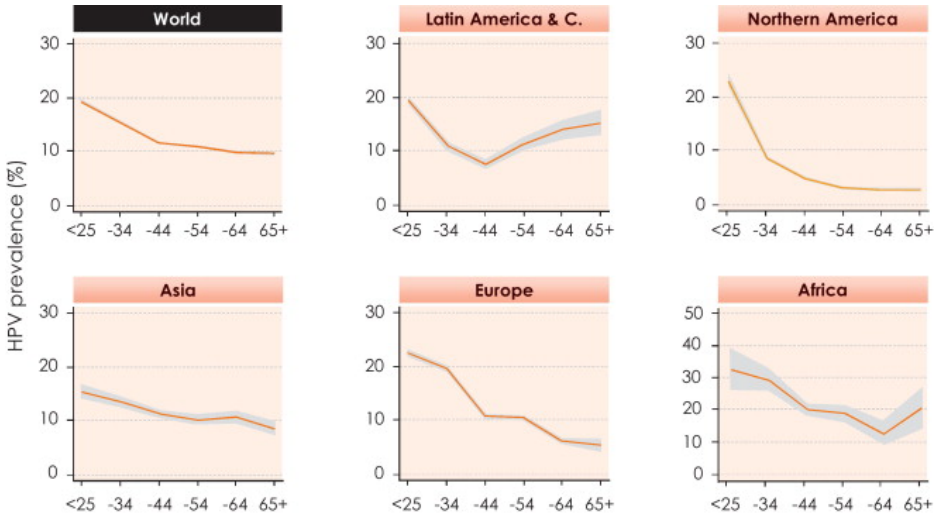

Figure 8: HPV prevalence among women with normal cytology by age group (years). Shaded area reflects 95% confidence intervals. C: Caribbean. Redrawn from Bruni L et al. [267].

Cervical Cancer Disease Burden

Human Papillomavirus (HPV) infection has been identified as a definite human carcinogen for six types of cancer: cervix, penis, vulva, vagina, anus and oropharynx (including the base of the tongue and tonsils). Cervical cancer, for which the HPV proportion attributable fraction is estimated to be 100% [275], accounted for 527 624 (86.9%) of the HPV attributable cases with the other five cancer types accounting for the residual 80,000 cancers [276, 277].

Cervical cancer (CC) is the third commonest cancer among women globally in terms of 5-year prevalence, with an estimated 527,624 new cases and 265,672 deaths globally in 2012, approximately 87% of the CC deaths occurred in less developed countries [277]. In terms of 5-year prevalence, globally, about 1.6 million women aged 15+ years are diagnosed with CC every 5 years, which is second only to breast cancer [277]. In East Africa, CC is the most prevalent cancer and the leading cause of cancer-related deaths among women [277]. In Uganda, the age-standardized incidence rate for CC was estimated at 44.4 per 100,000 women in 2012, which

was one of the highest in the world [277]. Cervical cancer is the leading cancer-related cause of morbidity among women in Uganda [278]. Survival from cervical cancer in Uganda is also not that good, with overall and relative 3-year survival rates standing at 52.4% and 59.9% [279].

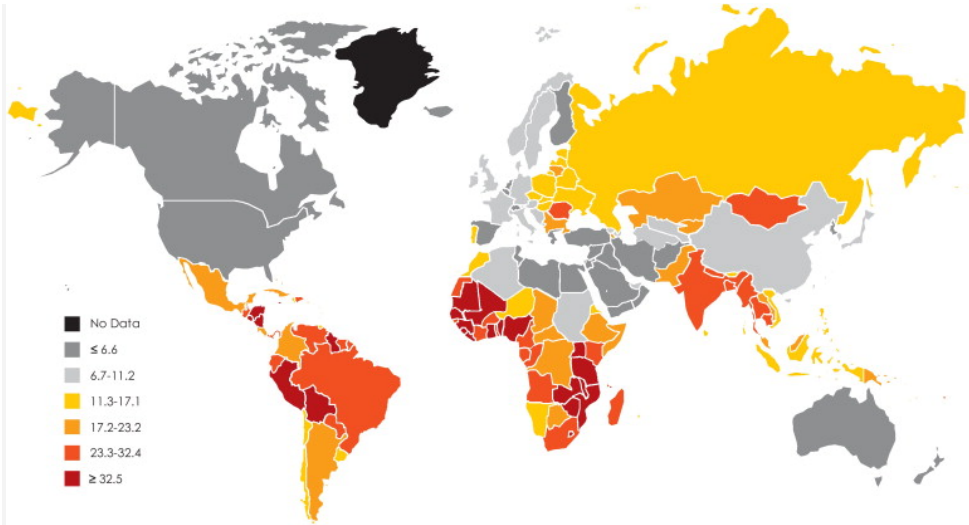

Figure 9: Cervical cancer, global map showing estimated age-standardized (world standard) incidence rate per 100,000 in 2008 (all ages). Data were based on GLOBOCAN 2008 [277].

Cervical Cancer Prevention

Cervical cancer prevention options or methods in the offing include HPV vaccination, screening coupled with treatment for precancerous lesions and HPV testing.

HPV vaccination

Human Papillomavirus (HPV) vaccines are virus-like particles (VLPs) derived from the L1 capsid proteins of selected HPV types that can induce immune response against HPV. Being VLPs, they are neither live nor attenuated vaccines. Until 2014, there are 2 types of HPV vaccines on the

market, the quadrivalent HPV-6/11/16/18 vaccine (Gardasil®, MSD, USA) and the ASO4-adjuvanted HPV-16/18 vaccine (Cervarix®, GlaxoSmithKline Biologicals, Belgium) [280-281]. Whereas Bivalent HPV vaccine (Cervarix®) targets HPV16/18, the Quadrivalent HPV vaccine Gardasil targets HPV6/11 the causative agent of genital warts in addition to HPV16/18. The third investigational HPV vaccine from MSD USA called 9-valent HPV vaccine (9vHPV) is in phase II clinical trial but has been approved by US Food and Drug Administration (FDA) [282]. The 9vHPV vaccine is basically the quadrivalent HPV 6/11/16/18 plus HPV 31/33/45/52/58. Put together, HPV vaccines are indicated for use in the prevention of cervical cancer, vulvar and vaginal precancer and cancers, precancerous lesions and genital warts associated with selected HPV types 6, 11, 16 or 18 infections in adolescents 9-15 years and young women 16-26 years [278-282].

In Uganda, the ASO4-adjuvanted HPV-16/18 vaccine (Cervarix®) was introduced in 2008 for young girls as a primary prevention strategy against cervical cancer. The first pilot project of ASO4-adjuvanted HPV-16/18 vaccination was implemented in Ibanda District, situated in southwestern Uganda. In 2008 alone, over 3000 young girls aged 10-18 years who were in primary school grade 5 (P5) received intramuscular injections of the vaccine as per the recommended schedule of 0, 1 and 6 months and a vaccination coverage of over 95% was achieved [283].

Whereas data regarding the efficacy, immunogenicity and safety of the HPV vaccines (both the ASO4-adjuvanted HPV-16/18 and quadrivalent HPV6/11/16/18) is beyond question and both vaccines have been licensed for use [284-285], there are growing concerns about potential epidemiological changes in disease patterns after vaccine implementation e.g. changes in age distribution of disease or changes in strains causing disease. Changes in distribution of disease are possible in settings like in sub Saharan African countries where there are high frequency of comorbidities among the subpopulation groups (e.g. HIV, AIDS and syphilis comorbidities) as well as inequitable access to healthcare interventions such as screening, condoms and safe male circumcision among others [286].

Similarly, changes in strains causing disease (also known as type replacement) are likely to happen in the virus as a result of biological or ecological interactions between the different strains of the virus in situations of

concurrent infections with multiple HPV strains or mutation [287-290]. Previous studies on changes on demographic distribution of disease per se are scarce in the post HPV vaccine introduction era particularly with ASO4-adjuvanted HPV-16/18 vaccine. One post ASO4-adjuvanted HPV-16/18 vaccination 4-years follow up study among 16-17 year old females from Finland found no increased occurrence of non-vaccine HPV types suggestive of type-replacement 1-4 years post-vaccination among HPV-16/18-vaccinated Finnish adolescents [291]. The wide range in the participant's follow up time of 1-4 years was a potential limition of the study. Other previous studies on HPV type replacement as a result of biological interactions between different strains of the virus in situations of concurrent infections with multiple HPV types were focused on HPV clustering patterns or intertype associations mostly among the non-vaccinated women population [292-294] without vaccinated comparison group as such have fallen short of providing explanatory models for the patterns of HPV clustering or intertype associations seen in concurrent infections with multiple HPV types rather than attributing it to random process contrary to other evidence that showed non-independence or preferential intertype associations or clustering [262, 291].

To address the aforementioned concerns about changes in disease distribution pattern or strains causing disease after HPV vaccination, World Health Organization (WHO) recommended monitoring of the HPV infections among young women shortly after sexual debut as one of the strategies to provide early indications of HPV vaccine impact on morbidity, to demonstrate vaccine effectiveness in real world settings, and to identify epidemiological changes in disease distribution patterns or strains causing disease [295]. Such WHO recommended HPV vaccine follow up studies are still scarce worse so from developing countries. Previous population-based HPV vaccine follow up studies of young women were conducted in the Germany [296], US [297], Australia [298] and Sweden [299-300] among others but all were conducted after quadrivalent HPV6/11/16/18 vaccine (Gardasil®) or a mix of ASO4-adjuvanted HPV-16/18 vaccine [296] implementation, making their findings difficult to compare with ASO4-adjuvanted HPV-16/18 vaccine on focus in this thesis. Nevertheless, pre- and post ASO4-adjuvanted HPV-16/18 vaccine (Cervarix®) surveillance studies were conducted in Scotland and England [366-367] but their findings are difficult to generalize to developing countries such as Uganda due to differences in the patterns of health problems and health hazards

between European countries and developing countries in Sub Saharan Africa. Furthermore, given that the ASO4-adjuvanted HPV-16/18 vaccine (Cervarix®) in use in Uganda does not target genital wart causing LR HPV-6/11 unlike the quadrivalent HPV-6/11/16/18 vaccine in use in other countries with some HPV vaccine follow up data, there was need for the 5-year post ASO4-adjuvanted HPV-16/18 vaccine follow up study in developing country Uganda to possibly address some of the aforementioned concerns about changes after HPV vaccine implementation.

Cervical Cancer screening

Cervical cancer screening is the intentional testing and follow-up treatment of women at risk of CC with the objective of early detection and treatment of cervical precancerous lesions or early linkage of the detected cancer cases to treatment. The available CC screening methods or options in Uganda and as is the situation in many developing countries include Papanicolaou smear, visual inspection with 5% acetic solution (VIA), visual inspection with Lugol Iodine (VILI), Colposcopy (although more of diagnostic procedure but is often indicated in many screening algorithms before treatment of detected precancerous lesions) and biopsy or histology (often used as the gold standard for cervical cancer screening methods).

Papanicolaou smear: Papanicolaou smear (Pap smear) is a cervical cancer screening set of procedures which involves collection of exfoliated cervical cells, fixing it on slide and visualizing it under microscopy for abnormal changes in the cells. This could be abnormal changes in the morphology or size of the cytoplasm and or nucleus of the cells. The severity or degree of the abnormal changes in the cells whether it involves basal, parabasal or suprabasal cell layers of the epithelium is important and often provides the basis for grading the lesions. The examination of the slides is often performed by specialist medical doctors (Pathologists).

Depending on the type and severity or degree of the epithelial changes, Pap smear test results are reported as Normal, low-grade squamous intraepithelial lesions (LG SIL), high-grade squamous intraepithelial lesions (HG SIL) and invasive cervical cancer according to the new Bethesda Pap smear system and its 1991 revisions [301-302]. This Pap smear result reporting system has replaced the old system of Normal, Mild Dysplasia or Cervical Intraepithelial Neoplasia grade-1 (CINI), Moderate Dysplasia or

Cervical Intraepithelial Neoplasia grade-II (CINII), Severe Dysplasia or Cervical Intraepithelial Neoplasia grade-III (CINIII), Atypical Squamous Cells of Undetermined Significance (ASCUS), Atypical Glandular Cells of Undetermined Significance (AGCUS), Carcinoma In situ (CIS) or Invasive Cervical Cancer (ICC). In the Bethesda System, Atypical squamous cells of undetermined significance (ASCUS) and atypical glandular cells of undetermined significance (AGCUS) need further qualification as to whether they favor either a reactive or neoplastic process [301-302].

Low-grade SIL means there are very minor changes to the cervical cells detected. High-grade SIL are more serious changes to the cells of the cervix which, if left untreated, have a greater chance of progressing to cervical cancer disease. The more severe the abnormality, the less likely it is to go away (regress) and the more likely it is to get worse (progress) and eventually turn into cancer.
Low and high-grade SIL often peak in the women's fourth decade while that for cancer is the fifth decade (303-305). However, they also occur in younger and older women. For many women, especially those with LG SIL, the problem will heal or regress on its own, with the cells of the cervix returning to normal. For HG SIL, it usually takes 8-10 years before it totally transform into invasive cancer if untreated [305]. It is impossible to predict whether the abnormality will regress to normal or progress into cancer. Further tests are always needed to confirm the diagnosis. World Health Organizations (WHO) recommends once in a lifetime Pap smear screening for a woman between the ages of 35 and 40 years for resource limited settings and after every 3 years for high income settings.

A multi-country study including Uganda put the sensitivity and specificity of Pap smear in range of 40.7%- 73.7% and 87.7% respectively [306]. Organized cytology-based cervical cancer screening has been shown to reduce cervical cancer incidence and mortality. For example in Norway, in just 2 years after implementation of an organized cytology-based cervical screening, the incidence of invasive cancer was 22% lower than in the period before the programme [307]. Similar drop in invasive cervical cancer was reported in Colombia after introducing cytology-based cervical cancer screening [308].

However, organized mass cytology-based cervical cancer screening programmes require frequent repeats of the screening tests, a functioning

healthcare infrastructure with laboratories for smear processing and interpretation, mechanisms for quality control, referral for colposcopy, treatment of precursors, and follow-up to detect failures of treatment. Although this cytology-based cervical screening approach has been successful in preventing cervical cancer where implemented correctly, it has proved complex and expensive for developing countries [309]. Consequently, no successful cytology-based cervical screening programmes have been established in poor countries including Uganda.

Visual Inspection with 5% acetic solution (VIA): Visual inspection with 5% acetic acid solution (VIA) is a cervical cancer screening procedure which involves visual examination of the cervix for spots of color changes plus other gross abnormalities on the surface of the cervical epithelial cells after application of 5% acetic acid solution. The spots of color changes referred to as acetowhite areas especially on the transformation zone of the cervix or the endocervix often occurs in areas with cervical precancerous lesions or cancer. The VIA screening may be performed with or without the aid of a magnification camera. The spots of acetowhite color changes are visible within 1 minute of application of the 5% acetic acid solution, making the test result readily available for the HCPs to use for treatment decision on the positive cases there and then.

VIA test result is often reported as Negative, Positive for cervical precancerous lesions and Positive Suspicious for Cancer. Negative result means absence of cervical precancerous lesions on the cervix, which is normal finding. A VIA positive results means there are cervical precancerous cells which if left untreated have higher chances of progressing to cancer. And positive Suspicious for Cervical Cancer means there is cancer. The VIA examination is often performed by a medical doctor and even nurses making it a scalable service with the potential to increase women's access to CC screening.

Cervical precancerous lesions can be treated for example by cryotherapy, which is an outpatient procedure that can be performed immediately after the VIA screening in a single visit approach (SVA). The readily available results and the possibility of combining VIA screening with cryotherapy treatment for test positive cases in a SVA eliminates the need for HCPs to recall the women with positive test results to receive their results and for treatment or the need for the women to make multiple visits to health

facility first for screening and second for test results and treatment if found positive and its associated risk of loss to follow up.

Pooled sensitivity, specificity, positive and negative predictive values of VIA were 80%, 92%, 10% and 99%, respectively, for detecting cervical intraepithelial neoplasia grade 2 (CINII) or worse lesions [310]. Realistic sensitivity of a quality- assured single VIA is around 50% [310]. A multi-country study including Uganda put the sensitivity and specificity of VIA in the range of 21.9-73.6% and 84.2% respectively [306]. A single round of VIA screening has been associated with a 25-35% reduction in cervical cancer incidence and the frequency of cervical intraepithelial neoplasia grade 2 or worse lesions in randomized-controlled trials [310].

Despite aforementioned limitations, VIA-based cervical screening remains the most feasible and pragmatic approach to cervical cancer prevention in resource limited settings in developing countries such as Uganda. WHO recommends CC screening by VIA for women from the age of 25 years. And the screening interval is every 3 years for an HIV-negative woman but annually for an HIV-positive woman. In Uganda, VIA cervical screening services are increasingly becoming popular and available in many lower and higher level public and private health facilities countrywide.

VILI: Visual Inspection with Lugol Iodine (VILI) is exactly like VIA described above except Iodine solution is used in place of 5% acetic acid solution. Instead of acetowhite, the positive color change after application of VILI will be golden yellow. The sensitivity, specificity, and positive and negative predictive values of VILI were 97.7%, 94.8%, 46.2%, and 99.9%, respectively for detecting CINII or worse [311]. The initiation age and interval of VILI cervical screening are exactly the same as that of VIA described above. Despite its similarity to VIA, VILI is less preferred to VIA because of the Iodine it contains which ends up undesirably staining linens and clothes used in the procedure.

HPV Testing and Genotyping

HPV testing and genotyping tests are used to detect the presence or absence of an HPV infection and the HPV genotype. Several molecular and non-molecular technologies for HPV testing and genotyping basically detect three classes of detectable markers directly derived from HPV infec-

tion: molecular markers based on detection of nucleic acid sequences (DNA or mRNA), serological markers based on detection of antibodies against viral proteins, and cellular markers based on detection of proteins expressed intracellularly, upon either HPV infection or carcinogenesis.

Molecular techniques

Most molecular techniques detect the nucleic acid (DNA or RNA) of the virus [312] and therefore are the gold standards for identification of HPV. The HPV-genome can be easily extracted from exfoliated cells from the genitals e.g. cervix and its detection by the molecular techniques involves a process called hybridization. There are essentially 3 methods of nucleic acid hybridization namely (i) non-amplified or direct hybridization assays such as Southern Transfer Hybridization (STH), Dot Blot hybridization (DB) and In Situ Hybridization (ISH); (ii) signal amplified hybridization assays such as hybrid capture assays (HC) and (iii) target amplification assays such as polymerase chain reaction (PCR) and in situ PCR [313-316].

The direct hybridization assays such as STH requires large amount of highly purified DNA, is laborious, time consuming and not reproducible. The direct hybridization assay STH, because of its laborious and time consuming procedures, and ISH because of its moderate sensitivity for HPV were abandoned [315].

The signal amplification assays such as Hybrid Capture® 2 (HC2 Digene Corp Gaithersburg, MD, USA) assay and Cervista® HPV (Hologic, USA) assay, both are approved by the US FDA [313-316]. Of the signal amplification methods, the most frequently used in previous HPV epidemiological studies is the HC2. Hybrid Capture 2 (HC2) uses a non-radioactive substance for amplification of the signals from the hybridization of the target HPV-DNA to cocktail of labeled RNA probes to detect 13 HR- and 5 LR-HPV types [317-318]. Although HC2 is highly sensitive, comparable to target amplification methods and correlates well with cytological and histological results [317-318], it has some limitations, for instance, it has an amplification and detection limit of approximately 5,000 genome equivalents [319] thus is not suitable for specimens with low DNA content like in normal cytology and the two probe cocktail it uses are prone to cross-reactivity thus affecting its clinical specificity [320, 321].

Target DNA amplification assays such as PCR or real-time PCR (RT-PCR) amplifies a unique region of the DNA so they can be detected and is both highly sensitive and specific. PCR can be used with either genotype-specific or consensus primers for HPV detection [312, 322]. Consensus primers target a conserved L1 region in the different HPV genotypes as such can detect a broad spectrum of HPV types in clinical specimens in a single PCR reaction [316]. Genotype-specific primer mediated PCR assays for example Anyplex™ II HPV28 (Seegene, Seoul Korea) and CLART HPV 2 (Genomica, Madrid Spain) targets and detects 28 and 35 different HPV genotypes respectively based on small variation in their L1-region [313-316].

CLART HPV2 has demonstrated performance comparable to Linear Array and HC2 in terms of concordance level, clinical sensitivity and specificity. The agreement between CLART HPV 2 and Linear Array was 88.7% concordance level [323]. The clinical sensitivity of CLART HPV2 versus Linear Array by positive predictive value of CIN2+ in ASCUS were 67.3% vs 57.1% [313]. The agreement between CLART HPV2 and HC2 was also very good with concordance level in the range of 98.6 - 98.8% [324]. The clinical sensitivity of CLART HPV2 against CIN2+ was in the range of 96-96.9% which were comparable with HC2 with 71.4% sensitivity [324]. The specificity of CLART HPV2 against CIN2+ was in the range of 71.9-73.6% [324].

Generally, during PCR reaction, the viral genome fragment is amplified through repeated cycles of denaturation, primer hybridization and primer extension. PCR assays are highly sensitive and specific and can be performed with small amounts of DNA from 10 to 100 DNA molecules in a specimen and then can produce as many as one million copies from a single stranded DNA molecule after 30 minutes of amplification cycles and therefore ideal for use on specimens with low DNA content. Nonetheless, PCR assays cannot detect very low HPV viral loads of less than 10 DNA molecules as a result such specimens would erroneously be classified as HPV DNA negative.

Additionally, there are also molecular assays for detection of HPV E6/E7 messenger RNA (mRNA) for example APTIMA (Gene-Probe Inc., San Diego, CA, USA) which can be used for both primary screening and triage

of women with borderline cytological abnormalities. It detects HPV E6/E7 mRNA for 14 high-risk HPV types. Studies have evaluated the performance of APTIMA against HPV16 DNA testing, repeat cytology and HC2 and have concluded that APTIMA has demonstrated high sensitivity to predict CIN2+ and CIN3+ in group of women with ASCUS, although its specificity was insufficient (<50%) in some women subgroups [325-327].

Furthermore, there are also new molecular assays for detection of surrogate markers for transforming activity of HR HPV such as $p16^{INK4a}$ and Ki-67 expressions which can be used for improving the accuracy of immunohistochemistry grading of tumors and possibly a biomarker of HPV positivity [328-329]. This when combined with the aforementioned molecular amplification methods has been reported to improve specificity in screening [329].

HPV serology

HPV serological tests detect serological markers such as antibodies against viral proteins. However, there are varieties of different substrates for the assays for HPV serology. Some assays involve the use of HPV virus-like particles (VLPs) as substrates, but the VLPs were from different sources and had been produced by several different methods. Majority of the assays on the market are direct EIAs, involving coating EIA plates with VLPs whereas others used capture assays. A few assays use radioimmunoassay (RIA) and or bacterially expressed and affinity-purified L1 capsid proteins fused to glutathione S-transferase as antigens. There are also HPV 16 neutralization assays and basically tests for the ability of serum samples to neutralize HPV 16 pseudovirions carrying a reporter gene.

Immunoassays

These are qualitative tests that provide positive/negative results based on antibody-antigen titers compared to cut-off values. Antibody-antigen titers are obtained following serial dilutions. The cut-offs are in a range of formats. Some assays results are based end-point dilutions or values of antibody units calculated using parallel line methods in relation to an "in-house" reference sample.

HPV 16 assays: Serums are scored reactive or negative according to the cut-off. However, the cut-off values vary considerably between assays depending on the manufacturers. HPV 16 assays have excellent sensitivity and specificity when tested against negative and positive control panels.

HPV 16 neutralization assays: HPV 16 neutralization test endpoints are based on antigen-antibody titers levels following serial dilution. Results are reported as positive/reactive or negative/non-reactive.

HPV 18 assays: HPV 18 immunoassays are also based on antibody-antigen titers levels following serial dilutions. The results are reported as positive/reactive or negative/non-reactive. HPV-18 assays have high sensitivity for antibodies generated from natural infection.

HPV 11/6 assays: HPV 11 and 6 assays also report test results based on antibody-antigen titer levels following serial dilutions. Test results are reported as positive/reactive or negative/nonreactive. HPV 11 assays are nonspecific.

Sensitivity and specificity of HPV serological assays: Standardization studies conducted by WHO have found that the currently used HPV serological assays are generally specific when considering samples from naturally infected individuals in relation to a negative individual with a known sexual history, *i.e.*, the negative is scored negative [330]. Causes of false negative results were attributable to the starting dilution used that may have been close to the titer of the serum and not appropriate for the level of reactivity in the samples. Notably, some assays are not type-specific.

Sensitivity of HPV serological assays is often assessed by type-specific antibody concentration potency relative to a serum with natural infection with the type-specific HPV type, which is arbitrarily assigned a unitage of 1.0. Available WHO conducted studies have used HPV 16 and 18 for assessing the potency relative to HPV 16 and HPV 18 natural infection antibody concentration. And one of the studies [330] has shown that the currently used EIAs HPV serological assays potency relative to HPV16 and HPV 18 antibody concentration from natural infection were high, implying high sensitivity. Similarly, the results obtained with currently used HPV neutralization assays corresponded well to those obtained in EIAs showing that the currently used neutralization assays equally have

high sensitivity on HPV16 and HPV18 antibodies [330]. False positive or negative results are often due to quality of VLPs and the definition of cut-off.

In view of the variety of HPV detection methods available, the choice of the test depends on the intended use of the results whether for screening purposes or follows up of established lesions or epidemiological investigations. In most epidemiological studies, PCR is the widely used HPV DNA detection method. Despite the above applications of molecular HPV detection techniques, they are not yet available in Uganda health sector as a cervical cancer screening option.

Treatment of Cervical Precancerous Lesions

Cryotherapy

Cervical precancerous lesions can be treated by cryotherapy, which is an outpatient procedure that can be performed immediately after the VIA or VILI screening in a single visit approach (SVA). The readiness of the test results from VIA or VILI-based cervical screening make it possible to combine VIA or VILI screening with cryotherapy treatment for test positive cases in a SVA. This eliminates the need for the women to make multiple visits to the health facility first for screening and second for test results and treatment if found positive in addition to reducing the risk of loss to follow up of test-positive women required for treatment.

Cryotherapy is a cervical precancerous lesion treatment procedure that involves freezing the cervix using either compressed carbon dioxide or nitrous oxide gas as the coolant. When the cryotherapy equipment is switched on, the coolant forms an ice ball with temperature as low as negative 65 degree Celsius on the cryotherapy probe tip which when applied on the cervix to freeze continuously for 3 minutes, allowing the lesion to thaw for the following 5 minutes and then reapplied to freeze for another 3 minutes (3-5-3 minutes freeze formula) twice (double freeze) kills the cervical precancerous cells and leave a wound which will heal later. Cryotherapy is indicated for women who are VIA or VILI positive but not suspicious for cancer, the lesion occupies less than 75% of the cervix, the lesion does not extend onto the vaginal wall or into the cervical canal beyond the reach of the tip of the cryoprobe and the lesion extends

less than 2mm beyond the diameter of the cryotherapy probe including the tip of the probe.

About efficacy, safety, acceptability and affordability of cryotherapy, systematic reviews and meta-analysis put cryotherapy cure rates at a range of 56.8-96.6% among prospective controlled trials and 70-95.5% among observational studies [331]. Cryotherapy has very low complication rates and serious complications requiring medical intervention or affecting future reproductive outcomes are extremely rare. Side effects including vaginal discharge and cramping are temporary, generally self-limited, and well tolerated after anticipatory patient counseling [331-332]. In surveys, women find cryotherapy highly acceptable [331-332]. Compared to other treatment modalities, cryotherapy is very affordable and feasible to integrate into cervical cancer screening and treatment programs [331-332]

Cold knife conization, laser conization and LEEP

Cervical precancerous lesions can be excised by Cold knife conization, laser conization or loop electrosurgical excisional procedure (LEEP). The resultant raw wound on the cervix is then cauterized using a ball-type electrode usually part of the LEEP system or left to heal if Cold Knife conization or laser conization were used. The aforementioned 3 excision procedures can be performed as an outpatient procedure. And are indicated in a VIA or VILI-positive women with large-sized or wide lesions for which cryotherapy is contraindicated.

Systematic reviews and meta-analysis have indicated that the pooled rates of cure from low-grade, high-grade, or combined squamous intraepithelial lesions were similar across the aforementioned different treatment modalities (range 85.2-94.7%), with substantial overlap among the 95% confidence intervals [333]. In the order of increasing frequency, significant hemorrhage were reported most frequently in subjects who received cone biopsy 4.6%, followed by laser ablation 1.75% and LEEP 1.35%. There were no reported cases of progression to invasive cancer, but duration of follow-up (median follow-up time for all eligible studies was 12 months) was not sufficient to evaluate long-term outcomes [333]. Prospective randomized studies also came up with the same effectiveness data for cone, laser and LEEP [334].

Electrocautery

Cervical precancerous lesions can also be treated by electrocautery. Electrocautery is the burning and removal of cervical lesions by use of electrocautery unit which passively transfer heat from a hot probe to the tissue. The procedure is performed under local anesthesia (paracervical block) to prevent uterine cramping and pain associated with the treatment. A recent innovation the Semm cold coagulation employs a different mode of heat transfer and causes minimal pain. Studies have shown that cervical lesion cure rate from electrocautery is greater than 90% [335-337].

Diagnosis of Cervical Cancer

Colposcopy

Colposcopy is the use of a special kind of microscope called Colposcope to visualize the microscopic features of cervical lesions such as the absence or presence of enlarged and bleeding blood vessels and necrotic tissue often associated with tumors. The procedure also allows for collection of biopsy samples from the most affected part(s) of the lesions for histological analysis. Depending on the degree of the lesions, Colposcopy results may be reported as Normal, LG SIL, HG SIL and ICC. In view of this, Colposcopy is not a screening test per se but rather a confirmatory test often performed after a positive VIA or VILI or abnormal Pap screening test results to grade the tumor. However, some cervical precancer screening and treatment algorithms in some developing countries stipulates Colposcopic examination of all abnormal Pap smear or VIA-positive or VILI-positive cases before treatment so Colposcopy is part and parcel of cervical cancer secondary prevention programmes.

Systematic review has revealed that only few studies have compared the test criteria of colposcopy with those of cytology for screening in cervical cancer. In all the available studies, both the sensitivity and specificity of colposcopy were lower than the sensitivity of cytology [338]. This does not support inclusion of Colposcopy in primary cervical cancer screening programmes.

In Uganda, Colposcopy services are only available within gynecology department of a few selected higher level hospitals known as national or

regional referral hospitals situated in major towns and the Capital city. And it is a referral only service.

Biopsy and Histology

Biopsy and histology is the gold standard for CC diagnosis and staging. Biopsy and histology involves punching and pinching a small piece of tissue often from the most affected part(s) of the cervical lesion, alcohol fixing, and paraffin embedding and then staining before examination under a microscope. Histology allows for visualization of changes in a thicker layer of cells possibly from apical, basal through sub basal cells. The advantage of this is that it allows for a better examination of the extent or degree of cellular changes across the different layers of epithelium. Depending on the degree of cellular changes, histology results are reported as Normal, CINI, CINII, CINIII and ICC.

Biopsy or histology is not a screening test in fact the gold standard for all cervical screening and diagnosis methods. In Uganda, biopsy and histology services are only available within Pathology department of only one national referral hospital (Mulago National Referral Hospital) and a few private pathology laboratories in the Capital city Kampala. Just like Colposcopy, being a referral-only service, biopsy and histology consultations are often sought for women with HG SIL or ICC Colposcopy findings or VIA-positive suspicious for cancer or VILI-positive suspicious for cancer test results.

Treatment and Palliation of Invasive Cervical Cancer

Invasive Cervical Cancer (ICC) can be treated with surgery (hysterectomy), radiotherapy and chemotherapy singly or in combination depending on the stage and grade of the cancer. Again depending on the stage and grade of the cancer, some ICC cases are managed by palliative care. In Uganda, radiotherapy and chemotherapy services are only available in the national regional hospital. More so, palliative care services are provided free of charge to cancer patients by a private non-profit organization called Hospice Africa, Uganda from the health facilities (both public and private) where the patient is receiving other medical treatment or from the patient's home.

INTEGRATION OF HIV AND CERVICAL CANCER PREVENTION

The justification for integration of HIV/AIDS and HPV/CC prevention services is based on the similarities and opportunities therein in the two viruses' biology and lifecycle, risk factors, pathogenesis and available prevention modalities.

Shared risk factors

HIV and human papillomavirus (HPV) are both sexually transmitted infections (STIs) and so share the same risk factors, such as early age at sexual debut, multiple sexual partners, and condom use [99-103, 216-218]. Taken together, this should provide justification for the integration of risk reduction interventions such as information, education and communication (IEC) materials in a single-visit approach.

HPV and risk of acquisition of HIV

Accumulating evidence including systemic reviews indicate that pre-existing genital HPV infection increases the risk of acquisition of HIV infection. A recent systematic review indicated that HIV incident infection was significantly associated with HR-HPV in five of six studies and with LR-HPV in two out of five [339]. The association was significant for HR-HPV and borderline for LR-HPV. An earlier systematic review and meta-analysis had indicated that the risk of HIV acquisition in women doubled with prevalent HPV infection with any genotype, although adjustment for confounders was often inadequate [340]. The effect was similar for high-risk and low-risk. Even studies among men showed an association between HPV infection and HIV acquisition. Proportion-attributable fraction (PAF) for HIV attributable to infection with any HPV genotype ranged between 21 and 37% [340]. This should provide justification for integrating HIV prevention services within existing cervical cancer prevention programs including screening and vaccination but this was not completely the case in Uganda.

HIV and risk of acquisition and progression of HPV

Systematic reviews and meta-analysis have consistently indicated the increased risk of acquisition of HPV among HIV-positive women than their

HIV-negative peers [234-235]. Similarly, an increasing body of literature also indicates that HIV-positive women have an increased risk of developing cervical precancerous neoplasia and CC in comparison with their HIV-negative counterparts [341-346]. A lot of explanations have been suggested including reactivation of silent HPV infections by HIV infection [236], the destruction of helper T cells (CD4 cells) and the resultant immunosuppressive state (ISS) from HIV infection providing immune unchecked environment for HPV infections and progression [236] and the HIV-associated tight junction disruption of mucosal epithelia potentiating HPV infection and penetration into the basal and parabasal epithelium [347]. This should have provided justification for integration of cervical cancer prevention services into HIV/AIDS programs but this is not completely the case in Uganda and many other developing countries.

WHO recommendations on integration of HIV and CC screening

In response to the accumulated evidence regarding the association between HIV/AIDS, HPV and cervical cancer, the World Health Organization (WHO) recommends a more aggressive CC screening and treatment schedule for HIV-positive women than for HIV-negative women (annually vs. 3 yearly respectively) [348]. Such scheduling of CC screening according to HIV status of the women implies that women should be screened for HIV within CC programs. Taken together, this should provide additional justification for the integration of interventions for HIV and CC prevention to include information, education, communication, screening, and treatment in a single-visit approach.

Integration approaches and models

Recent studies concluded that there is a large potential health gain if HIV and CC prevention services are integrated [349-350]. Policy makers (PM)s from disciplines outside the cancer domain—for example HIV care — were warranted to create CC prevention services for women attending those programs [350]. Other studies have demonstrated various models of integrating HIV and CC screening services. For example, in Zambia where CC prevention clinics were co-located within the public health clinics offering HIV/AIDS care and treatment, manifold advantages were observed including resource and infrastructure sharing, availability of a wider range of women's health services for HIV-infected/at risk women, and opportu-

nities for referral between the clinic systems and maximization of participation in both programs [351]. Similarly, in Kenya were CC screening services were integrated into mother, child health (MCH) and family planning (FP) clinics, it was reported that a high proportion of women who visited the MCH-FP clinics for well-baby or family planning services also benefited from CC and sexually transmitted infections (STIs) screening and treatment services in a single visit approach [352]. In Nigeria, where women seeking reproductive health (RH) including CC screening or HIV care services were bi-directionally referred to either HIV care or RH clinics co-situated within the same health facility, it was demonstrated that CC screening by visual inspection with 5% acetic acid (VIA) was highly acceptable and facilitated early detection and treatment of very many cases of cervical pre-cancerous lesions among women [353-354]. The aforementioned mentioned successful HIV and CC prevention integration models would provide justification and frameworks for wide scale integration in many other developing countries but it did not.

Health risks from unintegrated HIV and CC prevention services

As unintegrated clinics, almost all the HIV care programs in Uganda do not offer CC screening services and hence the female HIV patients miss CC screening opportunities despite the frequent visits they make to the HIV facilities for reviews and drug refills. This increases their risk of presenting late with advanced CC disease with poor prognosis. This kind of problem was confirmed in a study conducted in Nigeria among HIV-positive women attending post-HIV test counseling which indicated that none of the respondents were informed about CC and its screening during the posttest counseling sessions [355]. Similarly, almost all CC screening programs in Uganda do not offer HIV screening services to women and hence the women risk receiving inappropriate schedules for CC re-screening. Less aggressive CC re-screening interval for HIV positive women similarly increases the risk of them presenting late with advanced CC disease with poor prognosis. Consistent with this, a study conducted in Uganda among mainly cervical healthcare providers (HCPs) and policy-makers affirmed that much pessimism exists among them regarding the feasibility of HIV and CC screening integration [356].

AIMS

The overall aim of the thesis was to evaluate selected HIV, HPV and cervical cancer prevention services in Uganda. The specific aims were:

1. To determine the occupational exposures to human immunodeficiency virus (HIV) prevalence, predisposing factors, high-risk groups and post-exposure prophylaxis (PEP) among healthcare workers in a Ugandan hospital.

2. To explore perceptions of healthcare providers (HCP) and policy makers (PM) regarding the integration of HIV and cervical cancer screening services

3. To explore perceptions and preferences of communities in Uganda regarding the integration of HIV and CC screening services

4. To establish the prevalence, genotypes and risk factors for vaccine and non-vaccine types of HPV infections among Bivalent ASO4-adjuvanted HPV16/18 vaccinated and non-vaccinated young women in Uganda 5-years after vaccine implementation

Table 1: Summary of the sub-studies

Substudies	Population and sample	Study design	Data collection and analysis methods	Progress Status
I – Occupational exposure to HIV – a conflict situation to healthcare workers in Uganda.	Healthcare workers	Quantitative cross sectional survey	Self-administered questionnaire and statistical analysis.	Published Int. Nurs. Review 2011
II – Integration of HIV and cervical cancer screening percetpions of healthcare providers and policy makers in Uganda	Healthcare providers and policy makers	Qualitative content analysis	Individual Interviews (IDIs) and content analysis.	Published BMC Public Health 2014
III – Integrating HIV and cervical cancer screening perceptions and preferences of communities in Uganda	Women, men and village health teams	Qualitative content analysis	Focus group interviews (FGDs) and Individual Interviews (IDIs)	Published BMC Women's Health 2015
IV – Prevalence, genotypes and risk factors for vaccine and non-vaccine types of Human Papillomavirus (HPV) infections among young women 5-years after AS04-adjuvanted HPV-16/18 vaccinations in Uganda and the role of HIV and syphilis	HPV-vaccinated and non-vaccinated young women	Quantitative, comparative cross sectional study	1-Cervical samples for HPV typing and genotyping using CLART HPV2 PCR 2-Blood sample for CD4 count estimation by FACScount, HIV testing by Alere Determine and Stat-Pak HIV-1/2 RDTs and Syphilis testing using Human TPHA liquid GmBH, Wiesbaden, German.	Submitted

STUDY AREA

Study Sites, study population and sampling

The 5 sub-studies were conducted in 4 Ugandan districts namely Kampala, Nakasongola, Mbarara and Ibanda.

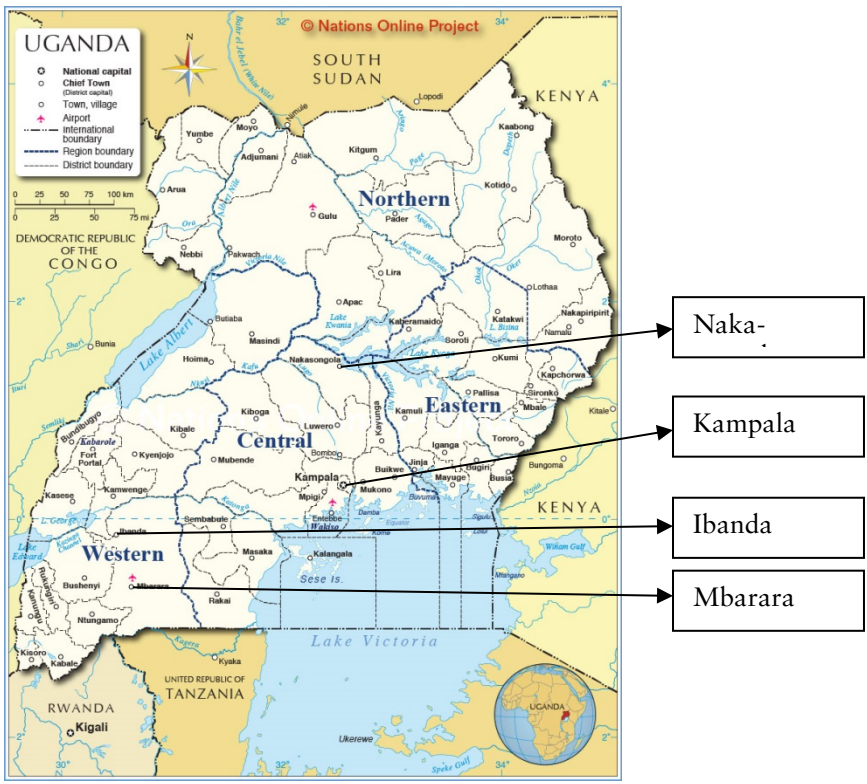

Figure 10: Map of Uganda showing the 4 Study Districts.

Paper I: Sub-study for paper I was conducted in Mbarara district, situated in southwestern Uganda. The study population comprised of healthcare workers including medical doctors, nurses, laboratory technicians and clinical students. The participants for the study were recruited from Mbarara Regional Referral Hospital and Mbarara University of Science and Technology. Mbarara Regional Referral Hospital was a University Teaching Hospital for medical, nursing and medical laboratory students and a

patient referral center for over 6 districts in the region. Stratified random sampling method was used to select participants from the total list of employees and students maintained by the Mbarara Regional Referral Hospital and Mbarara University of Science and Technology. The sample size was 224 participants. The sample size was calculated using Kish and Leslie formula (1967).

Paper II: Sub-study for paper II was conducted in 4 Ugandan districts namely Mbarara and Ibanda situated in the southwestern Uganda, Kampala the capital city of Uganda and Nakasongola situated in the central region of Uganda. The study population comprised of HCPs and policy makers. The healthcare providers were recruited into the study from cervical cancer clinics within the district where they were working. Similarly, the policy makers were recruited from the district health offices were they were working. The sampling method used was purposive sampling from the list of HCPs and district level policy makers working in the cervical cancer clinic and district health office respectively of each district. The sample size was 16 participants comprising of 12 HCPs and 4 policy makers who participated in the study. The sample size was determined by data saturation.

Paper III: Sub-study for paper III was conducted in 3 Ugandan districts namely Kampala the capital city situated in central Uganda, Mbarara and Ibanda districts situated in southwestern Uganda. The study population comprised of women, men and village health teams (VHTs). The study respondents were recruited from cervical cancer clinics situated within the districts, where they were registered as people who have ever visited the clinics for services or as a male partner/companion or as a village health team member working in the locality. The total of 88 respondents were purposively sampled and participated in the study. The sample size was determined by data saturation.

Paper IV: Sub-study for paper IV was conducted in only one district, Ibanda district which is situated in southwestern Uganda. This was the first Ugandan district to initiate Bivalent ASO4-adjuvanted HPV16/18 vaccination of young girls in 2008. The study population comprised of HPV-vaccinated and non-vaccinated young women. The study participants were recruited from secondary schools within the district where they were enrolled for secondary education. Multi-stage sampling procedure

was performed to select the study participants from the schools. The eligible girls were given appointment to visit a designated clinic at a health facility within the locality for data and sample collection. Power analysis method was used to calculate the sample size of 376 at a power of 85% for comparison of two vaccine HPV-16/18 prevalence of 1% vs 10.7% among the HPV-16/18-vaccinated and non-vaccinated groups respectively at 5% significance level. This was adjusted to 492 participants (i.e. 241vaccinated and another 241 non-vaccinated girls) to cater for potential loss to follow up. Figure 11 shows the number of participants approached and provided completed questionnaire, blood and cervical samples for paper IV.

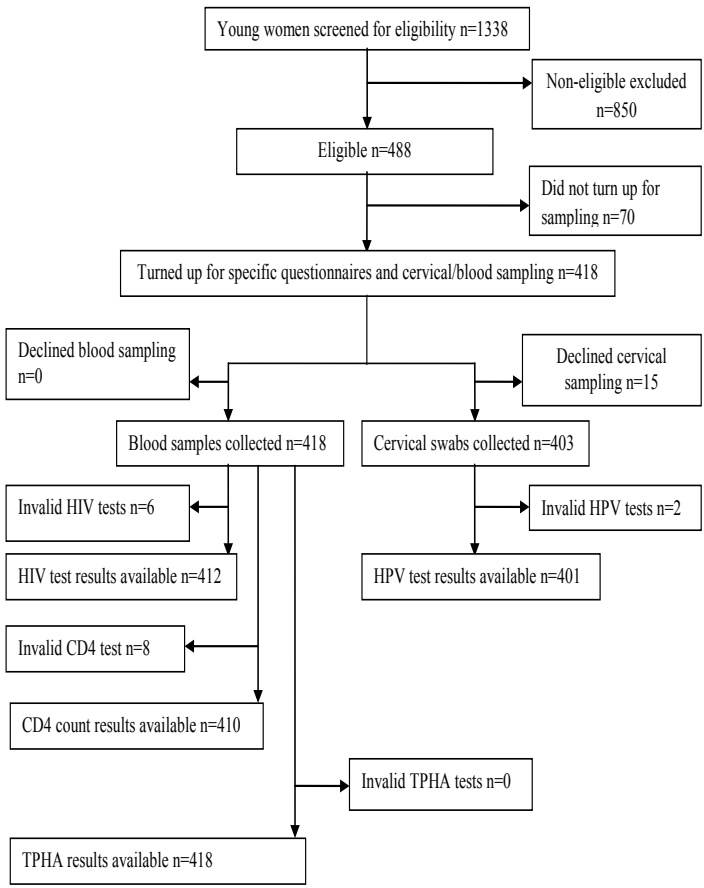

Figure 11: Showing participants flow and procedures for sub study IV.

MATERIALS AND METHODS

Research Instruments and Data Collection Methods

Paper I: The tool for data collection used for paper I was a quantitative questionnaire. The questionnaire was served as self-administered tool. The questionnaire was completed by a total of 224 participants (including 98 HCWs and 126 students) in Mbarara Regional Referral Hospital, Uganda. Data on the quantitative questionnaire were entered into the Statistical Package for Social Sciences version 12.0 (SPSS Inc, Chicago, IL, USA) for analysis. Descriptive statistics was used to determine frequencies and percentages of occupational exposure to HIV through the various means. The predisposing factors were identified from the participants' self-reports of the reasons why and the circumstances under which the injuries or contamination with patient's blood or body fluid occurred. Responses were compared using chi-square statistics for categorical data. The 95% confidence interval, 0.05 significance level and two-tailed tests were used for all the statistical tests.

Paper II: The tool for data collection used for paper II was an interview guide. Data were generated from the selected healthcare providers and policy makers by individual interviews. Interviews were conducted in English language and audio recorded for each respondent. The audiorecorded data were later transcribed verbatim for analysis. Content analysis was used to analyze data for themes and categories.

Paper III: The tools for data collection used for paper III were focus group discussions (FGDs) and individual interview (IDI) guides. Data were generated from the selected women from the community by focus group discussions. Similarly, FGDs were used to generate data from the selected village health teams (VHTs). As for the selected men, individual interviews (IDIs) were conducted. During the focus group discussions simultaneous translation took place from English to local language and back to English. All FGDs and IDIs were audio recorded. The data on the audio recording device were transcribed verbatim before analysis. Content analysis method was used to analyze the data for themes and categories.

Paper IV: The materials and tools used for generating data for paper IV included questionnaire, cervical swabs, blood, weight and height scales

among the key ones. Cervical swabs were used for HPV test and genotyping. Cervical swabs were collected using CareBrush® (QIAGEN, Gaithersburg, MD, USA) after inserting the right-sized speculum into the vagina and bringing the cervix to focus. Cervical swabs were stored in liquid nitrogen and shipped in cold chain to Örebro University Hospital Sweden for analysis. In Sweden, cervical swabs were analyzed with a HPV genotyping test, CLART® HPV2 (Genomica, Spain) which is based on consensus PCR followed by microarray for determination of genotype. CLART HPV2 (Genomica, Madrid, Spain) is a low-density microarray assay based on PCR amplification of genotype specific HPV L1 fragments from 35 individual HPV genotypes (6, 11, 16, 18, 26, 31, 33, 35, 39, 40, 42, 43, 44, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 68, 70, 71, 72, 73, 81, 82, 83, 84, 85 and 89), with analytical sensitivity calibrated against known copies of cloned plasmids. Notably, CLART HPV2 is not designed to detect infection with some HPV genotypes including HPV 7, 13, 30, 32, 34, 67, 69, 74, 86, 87 and 91.

250µl of the diluted careHPV sample was spun down (15 min, 14,000 revolutions per minute), with the supernatant removed and cell pellet re-suspended in a mix of 180 µl (ATL buffer Qiagen, http://www.qiagen.com/se/products/catalog/lab-essentials-and-accessories/buffer-atl-gpr/#orderinginformation) phosphate buffered saline (10x conc. pH 7.4, pharmacy product) and 20 µl Proteinase K (QIAGEN, http://www.qiagen.com/se/products/catalog/lab-essentials-and-accessories/qiagen-proteinase-k/#productdetails) (recombinant, PCR Grade, Roche Diagnostics). Samples were then vortexed and incubated for two hours at 56°C.

HPV DNA was purified using QIACube instrument (http://www.qiagen.com/se/products/catalog/automated-solutions/sample-prep/qiacube/) with the CIAcube mini kit (http://www.qiagen.com/se/products/catalog/sample-technologies/dna-sample-technologies/genomic-dna/qiaamp-dna-mini-kit/), Qiagen.

Five µlof purified DNA were used for the PCR amplification. During amplification the PCR products were labeled with biotin. Prior to visualization, the PCR products were denatured at 95°C for 10 minutes. Visualization was performed on the CLART microarray, using 5 µl of the denatured PCR products. Hybridization between the amplicons and their specific

probes on CLART results in formation of an insoluble precipitate of peroxidase when adding a Streptavidin conjugate that bind to the biotin-labeled PCR products. Precipitate is analyzed on the Clinical Array Reader (Genomica, Madrid, Spain). All samples returning an invalid outcome were retested, and the second result was considered definitive.

Another material used was venipuncture blood samples collected from each participant. Blood samples were used for CD4 count estimation, HIV and syphilis tests. CD4 count was estimated using BD FacsCount® (Immunocytometry Systems; Becton Dickinson, Franklin Lakes, NJ, USA) according to the manufacturer's protocol.

The HIV-1 testing was performed using first the Determine rapid test (Abbott Diagnostics, US). If the sample was not reactive, it was considered HIV-negative. Otherwise, the Statpak rapid test (ChemoBio Diagnostics Systems, US) was used to confirm HIV positivity. In case of disagreement between the 2 tests, the Unigold rapid test (Trinity Biotech PLC US) was used as a tie-breaker test.

The Syphilis tests were performed using a commercially available kit Human TPHA liquid GmBH (Wiesbaden, Germany). The tests were performed according to the manufacturer's protocol. The TPHA test is an indirect hemagglutination test for the determination of antibodies specific to *Treponema pallidum*.

Weights and height measurements were also taken from each participant to be used to compute body mass index (BMI), a surrogate marker of nutritional status. Each participant also provided data on risk factors for HPV infections such as demographic characteristics and high risk sexual behaviors on a specific questionnaire served as an interview tool.

Before analysis, the collected data were coded, entered and analyzed with Statistical Package for Social Sciences (SPSS) version 22.0 for windows software (SPSS Inc, Chicago, IL, USA). The primary outcome variable was the HPV test result which was a categorical data. The HPV test result was further categorized into vaccine type, non-vaccine types, HR HPV strains, MR HPV strains, LR HPV strains and others, all of which were categorical outcome variables. All the continuous numerical variables such as age, age at sexual debut, number of sexual partners, CD4 count, body mass

index (BMI) were left uncoded. The study groups i.e. the vaccinated and non-vaccinated groups were compared on categorical variables using Chisquare statistics and association between them and HPV infection were assessed univariate and multivariate analyses. On continuous numerical variables, the study groups were compared using t-test for independent samples. Hierarchical logistic regression was performed after controlling for participant's age, age at sexual debut and educational level to identify useful predictors for infections with the various categories of HPV infections. All significance testing were performed at 5% significance level, two tailed and 95% confidence intervals were computed whenever necessary.

Ethical considerations

The ethical review and approval for **sub study I** (**paper I**) was obtained from Mbarara University of Science and Technology in 2011. For **sub studies II-III** (**papers II-III**) and that for **sub study IV** (**paper IV**), the ethical approvals were obtained from Makerere University School of Biomedical Sciences Institutional Review Board in 2012 and 2013 respectively. The ethical approval reference numbers for **sub studies II-III** and that for **sub study IV** were SBS-030 and SBS HDREEC -131 respectively.

RESULTS

Occupational exposure to HIV among Healthcare providers

Of the 224 HCWs surveyed in **sub study I (paper I),** 20 (8.93%) were exposed to HIV through percutaneous injuries while 23 (10.27%) were exposed through muco-cutaneous contamination at least once during the previous 12 months.

In the category of percutaneous injury, injection needle stick injuries was the most frequently reported mode of HIV exposure with a prevalence of 19.2% in the previous year, of which 4.46% occurred with confirmed HIV-infected blood. Other reported percutaneous injuries were suture needle stick injuries (3.13%), cannula needle stick injury (0.89%) and scalpel cut injuries (0.45%). Exposure through scalpel injuries was significantly higher in nurses–midwives compared to other health professionals (66.7% vs. 33.3%, X^2 28.326, df 6, *P* 0.001). For other routes of exposures, no significant differences were observed between nurses–midwives and other health professionals. Similarly, a significantly higher rate of scalpel injuries was reported among students compared with qualified HCWs (66.7% vs. 33.3%, X^2 33.164, df16, p 0.007). For needle stick injuries no significant differences were however observed between students and qualified HCWs.

Occupational exposure to HIV through muco-cutaneous contamination was also common (10.27%). Exposure through contamination was significantly higher among the 21–35 years age group compared with older age groups (69.2% vs. 31.8%, X^2 9.116, df 2, p 0.010). For other routes of exposure, no significant differences were observed between the age group of 21–35 years and older groups.

Low clinical experience was found to be an important risk factor for occupational exposure to HIV. For example, significantly higher exposures through contamination were reported among participants with clinical experience of less than 15 years compared with those with experience of more than 15 years (90% vs. 10%, X^2 13.92, df 2, p 0.001). For exposures through stick and cut injuries, no significant differences were observed between highly experienced and less experienced participants.

Surgical units such as emergency ward, surgical theatre and surgical ward were found to carry an increased risk for exposure compared with other units. For example, exposure through muco-cutaneous contamination was found to be significantly higher among participants working in surgical units compared with other units (21.5% vs. 78.5%, X^2 28.498, df 8, $p < 0.001$). For exposures through injection, suture and cannula needle stick injuries, no significant differences were observed between participants working in surgical units and other units.

Participants who perceived a low risk of HIV sero-conversion from occupational exposures were found to be another high-risk group. For example, exposures through needle stick injuries were found to be significantly higher among participants who perceived a low risk of contracting HIV from needle stick injuries compared with those who perceived a higher risk of contracting HIV from needle stick injuries (70.4% vs. 29.6%, X^2 12.612, df 3, p 0.006).

As for the role of universal precautions, no significant differences were observed between participants who reported to always and rarely abide by the universal precautions regarding occupational exposure to HIV through the various routes. Similarly, we found no significant difference between participants with low and high personal concern for safety with respect to occupational exposure to HIV through the various routes.

Also, there was no significant difference between participants who felt that safety devices were inadequate in their workplace and those who felt that safety devices were adequate with respect to occupational exposures to HIV through the various means.

The setting and circumstances under which occupational exposure to HIV occurred included carelessness on the side of the HCP, poor clinical skills, poor analgesia or anesthesia of patients during painful procedures, inadequate restraints of patients during painful procedures, recapping of used needles, improper disposal of sharps and poor visibility during invasive procedures.

Post Exposure Prophylaxis (PEP) among Healthcare providers

All the participants (100%) in **sub study I** (**paper I**) knew of the benefits of using PEP for prevention of HIV sero-conversion after occupational exposure. However, 49 of the 104 participants who provided data on use of PEP (47.1%) did not report exposure because they believed that the risk of HIV infection from occupational exposure is low. Another 28 (26.9%) of the participants did not report exposure because they believed that the first aid treatment (washing the exposed area with plenty of running water and soap) was adequate to prevent HIV sero-conversion. Another eight (7.7%) of the participants held the assumption that the patient was HIV negative so they did not bother to report the exposure. Similarly, seven (6.9%) of the participants did not report exposure because they felt that the injury and exposure were very minor. Lastly, three (2.9%) participants did not report exposure because of fear of the long and tiresome process of reporting exposure.

Sub study I (**paper I**) data regarding barriers to initiating PEP were provided by 14 participants. Three (21.4%) of the participants did not initiate PEP because the physician responsible could not be reached to prescribe anti-retroviral drugs within 24 hours of exposure. Two (14.3%) of the participants did not initiate PEP after exposure because of fear of the side effects of anti-retroviral drugs. None of the HCP reported of having received HIV screening/testing after occupational exposure to confirm sero-conversion.

HIV infections among 15-24 year old women

Of the 412 young women tested for HIV in **sub study IV** (**paper IV**), the overall HIV prevalence was 1.7% (95% CI 0.5-2.9). In univariate analysis, young women with HPV infection and those with signs of immunosuppressive syndrome (that was CD4 count of <500 cells/μL) were significantly 12 times and 178 times more likely to be HIV positive compared to their counterparts. In multivariate analysis however, it was only CD4 count (p 0.004, OR 0.987, 95% CI 0.978-0.996) which was inversely associated with HIV positivity, the rest of the factors considered in the regression model were not.

Sexual behaviors and HIV infection among young women

Of the 381 young women in **sub-study IV (paper IV),** 69.8% reported to have initiated sexual activity from the age of 16 years, 40.9% ever used condom during sexual intercourse and 83.2% had only one sexual partner in a lifetime. However, none of the above sexual behaviors were significantly associated to HIV positivity in both univariate and multivariate analyses.

HIV screening/testing and HIV infection among young women

Of the 488 young women aged 15-24 years in **sub study IV (paper IV),** less than half (35.7%) reported of having ever tested for HIV before the time of **sub study IV.** The median age of those had ever tested for HIV was 18.0 years (IQR 2.0). Similarly, only 29.0% of them reported knowledge of the HIV status of at least one of their male sexual partner. Four of the 7 HIV-positive cases from the HIV test conducted in **sub study IV** had CD4 count of <500 cells/μL and so where eligible for evaluation for ART initiation but only 1 reported being on ART at the time of **sub study IV.** And there was not any statistically significant association between previous HIV testing behavior and current HIV status.

HPV infection Prevalence and Risk Factors

The result of **sub study IV (paper IV)** showed that out of the 401 cervical samples tested, there were 135 HPV DNA positive cases giving a general cervical HPV infection prevalence of 33.7% (95% CI 29.1-38.3). Figure 12 shows that HPV infections tended to be more prevalent among the older 20-24 year old women than the younger 15-19 year old group with exception of non-vaccine A7-HPV and LR-HPV infections although none of the differences were statistically significant.

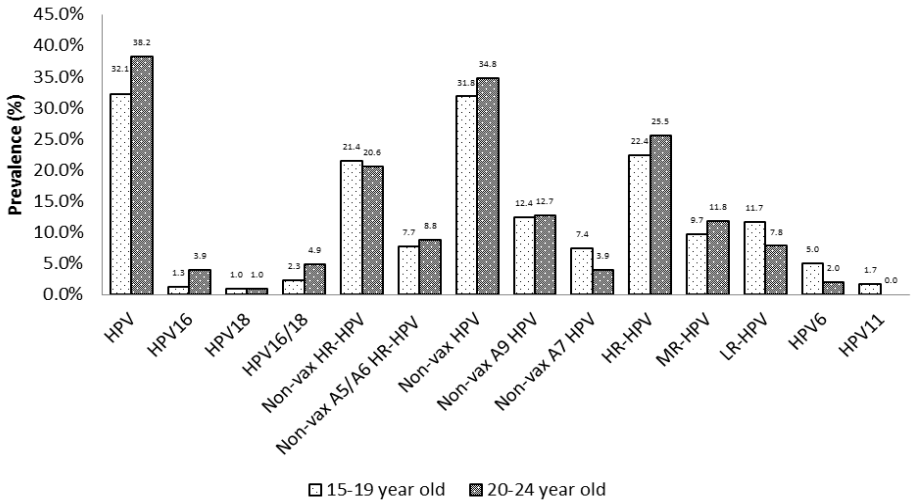

Figure 12 showing age distribution of HPV infections among the 15-24 year old women. Note: Non-vax means non-vaccine HPV-16/18; HR HPV types include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68/73; MR-HPV types include 26, 53, 70, 71, 81, 82, 83, 84 & 89; LR-HPV types include 6, 11, 40, 42, 43, 44, 54, 61 & 62; Non-vaccine A9-HPV types are phylogenetically HPV-16 close relatives and they include 31, 33, 35, 52, 58 and 67; Non-vaccine A7-HPV types are phylogenetically HPV-18 close relatives and they include 39, 45, 59, 68 and 70. Non-vaccine A5/A6-HR-HPV types phylogenetically are neither HPV-16 nor HPV-18 close relatives and they include 51, 56 and 66.

In univariate analysis of **sub study IV** data revealed that any cervical HPV infections were significantly more prevalent among participants from urban address than rural address (39.5% vs 22.2%, X^2 11.17, df1, p 0.001), more prevalent among young women with other STIs (HIV/syphilis) than those who tested negative for other STIs (75.0% vs 32.5%, p 0.004) and also more prevalent among young women with signs of immunosuppressive syndrome (in this case CD4 count of <500 cells/μL) than their counterparts with normal CD4 count (71.4% vs 33.2%, p 0.047).

Out of the 401 cervical samples tested in **sub study IV**, there were 12 vaccine-type HPV positive cases giving a vaccine-type HPV prevalence of 3% (95% CI of 1.3-4.7). In univariate analysis, the vaccine-type HPV16/18 infections were significantly more prevalent among young women with

other STIs (HIV/syphilis) than their counterparts without other STIs (16.7% vs 2.6%, p 0.046), more prevalent among young women with signs of immunosuppressive syndrome in this case CD4 count of <500 cells/μL than those without signs of immunosuppressive syndrome (28.6% vs 2.6%, p 0.016) and also more prevalent among young women attending advanced secondary education than those attending ordinary secondary education (5.9% vs 1.7%, p 0.036). In multivariate model excluding HPV vaccination from the covariates, it was only other STIs (in this case HIV and syphilis) that were useful predictors for vaccine HPV16/18 positivity after controlling for educational level, age and age at sexual debut.

Out of the 401 cervical samples tested in **sub study IV**, there were 130 non-vaccine-type HPV positive cases giving a non-vaccine-type HPV prevalence of 32.4% (95% CI 27.8-37.0). In univariate analysis, non-vaccine-type HPV infections were significantly more prevalent among young women from urban address than rural address (38.7% vs 20.0%, X2, df1, p 0.001) and also more prevalent among young women with other STIs (HIV/syphilis) than their counterparts without other STIs (75.0% vs 31.2%, X^2, df1, p 0.003). In multivariate analysis, after controlling for the effects of age, age at sexual debut and educational level, having urban address, BMI, number of lifetime sexual partners and other STIs were useful predictors of non-vaccine HPV infections.

Out of the 401 cervical samples tested in **sub study IV**, there were 93 HR HPV positive cases giving HR HPV infection prevalence of 23.2% (95% CI 18.6-27.8). In univariate analysis, HR HPV infections were significantly more prevalent among young women with other STIs (HIV/syphilis) than those without (50.0% vs 22.4%, p 0.037) and also more prevalent among young women with the sign of immunosuppressive syndrome (in this case CD4 count of <500 cells/μL) than their counterparts with normal CD4 count (57.1% vs 22.8%, p 0.055). In multivariate analysis, after controlling for the effects of age, age at sexual debut and educational level, the useful predictors for HR HPV infection were 12-months or lifetime number of sexual partners and other STIs.

Out of the 401 cervical samples tested in **sub study IV**, there were 40 MR HPV positive cases giving MR HPV infection prevalence of 10.2% (95% CI 7.2-13.2). In univariate analysis, MR HPV infections were more prevalent among young women with other STIs (HIV/syphilis) than those with-

out other STIs (33.3% vs 9.5%, p 0.026) and also more prevalent among young women who reported having multiple sexual partners in their lifetime than those who reported having only one sexual partner in their lifetime (20.3% vs 8.3%, X^2 7.123, df1, p 0.008). Similarly, in multivariate analysis, after controlling for the effects of age, age at sexual debut and educational level, the useful predictors for MR HPV infection were 12-months or lifetime number of sexual partners and other STIs.

Out of the 401 cervical samples tested in **sub study IV**, there were 43 LR HPV positive cases giving LR HPV infection prevalence of 10.7% (95% CI 7.7-13.7). In univariate analysis, LR HPV infections were more pronounced among young women from urban address than rural address (13.5% vs 5.2%, X^2 5.677, df1, p 0.017) and also more pronounced among young women who initiated sexual activity at an early age (<16 years) than those who initiated sexual activity at a later age from 16 years (15.2% vs 9.2%, X^2 2.250, df1, p 0.134) although the difference was not statistically significant. In multivariate analysis, after controlling for the effects of age, age at sexual debut and educational level, the useful predictors for LR HPV infections were having an urban address, BMI and lifetime number of sexual partners.

Categorizing the infections by phylogenetic relatedness, there were 50 other HPV16-related genotype infections (clade A9), 26 other HPV18-related genotype infections (clade A7) and 32 vaccine unrelated other HR HPV genotype infections (other clades rather than A7 and A9) which translated to the prevalence of 12.5% (95% CI 9.3-15.7) for other A9 or HPV16-related genotype infections, 6.5% (95% CI 4.1-8.9) for other A7 or HPV18-related genotype infections and 8.0% (95% CI 5.3-10.7) for other A5/A6 clades HR HPV infections.

Sexual behaviors and HPV infection prevention among young women

Of the 381 **sub study IV (paper IV)** participants with data on age at sexual initiation, 69.8% of them reported of having initiated sexual activity at a later age from 16 years, 40.9% reported of having ever used a condom during sexual intercourse and 83.2% reported of having only one lifetime sexual partner.
However, no significant associations were found between delayed initiation of sexual activity from age of 16 years and any of the categories of

HPV infections. Similarly, no significant associations were found between condom use and any HPV infection, even any multiple HPV infections. Instead, more of the HPV infections tended to occur among condom users than nonusers.

As for the association between number of sexual partners and HPV infection, having fewer lifetime number of sexual partners was associated with significantly lower MR HPV infections (8.3% vs 20.3%, X^2 8.379. df1, p 0.008) and also lower other A7 or HPV18-related genotypes infections (5.1% vs 12.5%, X^2 4.938, df1, p 0.043). For the rest of the categories of HPV infections, there were no statistically significant associations with lifetime number of sexual partners.

HPV-16/18 vaccination coverage among young women

Of the 488 sexually active young women aged 15-24 years in **sub study IV** (**paper IV**), 252 were vaccinated with the Bivalent ASO4-adjuvanted HPV-16/18 vaccine in 2008 alone, giving HPV vaccination coverage of 51.6%. Compared to the non-vaccinated group, the vaccinated group was significantly younger (15.56 vs 16.22, t -2.207, df 379, p 0.028), mostly attending ordinary secondary education (99.6% vs 43.1%, X^2 193.494, df1, p <0.001) and initiated sexual activity from a younger age (18.08 vs 19.08, t -8.560, df 482, p <0.001). Otherwise, there were no significant differences between the HPV-vaccinated and non-vaccinated groups regarding their demographic characteristics.

HPV-16/18 vaccination and any HPV infection

Results from **sub study IV** showed that of the 401 cervical samples tested, there were 135 HPV positive cases giving any cervical HPV infection prevalence of 33.7% (95% CI 29.1-38.3). There was no statistically significant difference between the Bivalent HPV-16/18 vaccinated and non-vaccinated young women regarding the general prevalence of HPV infection [29.8% vs 37.8%, x^2 2.524, df1, p 0.112].

HPV-16/18 vaccination, vaccine and non-vaccine types of HPV infections

Figure 13 generated from results of **sub study IV** shows that HPV infections were generally less prevalent among the HPV-16/18-vaccinated

group than the non-vaccinated group except for non-vaccine A7-HPV and LR-HPV types.

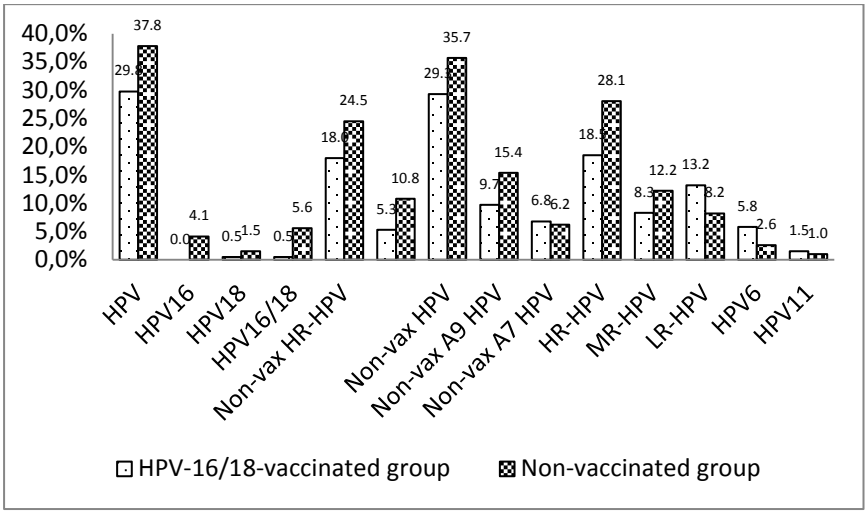

Figure 13: showing HPV-vaccination distribution of HPV infections among the 15-24 year old women. Note: Non-vax means non-vaccine HPV-16/18; HR-HPV types include HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68/73; MR-HPV types include 26, 53, 70, 71, 81, 82, 83, 84 & 89; LR-HPV types include 6, 11, 40, 42, 43, 44, 54, 61 & 62; Non-vaccine A9-HPV types are phylogenetically HPV-16 close relatives and they include 31, 33, 35, 52, 58 and 67; Non-vaccine A7-HPV types are phylogenetically HPV-18 close relatives and they include 39, 45, 59, 68 and 70. Non-vaccine A5/A6-HR-HPV types phylogenetically are neither HPV-16 nor HPV-18 close relatives and they include 51, 56 and 66.

Sub study IV results also showed that vaccine HPV16/18 prevalence were significantly lower among the HPV-16/18 vaccinated than the non-vaccinated group (0.5% vs 5.6%, p 0.006, OR 95% CI 0.08(0.01-0.64). At type-specific level, it was only HPV16 prevalence which was significantly lower among the HPV-vaccinated group compared to the non-vaccinated group (0.0% vs 4.1%, X^2, df1, p 0.003). In multivariate analysis, HPV-16/18 vaccination was significantly associated with decreased risk of vaccine-type HPV16/18 infections. However, other STIs (in this case HIV and syphilis) were significantly associated with an increased risk of vaccine HPV16/18 infections.

On the other hand, the prevalence of non-vaccine types of HPV infections was not significantly lower among the HPV-16/18 vaccinated group compared to the non-vaccinated group (29.3% vs 35.7%, X^2 1.697, df1, p 0.203). Similarly, in multivariate analysis too, HPV16/18 vaccination was not a significant predictor of non-vaccine types of HPV infection.

HPV-16/18 vaccination and other categories of HPV infections

Sub study IV results showed that high-risk-HPV infections were significantly less prevalent among vaccinated group compared to non-vaccinated group [18.5% vs 28.1%, p 0.032, OR 95% CI 0.6(0.4-0.9)]. For moderate-risk-HPV and low-risk-HPV genotypes, there were no significant differences between vaccinated and non-vaccinated groups. In multivariate analysis, HPV-16/18 vaccination was not significantly associated with other categories of HPV infections with exception of infection with other HPV16-related HR genotypes (clade A9 relatives) for which it was associated with a decreased risk of infection. Instead, it was other STIs (in this case HIV and syphilis) that were a useful predictor for infections with almost all other categories of HPV genotypes infections.

HPV-16/18 vaccination and concurrent multiple HPV types infections

Sub study IV results revealed that of the 135 cases of HPV infections, 49 [36.3%, 95% CI (28.2 – 44.4)] occurred as concurrent infection with multiple HPV types. There was no statistically significant difference between the HPV-16/18-vaccinated and non-vaccinated groups regarding the prevalence of concurrent multiple HPV types infections (41.0 vs 32.4%, X^2 0.720, df 1, p 0.396). A total of 29 different HPV genotypes were involved in the concurrent multiple HPV types infections. The number of HPV strains per concurrent multiple HPV-types infection ranges from 2-6 strains. Higher order concurrent multiple HPV-types infections such as those involving 5 – 6 strains tended to be more prevalent among the non-vaccinated group than the vaccinated group although the difference was not statistically significant (t -1.716, df 399, p 0.087). In multivariate analysis, HPV-16/18 vaccination was not significantly associated with the occurrence of concurrent multiple HPV-types infections.

Among the HPV-vaccinated group, the strains most frequently found in concurrent multiple HPV-types infections were HR HPV59, LR HPV6, HR HPV66, MR HPV53 and LR HPV61. Among the non-vaccinated

group on the other hand, the strains most frequently found in concurrent multiple HPV-types infections were HR HPV51, HR HPV16, HR HPV52, HR HPV58 and HR HPV66. With exception of HR HPV16, there were no statistically significant differences between the vaccinated and non-vaccinated groups regarding the type-specific frequency of involvement in concurrent multiple HPV-types infections for the rest of the strains.

HPV-16/18 vaccination and HPV clustering patterns

In sub study IV, of the 49 concurrent multiple HPV types infections, 25(41.0%) occurred among the HPV-16/18 vaccinated young women. Regarding clustering pattern, the majority of the multiple HPV-types co-infections among the vaccinated group clustered around HR HPV59 strain whereas that among non-vaccinated group clustered around HPV 52. In univariate analysis, there was no any statistically significant association between any two HPV genotypes. The useful predictors for multiple HPV-type co-infections were number of sexual partners and other STIs. HPV vaccination was not a significant predictor for multiple HPV-types coin-fection.

Cervical Cancer Screening Uptake among young women

From the **sub study IV** (**paper IV**) among the 15-24 year old young women, none of them reported of ever being screened for CC in their lifetime. The major reason for not seeking CC screening reported was lack of awareness of the age to initiate CC screening.

Healthcare Providers and Policy Maker's Perspectives of Integration of HIV and Cervical Cancer Screening

The views of HCPs and PM's about integration of HIV and CC screening services generated from **sub study II** (**paper II**) emerged in three themes namely appreciating the benefits of integration, worrying about the limited health system capacity and potential consequences of integration and feeling optimistic about integration under improved health system conditions. The themes and subthemes that emerged from the data for sub study II (paper II) is shown in figure 14. The benefits embraced the women – particularly the HIV-positive women- but also men, healthcare providers and the health system.

Benefits to the women from the perspectives of the HCPs and PMs would include: enabling women to receive more health services in a single visit approach, improving access to CC screening services among HIV-positive women who are the rightful target, reducing on the frequency of visits women make to the health facility for health services, facilitating early detection and treatment of other gynecological diseases that the woman may not even be aware of, minimizing loss to follow up of women from CC screening programs, enabling HCP to give proper or correct follow up schedule for the HIV-positive women who turn out to be positive for CC, increase the availability of CC screening sites for women.

Also, the HCPs and PMs were of the view that integration of HIV and CC screening services would provide some knowledge and skills benefits to HCPs as it will enable them to acquire more knowledge and skills on delivery of both HIV and CC screening services. More so, the HCPs and PMs were of the view that HIV and CC screening integration would enable men save both on transportation time and cost of their female partners to the health facilities for HIV or CC screening services. As to the funders of health services such as government, the HCPs and PMs explained that integration will benefit the health system by making it less expensive to deliver HIV and CC screening services than it would be in the unintegrated approach. There were worries that HIV stigma and shortage of healthcare workers would affect the effective delivery of the integrated program.

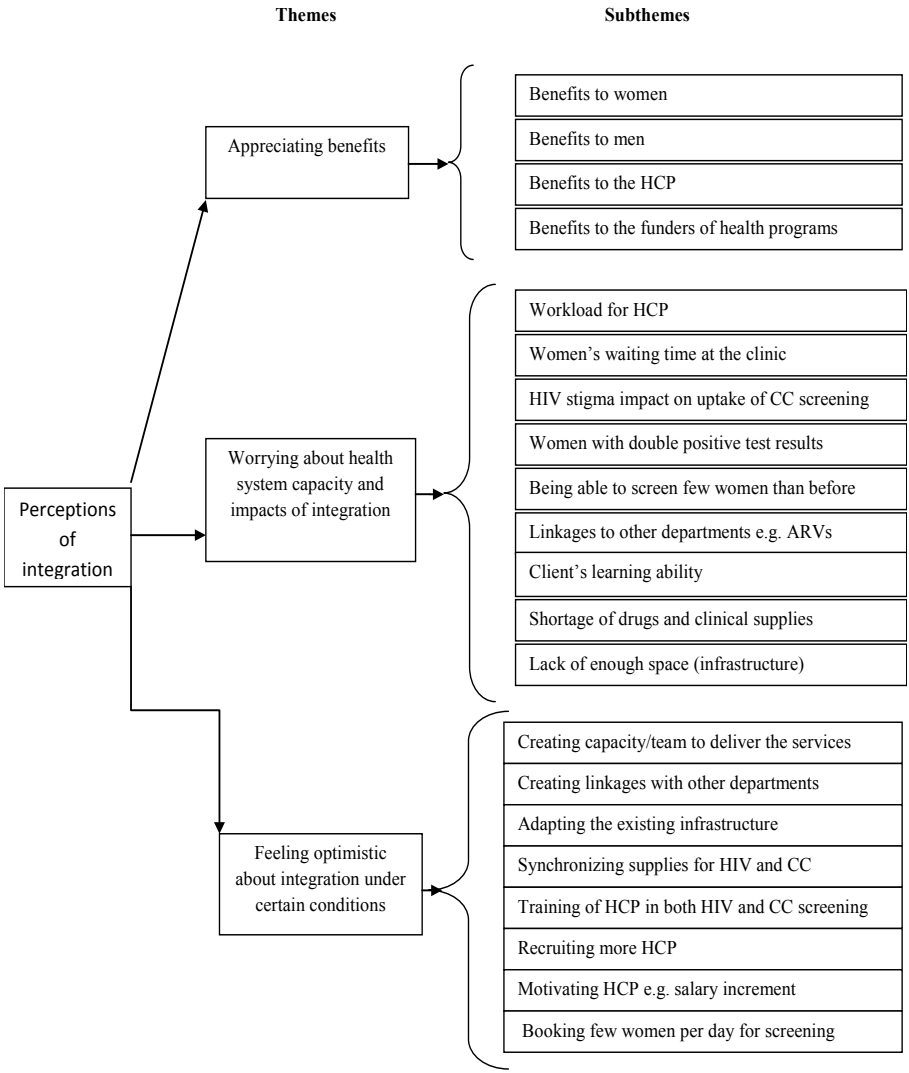

Figure 14: showing themes and subthemes from HCPs and PM's perceptions of integration of HIV and cervical cancer screening services.

Community member's Perspectives of Integration of HIV and Cervical Cancer Screening

The views of community member's regarding integration of HIV and CC screening services generated from **sub study III** (**paper III**) emerged in three themes namely appreciating the benefits of integration, worrying about the challenges of integration, and preferences for integration. The women endorsed the benefits. However, there were worries that integration would prolong the waiting time at the health facility and induce tiredness in both the healthcare providers and the women. There were also fears of being found positive for both HIV and CC and the consequences such as stress, self-isolation, and social conflicts. Participants, particularly the women, considered the challenges of screening integration to be manageable by, for example, taking a day off work to visit the hospital, delegating house chores to other family members, or taking a packed lunch on visiting the hospital. The views are shown in figure 15.

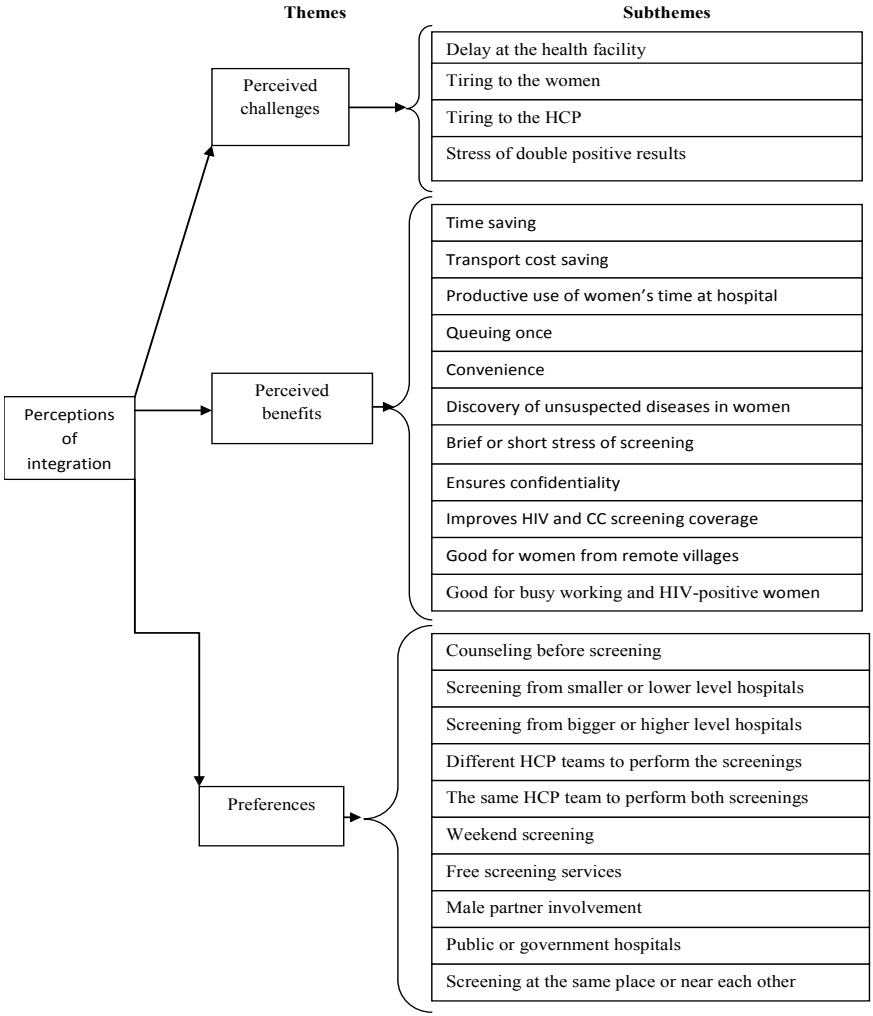

Figure 15: Themes and subthemes from community member's perceptions of integration of HIV and cervical cancer screening services.

GENERAL DISCUSSION

The prevalence of occupational exposure to HIV through needle stick injuries among health workers in **sub study I** (**paper I**) setting (Mbarara Regional Referral Hospital Uganda) of 8.5% was much lower than rates reported from other regions of Uganda. For example, prevalence of occupational exposure to HIV through needle stick injury with HIV-infected blood of as high as 27.7% was reported among health workers in a Ugandan hospital in Northern Uganda [357]. Prevalence rates for needle sticks injuries with HIV-infected blood reported from other African countries for example Ethiopia [358] and Kenya [358] of 31.5% and 19% respectively were also on the higher side compared to the rate found in the current study. The difference between **sub study I** (**paper I**) and other studies regarding the prevalence rates for occupational exposure to HIV through needle stick injuries could be due to differences in measurement methods used, health facility or education or experience of the health workers in question.

Consistent with other studies was the finding from **sub study I** (**paper I**) that nurses and junior clinical staffs including clinical students were the high-risk group for occupational exposure to HIV through stick injuries and muco-cutaneous contamination [357-359]. Also in line with other studies was the finding from **sub study I** (**paper I**) that poor clinical skills, inconsistent use of protective devices during clinical procedures, improper disposal of sharps and inadequate analgesia of patients during painful procedures were some of the predisposing factors to occupational exposure to HIV [357-359]. Also, consistent with other studies was the finding from **sub study I** (**paper I**) that use of PEP for prevention of HIV seroconversion after occupational exposure is hampered by poor reporting of exposures besides poor management of the reported cases [357-359].

The HIV prevalence of 1.7% among young women aged 15-24 years in **sub study IV** (**paper IV**) setting (Ibanda district situated in southwestern Uganda) was much lower than both the national HIV prevalence (4%) and southwestern region HIV prevalence (4.2%) estimates for young women aged 15-24 years in Uganda [360]. The difference could be due to differences in the composition of the young women population used in the two studies. The young women population for **sub study IV** (**paper IV**) were strictly never in union school going youths whereas that for

HIV/AIDS sero-behavioral survey 2004-2005) [360] in addition to never in union youths included a significant proportion of youths in union, widowed and divorced/separated who were more likely to be HIV positive than any other subpopulation of young women aged 15-24 years.

In **sub study IV** (**paper IV**), no significant associations were found between age at sexual debut, condom use and lifetime number of sexual partners and HIV infection. This contradicts the huge volume of previous studies that indicated associations between age at sexual debut, condom use, lifetime number of sexual partners and HIV infection [99-103]. The difference could be due to the small sample sizes for **sub study IV** (**paper IV**) which yielded only seven (7) HIV-positive cases.

Sub study IV (**paper IV**) showed increased rates of sexual risk behaviors among the young women compared to previous estimates in **sub study IV** setting (Ibanda district). Specifically, the finding that 30.2% of young women in **sub study IV** had initiated sexual activity by the age of 15 years was much higher than previous estimate of 5.1% for young women in that southwestern region of Uganda [360]. Similarly, the finding that 16.8% of the young women in **sub study IV** setting had multiple sexual partners was also higher than the previous estimate of 3% [360] for that region. Condom use prevalence of 40.9% and HIV testing/screening prevalence of 35.7% among youths in **sub study IV** setting were higher than the previously reported prevalence of 14.6% and 14.2% for that region [360]. The lower rates of sexual risk behaviors among youths observed in sub study IV compared to previous studies were expected in view of the widespread HIV prevention programs in Uganda over the recent years. However, the increased sexual risk behaviors among the young women call for the integration of sexual health education into existing health interventions targeting young women including HPV vaccinations.

Sub study IV (**paper IV**) established that the prevalence of vaccine-type HPV-16/18 infection was significantly lower among the HPV-16/18-vaccinated group compared to the non-vaccinated group with (0.5% vs 5.6%, p 0.006). This finding concurs with previous HPV vaccine (including ASO4-adjuvanted HPV16/18 vaccine) follow up study in Germany [361] although the vaccines in use in Germany was a mix of ASO4-adjuvanted HPV-16/18 and Quadrivalent HPV6/11/16/18 vaccines. This implies that ASO4-adjuvanted HPV-16/18 vaccination was protective

against infections with HPV 16 and 18. The findings of several other previous HPV vaccine follow up studies (in Sweden, Denmark, Australia, Netherlands and US) are difficult to compare with the current study because they were conducted after Quadrivalent HPV6/11/16/18 vaccine (Gardasil®) not the Bivalent HPV-16/18 vaccine (Cervirix®).

Sub study IV (**paper IV**) also found that the prevalence of HR HPV infection was significantly lower among the HPV-16/18 vaccinated group compared to the non-vaccinated group [18.5% vs 28.1%, p 0.032] although HPV-16/18 vaccination was not significantly associated with HR HPV infection in multivariate analysis. These findings concur with previous HPV vaccine (including ASO4-adjuvanted HPV16/18 vaccine follow up study in Scotland [366], England [367] and Germany [361] although the vaccines in use in Germany was a mix of ASO4-adjuvanted HPV-16/18 and Quadrivalent HPV6/11/16/18 vaccines. This could imply that ASO4-adjuvanted HPV-16/18 vaccine offered some limited protection against broad range of HR HPV types.

The findings from **sub study IV** (**paper IV**) that the prevalence of other categories of HPV types including non-vaccine genotypes, non-vaccine MR HPV and non-vaccine LR HPV, non-vaccine A7-HPV or HPV 18-related genotypes and non-vaccine A9-HPV types or HPV 16-related genotypes were not significantly lower among the HPV-16/18-vaccinated group compared to their non-vaccinated counterparts was consistent with previous HPV vaccine follow up study in Scotland [366], England [367] and Germany [361]. This therefore implies that protection generated by Bivalent HPV-16/18 vaccine was not sufficient enough to offer cross protection against non-vaccine HPV genotypes.

Sub study IV (**paper IV**) also established that apart from HPV16, there were no statistically significant differences between HPV-16/18 vaccinated and non-vaccinated groups regarding the type-specific prevalence of any of the HPV genotype. More so, the patterns of HPV clustering in concurrent infections with multiple HPV-types revealed no significant association between any two HPV genotypes although the majority of the multiple HPV-types co-infections among the non-vaccinated group frequently involved HPV 52 and 62 while that among the vaccinated group frequently involved HPV 59 and 6. Sub study IV (paper IV) also revealed that the occurrence of concurrent infections with multiple HPV-types was a ran-

dom process dependent on the circulating HPV genotypes in the population. This finding therefore supports previous suggestions that there is no specific HPV type-type association preference in concurrent infections with multiple HPV types after HPV-16/18 vaccinations [362-364].

The significant associations of other STIs (HIV and syphilis) with an increased risk of HPV infections including the vaccine-types, non-vaccine-types and HR HPV types observed in **sub study IV** (**paper IV**) was consistent with previous studies [237-240]. Although syphilis is a rare disease among young women in Uganda, this finding does suggest the need to consider syphilis interventions along with HIV and HPV interventions especially those targeted at young women in a developing country such as Uganda.

There were a number of perspectives from Ugandan HCPs and PMs in **sub study II** (**paper II**), that points to the possibilities of integration of HIV and HPV/CC prevention services in single visit approach. This included the HCPs and PM's views that an integrated HIV and CC screening program would be feasible with improvements in the health system and would benefit the women, men, HCPs themselves and the policy makers. The HCPs thought that the potential issues about integration of HIV and CC screening services are manageable by employing various strategies including recruitment and training of HCPs among others. Previous successful models for integrating HIV and CC prevention services such as from Kenya, Zambia and Nigeria could serve as motivation and opportunities for sharing worries, concerns and experiences [349-354].

Ugandan Community members' perspectives of integration of HIV and CC screening services in **sub study III** (**paper III**) were favorable and points to their preferences and likelihood of uptake in the future. The community members views of the benefits and challenges of implementing an integrated HIV and CC screening program concurs in many ways with the HCPs and PMs perspectives on the same [356]. Nonetheless, the fear expressed by the community members about double positive results (that was being found positive for both HIV and cervical cancer) and the likely social consequences would present a unique barrier to acceptability and uptake of services from an integrated HIV and CC screening program that needs to be simultaneously addressed with structural and programming issues presented by HCPs and PMs in **sub study II** (**paper II**). Furthermore, commu-

nity members desire to obtain the integrated HIV and CC screening services from a wide range of health facilities calls for coordinated efforts to scale up planning and implementation. More so, the community member's desire for a transdisciplinary team to deliver the integrated screening services calls for a critical review of curricula for pre-service and in-service education of healthcare professionals.

CONCLUSIONS & RECOMMENDATIONS

One of the sub studies in this thesis has pointed out the prevalence of occupational exposure of healthcare providers to HIV particularly through percutaneous injuries and muco-cutaneous contaminations with an HIV-infected blood or body fluids, the high-risk groups, the predisposing factors and use of PEP for the prevention of the potential risk of HIV transmission. This was perhaps the first study in Uganda to break the silence about the often ignored non-sexual transmission of HIV in occupational healthcare settings and the need to incorporate interventions and resources for its prevention into national HIV prevention and control programs despite the low transmission risk it poses. The findings concurred with other studies from other parts of the world regarding the high prevalence of occupational HIV exposure among healthcare providers particularly through percutaneous injuries and that nurses and poorly skilled junior clinical staffs and students are the high-risk groups. Nonetheless, this thesis uniquely identified that the predisposing and thus risk factors to occupational exposure and transmission of HIV among healthcare providers in Uganda are similar to that in other low resource settings in Africa. They included poor adherence to universal precautions, inappropriate healthcare waste disposal, the practice of recapping of used needles despite the ban, insufficient supply and thus nonuse of safety wears and devices when needed, inadequate analgesia or anesthesia of patients during painful invasive procedures, poor reporting and investigation of exposures and untimely access to PEP after exposure. Future studies in low income settings such as Uganda should evaluate interventions to improve HCP's compliance on universal precautions, reporting and investigations of occupational exposures to HIV and timely access to PEP.

Another sub study in this thesis has determined the prevalence and risk factors for cervical HPV infections among young women, the benefits and challenges of ASO4-adjuvanted HPV-16/18 vaccination as a primary pre-

vention of cervical cancer. This was perhaps the first real world follow up study of ASO4-adjuvanted HPV-16/18 vaccinated young women in Africa. The findings concurred with the existing literature from other parts of the world regarding the effectiveness of ASO4-adjuvanted HPV-16/18 vaccines in preventing vaccine-types HPV 16/18 infections and the additional cross protection benefits against any HR HPV infection in general. The sub studies in this thesis however did not find any clear evidence in support of the risk of HPV type replacement after the ASO4-adjuvanted HPV-16/18 vaccinations of Ugandan young girls. The role of other prevalent STIs particularly HIV and syphilis as a potential threat destined to undermine the lifesaving benefits of HPV-16/18 vaccination among adolescent girls in Africa need further research. There is also need for more bivalent HPV16/18 vaccine follow up studies especially those that corroborate anti-HPV antibodies levels with HPV infection status of the young women. The health consequences of the changes in HPV type distribution after bivalent HPV16/18 vaccination e.g. persistence and progression of HPV infections into cervical precancerous lesions and cervical cancer needs further reaearch.

Other sub studies in this thesis have explored stakeholders and community member's perceptions about integration of HIV and cervical cancer prevention services. The stakeholders and community members alike have appreciated the manifold benefits of integration to women, men, healthcare providers, and the health sector as a whole. Similarly, the stakeholders and community members alike have wondered and expressed worries and concerns about the feasibility, effectiveness and potential social impact of the integrated screening approach given the weak and inefficient health system in Uganda as is the situation in many developing countries. The stakeholders and community members alike have used different dimensions to express their desire for the integrated screening approach to incorporate beforehand effective mechanisms and procedures for clinic appointments management, waiting time management, patient education, social support, transdisciplinary training of healthcare providers, clinical supplies integration and management, integrated screening algorithms, community education and mobilization strategies and male involvement. Future studies could examine the health impacts of integrated HIV and cervical cancer screening in low resource settings such as Uganda.

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