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CERVICAL CANCER AND HIV/AIDS

INFLUENCE OF HIV/AIDS ON CERVICAL PRECANCEROUS LESIONS AND INVASIVE CERCICAL CANCER IN MOROGORO REGION, TANZANIA.

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CERVICAL CANCER AND HIV/AIDS:

INFLUENCE OF HIV/AIDS ON CERVICAL PRECANCEROUS LESIONS AND INVASIVE CERVICAL CANCER IN MOROGORO REGION, TANZANIA.

A dissertation submitted in partial fulfilment of the requirements for the degree of Master of Science in Oncology of the Institute of Biomedical Sciences Abel Salazar of the University of Porto, Portugal.

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ABSTRACT

Cervical cancer is the leading cause of cancer related morbidity and mortality in women in Tanzania. The impact of HIV/AIDS epidemic to cervical precancerous lesions and invasive cervical cancer has a significant implication, for any public health concern, especially in one of the most prevalent areas like Morogoro Region, in Tanzania. A comparative retrospective study of 536 women screened for cervical cancer disease by visual inspection methods at the Morogoro Regional Referral Hospital for 3 years and grouped according to their HIV status was done. The prevalence of precancerous lesions was 55.5% in HIV positive women and 14.7% in HIV sero negative women. Furthermore, the prevalence of extensive or large precancerous lesions was 52.5 % in HIV positive women and 25% in HIV sero negative women.In addition, the prevalence of invasive cervical cancer was 50.8% in HIV sero negative women compared to 26.7% in HIV positive women.The risk factors for the extensive/large cervical lesions and suspicious of cervical cancer disease were HIV positive status (OR 3.4, 95% CI 1.7-6.8, p<0.001), CD 4 T-cells count less than 300 cells/mm^3(OR 4.7,95% CI 0.86-31.07,p =0.0352), high parity (OR 3.3,95% CI 1.89-5.79, p <0.001), and age above 45 years (OR 3.25,95% CI 1.81-5.85, p <0.001) .Therefore, HIV/AIDS has a highly statistical significance association (p <0.001) and high influence on the development of cervical precancerous lesions in HIV positive women, however it's direct involvement in the progression to the invasive cervical cancer especially in the HAART era is questionable.

Key words (Cervical precancerous lesions, invasive cervical cancer, HIV, visual inspection methods)

RESUMO

O cancro do colo do útero é a principal causa de morbilidade e mortalidade associada ao cancro em mulheres na Tanzânia. A infecção por VIH/SIDA tem implicações significativas nas lesões pré-cancerígenas do colo do útero e na respectiva neoplasia invasiva. Dada a extensão que esta epidemia contabiliza, ela assume-se como um verdadeiro problema de saúde pública, particularmente grave nas áreas mais prevalentes da doença, como é o caso da região de Morogoro na Tanzânia. Foi realizado um estudo retrospectivo comparativo durante 3 anos, em 536 mulheres rastreadas para a presença de cancro do colo do útero, diagnosticado por métodos de inspecção visual no Morogoro Regional Referral Hospital. As participantes foram agrupadas de acordo com a sua condição para o vírus VIH. A prevalência de lesões pré-cancerígenas foi de cerca de 55,5% em mulheres positivas para a presença do vírus VIH e cerca de 14,7% em mulheres VIH seronegativas. Além disso, a prevalência de lesões pré-cancerígenas extensas ou grandes foram detectadas em cerca de 52,5% das mulheres VIH seropositivas e apenas em cerca de 25% das mulheres VIH seronegativas. No entanto, a prevalência de cancro do colo do útero invasivo foi reportada em cerca de 50,8% das participantes VIH seronegativas, enquanto ao passo que uma condição clínica idêntica foi verificada em cerca de 26,7% das mulheres positivas para a presença do vírus VIH. Os factores de risco identificados à existência de lesões extensas ou grandes no colo do útero e consequente suspeita da neoplasia que lhe está associada foram seropositividade para o vírus VIH (OR 3,4; 95% IC 1,7-6,8; p<0,001), contagem de células T $CD4^+$ menor que 300 células/mm^3 (OR 4,7; 95% IC 0,86-31,07; p=0,0352), elevada paridade (OR 3,3; 95% IC 1,89-5,79; p<0,001) e idade acima dos 45 anos (OR 3,25; 95% IC 1,81-5,85; p<0,001). De acordo com os nossos resultados, a influência do VIH/SIDA, nomeadamente entre mulheres VIH seropositivas, e o desenvolvimento de lesões pré-cancerígenas do colo do útero, representa uma associação estatisticamente relevante (p<0,001). No entanto, o envolvimento directo do vírus VIH na progressão para cancro invasivo do colo do útero, especialmente na era HAART, permanece não conclusiva.

(Palavras-chave: Lesões pré-cancerígenas do colo do útero; Cancro do colo do útero invasivo; VIH; Métodos de inspecção visual)

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LIST OF ABBREVIATIONS

ADI	Acquired Immunodeficiency Syndrome Defining Illness
AIDS	Acquired Immunodeficiency Syndrome
ASCUS	Atypical Squamous Cells of Undetermined Significance
ASR	Age Standardized Rate
CA	Cancer
CDC	Centers for Disease Control and Prevention
CI	Confidence Interval
CIN	Cervical Intraepithelial Neoplasia
CIS	Carcinoma Insitu
DNA	Deoxyribonucleic Acid
E	Early Protein
HAART	Highly Active Antiretroviral Therapy
HG	High Grade
HGAIN	High Grade Anal Intraepithelial Lesion
HGCIN	High Grade Cervical Intraepithelial Neoplasia
HGSIL	High Grade Squamous Intraepithelial Lesion
HIV	Human immunodeficiency Virus
HIV-VL	Human Immunodeficiency Virus Viral Load
HLA	Human Leukocyte Antigen
HPV	Human Papillomavirus
HPV DNA	Human Papillomavirus Virus Deoxyribonucleic Acid
HR-HPV	High Risk-Human Papillomavirus Virus
HSV-2	Herpes Symplex-2 virus
ICC	Invasive Cervical Cancer
IFN-γ	Interferon gamma
KS	Kaposi’s sarcoma

L1	Capsid protein 1
L2	Capsid protein 2
LCR	Non-coding regions
LEEP	Loop electrosurgical excision procedure
LETZ	Loop excision of the transformation zone
M&E	Monitoring and Evaluations
MC	Male Circumcision
MDG	Millennium Development Goal
MRRH	Morogoro Regional Referral Hospital
MSM	Men having sex with men
NGO	Non-Governmental Organization
NHL	Non-Hodgkin's Lymphoma
OR	Odds Ratio
ORCI	Ocean Road Cancer Institute
p or p^a	Statistical power
p53	Tumor protein 53
SD-Bioline	Standard Diagnostics-Bioline
SIL	Squamous Intraepithelial Lesions
SPSS	Statistical Package for Social Sciences
STA	See-and-Treat Approach
STI	Sexually Transmitted Infection
SVA	Single Visit Approach
TAH	Total Abdominal Hysterectomy
TB	Tuberculosis
Th_1	T Helper cell 1
Th_2	T Helper cell 2
TNF-α	Tumor necrosis factor
US	United States
VIA	Visual inspection with acetic acid

VILI	Visual inspection with Lugol’s iodine
VL	Viral load
WHO	World Health Organization
-ve	Negative
+ve	Positive

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CHAPTER 1

1.0 INTRODUCTION

1.1 Background

Each year, more than 530,000 women worldwide are diagnosed with cervical cancer and around 275,000 die from the disease, with 88% of deaths occurring in developing countries especially in sub Saharan Africa [1].

Cervical cancer disease is a fully preventable and curable disease at low cost and at low risk, when early screening to facilitate the timely detection of precancerous lesions in asymptomatic women is available together with appropriate diagnosis, treatment and follow-up [1, 2]. However, with the introduction of HPV vaccine, the burden of the disease is very low in developed countries, while the situation is quite the reverse in developing countries where it constitutes a major public health problem due to genital cancers in women [1-3].

Several cancers have been designated as AIDS related, with a significantly increased risk among people living with HIV [4]. Cervical cancer is recognized as a sexually transmitted disease [5]. The sexually transmitted aetiological agent has been identified as the human papillomavirus [4, 5]. Women with HIV infection are more likely to have a concurrent HPV infection [4]. There is firm evidence, however, that HIV is independently associated with an increased risk of cervical intraepithelial neoplasm [6]. Some studies suggest that HIV infection is associated with the rapid progression of HPV induced cervical pre-malignant lesions to invasive cervical cancer [7].

Tanzania has among the highest incidences of cervical cancer disease and HIV/AIDS cases in the world with concomitant high mortality affecting women at their prime [1, 8].The association between HIV and invasive cervical cancer is complex, with several studies clearly demonstrating an increased risk of precancerous cervical lesions and a more rapid progression to invasive cancer, amongst HIV infected women [7, 9].

Regular screening with a Pap smear has been shown to effectively lower the risk of developing invasive cervical cancer, by detecting precancerous changes earlier [10].However, in developing countries, only about 5% of eligible women undergo cytology-based screening in a 5-year period [11]. This is because there are too few trained and skilled professionals to implement such programs effectively in developing countries like Tanzania [12]. In addition, healthcare resources are not available to sustain such programs [11]. In virtually all developing countries, cytology-based services are confined to teaching hospitals or private laboratories in urban areas [13]. Furthermore, delays in reporting cytology results make it less likely that test positive women ever receive their results on time, hence delay in

treatment or loss in follow-up within a very short duration [13]. These are some of the barriers that prevent cytology-based screening programs from being effective in developing countries.

Recent studies have demonstrated that visual inspection with acetic acid or with Lugol's iodine are alternatives of sensitive cervical cancer screening methods [14]. The procedures are cheap and non-invasive, and can be done in a low level health facility like a health centre [14]. More importantly, both provide instant results and those patients eligible for treatment can receive the treatment of the precancerous lesions using cryotherapy on the same day and in the same health facility [15]. These "see and treat" methods ensure adherence to treatment soon after diagnosis, hence decreases the late presentation of the disease [11, 15].

Cryotherapy as a method of treatment for precancerous lesions is effective and easier to implement than other treatment modalities such as loop electrosurgical excision procedure , loop excision of the transformation zone and cone biopsy [16]. Furthermore, it has additional advantages, including the facts that it is affordable; there are no need for complicated equipment and it can be done by less specialized personnel and thus can be implemented in a primary health-care setting [16].Secondary prevention of cervical cancer through screening and treatment of cervical precancerous lesions is associated with an overall reduction in morbidity and mortality due to cervical cancer disease [1].

Moreover, lack of sustainable screening programs for early detection of precancerous lesions, HPV vaccination program and functional cancer registries in Tanzania is currently unforeseen situation which has to be directed [1, 3, 8]. Most screening activities are done as pilot studies or research projects which are discontinued on completion [1]. Generally, the coverage of cervical cancer screening remains very poor especially in the remote areas [17] like Morogoro region where the impact on invasive cervical cancer is very prominent while the prevalence of the disease is still unknown [1, 8, 18].

For example, despite the high burden of the disease, cervical cancer prevention services in the country, are not readily available to a majority of women [19] . Cytology based screening, HPV vaccination and HPV DNA self-sampling methods as used in high-income countries [17] [20], are currently not feasible in Tanzania, because of the financial, infrastructure, human resources and technological investments required[19]. Tanzania also lacks adequate histopathology laboratories, other diagnostic and treatment facilities for cervical cancer in regional hospitals [19] including Morogoro Regional Referral Hospital. Yet, opportunities do exist to prevent, cure and relieve suffering, by promoting primary and secondary prevention, as well as tertiary care [21]. In addition, with the onset of HIV/AIDS epidemic which is very

high in Tanzania and so the Sub Saharan Africa region as a whole, there is a growing evidence that it has elevated the problem of cervical cancer to a serious level and the exact prevalence and the outcome of the disease for the people living with HIV especially in upcountry areas is ambiguous [19].

For instance, in Uganda, where the incidence of HIV/AIDS is very high among East African countries, Parkin et al [22] reported a marked increase in the incidence of cervical cancer cases since the nineteen eighties, with stabilization in the nineteen nineties. In the WHO world cancer report 2012 [1] on HIV/AIDS, there were more than 8 million people living with HIV in low and middle income countries at the end of 2011. This was a 20-fold increase in the number of people living with HIV in developing countries between 2003 and 2011, and a 20% increase in just one year (from 6.6 million in 2010 to more than 8 million in 2011) [1].

Summing up, there must be an evaluation of available data on current cervical cancer screening programs in both HIV infected women and non HIV infected women in order to draw the conclusion and recommendations on the impact of HIV/AIDS pandemic [23] to the development of invasive cervical cancer disease so as to change the strategies to tackle the disease under new threat of increasing HIV infections in women especially in rural areas like Morogoro region [1, 24].

1.2 Problem statement

HIV/AIDS epidemic in sub-Saharan Africa, which equally affects Tanzania with high HIV prevalence rate of 6.8% in 2007 being women aged 15–49, has significant implication, for any public health concern, attempting to address the burden of the disease on the invasive cervical cancer in the country [19].

Tanzania, being one of the Sub Saharan regions, the country has among the highest incidences of cervical cancer disease and HIV/AIDS cases in the world with concomitant high mortality affecting women at their prime [1, 8].The association between HIV and invasive cervical cancer is complex, with several studies demonstrating an increased risk of precancerous cervical lesions and a more rapid progression to invasive cancer, amongst HIV infected women especially in young ages [7, 9]. The vast majority of cervical cancer patients is usually seen only at a late stage of evolution of the disease, which reduces considerably the chances of survival [19].

Currently, there are no published data on the burden of the disease in Morogoro region [25]. In addition, the impact of HIV/AIDS to cervical cancer has not been determined regardless of the increase in the number of invasive cervical cancer cases in young women in the eastern

zone of Tanzania, specifically in Morogoro region, one of the most HIV/AIDS prevalent regions [19].

1.3 Justification

Tanzania is a developing country, despite of a population of approximately 48 million people and a country size of 945,087 square kilometres, it has only one major cancer treatment centre with few documented cancer registries for more than 53 years of independence [19].

The determination and comparison of the current prevalence, risk factors, methods of screening, treatment protocols and their outcome in patients with cervical precancerous lesions, cervical cancer and their HIV status at MRRH will lead to the determination of the effects of the two diseases and hence initiation of a benchmark of continuous screening program, proper follow up of the patients and treatment protocols by using the minimum resources we have, which is a very crucial step in the implementation of a cancer registry in Morogoro region.

Another reason is to have concrete data, knowledge and research so that convey the new strategies to combat cervical cancer disease in the region, according to the new challenges of HIV/AIDS epidemic. The outcome seen in this study will help to shift the current focus towards more preventive strategies like implementation of continuous cervical cancer screening and treatment in all HIV/AIDS clinics, HPV vaccination for all girls before sexual debut and massive community cervical cancer screening campaign in Morogoro region as a whole.

Finally, the impact of this implementation will decrease the number of referrals, advanced cases of cervical cancer disease, increase awareness of the disease to the society and policy makers, hence gradually it will reduce the overall high mortality due to cervical cancer in the region.

1.4 Research Question

What is the influence of HIV/AIDS on cervical precancerous lesions and invasive cervical cancer in Morogoro region?

1.4.1 Objectives

1.4.2 Broad objective

To determine the influence of HIV/AIDS on prevalence and risk factors for cervical precancerous lesions and invasive cervical cancer among HIV infected women at MRRH.

1.4.3 Specific objectives

i) To determine the prevalence of cervical precancerous lesions among HIV infected women at MRRH.

ii) To determine the percentage and risk factors/determinants for extensive/large cervical precancerous lesions among HIV infected women at MRRH.

iii) To determine the prevalence of ICC among HIV infected women at MRRH.

CHAPTER 2

2.0 LITERATURE REVIEW

2.1 Epidemiology

2.1.1 Epidemiological link between the two pandemics

Tanzania suffers one of the highest cervical cancer burdens in the world and the highest in East Africa, with an age-standardized incidence rate of 50.9 cases per 100,000 women and an ASR mortality of 37.5 per 100,000 women[19]. In 2009, cervical cancer accounted for 35.3% of all cancer patients seen at the ORCI, the only specialized facility for cancer management in Tanzania [26].There is a lot of evidence that HIV infection enhances HPV carriage in a single individual [1].Cervical cancer incidence rates are lowest in Western Asia and highest in East Africa [1, 27]. As a consequence, HIV also has an impact on HPV infection within couples [27]. Among heterosexual couples living in South Africa, HIV negative male partners of HIV positive women had a significantly higher rate of HPV penile carriage than partners of HIV negative females (58 vs 32% respectively; p=0.001) and the prevalence was further increased to 72% when both partners were HIV positive [28].Furthermore, type-specific sharing of HPV within the couple was also associated with HIV co-infection [28].

According to two large meta-analyses, one performed in women from the general population and the other in HIV infected women, HPV prevalence was higher within the same region in women with HIV compared with women without HIV. When comparing the HPV infection rates and incidence rates from different world regions, the highest prevalence was found in regions where HIV infection was more prevalent, according to the WHO data, such as sub-Saharan Africa (Figure 1) [1, 28] .

The age-standardized incidence of cervical cancer is the highest in Sub-Saharan Africa and varies from 23/100,000 woman-years to 34.5 in Eastern Africa compared with <10 in Western Europe and North America [1, 27, 28]. For example the world's highest age-standardized death rates from invasive cervical cancer were 67 per 100 000 people in Harare, Zimbabwe and 40.8 per 100 000 in Kampala, Uganda in 1997 [15, 27].

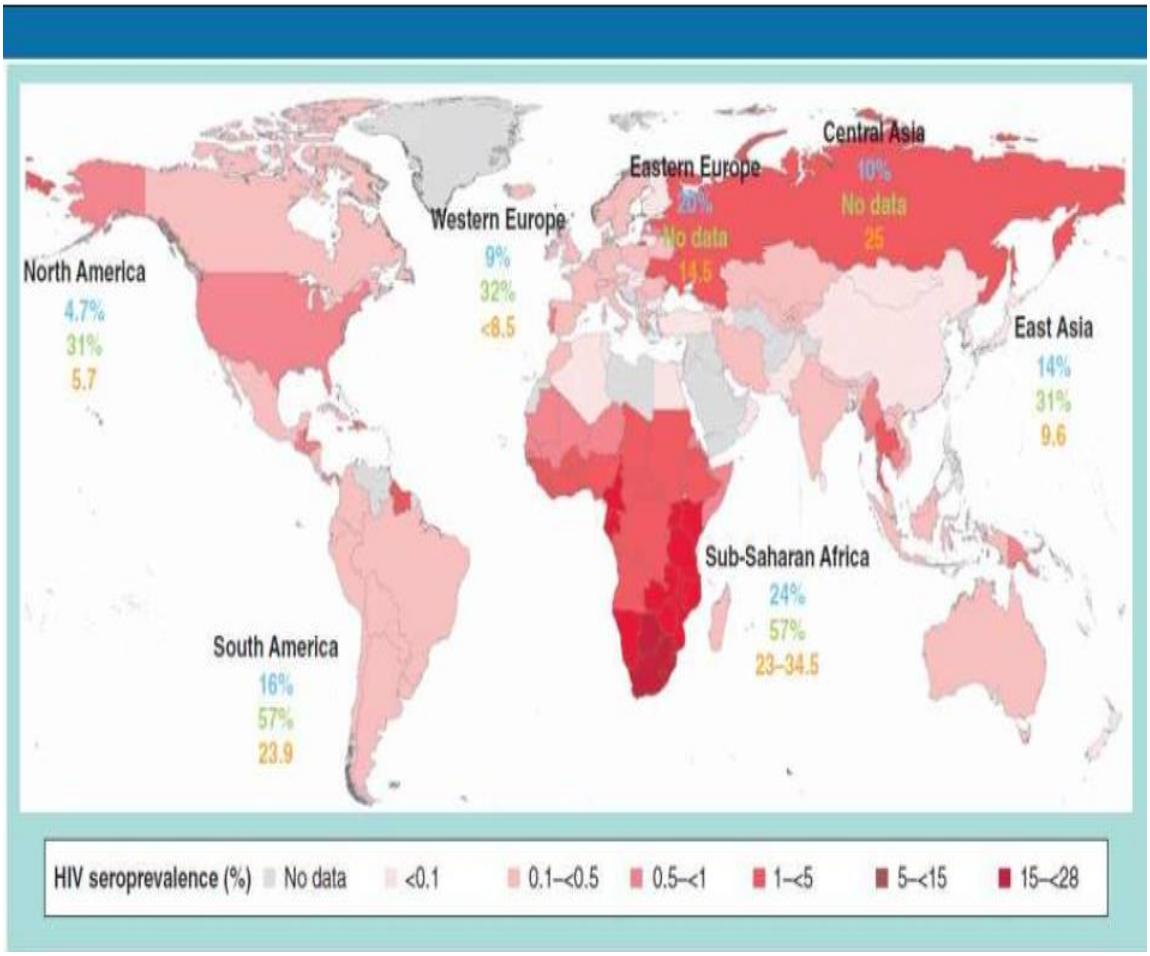


Figure 1: HPV infection prevalence in the cervix and invasive cervical cancer incidence according to WHO HIV sero-prevalence distribution in 2010 (Adopted from [1]).

2.1.2 HPV, HIV infection and cervical cancer

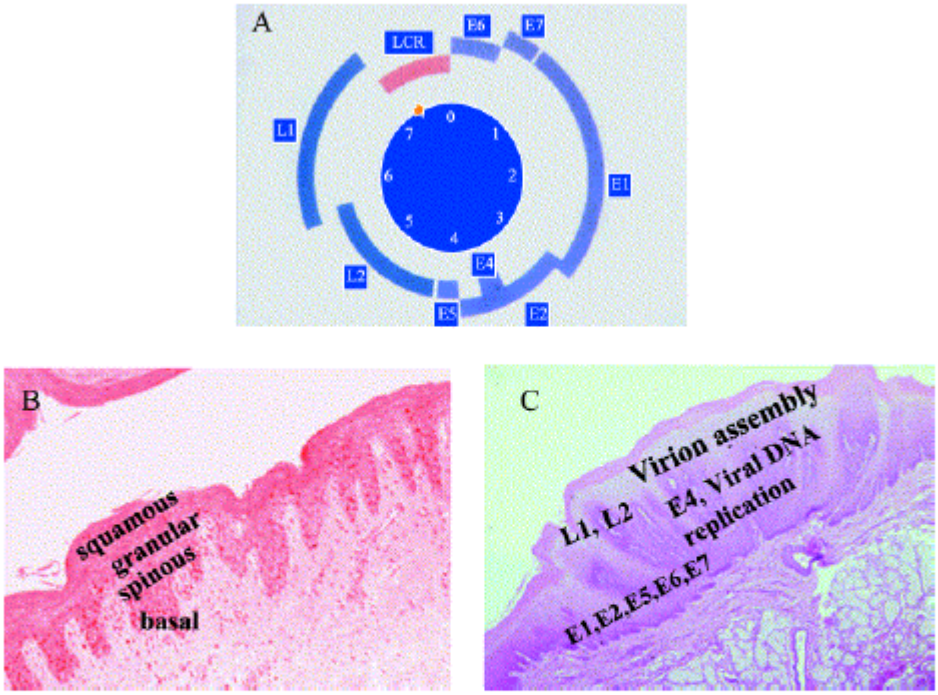
Persistence infection with oncogenic HPV has been established as a necessary causal factor for cervical cancer [29]. Harald zur Hausen, postulated in the early seventies that HPV infection was the causative agents and it were proved in the eighties with advanced technologies to detect the viral genome [28].

Apart from vertical transmission and blood transfusion, the main route of transmission of HPV and HIV is sexual intercourse [28, 30]. Many women get exposed to HPV, but very few develop into cervical cancer[31].This implies that there are other co-factors at play in the carcinogenic process [28].Behavioral and biological determinants include number of sexual partners, the sexual behaviour of male partner, the age at first sexual intercourse, high parity, smoking, long-term use of hormonal contraceptives, nutritional status and vaginal micro flora [18, 24]. HPV co-infection with other sexually transmitted diseases such as HIV, *Chlamydia trachomatitis* and *Herpes simplex* virus type 2 increases the risk of developing cervical cancer disease [28, 31].

However, there are some arguments on this causal role of HSV-2 in cervical cancer onset that many studies which concluded this did not control for the role of HPV [9]. Immunosuppression, which is caused by HIV and organ transplantation, has been associated with multiple infections with HPV persistence and progression from pre-malignant lesions to cancer [28, 32]. HPV oncoproteins E6 and E7 (Figure 2) inhibit the action of p53, and pRB preventing human DNA repair, as cellular replication is no longer controlled, DNA errors and chromosomal mutations accumulate that may cause the emergence of tumour cells [32].

On the other hand, HPV has the capability to escape from immune defences, as it does not induce cell death or viraemia and when it replicates, new virions are released far from the immune system in terminally differentiated epithelial cells of the mucosa [31]. This induces a weak or nonexistent antibody response [32]. There are approximately 40 different HPV genotypes affecting the human anogenital tract (cervix, vulva, vagina, penis and anus) [24, 32, 33]. These genotypes are divided in low-risk HPV (mostly types 6 and 11) responsible for benign warts or condylomas, which are nonmalignant tumours and high-risk (HR) HPV (mostly types 16 and 18, and less frequently types 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and 68) are responsible for malignant disease [32].Infection with HPV is very frequent in human beings, with a lifetime infection risk of at least 80% [33, 34]. Different type-specific HPV infections occur early during the first sexual intercourses and are transient for most individuals [32] .

Nonetheless, 5–10% of women harbour persistent cervical infection with HR HPV and will thus be at risk of developing high-grade (HG) squamous intraepithelial lesions (SIL) in their third or fourth decade of life. HG SIL, also called HG dysplasia, is the stage just before cancer on cytological samples [35, 36].



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

Figure 2: The Human papillomavirus life cycle (Adapted from [37]).

2.1.3 Cervical precancerous lesions

Richart classification uses cervical intraepithelial neoplasia (CIN) [5, 14].The Bethesda classification uses squamous intraepithelial lesions (SIL) [5].Lesions are classified histologically in accordance to the degree of neoplastic progression with a scale ranging from CIN 1 to CIN 3 or carcinoma in situ (CIS) by Richart classification [14]. The corresponding grades I Bethesda classification is low grade SIL(LGSIL), for CIN1 and high grade SIL(HGSIL) for CIN2 and CIN3 [14, 38].Atypical cells of unknown significance (ASCUS) refer to unclear abnormal findings which may be due to neoplastic changes, inflammations or non-infective processes [5, 14, 36].

Many studies of precancerous lesions of the cervix have reported regression in LGSIL in over 60% and progression to cancer about 1%, regression of CIN2 of 40% and progression to cancer about 5%, regression of CIN3 of 30% and progression to cancer by 12% [30].The average modal time from infection with HPV to CIN3 has been estimated to be 10 years (Figure 3) and from CIN3 to invasive cancer averaging 10 to 15 years [14].

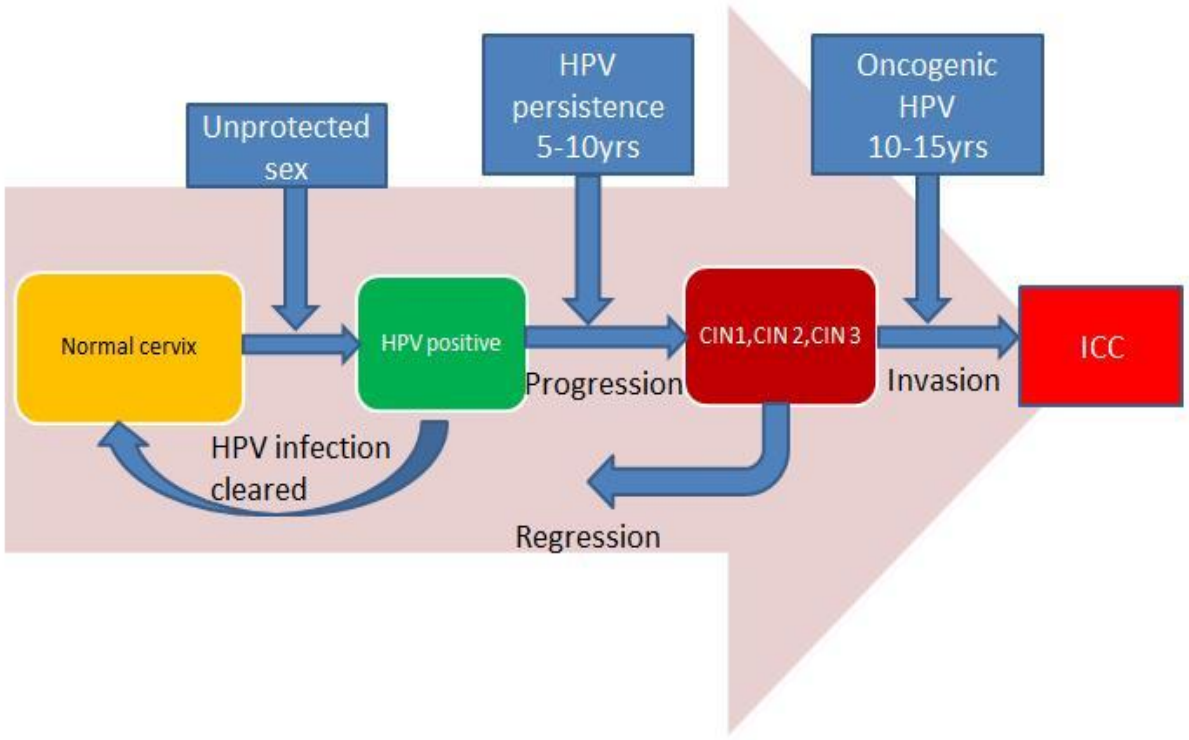


Figure 3: The diagram to show the natural history of HPV infection to precancerous lesions and the invasive cervical cancer.

2.2 HIV favouring HPV Infection

HIV infection favours HPV at the molecular and cellular levels such, as penetration of HPV in the target cells, HPV replication and HPV immune escape from host defences [31, 39].

In cervical epithelial cells from HIV-uninfected patients, the adjunction of HIV proteins (tat and gp120) with cytokines produced by HIV-infected cells (TNF-α and IFN-γ) induced the disruption of epithelial tight junctions and potentiated HPV penetration into the basal epithelial cells, which are HPV targeted cells [30, 31, 39].

HIV tat protein during HPV replication showed to significantly enhance HPV transcription and thus the expression of the HPV E oncogenes and L capsid proteins in cell cultures [28, 32, 33].To escape immune surveillance, HPV is able to induce a shift to T helper-type (Th) polarization from Th1 to Th2, with a parallel shift in cytokine production [39, 40].

Again, HIV favours HPV infection at the clinical level by increasing the new acquisition of HPV 16 [40]. HIV and HPV share a common route of transmission, unprotected sex with multiple partners [39]. Moreover, HPV infection is further increased by progressive immunosuppression during HIV course [40]. This results in HIV infected individuals with

higher HPV viral loads (VLs) than HIV uninfected individuals [41, 42], less HPV clearance and more frequent HPV persistent infection [6]. Reactivation of latent HPV infection is more frequent in HIV positive patients with lower immunity levels [6, 39, 40].

In heterosexual couples lived in South Africa, showed that HIV positive status was associated with a significantly increased risk (2–3 times) of new detection of HPV in both men and women [43]. Meanwhile, the chance to clear HPV infection was reduced in HIV positive men and women compared with HIV negative individuals (relative risk [RR]: 0.71; 95% CI: 0.55–0.93 in men and RR: 0.46; 95% CI: 0.34–0.62 in women) [28].

In another cohort study of 652 HIV positive women of African origin lived in Europe, the incidence rate of new HR HPV infections of the cervix was 13.4, contrasting with the five per 100 woman-years found in HIV-negative women with similar age and cytology [18]. Similarly, in the same study, for each increase of 100 CD4 cells/μl, there was a proportional significant decrease in the risk of carrying HR HPV with an odds ratio of 0.82 (95% CI: 0.76–0.89, $p < 0.001$) [18, 33, 39].

Many studies found that, higher HIV VL is associated with a significantly increased carriage of HPV and of SIL independently from CD4+ T-cell count decrease [43, 44]. HIV viraemia has shown to be an independent predictive factor for other opportunistic infections by inducing immune defects other than depletion of CD4+ T-cell count, such as cytokine production alterations which was notably modified in HIV positive patients [44].

Another recent study showed that, the risk of newly detected cervical HR HPV was increased by 2.5–5 times within 3–6 months after acquiring HIV infection [44]. This may reflect the highest rate of HPV latent infection reactivation already demonstrated in HIV positive patients [44].

In addition, another study showed that, HIV positive adolescents with >500 CD4+ T cells/μl had a significantly higher rate of persistent HR HPV than HIV-negative controls, illustrating that even slight changes in immune function might be responsible for higher HR HPV prevalence in HIV-positive patients [18, 28, 33].

HPV prevalence was 45% for any HPV genotypes and 31% for HR genotypes in 305 HIV-infected men who had sex with men (MSM) in Taiwan compared with 18% and 13%, respectively, in the HIV uninfected MSM. In the same study, decreasing CD4+ T cell count was strongly associated with increasing prevalence of HPV including HR types [44].

Similarly to what has been largely demonstrated in the genital area, HPV infection is also more important in the oral area of HIV infected individuals [43]. For instance, among 249 HIV positive patients from Australia, HPV prevalence was 19% for all HPV genotypes and 4.4% for HPV16 compared with HPV 7, and 0.8% in 251 HIV negative patients [43]. In another study, the mean HPV VL found in saliva samples was 20-times higher in HIV infected patients ($p < 0.001$) [45].

2.3 HIV favouring HPV-induced Lesions

The incidence of cervical biopsy-confirmed SIL was found to be 8.3/100 person-years in HIV-positive women compared with 1.8/100 person-years for HIV-negative women [39, 43].

Moreover, regression of warts or SIL can be achieved by a CD4+ T cell response against HPV antigen such as E6 and E7 oncoproteins, but this immune response is impaired during the course of HIV infection [32].In a case–control study, abnormal pap smear after conization was more frequent in women with HIV, 62% compared to 33% in HIV uninfected women and there was HG SIL recurrence in 20% compared with none in women without HIV [18, 28, 33, 39].

In MSM, the pooled prevalence of HG AIN proved by biopsy was 29% compared with 22% in HIV uninfected MSM [34]. In the same meta-analysis, anal cancer incidence was 46/100,000 HIV positive MSM per year versus 5.1 in HIV negative MSM [39, 40].

In a cohort of 500,000 HIV patients linked to the national US cancer registry, the incidence of cervical and anal cancer were significantly increased in HIV-positive patients (ICC is 6–10 times more frequent than in the general population) [18, 33, 43].

2.4 HPV infection favouring HIV acquisition

The risk of acquiring HIV was doubled when the infection by any HPV genotype was present prior to HIV infection [39, 40]. In addition, the risk of HIV acquisition increased significantly if there was a previous infection with two or more HPV genotypes in the same individual (hazard ratio: 3.5; 95% CI: 1.02–10.06; p= 0.002) [40].

As other STIs are known to be associated with increased HIV acquisition, HPV infection and induced lesions could disrupt the mucosal epithelia integrity, allowing HIV to enter more easily [18, 33].Similarly, the E7 protein of HPV16 has been shown to potentiate increased permeability of genital mucosa to HIV by down regulating an epithelial adhesion molecule called E-cadherin [32, 33].

HIV acquisition, on the other hand, was significantly higher in women with HPV detection loss just before acquiring HIV compared with women with persistently detectable HPV infection, odds ratio was 5.4 (95% CI: 2.4–9.9, p< 0.001) [33]. This hypothesis has been confirmed in a case–control study done in heterosexual males included in a circumcision trial [46].

In cases of other concomitant STIs, cells susceptible to HIV infection are recruited to the surface of the mucosa [46, 47]. The results of a case–control study in Zimbabwe, showed that, during the 22 months of follow-up, 154 women acquired HIV while 479 remained HIV uninfected [35]. After controlling for behavioural and biological covariates such as other STIs, the cervical infections with HPV raised the risk of acquiring HIV infection by a 2.4-fold (p<0.001, 95% CI, 1.5–4) and the risk was enhanced with increasing numbers of HPV types isolated in the same individual [35].

CHAPTER 3

3.0 METHODOLOGY

3.1 Study Area and Duration

This study was conducted at MRRH obstetrics and gynaecology department from 1st January, 2010 to 31st December, 2013.The hospital is situated in the eastern zone of Tanzania, about 1 Kilometre north of Morogoro municipality. It is the referral hospital for the whole region. The hospital has a bed capacity of 500 and serves about more than 15,000 patients per year. According to the 2012 national census, the region had a population of 2,218,492 with the region's 2.4 percent average annual population growth rate. It was also the 22nd most densely populated region with 31 people per square kilometre [8].

3.2 Study design

A comparative retrospective study of patients who were screened for cervical cancer disease by visual inspection using acetic acid or Lugol's iodine at the hospital, according to their HIV status and followed up for further pathological diagnosis for those who were referred to a tertiary hospital.

3.3 Data Source

Extraction of information from the original patients' files and other log books at the MRRH medical records was done with the assistance of the hospital gynaecologist and his team who did the screening. Women came to the health facility spontaneously as a result of the cervical cancer awareness campaign and others were followed in the HIV clinic, gynaecology clinic and outpatients' clinics. The following information was sorted out from the files: Socio-demographic characteristics, age at screening, cervical appearance at screening, treatment, referral status and outcomes, HIV status, HAART usage and CD4 T cells count if previously known case of HIV/AIDS, method of screening used, age at menarche, age at sexual debut and first delivery, marital status, education level, occupation, contraceptive history and parity [19].

3.4 Ethical Considerations

The ethical clearance was requested and permission was sought from the management of MRRH. Confidentialities of screened women were highly considered. The obtained information will be used for the approved research purposes only.

3.5 HIV 1/2 rapid test

Tanzania HIV rapid test algorithm for detection of HIV antibodies was used as a standard operating procedure [48]. Test one used SD-Bioline™ [49]HIV rapid test using venous blood either whole blood or serum as a sample[50]. If the results were non-reactive by seeing one

clear red band on the SD-Bioline™ [49], it was reported negative for HIV test[48]. On the other hand, if the results were reactive by seeing two or three separated bands on the SD-Bioline™ [49], then test two using Alere Determine™ [51]. HIV rapid tests were carried out for further evaluations [50]. Furthermore, if the results were reactive by seeing two separate red bands on the AlereDetermine™ [51]rapid test, it was confirmed positive for HIV test [48].

In addition, if the results were negative using Alere Determine™ [51] HIV rapid test by seeing only one red band[50], further evaluations using test three,Uni-Gold ™[52]HIV rapid test were carried out [48]. Moreover, if the results were non-reactive using Uni-Gold™[52]by seeing only one red band[50], the final report was negative for HIV test [48], and if the results were reactive by seeing two separate red bands using Uni-Gold™[52], the final report was positive for HIV test [48].

3.6 Visual Inspection Methods VIA and VILI

Visual inspection with acetic acid (VIA) involved naked-eye inspection of the cervix under bright light conditions at least 1 minute after the application of 3-5% diluted acetic acid. The screening tests were carried out by a gynaecologists or trained medical doctors, nurses and midwives [33].

A positive result was based on the appearance of well-defined, acetowhite areas in the transformation zone [16, 33]. This is the region of the cervix that undergoes metaplastic change from columnar epithelium to squamous epithelium [24, 33]. Reported sensitivity of VIA ranges from 52% to 79% and specificity of 49% to 88%, which are similar to those for cytology. However, VIA is inefficient in detecting lesions located in the cervical canal of the uterus [33].

Visual inspection with Lugol's iodine (VILI) used Lugol's iodine solution applied to the cervix and then it stained glycogen stored in cervical epithelial cells [16, 24]. Neoplastic and immature squamous metaplastic epitheliums have less glycogen than the normal mature squamous epithelium and so did not turn mahogany brown. Instead they appeared as mustard yellow changes, easily recognizable as the acetowhite changes associated with VIA [24, 33]. Sensitivity has been reported to vary from 78% to 98% and specificity of 73% to 91% [24].

Both VIA and VILI have an added advantage of giving immediate results [24, 33]. These two methods of visual inspection have been shown to be feasible as a primary means of screening for cervical cancer in low income settings and can be used by well trained nurses [33].Because of organizational constraints and reported high rates of loss to follow up, strategies aiming at screening and treatment in the same visit ('see and treat') had been

proposed [24]. Effectiveness and acceptability have been evaluated in several studies and found to be good [24, 33].

3.7 Small Biopsy Specimens

Small loop biopsies were performed mainly for pre invasive lesions, but occasionally early invasive carcinomas if highly suspicious were identified [53].Wedge biopsies are usually performed for the confirmation and typing of tumours [54] but in this study it was not performed due to lack of colposcopy device. Fixation in eosin-tinted formalin to facilitate their preservation and identification were performed and all of the biopsy fragments were equally processed to ensure there were no fragments lost [55].

The request forms incorporated the macroscopic description of the specimens and identify the areas of the cervix from which the biopsy has originated, i.e. ectocervix, endocervix, or transformation zone [56].When a biopsy failed to reveal the source of the abnormal cells, it was clearly stated in the request form in order for the pathologist to note and to differentiate between a biopsy that was technically adequate but fails to identify a lesion, and a biopsy that was technically inadequate [55]. If invasive disease was suspected on the basis of the gynaecological examination [57], further levels were advised to examine the patient and referral was considered.

Clinical information required on the specimen request form included full patient details, the date biopsy taken, cervical screening history (if available), clinical appearance of the cervix, site of the biopsy and the results of previous biopsies if any.

3.8 Treatment of Cervical Lesions and Cervical Cancer

Treatment of precancerous lesions was by either ablative method, the most common being cryotherapy, or excisional methods, such as cone biopsy and LEEP[19]. Unlike LEEP, cryotherapy does not avail a biopsy sample, and thus it was not possible to know if the whole lesion has been destroyed [24].Cryotherapy is easier to use and can be performed by nurses, making it more applicable in low income settings [33].However, it may not be enough to treat all lesions detected, for example, when the entire squamocolumnar junction cannot be visualized, when the lesion is too large for the cryotherapy probe to cover in one application, if the lesion extends into the endocervical canal and when there is severe cervical atrophy [14, 18, 33].

Treatment of cervical cancer is dependent on the stage of the disease, age and medical state of the patient, tumor characteristics, patients' preferences and resources within the health sector of each country [36]. Options can be monotherapy or combined, they range from conisation of the cervix, simple hysterectomy with or without lymphadenectomy, radical

hysterectomy with pelvic lymphadenectomy, pelvic exenteration, chemotherapy, radiotherapy with palliative chemotherapy [24].Treatment at an early stage has the best prognosis with the highest cure rates [7].

One disadvantage of 'see and treat' screening strategy is overtreatment which has been reported to range from 1.2 to 83.3% [33].This is likely to be worse in see and treat strategies without a colposcopy before treatment [24]. Colposcopy has been evaluated and could be an added asset in improving the effectiveness of 'see and treat' programs [24, 33].

The treatment of invasive cervical cancer continues to be a major challenge in many sub-Saharan African countries, due to the lack of surgical facilities, skilled providers and radiotherapy services [58]. Management of women with invasive cervical cancer requires a multidisciplinary approach, including: gynecologists, radiation oncologists, medical oncologists, pathologists, medical physicists, technicians, nurses and counsellors [24, 58]. As the pelvic tumour bulkiness increases the proportion of patients with disease recurrence in the pelvis as the only site of treatment failure increases than the proportion of developing distant metastases [58].

3.9 The Operational Definitions

1. Exclusion criteria; Patients who were out of the study time frame and those with unknown HIV status.
2. Live: patients who were alive at the end of treatment or after referral and at the end of the study follow-up [19].
3. Dead: patients who died during or at the end of cervical cancer treatment and at the end of the study follow up [19].
4. Recurrence: This is defined as the occurrence of the disease symptoms and clinical signs six months after optimal treatment. Any occurrence before six months was regarded as persistence of the disease[19].
5. Small precancerous lesion: if the entire lesion was visible, the squamo-columnar junction was visible, and the lesion was not cover more than 75% of the ectocervix [59].
6. Extensive/large lesion: if the lesion extends beyond the cryoprobe being used, or extends more than 2 mm into the cervical canal or into the vaginal wall [59].
7. Suspicious of cervical cancer; Any abnormal mass around the cervical os or a cauliflower-like growth or an ulcer; fungating mass with postmenopausal bleeding, post coital bleeding, vaginal bleeding, nonspecific vaginal bleeding, abdominal pain with foul smelling vaginal discharge, severe weight loss, obstructive uropathy, or ascites in the 12 months prior [19, 60]

3.10 Follow-up of Women after Treatment

Follow-up of patients who had cryotherapy or LEEP was passive, in that they were encouraged and expected to return to the clinics if they experienced symptoms such as fever for more than 2 days, severe abdominal pain, heavy bleeding unrelated to menses, or bleeding with clots[19].Clients were given appointment cards to remind them about the next appointment. Those who had a biopsy taken during LEEP treatment were scheduled to return of their results after diagnosis at a tertiary hospital. Those who were VIA/VILI negative were advised to seek re-screening after 3 years, except for HIV positive women and those with positive VIA/VILI results who were scheduled to return yearly [19]. All women who were suspicious of cervical cancer disease were referred to a tertiary hospital for further pathological evaluations[19].

3.11 Data Management and Statistical Analysis

The record audits were done from the data logbook with follow up details of all patients' information and other details were noted from the original files, coded and then entered into the SPSS^{TM} computer program version 20 (SPSS Inc.Chicago, Illinois) to create a data base.Further analysis of the coded data base and categorical comparisons were performed with chi-square test and fisher's test on different occasions.Test statistics were evaluated with a significance level of $p<0.05$.

CHAPTER 4

4.0 RESULTS AND DISCUSSION

4.1 Results

A total of 536 women were screened for cervical cancer using VIA or VILI methods at MRRH for a period of 3 years. The baseline characteristics of these women according to their social demographic status are summarized in Table 1.

Table 1: Socio-demographic characteristics of study subjects (N=536)

Parameter	Characteristics	n	%
Age Groups	21-45	416	77.6
	46-70	114	21.3
	≥ 71	6	1.1
Marital status	Married	298	55.6
	Widowed/Single	170	31.7
	Divorced/Separated	68	12.7
Occupation	Business	171	31.9
	Peasant	129	24.1
	Employed	123	22.9
	Farmer	65	12.1
	Student	17	3.2
	Others	31	5.8
Education level	Non Formal	197	36.8
	College	115	21.5
	University	102	19
	Secondary	70	13.1
	Primary	52	9.7
Parity	0-3	360	67.2
	4-7	145	27.1
	≥8	31	5.2
Age of sexual debut	13-16	248	46.3
	17-20	225	42
	≥21	63	11.7

Table 1 shows the socio-demographic features of the study population. The age of the study population ranged from 21 to 79 years, with a median age of 39 years (± 10.5SD); majority of the study subjects 416 (77.6%) were in the age group 21-45 years. Most of the patients, 171(31.9%) were doing small scale businesses while majority 197 (36.8%) had no formal education. In addition, 360 (67.2%) women had a number of children from 0 to 3 while 31 (5.2%) women had equal or more than 8 children. Of all women screened, 248 (46.3%) had the age of sexual debut between 13 and 16 years.

Women who were suspected for ICC were all referred to a tertiary hospital and underwent further pathological investigations. Among the 121 women with positive cervical visual inspection with VIA or VILI, extended/large lesions were found in 52.5% of HIV-positive women and 25% of HIV-negative women (Figure 4). The prevalence of cervical precancerous lesions in HIV positive women was 55.5%, which was higher than the 14.7% found in HIV negative women. Cervical cancer screened positive results according to the age groups of all screened women are summarized in (Figure 5).

In multivariate analysis, an association was found between the HIV positive status and the VIA/VILI positive results ($p<0.001$, OR 9.7, 95% CI 5.6-16.8).In addition, there was an association between the HIV status and suspicious of cervical cancer disease ($p<0.001$, OR 3.4, 95% CI 1.7-6.8).There was no statistical association found between HIV status and late cervical cancer stage ($p >0.05$) (Table 2).

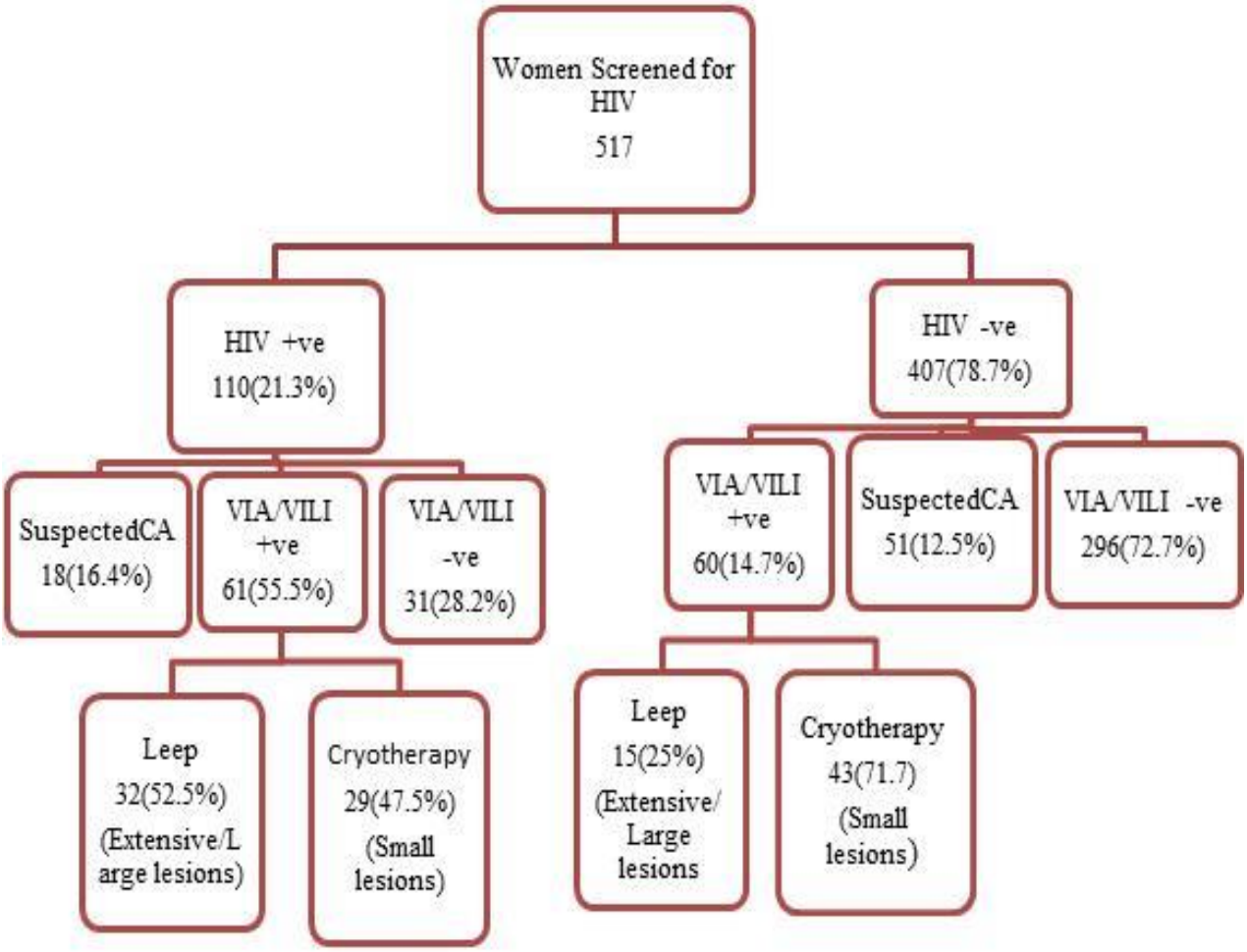


Figure 4: A flow chart summary showing all screened women distributed according to their HIV status, VIA/VILI results and methods of treatment used. (19 women with unknown HIV status were removed).

Figure 4 depicts the flow chart summary of the screened women according to their HIV status, VIA/VILI results and the method used for the treatment of precancerous lesions. Of all screened women, 110(21.3%) were HIV positive and 407(78.7%) were negative for HIV. Among HIV positive women, 61(55.5%) had VIA/VILI positive results. Moreover, in HIV positive women, 32(52.5%) had extensive precancerous lesions and were treated with LEEP while 29(47.5%) had small precancerous lesions and were treated with cryotherapy procedure.

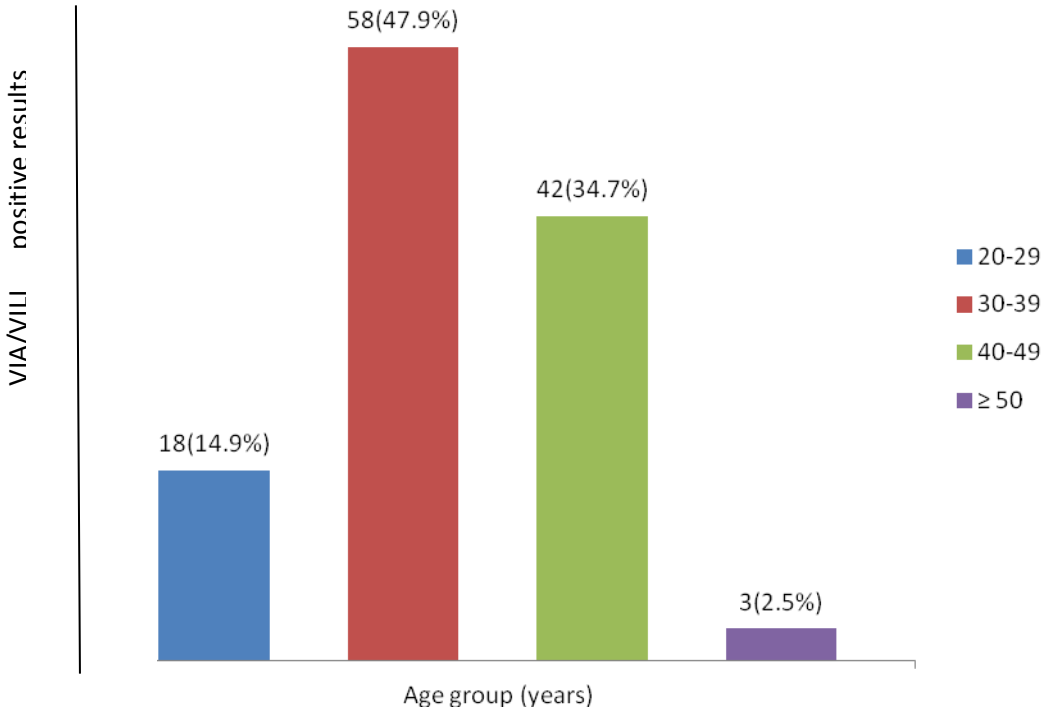


Figure 5: Cervical cancer screening positive results distributed according to the age groups of all screened women.

Figure 5 shows the VIA/VILI positive results according to age groups. Of all VIA/VILI screened positive women, 58(47.9%) were in the age group between 30 and 39 years and 42(34.7%) women were in the age group between 40 and 49 years.

Table 2: Summary of the VIA results, treatment used, HIV status and cervical cancer status with the p-values, confidence intervals and Odds Ratios

	HIV status				
	HIV negative (%)	HIV positive (%)	P[a]	C.I	OR
VIA results					
VIA/VILI negative	296(72.7)	31(28.2)	Ref.		
VIA/VILI positive	60(14.7)	61(55.5)	<0.001	5.6-16.8	9.7
Suspected cancer	51(12.5)	18(16.5)	<0.001	1.7-6.8	3.4
Cervical cancer stage					
No cancer	14(38.9)	22(61.1)	Ref.		
Stage I&II	24(68.6)	10(28.6)	0.0078	0.09-0.8	0.27
Stage III&IV	9(81.8)	2(18.8)	0.0127	0.02-0.88	0.14
Cervical Appearance					
Normal	299(73.5)	36(32.7)	Ref.		
Cervicitis	4(0.98)	2(1.8)	0.0814	0.51-27.7	4.2
Pre-cancerous lesions	53(13)	54(49.1)	<0.001	4.91-14.62	8.5
Suspected cancer	51(12.5)	18(16.4)	<0.001	1.47-5.81	2.9
Treatment used					
No treatment	349(83.9)	49(11.8)	Ref		
Cryotherapy	43(71.7)	29(47.5)	<0.001	2.65-8.71	4.8
Leep	15(25)	32(52.5)	<0.001	7.32-31.88	15.2
	Age at screening				
	21-45 years	>45 years			
Positive results					
VIA/VILI negative	273(65.4)	70(61.4)	Ref.		
VIA/VILI positive	107(25.9)	14(12.3)	0.0276	0.26-0.97	0.51
Suspected cancer	36(8.7)	30(26.3)	<0.001	1.81-5.85	3.25
	CD 4 T-cells count				
	CD4 ≥300	CD4 <300			
VIA/VILI negative	21(67.7)	10(32.3)	Ref		
VIA/VILI positive	36(59)	22(36.1)	0.5952	0.47-3.57	1.28
Suspected cancer	3(16.7)	7(38.9)	0.0352	0.86-31.07	4.9
	Parity				
	0-3 Children	>3Children			
VIA negative	245(68.1)	99(56)	Ref.		
VIA positive	85(23.6)	36(21)	0.7465	0.67-1.73	1.08
Suspected cancer	30(8.3)	40(22.7)	<0.001	1.89-5.79	3.3
	Age at sexual debut				
	≥21	< 21			
VIA/VILI negative	155(56.4)	156(56.4)	Ref.		
VIA/VILI positive	70(25.5)	119(43.3)	0.005	1.15-2.49	1.69

*Fisher exact test was considered in cells values less than 5

*All cases with unknown status and none confirmed cervical cancer diagnosis were disregarded

Table 2 shows the summary of the findings in comparison with the p-values,confidence intervals and odds ratios. Apart from HIV positive serostatus, other more common risk factors for having positive VIA/VILI test or suspicious of cervical cancer found in our study were early age at sexual debut (OR 1.69, 95% CI 1.15-2.49, p=0.005), high parity [61] (OR 3.3, 95% CI 1.89-5.79, p <0.001), age above 45 at screening [60, 62] (OR 3.25, 95% CI 1.81-5.85, p< 0.001) and CD4 T-cells count of less than 300 cells/mm^3 (OR 4.7, 95% CI 0.86-31.07, p =0.0352).

Table 3: Summary of the study objectives and the findings

Objectives	HIV positive	HIV negative
Prevalence of precancerous lesions (%)	55.5(n=110)	14.7(n=407)
Prevalence of exensive/large precancerous lesions(%)	52.5(n=61)	25(n=60)
Prevalence of Invasive Cervical cancer(%)	35.3(n=34)	70.2(n=47)
Risk factors for the extensive cervical lesions and suspicious of cancer	Low CD 4 T-cells count,high parity,early age at sexual debut	Early age at sexual debut,high parity,age at screening

Table 3 shows the summary of findings, according to the study objectives.The prevalence of precancerous lesions was greater in HIV positive women than in HIV negative women.Moreover, the prevalence of extensive cervical precancerous lesions was twice in HIV positive women compared to HIV negative women.However,the prevalence of invasive cervical cancer is lower in HIV positive women than in HIV negative women.

4.2 Discussion

The present study provides the first comparative retrospective analysis on the influence of HIV/AIDS on the precancerous lesions and ICC between HIV positive and HIV negative women in Morogoro region, primarily using VIA or VILI screening and then pathological evaluations after referral to a tertiary hospital. Considering the spread of HIV epidemic among women in Tanzania [25], an increase in cervical abnormalities could be expected, and our observations support the policy of integrating routine screening of HIV infected women for cervical cancer in all hospitals in the country.

The prevalence of cervical precancerous lesions was 55.5% in HIV positive women and only 14.7% in HIV negative women.This shows and supports the fact that HIV positive women develop more cervical precancerous lesions compared to HIV negative women ($p<0.001$, OR 9.7, 95% CI 5.6-16.8).This finding reinforces the previous evidences adding results from a different study population to the current existing similar findings.
Our findings on the prevalence of precancerous lesions are higher than the previous studies done in Tanzania so far.In the study done in HIV positive women in 2013 by Kafuruki et al [63], found the prevalence of precancerous lesions by 26%, Mwakionja et al [64] found the incidence of precancerous lesions by 38.3% in HIV positive women and 34% in HIV negative women while Balandya et al [65], found a prevalence of precancerous lesions by 42.2% in HIV positive women. Alltogether, these results show that the impact of the cervical precancerous lesions is increasing in HIV positive women in different study populations with time.

The high prevalence of cervical precancerous lesions in the present study could also be explained by the fact that our study used lower CD4 count up to 200 cells/mm^3 compared to others who used CD4 count of 351 cells/mm^3 [64, 65] or 450 cells/m^3 [63]. The lower CD4 cell count and advanced stage of HIV could be responsible for the higher prevalence of cervical precancerous lesions seen in our study [65, 66]. Kafuruki et al [63], however,found a reduced prevalence of cervical precancerous lesions from 26.8% to 16% when further proved by cytology.This might be due to high sensitivity and overdiagnosis hence overtreatment of VIA/VILI screening in the detection of cervical precancerous lesions [60, 62, 67]. The same effect like this has been seen also in a study done in HIV positive women screened initially by VIA and later by cytology in Cambodia by Raguenaud et al [68].

In the present study,also the prevalence of extensive/large cervical precancerous lesions was twice in HIV positive women compared to HIV negative women [64, 69]. In Africa, a high prevalence of CIN of 76% among HIV infected women has been observed in Lusaka, Zambia [70, 71]. The probable explanation could be HIV induced immune-suppression as

the CD4 count was as low as 165 cells/mm^3 compared to our study of which the lowest baseline CD4 count was 200 cells/mm^3.

However, the prevalence of invasive cervical cancer was the reverse, lower in HIV positive women than in HIV negative women (Table 3).This might be probably due to long duration of disease development as it takes 10 to 15 years from precancerous lesion to develop into ICC.Therefore, for a screening program like this and others, it would be very difficult to find a high prevalence of the ICC in screened women unless a clear follow up of positive women could be done in 10 to 15 years which is very difficult in normal settings. For instance,in a HIV clinic based case control study conducted by Mwakigonja et al [64] found the prevalence of the ICC was higher in HIV positive women (5.8%) and lower (2%) in HIV negative women but the p-value was not statistically significant (p=0.6). Furthermore, they used cases in HIV clinics and many of the HIV positive patients had been at the clinics for more than 25 years on HAART compared to the controls who were just HIV negative from the general population in a cervical cancer screening program like ours.

The pathology results of invasive cervical cancer seen in this setting emphasize the importance of introducing a continuous screening program and cancer registry for cervical cancer. Despite of being at risk of cervical cancer, none of the participants in this study had previously been screened for the disease. This is a major concern in many developing countries, including Tanzania, where continue cervical cancer screening is largely unavailable, leading to late presentation with advanced cervical cancer disease [60, 72, 73]. In addition, the comparison with other similar studies reported from different study populations in the world (Table 4) found out that the prevalence and incidence of precancerous cervical lesions were higher in HIV positive women than in HIV negative women.

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Aims	Our study (Tanzania)		Mwakigonja et al (Tanzania)		Horo et al (Ivory coast)		van Bogaert (South Africa)		Moodley et al (South Africa)		Chalermchoclecharoe et al (Thailand)	Park et al, et al (Korea)		Salascrabuddhe et al (India)	Teixeira et al (Brazil)	Liu et al et al (China)		Isaakidis, et al (India)	Raguenaud et al (Cambodia)
HIV Status	+	-	+	-	+	-	+	-	+	-	+	+	-	+	+	+	-	+	+
Incidence of Precancerous lesions(%)	-	-	38.3	34	9	3.9	23.1	14.3	59.9	31.6	37	18.3	1.8	10.9	-	-	-	17	-
Prevalence of Precancerous lesions(%)	55.5	27.3	-	-	-	-	-	-	50	13	15.4	30	4.9	-	23.6	23	11	16	17
Extended Precancerous lesions(%)	52.5	25	-	-	15	-	-	-	-	-	68.7	38.9	0	16.1	6	30	6	5	15.5
Prevalence of ICC(%)	26.7	50.8	5.8	2	2.3	15	30.6	57	13.6	-	-	-	-	0.3	0.1	-	-	2.1	2.1

*In HIV status column, + means positive for HIV test and - means negative for HIV test

Table 4 shows the comparison of the findings with other 11 similar studies according to the objectives of the current study.The present study has similar findings with van Bogaert et al [74] and Horo et al [69] in terms of prevalence of the ICC being higher in HIV negative women than HIV positive women.

Comparing our findings with Mwakigonja et al [64], Kafuruki et al [63] and Balandya et al [65] all done in Tanzania as well, it shows that the incidence of precancerous lesions were higher in HIV positive women while decreased in HIV negative women [47, 75].This further shows that generally the amount of new cases of women with cervical precancerous lesions are increasing in Tanzania.It has been hypothesized that since more women with HIV may survive longer due to access and use of HAART therefore the incidence of cervical cancer is likely to be increased in the HIV epidemic areas [74, 76].

In a study done in the USA in 1994, after 6 months of follow-up, cytology and biopsy confirmed that pre-invasive cervical squamous intraepithelial lesions (CIN) were detected in 13% of the 398 HIV-infected women, compared with 4% of the 307 uninfected women [77]. In the same study, high-grade SIL (CIN II, III) was detected in 7% of the HIV infected group and in 1% of the uninfected group. However, it is difficult also to ascertain the actual incidence of cervical cancer in HIV infected women in HIV endemic countries where the burden of cervical cancer is higher, but cancer registries for proper documentation, data source and follow up is still very scanty [75, 78] ,this was one of the obstacles we faced in our study.

Of the numerous cervical cancer screening studies that have been performed among HIV infected women in industrialized settings, most have found significantly higher rates of pre-invasive disease when compared to HIV negative women [7, 47, 70].For example in Pune, India, where 1,044 incident cases of cervical cancer were reported during 1996–2000, the highest prevalence of 33% of HPV 16/18 which are high risk HPV genotypes known to cause cervical cancer was found in HIV infected women [73, 79].

However, so far the incidence of cervical cancer among HIV infected women in the industrialized world has been unchanged since the introduction of HAART [80, 81]. The incidence of HPV induced diseases has increased rather than decreased since the introduction of HAART [75, 82]; HAART restores the immune response to AIDS-defining opportunistic infections such as Cytomegalovirus and Kaposi's sarcoma associated virus; 25% of the HIV infected CIN1 subjects on HAART still progressed to CIN2+, and HIV-infected patients with CIN2+ often do not show regression when treated with HAART [75,

80, 83]. Moreover, currently there are no data available to shed light on the effect of HAART on the incidence of invasive cervical cancer in HIV positive women [75, 84].

Regression of CIN1 and CIN2+ depends on the type of HPV (oncogenic or not) rather than on immunocompetence [85]. The longer survival of HIV infected patients related to HAART may have a proportionally greater impact on the risk of HPV related cancers than the partial reversal of immunosuppression that occurs with HAART [41, 68, 83].

In the present study, the duration since HIV diagnosis was not determined, in contrary to a study done by Kreiss et al [86] which showed that women living with HIV infection for more than two years were more likely to carry high risk HPV infections, which could result in CIN and hence ICC, compared to women who had been HIV infected for less than a year.

In comparison (table 4), a study done in Ivory coast by Horo et al [69] had the lowest findings on the incidence of precancerous lesions in both HIV positive (9%) and HIV negative (3.9%) although the incidence was almost doubled in HIV positive women. Another study by Moodley et al [18] , done in South Africa, reports the highest incidence of precancerous lesions in both HIV positive women (59.9%) and HIV negative women (31.6%).This might be due to more cervical cancer endemic study population, study design and sample size in their study compared to others. The second higher incidence in our comparison (table 4) was observed in a study done in Thailand by Chalermchockcharoenkit et al [87] which found an incidence of cervical precancerous lesions by 37% although only in HIV positive women.

In our study, in Tanzania, the prevalence precancerous lesions is very high if compared to the studies by Moodley et al [18] reported in South Africa, Park et al [88] reported in Korea ,Teixeira et al [89] reported in Brazil and Chalermchockcharoenkit et al [87] reported in Thailand. However, all show high prevalence of precancerous lesions in HIV positive women.The high prevalence of precancerous lesions in HIV positive women in the present study shows that the lesions are widespread in the population and there is a risk factor that plays a role in the high findings which might be the HIV infection although any other confounding factors like early age of the debut, very low CD4 count used and presence of high risk HPV genotypes might add an effect. Also, this indicates that invasive cervical cancer is a chronic disease since it takes longer time to develop [74] that might be why we have found a high prevalence of cervical precancerous lesions in HIV positive women but low prevalence of ICC in the same study population. Another reason might be due to the fact that we have conducted our study in a very high HIV endemic area in the region [19].

Another important comparative observation noted was in a study reported in South Africa by Moodley et al [18] where they got high incidence (59.9% to 31.6%) of cervical precancerous lesions, but low prevalence (50% to 13%) in both HIV positive and HIV negative women,respectively while in our study we have got high prevalence (55.5% to 14.7%) of precancerous lesions, in HIV positive and HIV sero negative women, respectively.This could be explained probably due to extensive development of well organized cancer registries and continue cervical cancer screening and treatment of precancerous lesions present in South Africa compared to the lack of these services in Morogoro,Tanzania hence there are accumulations of precancerous lesions in both HIV positive and HIV negative women. The implementation of cancer registries and continuous screening and treatment of cervical precancerous lesions in women should be invented in Tanzania especially in Morogoro region so as to reduce the observed high prevalence.

According to our study, HIV positive women were 15.2 times more at risk to develop extended/large precancerous lesions ($p< 0.001$, 95% CI 7.3-31.9) which keeps them in a very high risk to develop invasive cervical cancer if they are also co-infected with a high risk HPV [12]. In a similar study reported in Ivory coast by Horo et al [69] found the risk of developing extended/large precancerous lesions was 2.28 times more ($p< 0.001$, 95% CI 1.6-3.3) in HIV positive women than in HIV negative women.This risk is low compared to our study findings and different factors may account for this observation, such as study population, sample size, HAART use and amount of CD4+ T-cell count used [74].

In Thailand, Chalermchockcharoenkit et al [87] found the prevalence of extensive precancerous lesions in HIV infected women was 68.7% which is very high compared to 52.5% in our findings.This might be due to the fact that they used lower CD4 count. For example, more than 230 (28.9%) women had CD4 count less than 200 cells/mm^3.This also was observed in a study reported in Dar Es Salaam,Tanzania by Kapiga et al [72] where they found that the risk of SIL was significantly increased among women with CD4+ cell count of less than 200 cells/mm^3 ($p<0.001$, OR 6.15, 95% CI 1.19-41.37).The relationship between HIV and cervical cancer is unique in that women at risk of both conditions share common socio-behavioral patterns, such as early onset of sexual intercourse, high number of sexual partners and smoking [75, 90].

In our study, the prevalence of invasive cervical cancer was almost doubled in HIV negative women (50.8%) compared to HIV positive women (26.7%) . This finding is similar to the study done in South Africa by van Bogaert [74] in which the prevalence of the ICC was 30.6% in HIV positive women and 57% in HIV negative women.One hypothesis is that HIV infected women might die early from HIV-related opportunistic infections before the period of time needed for development of invasive cervical cancer. In another study reported in China,

by Chan et al [91], there was no patient with newly diagnosed cervical cancer found to have HIV infection by screening. In addition, Chan et al [91] and Branca et al [92] both found no cervical cancer patient who was found to have undiagnosed HIV through screening in their study, in which all HIV cases were previously identified as HIV positive before the diagnosis of cervical cancer or precancerous lesions, findings that are different with our study. The reason might be due to different study design, study population and the study area.

ICC has been recently included among AIDS-defining conditions, even though it has not significantly increased in the HIV positive female population [76, 90], contrary to what happens to other tumours such as KS and NHL [75, 83].The incidence of invasive cervical cancer is expected to increase over time due to the fact that HIV infected women will survive long enough to exceed the latency time of these tumours [75, 83, 90].

For instance, in the study by Kahesa et al [90] reported in Tanzania, it was concluded that HIV-1 is associated with ICC ($p<0.001$). However, as previously mentioned, in this way, a careful surveillance is needed because of a significant increase in pre-neoplastic lesions and survival improvement among HIV infected women in the last decade [93, 94]. For example, in an American series study, 16 of 84 (19%) patients with invasive cervical carcinoma were HIV seropositive, of which 14 (85%) were asymptomatic for HIV infection according to current CDC definitions [83].

Another example similar to our findings is published in a report from western Kenya from a population study of 4308 HIV infected women who underwent cervical cancer screening , the prevalence of the ICC was only 1.3%, with the majority having had stage IA1 disease at the time of diagnosis [95]. On the other hand, as mentioned earlier, a study done also in Tanzania by Mwakigonja et al [64] found the reverse of ours as the prevalence of the ICC was 5.8% in HIV positive women and 2% in HIV negative women, although the p-value was not statistically significant [64].This might be explained further as due to the small sample size, they used only170 women compared to our study sample size which was 536 women. Also they conducted their study in the HIV clinics only which could lead to a bias since the women were known HIV positive patients for long periods.

Again, another study reported in Ivory coast by Horo et al [69], found the similar results to ours as the prevalence of the ICC was 2.3% in HIV positive women and 15% in HIV negative women. This might suggest that immunosupresion from HIV infection is not the only determinant of invasiveness of cervical cancer development, hence the involvement of the ICC as ADIs is still debatable [74]. According to van Bogaert et al [74],data suggest that immunosuppression may lead to higher levels of HPV replication with resulting higher HPV DNA levels and increased incidences of LGSIL and HGSIL. However, there have been no

similar findings in ICC; cellular genetic changes rather than immunosuppression may be the driving force of progression into ICC.

Moreover, as HIV infection is much more common among young people, one could expect an earlier onset of the process of cervical cancer in this group [66]. For example, in our retrospective study, we found one woman with stage II cervical cancer disease at the age of 28, however, this is the first youngest case ever to be reported from Tanzania to date. Our findings are similar to those of Lomal-isa et al [96] and Gichangi et al [4] who reported that HIV seropositive women in South Africa and Kenya, respectively presented with invasive cervical cancer 10 years earlier than HIV negative women.

Immunosuppression is strongly associated with preinvasive cervical lesions, but the progression to the ICC is not solely associated with immunosuppression [74]. In HIV-infected women study by Harris et al [42] found the hazard ratio of developing any preinvasive lesion was 1.2 if the CD4+ T-cell count was >500/μL, which was similar to HIV negative controls. On the other hand, a Zimbabwean study, however, concluded that only HR HPV, and not HIV, was associated with CIN [97]. The rate of ICC in women with AIDS might be unrelated to immunosuppression only and therefore, not biologically associated with HIV [41].

The reversal of immunosuppression following HAART would have a dramatic effect on the natural history of cervical preinvasive lesions [98], but data on the effects of HAART in this regard are mixed [30, 99]. The increased risk of HIV infected women to CIN2 may be due to different factors such as high exposure to risk factors like coitarche, number of sexual partners, oral contraceptive use, a direct effect of HIV or a molecular interaction between HIV and HPV [92, 100].

The study of HPV induced cervical neoplasia in HIV infected women is complex and is riddled with contradictory reports [74].For instance, it has been reported, as already mentioned, that the incidence of cervical cancer has not decreased in the HAART era and that clinical research has not shown a clear benefit of HAART (Figure 6) in decreasing HPV related cervical disease in HIV infected women [99, 101].

However, contrary to Hawes et al [102], no impact on the development of SIL was evidenced for CD4+ T-cell counts by Heard et al [103], although in our study, we observed the increase of cervical precancerous lesions in HIV positive women with CD4 T-cell count of less than 300 cells/mm^3 (p=0.0352, 95% C.I 0.86-31.07, OR 4.9).

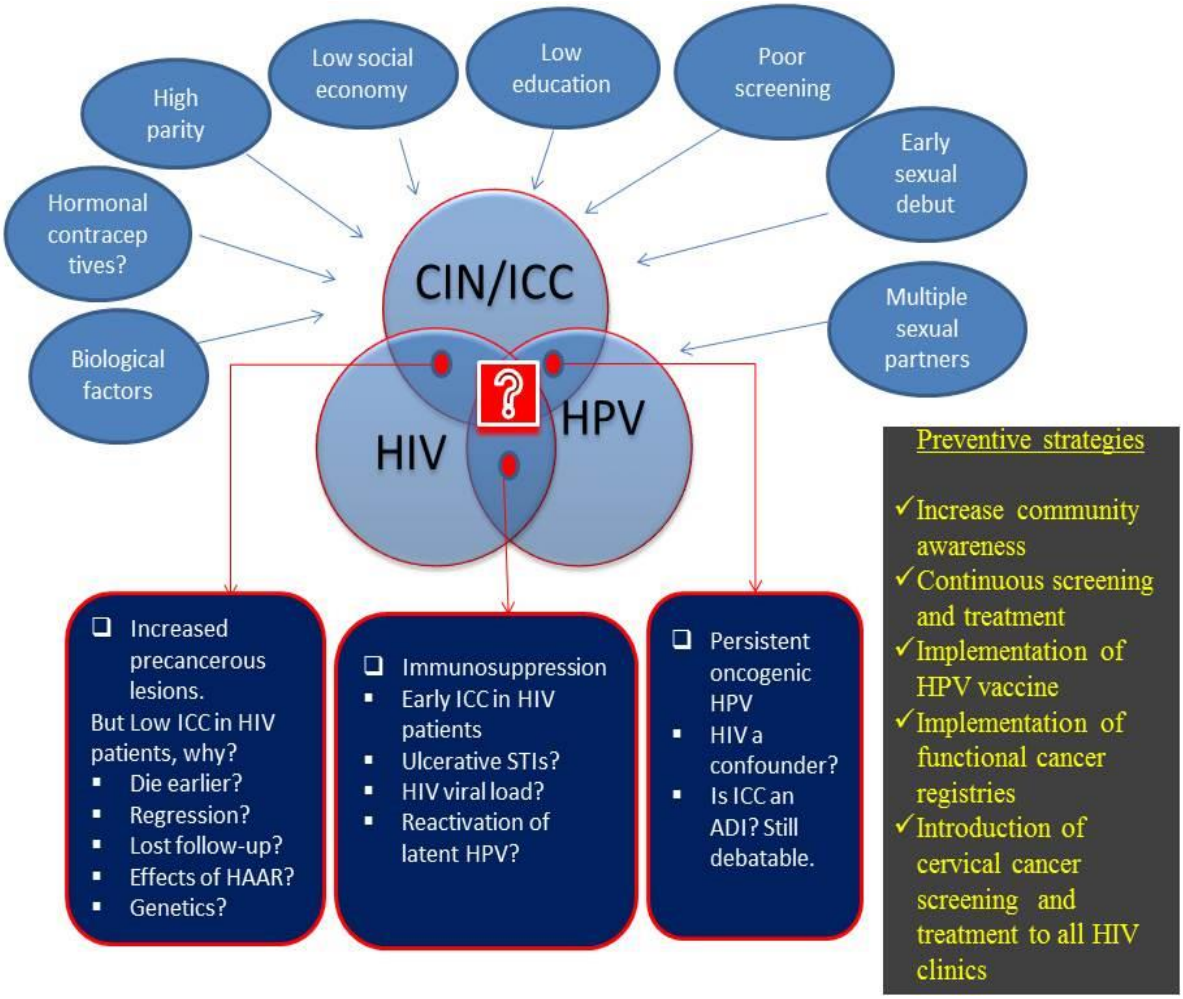

Figure 6: A diagram to summarize the findings and further suggestions

Figure 6 depicts the relationship between HIV, HPV infection and the cervical precancerous lesions and cervical cancer with the risk factors which contribute to the development of the interaction. The actual intersection between the two co-infections and the development of the ICC is still questionable with a lot of schools of thoughts from many studies. For instance, van Bogaert et al [74] clearly questioned the involvement of ICC among acquired immune deficiency syndrome-defining illnesses.

Human papillomavirus (HPV), the necessary cause of cervical cancer, is endemic in Africa [104]. Although our study did not evaluate the involvement of HPV subtypes in the precancerous lesions, it has been isolated in most cases of precancerous lesions and cervical cancer in all parts of the world. Epidemiological studies have shown that the association of genital human papilloma virus with cervical cancer is strong, independent of other risk factors, and consistent in several countries [105].

HPV 16/18 is estimated to account for 70% of all cervical cancers worldwide, although the estimated HPV 16/18 fraction is slightly higher in more developed (72–77%) than in less developed (65–72%) regions [106]. About 41–67% of high-grade squamous intraepithelial lesion (HSIL), 16–32% of low-grade squamous intraepithelial lesion (LSIL) and 6–27% of atypical squamous cells of undetermined significance (ASCUS) are also estimated to be HPV 16/18 positive, thus highlighting the increasing relative frequency of HPV 16/18 with increasing cervical lesion severity [106].

Many of the factors that increase both HPV acquisition and promote the oncogenic effect of the virus are also very widespread in Africa [107]. These include: early marriage, polygamous marriages and high parity. Polygamy is reported to increase the risk of cervical cancer two-fold and the risk increases with increasing number of wives [108]. This is another part of the male factors in addition to prostitution and circumcision that leads to the high prevalence of HPV in Sub Sahara Africa including Tanzania [109].

High parity, which is the norm in some cultures in Africa, is also a recognized HPV-related co-factor for the development of cervical cancer [110].The prevalence of HPV has been shown to be higher in uncircumcised men than in circumcised men [109]. In a study to investigate the association between male circumcision (MC) and high risk human papilloma virus (HR-HPV) prevalence, Auvert et al [111] using urethral swabs collected during a period of 262 consecutive days among participants from the intervention (circumcised) and control (uncircumcised) groups who were reporting for a scheduled follow-up visit reported that HR-HPV prevalence among intervention and control groups were 14.8% (94/637) and 22.3% (140/627),respectively. Further, the multiple HR-HPV prevalence was significantly lower among men of the intervention group compared with men of the control group, 4.2% and 9.9%, respectively.

Male circumcision was strongly associated with lower cervical cancer rates and fewer HIV cases, independent of religion [112]. Furthermore, in the same study, male circumcision was independently associated with HIV among countries with primarily heterosexual HIV transmission, and not among countries with primarily homosexual or injection drug use HIV transmission ($p<0.001$). All these findings suggest that male circumcision is a true protective factor that reduces the sexual transmission of HIV and possibly HPV [112].

Socio-economic factors, worldwide women of low socio-economic status have a greater risk of having cervical cancer [113]. Cervical cancer is often referred to as a disease of poverty

and of poor women. A recent study in Mali in West Africa showed that within a population widely infected with HPV, poor social conditions, high parity and poor hygienic conditions were the main co-factors for cervical cancer [17].

Morogoro region also has widespread conditions that encourage substandard living conditions [25]. These include natural disasters, famine and drought. These often lead to large populations being displaced externally and internally for long periods of time. Under these refugees- like conditions, social vices like rapes, prostitution, multiple marriages and cohabitation prevail encouraging the transmission of HPV [10]. Most villages of Morogoro region are located within the tropical rain forest with difficult terrain as there are lots of swampy areas and thick and mountainous forests. This makes access to screening for cervical cancer, health education and treatment to be difficult [25].

Biological factors like poor nutritional status and infections, for example, malaria, HIV and TB, are ravaging in Morogoro region as well as the whole sub-Saharan Africa and have made many people immuno-compromised [25]. Several studies have demonstrated the association of HIV with HPV. The prevalence of CIN has been estimated to be as high as 20–40% in HIV positive women [114]. HIV-positive women are more likely to have persistent HPV infections than HIV negative women [47]. In a similar study to ours, of 2,198 women who attended gynaecological clinics in Abidjan, Côte d'Ivoire, HIV positive women had a significantly higher prevalence of squamous intraepithelial lesions (SIL) [9]. Temmerman et al [115] reported a five-fold increased risk of high-grade SIL among 513 HIV positive women in a family planning clinic in Kenya. Another recently published study from Tanzania showed that the prevalence of HIV-1 was much higher among the cervical cancer patients (21.0%) than among the controls (11.6%). HIV-1 was a significant risk factor for cancer of the cervix ($p<0.001$, OR 2.9, 95% CI=1.4–5.9) [18].

Low education on awareness and knowledge of cervical cancer in Morogoro region has been observed as another risk factor for the disease. Cervical cancer is yet to be recognized as an important public health problem in the region [25]. In Tanzania, priority is given to infectious diseases such as malaria, tuberculosis, leprosy, diarrheal diseases, acute respiratory infections and HIV/AIDS all of which have preventive and management strategies [25]. Several studies have shown poor knowledge of cervical cancer in Sub Saharan Africa, which cuts across different literacy levels [116]. For example, among 500 attendees of a maternal and child health clinic in Lagos, Nigeria only 4.3% were found to be aware of cervical cancer [117]. In 2004, also in Lagos-Nigeria, 81.7% of 139 patients with advanced cervical cancer had never heard of cervical cancer before, and 20%, 30% and 10% respectively thought the symptoms they had were due to resumption of menses, lower genital infection and irregular menses [118]. On the other hand, similar studies in Kenya

and Tanzania also reported very poor knowledge of the disease in patients [5, 23]. Poor knowledge is not limited to patients alone, however, health care workers who are supposed to be better informed do not have good knowledge of the disease either. Education improves knowledge and acceptance of preventive measures against cervical cancer [15].

Poor cervical cancer screening services were another problem seen in Morogoro region. All 536 women in our study were never screened for cervical cancer before. In a study among medical workers in a Ugandan hospital (doctors, nurses and medical students) only 19% of the female medical workers had ever had a cervical cancer screening test done. The reasons for not having been screened included: not feeling at risk, lack of symptoms, carelessness, fear of vaginal examination, lack of interest, test being unpleasant and not yet being of risky age. Moreover, 25% of the female respondents said that they would only accept a vaginal examination by a female health worker [15].

In a similar study among nurses in a Tanzanian hospital concerning nurses' own cervical cancer screening practices most (116/137) of the respondents had never had a Pap smear. The most common reasons (54.7%) was not knowing where to go for the test, followed by seeing no reason for the test (13.1%), being afraid of the procedure (9.5%) and being afraid of bad results (7.3%)[24] .Furthermore, there are very few cervical cancer screening services in Tanzania and many of them are based on secondary and tertiary health care facilities located in urban areas. Screening in most developing countries like Tanzania is mainly opportunistic, characterized by an estimated low coverage, coexisting with over-screening of women with access to health services, and an absence of quality control procedures [24].Policies for cervical cancer screening in most African countries vary and, most often nonexistent [15] .

In our study, access to treatment for ICC was limited for patients who were referred, only one third of the women with ICC received a TAH, with more than a half having received no treatment or being lost to follow-up due to long waiting times, sizeable co-payments, and lack of staff availability all contributed to the lack of treatment access. Likewise, in a cervical cancer screening program in Kenya for example, when women offered LEEP in the clinic or referral for the treatment of suspected cervical cancer or extensive lesions, all eligible women chose LEEP performed in-clinic at no cost [95]. In other field reports suggest that LEEP referral rates are higher in HIV positive women due to more large lesions seen in this population [11, 119].

The strength of our investigation was that we carried out a large comparative retrospective study compare to others already done in Tanzania. The weakness was that we have had to rely on the information documented by others like the results of screening and the biopsy

diagnosis in case of referred patients.Also, there was no history of behavioural risk factors, for example lifetime sex partners and limited availability of post-treatment information because of loss to follow-up. Additionally, the clinical protocol did not include an immediate post-treatment biopsy to ascertain whether disease found at follow-up was truly new, recurrent or attributable to treatment failure. Also the margin status was not assessed as this is a known predictor of disease recurrence in the referred LEEP specimens. However all these minor shortcomings have no or little confounding effects on our results.

CHAPTER 5

5.0 CONCLUSION AND RECOMMENDATIONS

5.1 Conclusion

These results indicate the crucial importance of continuous screening of HIV-infected women who live in resource constrained settings like Morogoro region in Tanzania. Also, it shows that prevention of cervical cancer in HIV positive women must be focused towards early detection of cervical abnormalities by VIA or VILI followed by an appropriate treatment. Our findings may be an important incentive for all HIV-infected women to be engaged in a gynaecological care program. The high prevalence of precancerous lesions in our study is one of the highest reported findings in any population worldwide. Therefore, continuous cervical cancer screening and treatment services of HIV infected women in Tanzania should be implemented to prevent development of HPV-induced ICC. Despite a probable bias and minimal confounding factors, our study suggests that HIV seropositive women present significantly younger with cervical cancer disease than HIV negative women. This study also shows a significant increase in proportion of extended cervical precancerous lesions among the reproductive tract cancers of women and changes in mean age of presentation or severity of invasive cervical cancer over time. In our study, ICC prevalence in HIV infected women has decreased compared to their counterpart, HIV non- infected women. Because our sample was somehow representative of HIV infected women seeking antiretroviral therapy in the public sector clinics in Morogoro, it strongly suggests the critical need for undertaking a concerted effort towards the provision of a cervical cancer screening and treatment program that will benefit the average woman accessing these clinics. HPV vaccination shows a great promise for the control of cervical cancer in the developing countries which have a significant burden of morbidity and mortality due to cervical cancer and where difficulties in implementing and sustaining cytology based screening methods also do exist .Further studies are needed to determine the crucial relationship of co-infections between HIV and HPV with the development of invasive cervical cancer, however to current many studies show an open debate for the involvement of cervical cancer as an AIDS defining illness. Therefore, HIV/AIDS has a highly statistical significant association ($p<0.001$) and high influence on the development of cervical precancerous lesions in HIV positive women, however its direct involvement in the progression to ICC could be questionable, especially in the HAART era as we have also found in our study, as others already did, the number of women with ICC were almost doubled in HIV negative women compared to HIV positive women.

5.2 Recommendations

There is a need to improve the outlook of this condition in Tanzania. There should be improvement in attitude towards education needs of the population so as to create and improve awareness and knowledge about the disease. Disease prevention requires social change, including demographic groups at high risk for disease, appropriate governmental authorities, and essential medical personnel. There is a need for sensitization of healthcare workers about cervical cancer and the importance of screening for cervical cancer disease. Based on studies carried out in countries where organized screening and cancer registries are available, it is known that screening tendencies can be influenced by cultural beliefs, the social position of women, characteristics of the health care system, the health workers' attitudes towards screening and society comprehension of the screening process [120].

Embarrassments about undergoing a pelvic examination, fear of the procedure and society local believes that little can be done to prevent cancer diseases are other factors that might decrease screening participation [13]. Lower socio-economical background, lack of feasible health facilities and low literacy also compromise participation in the voluntary screening for cervical cancer [13, 23]. The gender of health care professionals and the limited time that they allocate to community education on the importance of screening for cervical cancer may negatively influence screening participation as well [121].

Formulation and ensuring compliance with national program guidelines is an essential step towards significantly reducing the burden of cervical cancer [122]. This type of service does not reach women most at risk for example, older women aged 35–60 years or HIV infected women especially to those who live in rural areas [70]. Cytology-based screening, which is used in developed countries, is a resource intensive, and difficult to apply in many countries in sub-Saharan Africa because of poor health care infrastructure and lack of resources [123]. For example, in Morogoro region where we conducted our study, there are no cytopathologists, cytoscreeners, cytotechnicians and even in other nearby regions some of them have inadequate training and quality control as well. Histopathological services are extremely limited in many sub-Sahara African countries [102]. Malawi, for example, a country with a cervical cancer incidence rate of 47 per 100,000 women, had one pathologist, one colposcope, no cyto-technicians and no facilities for cervical cancer screening or treatment in 5 regions of the country in 2001 [124]. This situation in Malawi is not much different compared to Tanzania.

A good example also was seen in Botswana, where a national cervical cytology screening committee was formed in 1996 with a role in planning and advising the government. Just six years later a national guidance document for the national cervical cytology program was drawn up and implemented. In this short duration of time public awareness has been

successfully raised, resulting in an increased uptake of Pap smears from 5000 per year before the start of the program with an impressive 32,000 per year in 2009 [125].This problem was not limited to Botswana as many African countries like Tanzania have similar inadequate resources to treat patients even when abnormal cervical cytology is detected.

Training curricula medical personnel need to be improved in order to include more practical cervical cancer screening skills [126]. There is a need to change attitudes that screening is only for gynaecologists. For opportunistic screening to work, health care workers in other departments need to be sensitized on the importance of cervical cancer screening and they need to refer all eligible women who come into their care for screening without much delay [15]. Nurses in developed countries for example, play a role in cancer prevention and participate in cervical cancer screening by carrying out Pap smear tests. Due to the lack of logistics and scarcity of gynaecologists and pathologists in Tanzania, nurses should be used effectively and in all centres in the prevention of cervical cancer, by being enabled to collect biopsy specimen and the use of the visual inspection technique which is less costly and does not require high expertise [24].

Visual inspection methods are, however, subjective methods and prone to different interpretations by different observers, depending on experience and competence [119, 127]. Other screening methods include HPV DNA assay which has been shown to be even superior to Pap smear test and colposcopy [128] but its implementation in Tanzania is still not feasible probably due to limited availability of funds, pathology laboratories and expertise in the diagnostic process [25].For example in a study published in Portugal by Silva et al [129] shows that self-sampling using HPV DNA testing may be a good alternative in substitution of the vaginal speculum examination by a clinician or dependence on highly trained pathologists instead self-sampling can be used as a tool for primary screening of HPV infection in young asymptomatic women.

However, VIA and VILI, as a form of population-based screening has shown better assistance so far across the continent [130]. VIA is similar to colposcopy in that 4% acetic acid is applied to the cervix and any acetowhite lesion is visualized, although with VIA there is no magnification [131]. Candidates with suspicious lesions are selected for treatment. Studies have shown the sensitivity of VIA to be the same as that of Pap smear while its specificity is lower than 85% [132].The specificity is also noted to be even lower among HIV positive patients possibly due to high rates of co-infections in the lower genital tract [84].

In developed areas with low mortality rates from cervical cancer, organized screening programs had great impact on the reduction of cervical cancer mortality. Time trends in mortality from cervical cancer in Denmark, Finland, Iceland, Norway, and Sweden since the early nineteen fifties were investigated in relation to the extent and intensity of organized screening programs in these countries [133]. In all five countries, the cumulative mortality rates (0-74 years) fell between 1965 and 1982. In Iceland, where the nationwide program has the widest target age range, the fall in mortality was greatest (80%)[133].

Finland and Sweden have nationwide programs also; the mortality fell by 50% and 34%, respectively. In Denmark, where about 40% of the population is covered by organized programs, the overall mortality fell by 25%, but in Norway, with only 5% of the population covered by organized screening, the mortality fell by only 10% [133]. The results support the conclusion that organized screening programs have had a major impact on the reduction in mortality from cervical cancer in the Nordic countries [133].For instance, in the majority of low-income countries like Tanzania, cervical cancer screening program has proven difficult to sustain, in large part probably because of its reliance on highly trained cytotechnologists, high-quality laboratories and well established infrastructures to support up to 3 visits for screening, colposcopic evaluation of abnormalities and treatment [25].

Recent studies suggest that alternate screening strategies that use HPV DNA testing or simple visual screening methods may be more practical in some areas of the world like Sub Saharan Africa including Tanzania. Also regardless of initial screening test strategies that enhance the linkage between screening and treatment, there should be functional cancer registries in order to minimize loss to follow-up, lack of organized data, inconsistency of the screening activities and lack of specialized units for the task force as it was observed in our study. Additionally, economic evaluations of all the above alternatives have concluded that they are promising [134].

HPV vaccination to the recommended target population will reduce the disease incidence in the future [25]. The high cost of the vaccines is a constraint on low resource countries that have a high incidence of the disease like Tanzania. It has been submitted that the vaccines if given to girls before onset of sexual activity, have the potential to dramatically reduce the incidence of HPV infection and therefore cervical cancer[135]. However, the vaccine is of little value to the population of women who have already become sexually active as it cannot eradicate the virus if already present or retard the growth of incipient cancer[135].

Prevention and early diagnosis through vaccination and screening requires effective mobilization of the target groups and this has been a problem in developing countries like

Tanzania since cultural, emotional barriers and practical needs are among the main reasons as to why women choose not to be screened [25]. Addressing these barriers and needs will help to increase women's awareness and willingness to seek services. Screening, treatment, and follow-up services need to address women's cultural, emotional, practical needs and concerns.

Community-based education approach as a way of building capacity to all women and promote their participation will help to reduce fear and misunderstandings about cervical cancer screening and treatment. Making women's experiences with screening services more positive, ensures greater follow-up rates and increases the likelihood that they will share information about their good experience with peers.

Effective public messages to improve cervical cancer awareness are needed. Messages should be targeted to reach community at higher risk of cervical cancer. Recognized barriers to women's participation in screening may include: little understanding of the disease, limited access to screening services, shame and fear of a vaginal exam, fear of death from cancer, lack of community and family support [19]. Common misconceptions about cervical cancer include the fact that people often do not know that it is preventable.

Community meetings, posters, pamphlet newspaper advertisements or articles, radio and television messages can help to spread cervical cancer awareness to the society. Key sources of information include peers who have received messages or been screened, leaders or members of women's groups, midwives and traditional healers, community health promoters, community leaders, nurses, nurse practitioners and medical doctors.

Places to reach women such as local women's groups community centers, women's workplaces, places of worship, health facilities, maternity clinics, schools and markets should be targeted to spread key cervical cancer prevention messages which may include the facts that good health practices can help prevent cancer, cervical cancer develops slowly and is preventable, screening can detect and treat the precancerous cervical lesions before they progress to invasive cervical cancer, women aged 30 and older or HIV positive are more likely to develop cervical precancerous lesions hence invasive cervical cancer than younger women, women in their thirties and forties should be screened and that the screening procedure is relatively simple, quick, and not painful. Screening test positive is not a death sentence, rather it provides the opportunity to eliminate abnormal cells before they become invasive.

Community health or outreach workers who can facilitate communication at the community level should be highly encouraged. Counselling by health care providers can both inform

women and help them talk to their families. In order to ensure women's positive experiences with screening, there is a need to build and maintain positive provider-client relationships. This is needed because women are more likely to participate when they are treated well, health care providers are sensitive, responsive and respectful.

Screening services and procedures should be friendly to women to ensure that women with positive experiences will become advocates when talking to other women. Important counselling tips include listening and encouraging women to express their concerns being sensitive to cultural and religious considerations expressing support through non-verbal communication, such as nodding, keeping messages simple and answering questions directly, calmly, and in a reassuring manner and providing adequate information to remind them of the instructions.

Having female health care providers in settings where women are uncomfortable with male health care providers, if possible could help. Ensuring affordability of the services and confidentiality are essential practice. Local languages should be incorporated into the counselling medium. Advisory teams of women and other key community leaders can be consulted in order to deliver a package that will meet women's cultural, emotional and practical needs.

High parity is associated with increased incidence of HPV infection leading to increased cases of cervical cancer. Education on the effective family planning methods can help in improving the general living condition of the people. This will eventually lead to a sizeable population that can be easily reached with cervical cancer control programs.

Early onset of sexual activities among teenage girls in Morogoro region is high. This predisposes such girls to high risk of developing cervical cancer. The promotion of the proper use of condoms through sex education programs in early school days can also help in reducing the transmission of HPV in the region. Closely linked to this is the issue of male circumcision, which should be encouraged among those with low level of practice.

Pre-requisites for cervical cancer screening, infrastructure, funding and training needs in Morogoro region might include; screening points have to be as many as possible and, the implementation of continuous cervical cancer screening in all HIV clinics in Tanzania. Sustainable funding for screening programs is important and, one way to achieve this could be for donors to insist that they would only fund a cervical cancer screening program if the government agreed to provide funding support for the program to continue after set-up funding ended.

Building up political will and support for national funding support towards cervical cancer screening would be required. Community advocates and the media would also need to be mobilized in a public health approach. Raising public awareness about the causes of cervical cancer, the impact of HIV/AIDS on the precancerous lesions and why treatment would be important.

If possible, screening should be integrated into existing programs, such as programs for breast cancer screening, sexual health programs and many others. This might go hand in hand with the training of health professionals to take smear tests and/or HPV tests and training of pathologists to read smear tests would also be critical.

Evaluation and cost effectiveness of cervical screening should focus on; training, are programs easy to staff? Should pap smears be abandoned for developing countries due to lack of people to read them? Work must be done by another health care level other than doctors? Brain drain is another issue; we must address the retention of health care personnel. Cost, need more interaction with health economists, sustainability of the project?

We must look into different financial flows, the idea of marketing campaigns and alternate financing schemes to raise substantial funds. Is coverage feasible of “mother-daughter” screening and vaccination project? Feasibility of self-screening? Political will; is there a need to sell a product to the policy makers? Screening must be made part of country’s health policy. Demonstration projects are a good way to show political leaders the efficacy of screening programs. MDG goals 4 and 5 (reduction in child mortality and improvement in maternal health) are a useful platform to use or argue that HPV is part of maternal health.

Creating a climate for informed decision-making in screening and vaccination program, the following must be considered; informed consent has to be tailored appropriately to population. Informed consent means different things in different situations and with different people, so it has to be appropriate to the population. Cervical cancer screening should become part of routine health care. Most policy makers are not yet aware of the cost/toll of cervical cancer in their countries. Consequently, the development of cancer registries is important. There is a need to show politicians that the impact of screening would be seen now and it is ideal.

Survivors’ stories and the family stories of the women who have died of cervical cancer need to be told. Doctors and nurses should also tell stories about cervical cancer. We should not be afraid to tell individual stories; these are the ones that touch people.

Policy and advocacy: raising awareness , supporting champions, advocates and funders to take action and assisting in the development of policies and guidelines to create an environment supportive of a practical, sustainable and cost-effective national strategy for comprehensive prevention of cervical cancer [120]. Developing training resources and conducting competency-based training for health care providers and supervisors.

Initiation of the sustainable service delivery system that will address integration, procurement, repair and maintenance of equipment, information systems for monitoring and evaluation (M&E), consistent supportive supervision, high-quality services and care of the screening programs should be highly encouraged [136]. Also, referral systems should be strengthened within the health systems to ensure that women receive the appropriate follow-up and treatment.

Capacity building which involves initial training, investments must be made in supportive supervision, coaching and quality assurance. Mid-level providers (nurses, midwives) should be used for effective delivery of VIA/VILI and cryotherapy services. Strengthening of M&E in all healthcare facilities that conduct cervical cancer screening must be supported to collect quality data and more important the use that data regularly to improve service provision. Lastly, the maintenance of cryotherapy equipment and other necessary supplies should be included in project planning.

Efforts to improve awareness of the target population can result in early detection of precancerous lesions, leading to improved survival from cervical cancer in Tanzania. A combination of community-based, facility-based and media-based strategies can be used to create awareness and inform women [137].

References

1. Cancer, I.A.f.R.o. *WHO world cancer report*. 2012 [cited 2013.
2. Arrossi, S., M. Paolino, and R. Sankaranarayanan, *Challenges faced by cervical cancer prevention programs in developing countries: a situational analysis of program organization in Argentina.* Rev Panam Salud Publica, 2010. **28**(4): p. 249-57.
3. Abotchie, P.N. and N.K. Shokar, *Cervical cancer screening among college students in ghana: knowledge and health beliefs.* Int J Gynecol Cancer, 2009. **19**(3): p. 412-6.
4. Gichangi, P., et al., *HIV and cervical cancer in Kenya.* Int J Gynaecol Obstet, 2002. **76**(1): p. 55-63.
5. Gichangi, P., et al., *Knowledge and practice about cervical cancer and Pap smear testing among patients at Kenyatta National Hospital, Nairobi, Kenya.* Int J Gynecol Cancer, 2003. **13**(6): p. 827-33.
6. Averbach, S.H., et al., *The association between cervical human papillomavirus infection and HIV acquisition among women in Zimbabwe.* AIDS, 2010. **24**(7): p. 1035-42.
7. Louie, K.S., S. de Sanjose, and P. Mayaud, *Epidemiology and prevention of human papillomavirus and cervical cancer in sub-Saharan Africa: a comprehensive review.* Trop Med Int Health, 2009. **14**(10): p. 1287-302.
8. Kahesa, C., et al., *Association between invasive cancer of the cervix and HIV-1 infection in Tanzania: the need for dual screening.* BMC Public Health, 2008. **8**(1): p. 262.
9. La Ruche, G., et al., *Squamous intraepithelial lesions of the cervix, invasive cervical carcinoma, and immunosuppression induced by human immunodeficiency virus in Africa. Dyscer-CI Group.* Cancer, 1998. **82**(12): p. 2401-8.
10. Suba, E.J., et al., *Systems analysis of real-world obstacles to successful cervical cancer prevention in developing countries.* Am J Public Health, 2006. **96**(3): p. 480-7.
11. Bradley, J., et al., *Delivering cervical cancer prevention services in low-resource settings.* Int J Gynaecol Obstet, 2005. **89 Suppl 2**: p. S21-9.
12. Obure, J., et al., *Prevalence and severity of cervical squamous intraepithelial lesion in a tertiary hospital in northern Tanzania.* Tanzan J Health Res, 2009. **11**(4): p. 163-9.
13. Bingham, A., et al., *Factors affecting utilization of cervical cancer prevention services in low-resource settings.* Salud Publica Mex, 2003. **45 Suppl 3**: p. S408-16.
14. Denny, L., et al., *Direct visual inspection for cervical cancer screening: an analysis of factors influencing test performance.* Cancer, 2002. **94**(6): p. 1699-707.

15. Mutyaba, T., F.A. Mmiro, and E. Weiderpass, *Knowledge, attitudes and practices on cervical cancer screening among the medical workers of Mulago Hospital, Uganda.* BMC Med Educ, 2006. **6**: p. 13.
16. Belinson, J.L., et al., *Cervical cancer screening by simple visual inspection after acetic acid.* Obstet Gynecol, 2001. **98**(3): p. 441-4.
17. Palacio-Mejia, L.S., et al., *Cervical cancer, a disease of poverty: mortality differences between urban and rural areas in Mexico.* Salud Publica Mex, 2003. **45 Suppl 3**: p. S315-25.
18. Moodley, J.R., et al., *HIV and pre-neoplastic and neoplastic lesions of the cervix in South Africa: a case-control study.* BMC Cancer, 2006. **6**: p. 135.
19. *Tanzania Service Delivery Guidelines for Cervical Cancer Prevention and Control, (2011), Dar es salaam.Tanzania Ministry of Health and Social Welfare,*2011: Dar es Salaam.
20. Medeiros, R. and D. Ramada, *Knowledge differences between male and female university students about human papillomavirus (HPV) and cervical cancer: Implications for health strategies and vaccination.* Vaccine, 2010. **29**(2): p. 153-60.
21. Matasha, E., et al., *Sexual and reproductive health among primary and secondary school pupils in Mwanza, Tanzania: need for intervention.* AIDS Care, 1998. **10**(5): p. 571-82.
22. Parkin, D.M., et al., *Changing cancer incidence in Kampala, Uganda, 1991-2006.* Int J Cancer, 2010. **126**(5): p. 1187-95.
23. Kidanto, H.L., C.D. Kilewo, and C. Moshiro, *Cancer of the cervix: knowledge and attitudes of female patients admitted at Muhimbili National Hospital, Dar es Salaam.* East Afr Med J, 2002. **79**(9): p. 467-75.
24. Urasa, M. and E. Darj, *Knowledge of cervical cancer and screening practices of nurses at a regional hospital in Tanzania.* Afr Health Sci, 2011. **11**(1): p. 48-57.
25. *National Cervical Canc er Prevention and Control Strategic Plan (2011-2015). Reproductive and Child Health Section, Tanzania Ministry of Health and Social Welfare*2011: Dar es Salaam.
26. Shetty, P., *Twalib Ngoma: creating cancer care in Tanzania.* Lancet, 2008. **371**(9625): p. 1657.
27. Jemal, A., et al., *Global cancer statistics.* CA Cancer J Clin, 2011. **61**(2): p. 69-90.
28. Mbulawa, Z.Z., et al., *Impact of human immunodeficiency virus on the natural history of human papillomavirus genital infection in South African men and women.* J Infect Dis, 2012. **206**(1): p. 15-27.
29. Bower, M., D. Mazhar, and J. Stebbing, *Should cervical cancer be an acquired immunodeficiency syndrome-defining cancer?* J Clin Oncol, 2006. **24**(16): p. 2417-9.

30. Palefsky, J.M., *Cervical human papillomavirus infection and cervical intraepithelial neoplasia in women positive for human immunodeficiency virus in the era of highly active antiretroviral therapy.* Curr Opin Oncol, 2003. **15**(5): p. 382-8.
31. Palefsky, J., *Biology of HPV in HIV infection.* Adv Dent Res, 2006. **19**(1): p. 99-105.
32. Laurson, J., et al., *Epigenetic repression of E-cadherin by human papillomavirus 16 E7 protein.* Carcinogenesis, 2010. **31**(5): p. 918-26.
33. Nowak, R.G., et al., *Increases in human papillomavirus detection during early HIV infection among women in Zimbabwe.* J Infect Dis, 2011. **203**(8): p. 1182-91.
34. Tugizov, S.M., et al., *HIV-associated disruption of mucosal epithelium facilitates paracellular penetration by human papillomavirus.* Virology, 2013. **446**(1-2): p. 378-88.
35. Ndiaye, C., et al., *Human papillomavirus distribution in invasive cervical carcinoma in sub-Saharan Africa: could HIV explain the differences?* Trop Med Int Health, 2012. **17**(12): p. 1432-40.
36. Mbulawa, Z.Z., et al., *Genital human papillomavirus prevalence and human papillomavirus concordance in heterosexual couples are positively associated with human immunodeficiency virus coinfection.* J Infect Dis, 2009. **199**(10): p. 1514-24.
37. O'Brien, P.M. and M. Saveria Campo, *Evasion of host immunity directed by papillomavirus-encoded proteins.* Virus Res, 2002. **88**(1-2): p. 103-17.
38. Chin-Hong, P.V., et al., *Comparison of patient- and clinician-collected anal cytology samples to screen for human papillomavirus-associated anal intraepithelial neoplasia in men who have sex with men.* Ann Intern Med, 2008. **149**(5): p. 300-6.
39. Houlihan, C.F., et al., *Human papillomavirus infection and increased risk of HIV acquisition. A systematic review and meta-analysis.* AIDS, 2012. **26**(17): p. 2211-22.
40. Chin-Hong, P.V., et al., *Anal human papillomavirus infection is associated with HIV acquisition in men who have sex with men.* AIDS, 2009. **23**(9): p. 1135-42.
41. Strickler, H.D., *Does HIV/AIDS have a biological impact on the risk of human papillomavirus-related cancers?* J Natl Cancer Inst, 2009. **101**(16): p. 1103-5.
42. Harris, T.G., et al., *Incidence of cervical squamous intraepithelial lesions associated with HIV serostatus, CD4 cell counts, and human papillomavirus test results.* JAMA, 2005. **293**(12): p. 1471-6.
43. Chaturvedi, A.K., et al., *Risk of human papillomavirus-associated cancers among persons with AIDS.* J Natl Cancer Inst, 2009. **101**(16): p. 1120-30.
44. Cheng, S.H., et al., *Influence of age and CD4+ T cell counts on the prevalence of genital human papillomavirus infection among HIV-seropositive men who have sex with men in Taiwan.* J Med Virol, 2012. **84**(12): p. 1876-83.
45. Gillison, M.L., et al., *Eurogin Roadmap: comparative epidemiology of HPV infection and associated cancers of the head and neck and cervix.* Int J Cancer, 2014. **134**(3): p. 497-507.

46. Chaturvedi, A.K., *Beyond cervical cancer: burden of other HPV-related cancers among men and women.* J Adolesc Health, 2010. **46**(4 Suppl): p. S20-6.
47. Mayaud, P., et al., *The interrelation of HIV, cervical human papillomavirus, and neoplasia among antenatal clinic attenders in Tanzania.* Sex Transm Infect, 2001. **77**(4): p. 248-54.
48. *Tanzania HIV Rapid Test Algorithm.Tanzania Ministry of Health and Social Welfare,*2007: www.ihi.eprints.org.
49. Inc., S.D., *Diagnostic Rapid Testing Kit,HIV*. 1999: Gyeonggi-Do,South Korea.
50. Lyamuya, E.F., et al., *Evaluation of simple rapid HIV assays and development of national rapid HIV test algorithms in Dar es Salaam, Tanzania.* BMC Infect Dis, 2009. **9**: p. 19.
51. HIV.com, A. 2000 [cited 2014 04/05].
52. Biotech, T., *Uni-Gold Recombigen HIV-1/2 Test*. 2002, IDA Business Park: Bray,Ireland.
53. Pecorelli, S., L. Zigliani, and F. Odicino, *Revised FIGO staging for carcinoma of the cervix.* Int J Gynaecol Obstet, 2009. **105**(2): p. 107-8.
54. Scurry, J., K. Patel, and M. Wells, *ACP Broadsheet No 138: May 1993. Gross examination of uterine specimens.* J Clin Pathol, 1993. **46**(5): p. 388-93.
55. Heatley, M., *Distribution of cervical intraepithelial neoplasia: are hysterectomy specimens sampled appropriately?* J Clin Pathol, 1995. **48**(4): p. 323-4.
56. Heatley, M.K., *A comparison of three methods of orienting cervical punch biopsies.* J Clin Pathol, 1999. **52**(2): p. 149-50.
57. McCluggage, W.G., et al., *Which staging system to use for gynaecological cancers: a survey with recommendations for practice in the UK.* J Clin Pathol, 2010. **63**(9): p. 768-70.
58. Kalliala, I., et al., *Risk of cervical and other cancers after treatment of cervical intraepithelial neoplasia: retrospective cohort study.* BMJ, 2005. **331**(7526): p. 1183-5.
59. Chinchai, T., et al., *Prevalence of human papillomavirus genotypes in cervical cancer.* Int J Gynecol Cancer, 2012. **22**(6): p. 1063-8.
60. Rustagi, A.S., et al., *Cervical screening and cervical cancer death among older women: a population-based, case-control study.* Am J Epidemiol, 2014. **179**(9): p. 1107-14.
61. Thomas, D.B., et al., *Risk factors for progression of squamous cell cervical carcinoma in-situ to invasive cervical cancer: results of a multinational study.* Cancer Causes Control, 2002. **13**(7): p. 683-90.
62. Rustagi, A.S., A. Kamineni, and N.S. Weiss, *Point: cervical cancer screening guidelines should consider observational data on screening efficacy in older women.* Am J Epidemiol, 2013. **178**(7): p. 1020-2.

63. Kafuruki, L., et al., *Prevalence and predictors of Cervical Intraepithelial Neoplasia among HIV infected women at Bugando Medical Centre, Mwanza-Tanzania.* Infect Agent Cancer, 2013. **8**(1): p. 45.
64. Mwakigonja, A.R., et al., *Cervical cytological changes in HIV-infected patients attending care and treatment clinic at Muhimbili National Hospital, Dar es Salaam, Tanzania.* Infect Agent Cancer, 2012. **7**: p. 3.
65. Balandya, B.S., A.B. Pembe, and H.A. Mwakyoma, *Cervical pre-malignant lesions in HIV infected women attending Care and Treatment Centre in a tertiary hospital, Dar es Salaam, Tanzania.* East Afr J Public Health, 2011. **8**(3): p. 185-9.
66. van Bogaert, L.J., *Age at diagnosis of preinvasive and invasive cervical neoplasia in South Africa: HIV-positive versus HIV-negative women.* Int J Gynecol Cancer, 2011. **21**(2): p. 363-6.
67. Ghaemmaghami, F., et al., *Visual inspection with acetic acid as a feasible screening test for cervical neoplasia in Iran.* Int J Gynecol Cancer, 2004. **14**(3): p. 465-9.
68. Raguenaud, M.E., et al., *Screening and treating cervical cancer in HIV-positive women in Cambodia.* J Acquir Immune Defic Syndr, 2009. **51**(5): p. 644-6.
69. Horo, A., et al., *Cervical cancer screening by visual inspection in Cote d'Ivoire, operational and clinical aspects according to HIV status.* BMC Public Health, 2012. **12**: p. 237.
70. Parham, G.P., et al., *Prevalence and predictors of squamous intraepithelial lesions of the cervix in HIV-infected women in Lusaka, Zambia.* Gynecol Oncol, 2006. **103**(3): p. 1017-22.
71. Abdus-Salam, A., O. Ogunnorin, and R. Abdus-Salam, *HIV Seroprevalence in Patients with Carcinoma of the Cervix in Ibadan, Nigeria.* Ghana Med J, 2008. **42**(4): p. 141-3.
72. Kapiga, S.H., et al., *Risk factors for cervical squamous intraepithelial lesions among HIV-1 seropositive women in Dar es Salaam, Tanzania.* Int J Gynaecol Obstet, 1999. **67**(2): p. 87-94.
73. Isaakidis, P., et al., *HPV infection, cervical abnormalities, and cancer in HIV-infected women in Mumbai, India: 12-month follow-up.* Int J Womens Health, 2013. **5**: p. 487-94.
74. van Bogaert, L.J., *The impact of human immunodeficiency virus infection on cervical preinvasive and invasive neoplasia in South Africa.* Ecancermedicalscience, 2013. **7**: p. 334.
75. Chirenje, Z.M., *HIV and cancer of the cervix.* Best Pract Res Clin Obstet Gynaecol, 2005. **19**(2): p. 269-76.
76. Ellerbrock, T.V., et al., *Incidence of cervical squamous intraepithelial lesions in HIV-infected women.* JAMA, 2000. **283**(8): p. 1031-7.

77. Wright, T.C., Jr., et al., *Cervical intraepithelial neoplasia in women infected with the human immunodeficiency virus: outcome after loop electrosurgical excision.* Gynecol Oncol, 1994. **55**(2): p. 253-8.
78. Sahasrabuddhe, V.V., et al., *Prevalence and predictors of colposcopic-histopathologically confirmed cervical intraepithelial neoplasia in HIV-infected women in India.* PLoS One, 2010. **5**(1): p. e8634.
79. Joshi, S.N., et al., *Cervical squamous intra-epithelial changes and human papillomavirus infection in women infected with human immunodeficiency virus in Pune, India.* J Med Virol, 2005. **76**(4): p. 470-5.
80. Stier, E.A. and A.S. Baranoski, *Human papillomavirus-related diseases in HIV-infected individuals.* Curr Opin Oncol, 2008. **20**(5): p. 541-6.
81. Launay, O. and L. Guillevin, *[Epidemiology of HIV-associated malignancies].* Bull Cancer, 2003. **90**(5): p. 387-92.
82. Denslow, S.A., et al., *Incidence and progression of cervical lesions in women with HIV: a systematic global review.* Int J STD AIDS, 2014. **25**(3): p. 163-77.
83. Boccalon, M., et al., *Intra-epithelial and invasive cervical neoplasia during HIV infection.* Eur J Cancer, 1996. **32A**(13): p. 2212-7.
84. Womack, S.D., et al., *HPV-based cervical cancer screening in a population at high risk for HIV infection.* Int J Cancer, 2000. **85**(2): p. 206-10.
85. van der Burg, S.H. and C.J. Melief, *Therapeutic vaccination against human papilloma virus induced malignancies.* Curr Opin Immunol, 2011. **23**(2): p. 252-7.
86. Kreiss, J.K., et al., *Human immunodeficiency virus, human papillomavirus, and cervical intraepithelial neoplasia in Nairobi prostitutes.* Sex Transm Dis, 1992. **19**(1): p. 54-9.
87. Chalermchockcharoenkit, A., et al., *Prevalence and cumulative incidence of abnormal cervical cytology among HIV-infected Thai women: a 5.5-year retrospective cohort study.* BMC Infect Dis, 2011. **11**: p. 8.
88. Park, E.K., et al., *Human papillomavirus prevalence and genotype distribution among HIV-infected women in Korea.* J Korean Med Sci, 2014. **29**(1): p. 32-7.
89. Teixeira, N.C., et al., *Prevalence and risk factors for cervical intraepithelial neoplasia among HIV-infected women.* Braz J Infect Dis, 2012. **16**(2): p. 164-9.
90. Kahesa, C., et al., *Association between invasive cancer of the cervix and HIV-1 infection in Tanzania: the need for dual screening.* BMC Public Health, 2008. **8**: p. 262.
91. Chan, Y.M., et al., *Screening for HIV infection in women with newly diagnosed cervical cancer.* Gynecol Oncol, 2004. **92**(1): p. 300-3.
92. Branca, M., et al., *Assessment of risk factors and human papillomavirus (HPV) related pathogenetic mechanisms of CIN in HIV-positive and HIV-negative women.*

Study design and baseline data of the HPV-PathogenISS study. Eur J Gynaecol Oncol, 2004. **25**(6): p. 689-98.

93. Liu, J.H., et al., *[Study on cervical intraepithelial neoplasia in relation to human papillomavirus and co-factors: a case-control study in China and Australia].* Zhonghua Liu Xing Bing Xue Za Zhi, 2007. **28**(10): p. 958-63.
94. Zhang, W.Y., et al., *[Epidemiological study on the prevalence of cervical intraepithelial neoplasia among married women aged 25-54 years in Beijing between 2007-2008].* Zhonghua Fu Chan Ke Za Zhi, 2010. **45**(3): p. 161-4.
95. Mungo, C., et al., *Prevalence, characteristics, and outcomes of HIV-positive women diagnosed with invasive cancer of the cervix in Kenya.* Int J Gynaecol Obstet, 2013. **123**(3): p. 231-5.
96. Lomalisa, P., T. Smith, and F. Guidozzi, *Human immunodeficiency virus infection and invasive cervical cancer in South Africa.* Gynecol Oncol, 2000. **77**(3): p. 460-3.
97. Baay, M.F., et al., *Human papillomavirus in a rural community in Zimbabwe: the impact of HIV co-infection on HPV genotype distribution.* J Med Virol, 2004. **73**(3): p. 481-5.
98. Ahdieh, L., et al., *Cervical neoplasia and repeated positivity of human papillomavirus infection in human immunodeficiency virus-seropositive and -seronegative women.* Am J Epidemiol, 2000. **151**(12): p. 1148-57.
99. De Vuyst, H., et al., *HIV, human papillomavirus, and cervical neoplasia and cancer in the era of highly active antiretroviral therapy.* Eur J Cancer Prev, 2008. **17**(6): p. 545-54.
100. Mandelblatt, J.S., et al., *Is HIV infection a cofactor for cervical squamous cell neoplasia?* Cancer Epidemiol Biomarkers Prev, 1999. **8**(1): p. 97-106.
101. Adler, D.H., *The impact of HAART on HPV-related cervical disease.* Curr HIV Res, 2010. **8**(7): p. 493-7.
102. Hawes, S.E., et al., *Incident high-grade squamous intraepithelial lesions in Senegalese women with and without human immunodeficiency virus type 1 (HIV-1) and HIV-2.* J Natl Cancer Inst, 2006. **98**(2): p. 100-9.
103. Heard, I., V. Potard, and D. Costagliola, *Limited impact of immunosuppression and HAART on the incidence of cervical squamous intraepithelial lesions in HIV-positive women.* Antivir Ther, 2006. **11**(8): p. 1091-6.
104. Keita, N., et al., *HPV infection in women with and without cervical cancer in Conakry, Guinea.* Br J Cancer, 2009. **101**(1): p. 202-8.
105. Dames, D.N., et al., *The prevalence of cervical cytology abnormalities and human papillomavirus in women infected with the human immunodeficiency virus.* Infect Agent Cancer, 2009. **4 Suppl 1**: p. S8.
106. Clifford, G., et al., *Chapter 3: HPV type-distribution in women with and without cervical neoplastic diseases.* Vaccine, 2006. **24 Suppl 3**: p. S3/26-34.

107. Schmauz, R., et al., *Multiple infections in cases of cervical cancer from a high-incidence area in tropical Africa.* Int J Cancer, 1989. **43**(5): p. 805-9.
108. Bayo, S., et al., *Risk factors of invasive cervical cancer in Mali.* Int J Epidemiol, 2002. **31**(1): p. 202-9.
109. Drain, P.K., et al., *Male circumcision, religion, and infectious diseases: an ecologic analysis of 118 developing countries.* BMC Infect Dis, 2006. **6**: p. 172.
110. Brinton, L.A., et al., *Parity as a risk factor for cervical cancer.* Am J Epidemiol, 1989. **130**(3): p. 486-96.
111. Auvert, B., et al., *Effect of male circumcision on the prevalence of high-risk human papillomavirus in young men: results of a randomized controlled trial conducted in Orange Farm, South Africa.* J Infect Dis, 2009. **199**(1): p. 14-9.
112. Brinton, L.A., et al., *The male factor in the etiology of cervical cancer among sexually monogamous women.* Int J Cancer, 1989. **44**(2): p. 199-203.
113. Reeves, W.C., et al., *Human papillomavirus infection and cervical cancer in Latin America.* N Engl J Med, 1989. **320**(22): p. 1437-41.
114. Wright, C.J. and L. Milot, *Promoting nutritional health in the HIV-infected client with stomatitis.* Medsurg Nurs, 1994. **3**(3): p. 228-9, 238.
115. Temmerman, M., et al., *Risk factors for human papillomavirus and cervical precancerous lesions, and the role of concurrent HIV-1 infection.* Int J Gynaecol Obstet, 1999. **65**(2): p. 171-81.
116. Ley, C., et al., *Determinants of genital human papillomavirus infection in young women.* J Natl Cancer Inst, 1991. **83**(14): p. 997-1003.
117. Anorlu, R.I., et al., *Late presentation of patients with cervical cancer to a tertiary hospital in Lagos: what is responsible?* Eur J Gynaecol Oncol, 2004. **25**(6): p. 729-32.
118. Ajayi, I.O. and I.F. Adewole, *Knowledge and attitude of general outpatient attendants in Nigeria to cervical cancer.* Cent Afr J Med, 1998. **44**(2): p. 41-3.
119. Gaffikin, L., et al., *Safety, acceptability, and feasibility of a single-visit approach to cervical-cancer prevention in rural Thailand: a demonstration project.* Lancet, 2003. **361**(9360): p. 814-20.
120. Sellors, J., et al., *Screening and management of precancerous lesions to prevent cervical cancer in low-resource settings.* Asian Pac J Cancer Prev, 2003. **4**(3): p. 277-80.
121. Mutyaba, T., et al., *Influences on uptake of reproductive health services in Nsangi community of Uganda and their implications for cervical cancer screening.* Reprod Health, 2007. **4**: p. 4.
122. Newton, R., et al., *Cancer and HIV infection in Rwanda.* Lancet, 1995. **345**(8961): p. 1378-9.

123. Ng'andwe, C., et al., *The distribution of sexually-transmitted Human Papillomaviruses in HIV positive and negative patients in Zambia, Africa.* BMC Infect Dis, 2007. **7**: p. 77.

124. Hawes, S.E., et al., *Increased risk of high-grade cervical squamous intraepithelial lesions and invasive cervical cancer among African women with human immunodeficiency virus type 1 and 2 infections.* J Infect Dis, 2003. **188**(4): p. 555-63.

125. Parkin, D.M., et al., *Cancer in Africa 2012.* Cancer Epidemiol Biomarkers Prev, 2014.

126. Jemal, A., et al., *Cancer burden in Africa and opportunities for prevention.* Cancer, 2012. **118**(18): p. 4372-84.

127. Sankaranarayanan, R., et al., *Effect of visual screening on cervical cancer incidence and mortality in Tamil Nadu, India: a cluster-randomised trial.* Lancet, 2007. **370**(9585): p. 398-406.

128. Sankaranarayanan, R., et al., *Accuracy of human papillomavirus testing in primary screening of cervical neoplasia: results from a multicenter study in India.* Int J Cancer, 2004. **112**(2): p. 341-7.

129. Silva, J., et al., *Oncogenic HPV Types Infection in Adolescents and University Women from North Portugal: From Self-Sampling to Cancer Prevention.* J Oncol, 2011. **2011**: p. 953469.

130. Miller, A.B., *The cost effectiveness of cervical cancer screening.* Ann Intern Med, 1992. **117**(6): p. 529-30.

131. Gaffikin, L., et al., *Visual inspection with acetic acid as a cervical cancer test: accuracy validated using latent class analysis.* BMC Med Res Methodol, 2007. **7**: p. 36.

132. Blumenthal, P.D., et al., *Cervical cancer prevention: safety, acceptability, and feasibility of a single-visit approach in Accra, Ghana.* Am J Obstet Gynecol, 2007. **196**(4): p. 407 e1-8; discussion 407 e8-9.

133. Laara, E., N.E. Day, and M. Hakama, *Trends in mortality from cervical cancer in the Nordic countries: association with organised screening programmes.* Lancet, 1987. **1**(8544): p. 1247-9.

134. Herdman, R., W. McGuire, and J. Simone, *Influencing cancer policy.* Health Aff (Millwood), 2006. **25**(3): p. 800-7.

135. Sankaranarayanan, R., *HPV vaccination: the promise & problems.* Indian J Med Res, 2009. **130**(3): p. 322-6.

136. Sherris, J., C. Herdman, and C. Elias, *Cervical cancer in the developing world.* West J Med, 2001. **175**(4): p. 231-3.

137. Sherris, J., et al., *Advocating for cervical cancer prevention.* Int J Gynaecol Obstet, 2005. **89 Suppl 2**: p. S46-54.