Towards improved cervical cancer screening in Ethiopia

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Towards improved cervical cancer screening in Ethiopia

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CLINICAL SCIENCES, LUND | FACULTY OF MEDICINE | LUND UNIVERSITY

SELAMAWIT MEKURIA is a consultant in Obstetrics and Gynaecology who while travelling back and forward between Sweden and Ethiopia for her research, has worked clinically at both Skåne University hospital and Halmstad county hospital. She decided on her specialisation after learning of how common cervical cancer is in low-resource settings despite it being a preventable disease. Researching and working clinically has been vital to reaching certain discussions and ideas regarding cervical cancer screening in Ethiopia. It has been possible because of the support of Mrs. Kamprad's cancer foundation and colleagues from Ethiopia, Sweden, Denmark and Canada.

This thesis wants to move the conversation and implementation of more efficient screening strategies forward for low- and middle-income countries.

"Being defiant can be a good thing sometimes"

- Chimamanda Ngozi Adichie

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Abstract:

Cervical cancer is the number one cause of cancer-related death in Sub-Saharan Africa. Prevention is through screening and vaccination. This thesis focuses on efforts to improve screening in Ethiopia.
In paper 1, a systematic review and meta-analysis was done based on six RCTs from LMIC that compared HPV self-sampling with health-provider dependent screening. The aim was to compare the uptake of screening as well as associated costs. HPV self-sampling improved uptake significantly compared to health-provider screening. One study included cost-data, signifying the need for further cost-research.
In paper 2, the prevalence of HPV and acceptance of self-sampling was evaluated in a cross-sectional trial at a workplace in Addis Abeba. For three days, 3.1% of all (N=5950) female employees were screened. The prevalence of HPV mRNA was 20.6% (37/180). HPV self-sampling improved participation when compared to historically low screening attendance.
In Paper 3, the aim was in a RCT compare the sensitivity and specificity for CIN2+ of visual inspection with acetic acid (VIA), with or without Iodine, as a triage for HPV self-sample positive women. VIA with Iodine had better sensitivity but similar specificity as without Iodine. The difference was statistically not significant. HIV positivity was a better at detecting CIN2+ than both triage arms.
In paper 4, the feasibility of including pregnant women in screening described in paper 3, was evaluated. Pregnant women accepted screening similarly to non-pregnant women. They had the same knowledge about cervical cancer but had a lower history of previous participation in screening (p=0.07).
In conclusion, HPV self-sampling allows screening in a wider population previously excluded. Triage using VIA with Iodine is possible, but further research on cost-sensitive methylation triage is warranted. Incorporating screening services into existing health systems and establishing registers for HPV genotypes existing in cancers that are diagnosed should be the next research steps.

Key words: HPV, cervical cancer, self-sampling, screening, meta-analyses, pregnancy

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To my parents, Eleni and Fisseha,
for always keeping our connection with Ethiopia.
To my sister, Bemnet, for holding me accountable.
Everyone needs a friend like that.

Table of Contents

Abstract

Cervical cancer is the number one cause of cancer-related death in Eastern Africa. Prevention is through screening and vaccination. This thesis focuses on efforts to improve screening in Ethiopia.

In paper 1, a systematic review and meta-analysis was done based on six RCTs from LMIC that compared HPV self-sampling with health-provider dependent screening. The aim was to compare the uptake of screening as well as associated costs. HPV self-sampling improved uptake significantly compared to health-provider screening. One study included cost-data, signifying the need for further cost-research.

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Populärvetenskaplig sammanfattning

Livmoderhalscancer är en cancerform som går att utrota genom screening och vaccination. I länder med lång historik av organiserad screening som Sverige, är livmoderhalscancer ovanlig (12e plats), medan i östra Afrika, så är det den vanligaste orsaken till cancerrelaterad död. Livmoderhalscancer orsakas av Humant Papillomvirus (HPV). Det finns flera sorters HPV, och man kallar dem som orsakar cancer för hög-risk. Högrisktyperna har olika potential att läka ut spontant, och det är typerna som är bäst på att kvarstå i vävnaden som har en högre risk för cellförändringar och cancer. Det tar tid att utveckla både cellförändringar och senare cancer, vilket ger oss möjligheten att arbeta förebyggande. Man vet däremot också att de flesta med HPV läker ut spontant, och att en majoritet av måttliga men även ca 30% av svåra cellförändringar går i regress.

Det finns ett antal risk-faktorer för livmoderhalscancer och dessa kan delas in i de som förenklar för HPV att integreras i livmoderhalstappens slemhinna och de som gör det svårare för immunförsvaret att läka ut infektionen. Att föda flera barn vaginalt ökar risken och man tror det beror på traumat som uppstår i livmoderhalsen vid födsel. HIV och rökning försvårar för immunförsvaret att göra sitt jobb, vilket är att läka ut HPV infektionen. Det sagt, att inte gå på screening är den huvudsakliga anledningen till att kvinnor får sin cancer.

I denna avhandling presenterar jag hur man kan förbättra screeningen i ett land som Etiopien där våra studier har genomförts. Den första studien är en sammanställning av tidigare publicerad forskning där man jämförde deltagandet i screening mellan att kvinnan tog ett självprov och att vårdpersonal genomförde screeningen. Ett självprov innebär att kvinnan använder sig av t.ex en bomullspinne och roterar denna i slidan 3-4 gånger. När vårdpersonal tar ett test, så innebär det allt från att lämna ett cellprov till det som kallas ”Visual Inspection with Acetic Acid” (VIA). Det innebär att man penslar med ättika på livmoderhalstappens yta, väntar en minut och ser ifall något område blir vitt. Blir det vitt, så behandlar man området direkt. Denna metod är det som rekommenderats länge av WHO för just låg-inkomst länder och använts i Etiopien. Sammanställningen fokuserade enbart på studier från låg och medelinkomstländer (LMIC). När man slår ihop studier med liknande utfallsmått, kan man i vad som kallas en meta-analys, få ett tydligare svar på forskningsfrågan eftersom antalalet deltagare blir fler. Meta-analysen visade att deltagandet i screening var högre för gruppen som erbjöds själv-prov än de som erbjöds screening gjord av vårdpersonal.

I den andra studien, ville vi undersöka deltagande i screening med HPV själv-prov och förekomsten av HPV på en arbetsplats i Addis Abeba som under de senaste åren försökt genom cellprov att få fler kvinnor att gå på sin screening. Fler kvinnor deltog i vår självprovstudie som pågick under tre dagar, än antalet kvinnor som lämnat

cellprov de senaste tre åren. En femtedel testade positivt för högrisk HPV. Vi fann att bättre uppföljning av HPV-positiva kvinnor var något som behövde förbättras och den lärdomen tog vi med oss i studie tre.

I den tredje studien, undersökte vi två sätt att följa upp de som är HPV positiva. Vi jämförde ifall VIA tillsammans med jod var bättre på att hitta svåra cellförändringar och cancer än enbart VIA. Alla kvinnor från en kohort i Adama blev erbjudna screening med HPV självprov från slidan. Först fick dem information i form av en kort film som förklarade varför det är viktigt med förebyggande screening då man inte har symptom av cellförändringar eller HPV. Sedan fick de svara på ett antal frågor och slutligen lämna ett självprov. Om kvinnan testade positivt för hög-risk HPV, blev hon inbokad till ett barnmorskebesök där hon antingen genomgick VIA med eller utan jod. Som en del av studien, togs biopsier från livmoderhalstappen på alla HPV positiva kvinnor som inte var gravida. På så sätt kunde vi räkna ut hur bra känslighet de två metoderna har för att upptäcka måttliga, svåra cellförändringar och cancer. VIA med jod var bättre men kunde inte statistiskt skiljas från VIA utan jod. En stor anledning till att vi inte kunde skilja på utfallen i de två grupperna vi jämförde var att tiden mellan screening och uppföljning var så pass lång att många hade hunnit läka ut sin HPV infektion, men sannolikt också eventuella cellförändringar. Alltså hade vårt material inte tillräckligt många med cellförändringar för att kunna med säkerhet uttala sig om skillnaden mellan grupperna var statistisk signifikant. Däremot kunde vi se att jämfört med en HIV negativ kvinna, ökade oddsen för cellförändringar/cancer om kvinnan var HIV positiv och samtidigt testade positivt för HPV.

I den fjärde studien undersökte vi genomförbarheten att inkludera gravida kvinnor i ett screeningprogram med HPV självprov. Gravida har alltid varit utelämnande från screening i LMIC eftersom metoden inneburit VIA screening och behandling samma dag. Det är också mycket svårare att bedöma VIA hos en gravid kvinna på grund av de hormonella förändringar som sker i livmoderhalsen. Då median antalet födslar per kvinna är ca fyra i Etiopien, är det många år då man inte anses vara berättigad till screening. HPV självprov erbjöds till alla kvinnor och genom att separera de som var gravida, kunde vi jämföra dem med de som inte var gravida. Kunskapen om livmoderhalscancerscreening var likvärdig mellan grupperna, men det var fler gravida som aldrig varit på screening tidigare. Gravida accepterade självprovet i vår studie i samma utsträckning som de icke-gravida. Många var inte längre gravida vid uppföljningen.

Sammanfattningsvis, så finns det tillräckligt med evidens för att genomföra HPV baserad screening med självprov i Etiopien. Det ger oss möjligheten att inkludera kvinnor som bor långt från sjukvårdsinrättningar samt gravida som alltid varit exkluderande. Självprov gör att man kan utöka screeningen på ett sätt som inte varit möjlighet med VIA som kräver mycket sjukvårdspersonal. Till dess att det finns

bättre uppföljningsmetoder, som kan skilja på cellförändringar som kommer läka spontant. Anser vi, att tillsammans med annan forskning som finns tillgänglig, att man kan rekommendera användning av VIA med jod som uppföljning test för HPV positiva. För HIV positiva kvinnor kan det räcka att de är HPV positiva för specifika typer av högrisk HPV för att erbjudas behandling. Screeningen kan integreras med andra sjukvårdsystem, t.ex mödravård, HIV-vård, familjeplanering, och detta hoppas vi är ett framtida implementeringsprojekt. Därtill behövs studier som tittar på kostnaderna för olika screeningmetoder. Studie 1 visade att det finns få studier som jämför kostnader mellan screeningmetoder i LMIC, vilket är vårt nästa projekt som börjar i Mars 2024.

List of original Papers

I

Mekuria SF, Timmermans S, Borgfeldt C, Jerkeman M, Johansson P, Linde DS. HPV self-sampling versus healthcare provider collection on the effect of cervical cancer screening uptake and costs in LMIC: a systematic review and meta-analysis. Syst Rev. 2023;12(1):103.

II

Mekuria S, Jerkeman M, Forslund O, Fikru S, Borgfeldt C. Detection of HPV mRNA in Self-collected Vaginal Samples Among Urban Ethiopian Women. Anticancer Res. 2020;40(3):1513-7.

III

Mekuria SF, Biazin H, T Abebe, Borgfeldt C, Assegid N, Mihret A, Nemomosa Obsi R, Forslund O, Jerkeman M. Comparing visual inspection with acetic acid, with and without Lugol's Iodine for triage of HPV self-sample positive women in Ethiopia – A randomised controlled trial. Submitted.

IV

Mekuria SF, N Assiged, Borgfeldt C, T Abebe, Biazin H, Mihret A, Forslund O, M Jerkeman. Is it time to include pregnant women in HPV-based cervical cancer screening programs? A feasibility study in Ethiopia. Manuscript.

Author's contribution to the papers

Paper I

The author has together with colleagues from Denmark and Canada performed a systematic review and meta-analysis for randomised studies from low-and-middle-income countries, that compare HPV self-sampling with standard procedure for cervical cancer screening. She has written the manuscript and incorporated comments from co-authors. She has also responded to comments during the publication process.

Paper II

In this study the author independently wrote a project plan and sought ethical clearance in Sweden and in Ethiopia. Together with colleagues from the Ethiopian Airlines employee medical facility, she planned the logistics and collected the data. study. The HPV analyses were done at the Microbiology department in Lund. The author has analysed the data, written the manuscript, incorporated comments from co-authors and responded to comments during the publication process.

Paper III

The author has performed a randomised study between two diagnostics tests to identify pre-malignant lesions in cervix of hrHPV self-sample positive women. The project plan was written by the author with input from collaborators in Ethiopia. Ethical approval needed to be applied for at three locations The author was responsible for this moving forward. The HPV analyses were performed at Black Lion Hospital, Addis Abeba. The author had a leading role in the coordination of the study, the logistics, and performed most of the cervical biopsies. She independently analysed the data, wrote the manuscripts, and incorporated comments from co-authors.

Paper IV

It was the author's idea to evaluate the feasibility of including pregnant women in the screening cohort that is paper 3. She independently analysed data, wrote the manuscript, and incorporated comments from co-authors.

Abbreviations

HPV	Human PapilommaVirus
LMIC	Low- and Middle- Income Countries
HIV	Human Immunodeficiency Virus
CpG island	Parts of DNA rich in cytosine and guanine
CIN	Cervical Intraepithelial Neoplasia
Tz	Transformation zone
RR	Relative Risk
RB	Retinoblastoma
YLL	Years of Life Lost
LBC	Liquid Based Cytology
VIA	Visual Inspection with Acetic acid
VILI	Visual Inspection with Luogol's Iodine
RCT	Randomised Controlled Trial
AUC	Area Under the Curve
GRADE	Grading of Recommendations Assessment, Development and Evaluation

Preface

Cervical cancer, in countries like Ethiopia, is the most common cancer seen in a gynaecological outpatient clinic. It is also, in low-resource settings, considered to be the first or second most common cancer amongst women. In 2015, before I began my resident training, I spent six weeks at an Obstetrics and Gynaecology department in Addis Abeba. I witnessed the high number of women, usually in their 30's and 40' with children at home, worn of late disease. They sought care at a stage where most could only be offered palliative care in the form of pain medicine. Radiation and chemotherapy were scarce. Even though access has improved since then; both treatment methods and surgery, which is only done for early disease, are costly, particularly for low-and-middle income countries (LMIC). The way forward is therefore prevention in the form of vaccination and screening. This is the aim of the World Health Organisation (WHO) and they have set three strategic goals to be reached by 2030; vaccinating 90% of eligible women/girls, screening 70% of women above 30 years and treating 90% of those with precancer and managing those with cervical cancer(1).

ln this thesis, I will cover screening as a prevention for cervical cancer, a topic that led me to work as a gynaecologist.

Introduction

Anatomy

The cervix is the most distal portion of the uterus and is commonly referred to as the “neck of the uterus” and is a hollow organ. The lower part of the cervix can be visualized in a gynaecological exam, which allows examination and screening in a simple manner. This part ends in the vagina, whereas the proximal part of the cervix lies in the abdominal cavity, a total of four centimetres in length and three centimetre in diameter. The size of the cervix may change based on factors that all have to do with hormonal changes in the woman i.e. being pregnant, previous pregnancies, pre/postmenopausal state.

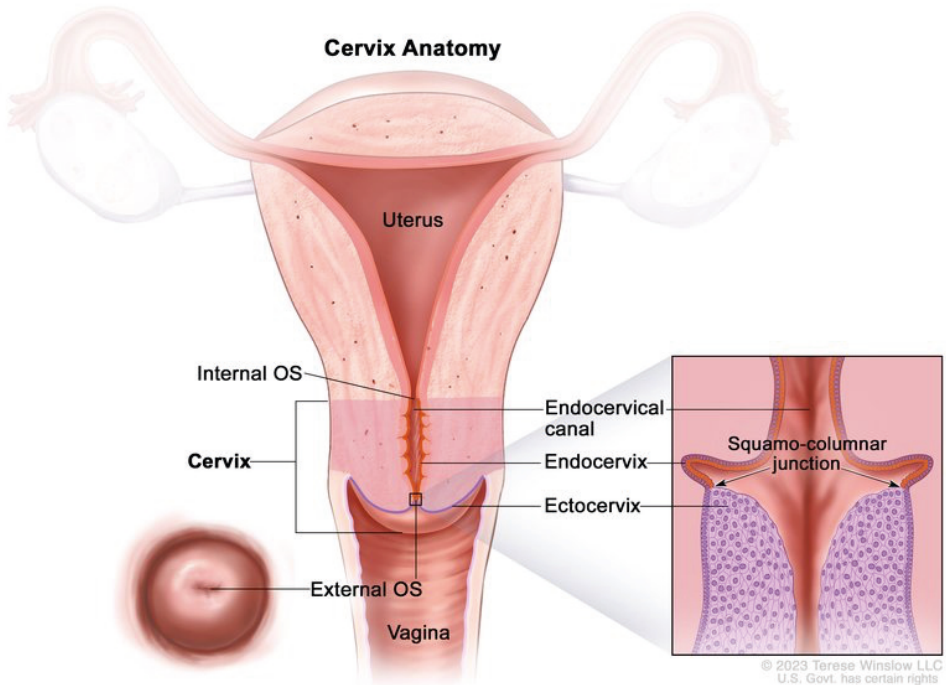


Figure 1. Cervical anatomy
Credit: © 2023 Terese Winslow LLC, U.S. Govt. has certain rights

Histology

The stroma of the cervix is made of fibromuscular tissue, whereas the cervical canal is lined with a single layer of columnar epithelium(2). Columnar epithelium produces secretions based on the woman's hormonal changes and are also called glandular epithelium. The glandular cells do not contain glycogen. The exterior surface of the cervix is lined by stratified non-keratinized squamous epithelium. The basal layer will be of importance when describing cervical disease development. The remaining layers have a high glycogen content, which is knowledge used for screening purposes discussed later.

Transformation zone

Squamocolumnar junction, where the squamous and columnar epithelium meet, is located at the external cervical os in early childhood. With arrival of puberty, leading to an increase in oestrogen, the original squamocolumnar junction gets pushed outwards by growing columnar epithelium. As the columnar epithelium migrates onto the exterior surface of the cervix (ectocervix), they encounter the acidic environment of the vagina. This causes them to change into immature squamous metaplastic epithelium, and the area is now called the transformation zone (Tz). With time, a maturation process ensues, which entails a stratification of the squamous metaplastic epithelium. The basal layer in the transformation zone is particularly sensitive to human papillomavirus infection, which will be covered in the next section.

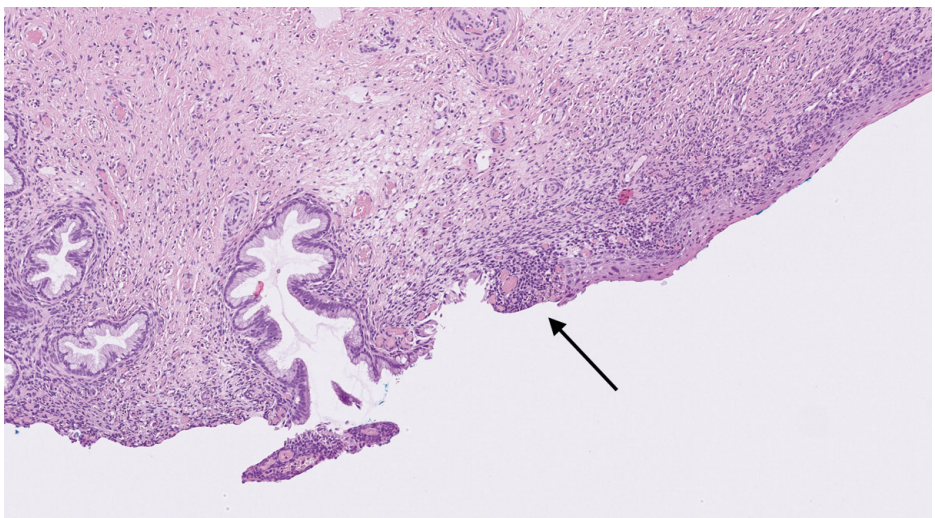


Figure 2. Transformation zone containing a small area with HPV infection in the basal layers
Credit: Anna Måsbäck MD. PhD, Department of Pathology, Lund University

Epidemiology

Cervical cancer is the eight most common cancer in women worldwide(3) but in many LMIC it ranks first or second(4). This despite cervical cancer being the only cancer that we know how to eliminate (less than four cases/100,000women). Cervical cancer incidence is on one hand decreasing in many developed countries(5). Whereas predictions are that in several African and Asian countries, cases will double(6). In Africa, the age-standardised rate of cervical cancer is the highest in Eastern Africa 40.4/100.000, and lowest in Western Africa 27.0/100.000(3). Moreover, the number one cancer-related death among women in Africa is cervical cancer.

Cervical cancers can be divided into two major histological groups, squamous carcinoma, and adenocarcinoma(7). Squamous carcinoma arises from the squamous epithelium on the exterior surface of the cervix and comprise 70-75% of all new cancer diagnoses in the cervix. Adenocarcinoma is diagnosed in 10-25% of cervical cancers and arises from the glandular epithelium of the cervical canal. The remaining histological types are rare, such as small cell carcinoma, a neuroendocrine tumour, carcinosarcoma and gastric type mucinous(8).

Cervical cancer is caused 95% of the time by the human papillomavirus (HPV). HPV is a sexually transmitted asymptomatic infection. It is a common virus in younger women (under 30 years), and thereafter its prevalence decreases with age until it reaches a steady-state(9). HPV alpha, hereafter written HPV, have 12 high-risk genotypes (16,18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59), all with the ability to cause cancer at varying degree(10). An intact immune system allows most women to clear the virus by themselves(10). It is the persistence of HPV that can lead to precancerous lesions and cancer.

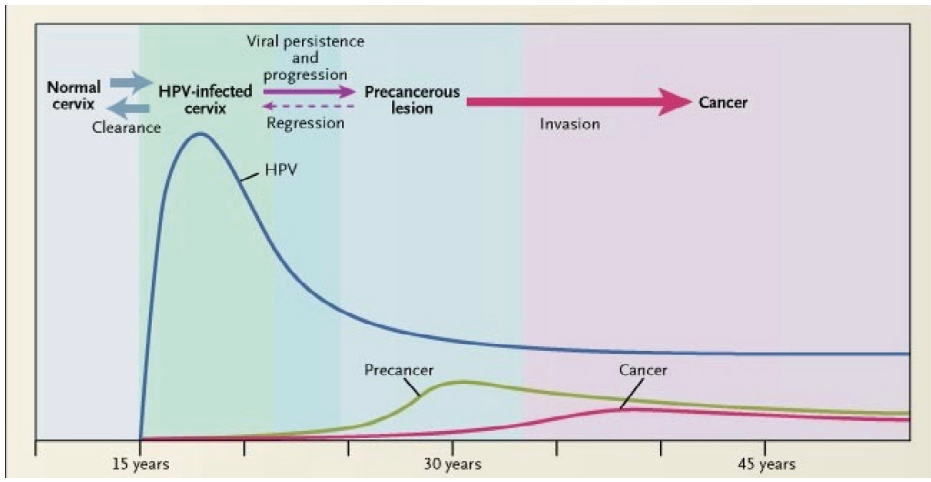


Figure 3. The natural history of HPV infection and cervical cancer
Reproduced with permission from Schiffman M, Castle PE. The promise of global cervical-cancer prevention. N Engl J Med. 2005;353(20):2101-4, Copyright Massachusetts Medical Society.

Human immunodeficiency virus (HIV) plays a synergistic role in the development of cervical cancer. The immunosuppression brought by HIV, makes it difficult for the woman to clear the HPV infection, leading to persistence. In southern and eastern Africa, the rates of HIV are the highest in the world (up to 40%), leading to a 6-fold risk of being diagnosed with cervical cancer(11). This major risk factor contributes to the high prevalence of cervical cancer in the region.

Other risk factors for cervical cancer include smoking, multiparity and oral contraceptive use. Being a smoker has relative risk (RR) of 1.7 (95%CI:1.53-1.88) for developing invasive cancer(12). The mechanisms are not fully understood but known carcinogens from tobacco cause DNA damage in cervix. At the same time tobacco can help HPV turn off tumor suppressor genes i.e p53 and pRB. Moreover, smoking decreases immune system surveillance by decreasing natural killer cells and antigen presenting cells, such as Langerhans' cells(13).

High parity (the number of births per woman) is associated with an increased odds of 2.65 (95%CI: 2.08-3.38) for developing cervical cancer(14). The exact mechanism is also here, not clearly defined, but to give birth, is a trauma to the cervical canal. In theory, this trauma would make it easier for HPV to integrate into the transformation zone. One might therefore argue that the decreased parity in high-income settings (1.5 births)(15), also is a co-factor in the decreasing trend of this disease. On the other hand, in many low-income settings, such as Ethiopia, the average number of births which is decreasing is still 4.6 births per woman, contributing to the prevalence of cervical cancer. Moreover, being pregnant has been a contraindication for screening in most LMIC. High parity excludes many women from screening during their reproductive years.

Human Papillomavirus

HPV biology

HPV is a double stranded DNA consisting of three different regions(10). The late region codes for capsid proteins, L1 and L2 that are used by the virus to infect the transitional epithelial cells(16). The L1 L2 protein-capsid binds to surface receptors, allowing the virus to integrate with the basal membrane of the epithelial cells. Here at the basal layer the virus replicates. Thanks to the integration of the virus, the cytokines that would have been released in a lytic process are not. Thus, the antigen presenting cells will not know of the infection(10), and it is one of the ways that the infection goes un-noticed by the immune system. This allows the virus to continue with its next step.

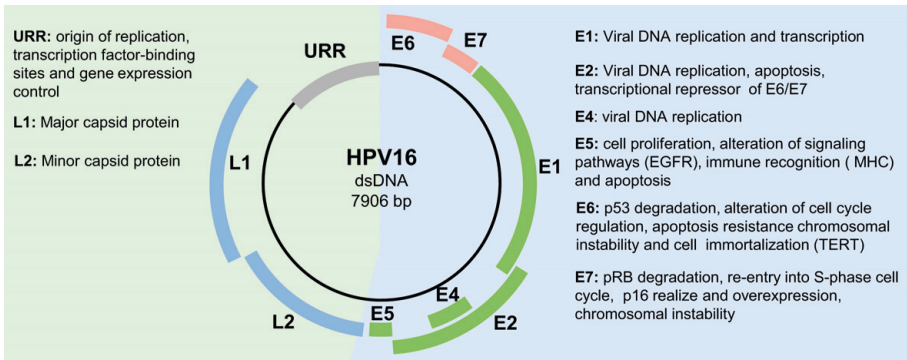


Figure 4. HPV 16 structure and viral proteins.
With permission from: de Sanjose S et al. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol. 2018;47:2-13. Copyright Elsevier 2024.

The early region transcribes the necessary genes for the viral cycle and cell differentiation(10). The infected cells differentiate while moving towards the surface of the epithelial layer. E6 and E7 oncoproteins exert their effect by transforming the harbouring epithelium to accumulate genetic defects. They also prevent the defective cells to undergo apoptosis.
Moreover, they both inhibit tumor-suppressor genes, specifically p53 and retinoblastoma (Rb), which can lead to uncontrolled replication of HPV transformed cells. One mechanism that this is done through, and which has promises for future screening techniques, is methylation. Methylation is an epigenetic process, where the gene expression is affected by modification to the genes we inherit(17). It occurs by the addition or removal of a methyl group to cytosine, usually cytosine found in DNA groups of cytosine and guanine (CpG islands). CpG islands are common in promoter regions of genes coding for proteins. This is one way of turning on and of

protein production in cells. In cervical cancer, turning off tumor suppressor genes is done by methylating these CpG islands in the promoter region of the tumor suppressor gene. This leaves the squamous epithelium infected by HPV, to continue rapidly multiply without any suppression.

The last and third region of HPV genome is called the Long control region (LCR)(10). It is a non-coding part that modulate the genes in the E and L region of the HPV genome.

Genotypes

Genotypes 16 and 18 are the most common ones found in cervical cancer patients. However, thereafter there are regional variabilities(18), and the 9-valent vaccine available covers these high risk types: 16, 18, 31, 33, 45, 52, 58. In North America and Europe, HPV 16 cause 72% and 66% of all cervical cancers respectively(18). Whereas according to available evidence, in Africa less than 50% of cervical cancer are caused by HPV 16. It is believed that the more common HPV types, such as 16 and 18 are better at persisting and through this cause progression of disease(19, 20). However, the reason why HPV 16 is better at persisting is not completely understood. Probably it has something with its ability to evade immune system recognition.

Many studies from LMIC focus on circulating genotypes, showing a high prevalence and diversity that isn't related to a cancer diagnosis(21). This may confuse implementation work, as the aim is to screen and vaccinate the genotypes found in cervical cancer, not its precancer.

One multicenter from 2010 study describes the genotypes common in cervical cancer in the different world regions and summaries the following in descending order(18):

Africa: 16,18, 45, 35, 52, 51, 31, 33.
Asia: 16,18, 45, 58, 52, 33, 31, 59.
Europe: 16, 18, 33, 45, 31, 35, 52, 51.
Central and South America: 16,18, 45, 52, 39,35, 58, 51.
North America: 16, 18, 45, 52, 31, 33, 58.

Focusing on studies coming out of Africa, one study from 2012 in Ethiopia and Sudan, demonstrated that 16, 18, 52, 58 were the most common genotypes causing cervical cancer(22). Another study from sub-Saharan Africa (Ghana, Nigeria, South Africa) concluded that the most common genotypes in cervical cancer patients were in descending order 16,18, 35, 45, 33 and 52(23). In Eastern Asia on the other hand, HPV 58 cause almost 10.2% ± 3.2 of all invasive cancers, even though this is a very rare type in the rest of the world(21).

The significance of the presence of multiple HPV genotypes in one person is still being studied. However, the ones that end up with cervical cancer often have HPV 16 as one of their multiple infections(24). Moreover, the presence of multiple HPV genotypes seems to decrease with increasing disease severity CIN 1 to Cancer)(25, 26). An observation regarding finding multiple genotypes in screening is that if they are not part of the same phylogenetic branch, it seems to have a negative correlation with cervical carcinoma(27) At the same time, multiple infections in cervical cancer has been significantly associated with worse prognosis in cervical cancer(28).

Pathology

The transformation zone undergoes changes caused by the integration of HPV into its basal layers. The cells infected by HPV have an increased nucleus to cytoplasm ratio (larger nucleus in relation to cytoplasm)(29). Depending on the how far these atypical cells extend and stratify from the basal layer to the epithelial surface, the severity of the lesion is decided. The term “crowded sheet” depicts the aggregation of cell layers seen with increasing dysplastic lesions(30). Squamous carcinoma is diagnosed when not only the epithelium is affected but the basement membrane has been breached.

The glandular cells may also undergo dysplastic change caused by HPV integration. This is seen similar to metaplastic squamous cells, that the nuclei of the glandular cells enlarge and there is crowding of cells(30). Moreover, because glandular cells are both found in the endocervix as well as the endometrium, further sampling higher up in the canal is normally warranted when glandular dysplasia is suspected. However, endometrial pathology is not caused by HPV. Adenocarcinoma is the potential end-stage of dysplastic changes caused to the glandular epithelium.

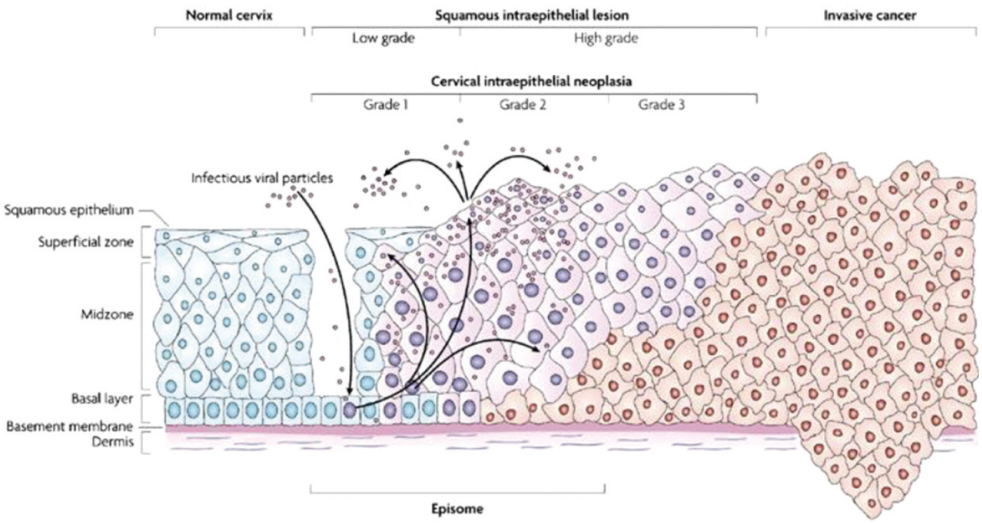


Figure 5. HPV integrating with the cervical mucosa, and causing different potential squamous intrepitelial lesions

With permission from: de Sanjose S et al. The natural history of human papillomavirus infection. Best Pract Res Clin Obstet Gynaecol. 2018;47:2-13. Copyright Elsevier 2024.

The cost of disease on the individual and society

In countries like Sweden, where the public sector covers all citizens' cancer treatments, the cost of HPV related cancers has been a driving reason for gender-neutral vaccination(31). Cervical cancer as a disease is a devastation for the individual's and family's economy by contributing the most to years of life lost (YLL) in LMIC(32). In countries like Ethiopia, the individual's economic hardship of a cancer diagnosis comes from both medical and non-medical factors, especially since the majority of cancer cases are found at an advanced stage(33). Travel, accommodation costs in addition to other out-of-pocket expenses are a reality for many patients who do not live in a city with cancer treatment. Loss of income and not being able to take care of the family on one salary is another important burden for the patient. These reasons may also lead to the sale of property to finance the out of pocket-expenses mentioned above. Cancer treatment is expensive for hospitals as it not only needs personnel but medications and surgical equipment to treat, manage and follow the disease. The factors above and more, make up what is called financial toxicity(34).

Financial toxicity is described as "the possible outcome of perceived subjective financial distress resulting from objective financial burden"(35). It leads some patients to discontinue treatment because of the financial strains it puts on the

family(36). Society can avoid these economic hardships if they invest in preventative measures.

Screening

History

Cervical cancer screening underwent a revolution with the introduction of the pap test named after its inventor, George Nicholas Papanicolaou(37). Dr. Papanicolaou, a Greek physician, received his medical degree in 1904, worked as a surgeon for the military before he left for Munich, Germany. There he earned his PhD in zoology in 1910. Shortly after returning to Greece, him and his wife emigrated to the USA.

The couple had no planned jobs waiting for them and did not know English. Thus, Mary worked as a seamstress and George went from rug salesman to violin player to a clerk at a Greek newspaper. Finally in 1914 he was hired at the New York University Pathology department, his wife Mary working with him as a technician. It was his research on the reproductive cycles of animals seen in vaginal smears that made him notice the difference in normal and malignant cells from simply looking in the microscope. The findings were published in 1928 but did not draw attention. The book that him and his college at New York University published in 1943 is what allowed the Pap test to be accepted and widely used around the world(38).

Primary screening

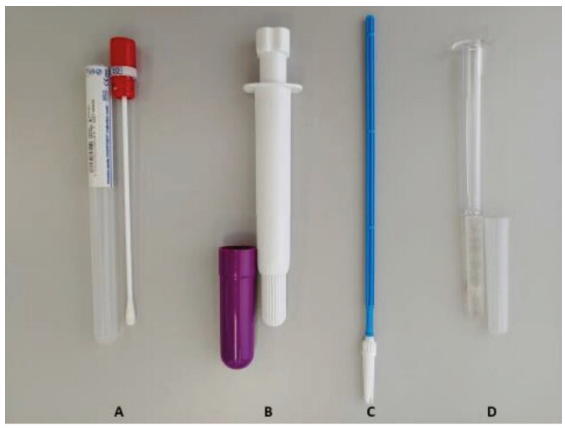
The second WHO goal for the elimination of cervical cancer is screening 70% of eligible women. WHO recommends screening starting at age 30 and depending on resources, stopped between 50-65 years(39). The recommended primary screening method is HPV testing. This is a molecular method, and the material can be collected from both cervical and self-samples(40). A cervical sample needs to be taken by a health-provider with the woman in lithotomy position using a small brush. A self-sample collects material mainly from the vaginal walls and can be done in the woman's private setting. The material can be collected in test-tube containing special media to preserve the DNA.

HPV testing

The HPV detecting molecular methods are divided into DNA or RNA target assays(41). The specific genes targeted in the HPV virus can be L1, E6/E7 or the entire E region. The laboratory techniques used to test the samples commonly include polymerase chain reaction (PCR) or signal amplification. The assays range from those supplying a negative/positive result, to full genotyping of high-risk HPV.

HPV testing can also be done on Point of care (POC) platforms, which are being used for both Tuberculosis diagnostics and HIV viral load(42). There are four main platforms, three which use PCR (GeneXpert, Ampfire and MA-6000) and that have been evaluated for use in low-resource settings(43). They differ in their ability to allow genotyping, turn-around time for testing as well as if both cervical and self-sample specimens can be used. The use of POC testing as part of screening algorithms allowing treatment the same day, are being researched in different countries(44, 45), with good outcome.

The pooled sensitivity of several different assays tested on cervical samples, revealed a sensitivity of 89-100%(41). Self-sampling means the woman collect the sample herself. Women can collect material from the vaginal wall using simple swabs for example the *Dry flocked swab* but also different types of brushes, such as the *Evalyn brush(46).*



A.Sterile viscose swab with a polystyrene stem into a sterile polypropylene tube.

B. Iune HPV sterile test cannula.

C. Viba-Brush®.

D.Mía by XytoTest®

Figure 6. Different self-sampling devices.
Creative common license(47)

Vaginal self-samples and cervical samples using PCR had no difference in sensitivity for cervical intraepithelial neoplasia 2 (CIN 2) or more(48, 49). HPV testing can be done using urine-samples and shows promise(50). However, the variations seen in the sensitivity of HPV testing in first-void urine need to be improved before clinical use(51). Suggestions for improvement include preferably a morning void, and that the urine is collected using transport/conservation media from the beginning.

It is common to report specificity of HPV testing and it is normally above 90%. This is not necessarily clinically relevant, as it answers the question if anyone who

is HPV-negative could have CIN. At this time, we know that HPV is a pre-requisite for virtually all cervical cancers and that HPV testing is more sensitive than the following methods covered, which are all subjective in one way or another.

Cytology

Liquid based cytology (LBC) or Pap smear have for many years been the primary screening method of choice, primarily for high-resource settings. It requires a health-professional to take a cervical specimen using a spatula or a small brush. HPV diagnostics can be done on this sample as well. The material is collected in media that will preserve the cells for them to be fixated on a slide for microscopic evaluation. The sample is considered adequate when both squamous and endocervical or metaplastic cells can be found. Depending on country, it is a cyto-diagnostician or a pathologist that evaluates these slides. Cytological result is commonly written according to the Bethesda classification or CIN classification(52). Pathology reports commonly with the same classification system.

Table 1. Bethesda and CIN classification

ASC-US: Atypical squamout cells of undefined significane. ASC-H: Atypical squamous cells of undefined signficance, but can exclude HSIL. LSIL: Low-grade squamous intraepithelial lesion. HSIL: High-grade squamous intraepithelial lesion. (52)

Bethesda	CIN
ASC-US	N/A
ASC-H	N/A
LSIL	HPV effect
LSIL	CIN 1
HSIL	CIN 2
HSIL	CIN 3

Cytology has a pooled sensitivity for CIN 2 of 62.8% (95% CI: 46.8 to 76.5%) and for CIN 3 74.4%, (95% CI: 67.8 to 80.1%). The pooled specificity is 97.7% (95% CI: 96.1-98.7), 96.9% (95%CI: 94.9-98.1%) for CIN 2 and CIN 3 respectively (53).

VIA and VILI

Visual inspection with 3-5% acetic acid (VIA) or Lugol's iodine (VILI) was until recently recommended for LMIC by the WHO(54). In contrast to the above-mentioned screening methods, they are single visit approaches where the woman is examined in lithotomy position and treated the same day. Acetic acid is sprayed on the ectocervix and then after 1 minute the health-provider assesses the patient with a naked eye. The transformation zone (TZ) is evaluated as fully visible (Tz1), visible with manipulation (Tz2) or inconclusive (Tz3), which means the TZ is drawn into cervical canal(55). This can be seen in post-menopausal women or patients on certain progesterone contraceptives. Normal squamous epithelium is light pinkish.

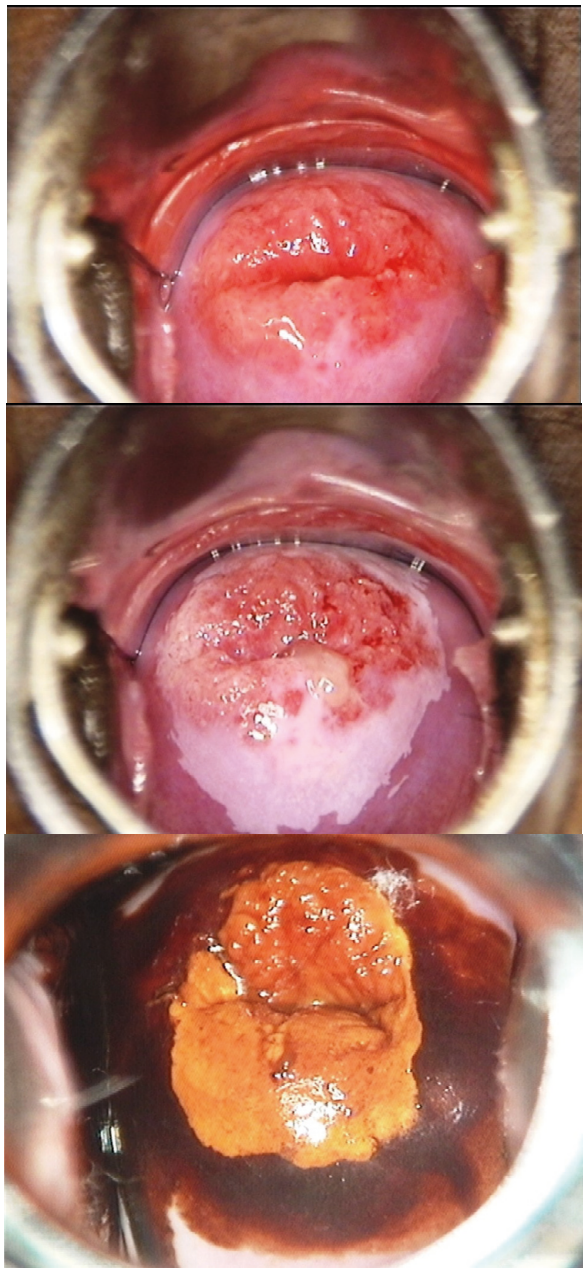


Figure 7. Image 1: Before. Image 2: Positiv with Acetic acid. Image 3: Positive with Lugol's Iodine
© International Agency for Research on Cancer] Mittal S, Basu P, Lucas E (2020). Atlas of visual inspection of the cervix with acetic acid for screening, triage, and assessment for treatment: IARC CancerBase No. 16 [Internet]. Lyon, France. Available from: https://screening.iarc.fr/atlasvia.php, accessed on 18Feb2024

Acetic acid turns white if the cells on the ectocervix have a high chromatin content because the proteins coagulate(56). High protein content is indicative of dysplasia. However, white coloration of the ectocervix may be a result of inflammation as well. Lugol's iodine stains cells with high glycogen content brown(57). As described in the histology section, squamous cells have a high glycogen content, thus they stain brown, whereas columnar cells and dysplastic cells are low in glycogen and stain yellow.

There are two common ways of assessing the exam, the IARC or the ABCD criteria. The IARC criteria(58) reports a positive test if there are dense white areas with clear borders on the exctocervix. The ABCD method is a co-testing method where A stands for Acetowhite, B for Bleeding on touch, C for Coloring with Lugol's Iodine, D for Diameter. ABCD gives an improved sensitivity but with more women being overtreated(59). The pooled sensitivity and specificity of VIA is 78% (95%CI: 73-83%), 88% (95%CI: 85-91%)(60). However, the authors of this meta-analysis report that verification of the VIA/VILI/co-testing was done mainly using colposcopy. Only in one study did they biopsy 20% of the colposcopy negative women. Hence, the sensitivity may be inflated.

Table 2. Diagnostic accuracy of primary screening tests (CIN 2 threshold)
*cervical samples(53). **(60).

Screening test	Pooled sensitivity % (95% CI)	Pooled specificity % (95% CI)
HPV DNA* (PCR)	95.1 (89.5-97.8)	91.9 (83.8-96.1)
HPV mRNA* (APTIMA)	92.66 (31.77 to 99.71)	93.31 (47.30 to 99.54)
Cytology* (Pap)	62.84 (46.79-76.50))	97.73 (96.09-98.70)
Cytology (LBC)	70.33 (59.73 to 79.11)	96.20 (94.57 to 97.36)
VIA**	78.0 (73.0-83.0)	88.0 (85.0-91.0)
Co-testing**	90.0 (85.0–94.0)	83.0 (79.0–86.0)

Triage

Triage in today's screening algorithm pertains to the triage of those that test positive on a high-risk HPV test. The need for triage is because the majority of HPV positive women will have no disease, and in comparison to both VIA and cytology as primary screening, HPV testing leads to more positive tests(61). Nevertheless, HPV test and treat(45) is one of the recommended screening algorithms. This strategy does not however consider that most HPV infections heal in addition to that approximately 50 % of CIN 2 regress(62).

As discussed previously in the background but also in paper three, it is important to risk-evaluate the woman as factors such as smoking, parity and most importantly HIV increases the risk of being diagnosed with CIN 2 or more. Moreover, smoking

and immunomodulating diseases decrease the likelihood of the woman being able to clear her dysplasia(11, 12).

Cytology and VIA/VILI

Cytology and VIA/VILI are both methods used for triage of HPV positive women(63). Their diagnostic accuracy shows some difference when used as a triage instead of primary screening. However, this observation also has to do with what the studies have used as gold standard. Cytology has in LMIC particularly had difficulties in keeping up performance(64). WHO data informs us that 8-15% of HPV positive women will be VIA/VILI positive and from those, only 20-30% have some type of grade of dysplasia(58).

Extended genotyping

Genotyping as a triage method is using the knowledge of what genotypes more commonly cause cancer, to decide which ones need follow-up and/or treatment directly(65). In a study from Cameroon, using HPV positive self-samples, the sensitivity for CIN2+ went from 38.6% to 93.2% when comparing triage genotyping for 16/18/45 (pool 1) and 16/18/45/31/33/35/52/58 (pool 2)(66). But the number of false triage positive increased, resulting in a specificity of 35.0% for pool 2.

In women living with HIV (WLHIV) in China who test HPV positive, triage in the form of genotyping (16, 18, 31, 33, 45, 52,58) revealed a sensitivity for CIN2+ of 94.7% and a specificity of 77.3%(67). The specificity for extended genotyping as triage became better when used in WLHIV. The potential of extended genotyping in this risk group is echoed from a South African study(68).

Dual stain

Dual stain is essentially a histochemistry method to interpret cytological specimens(61). p16 is a cellular protein that is present through the activity of the E7 oncogene, and if present the cytoplasm stains brown. This protein disturbs the retinoblastoma pathway, which is important in tumor suppression. The red staining of the nucleus is indicative of ki67 presence and informs us of the proliferative nature of the cell(69), which is a normal cell characteristic. It is the staining of both the nucleus and the cytoplasm that demonstrates the oncogenic nature of the cell.

Colposcopy

Colposcopy is like VIA/VILI with the exception that the assessment is done through a magnification-instrument instead of the naked eye. To standardize the method, assessment is commonly through Swedescore(70). The role of colposcopy has been to follow cytology done with or without HPV-testing and allow for directed biopsy. This has been standard in most high-income settings. However, the need for equipment, specific training and infrastructure has made it difficult to implement in LMIC(39).

In a recent multi-centric trial from Latin America, a high sensitivity was achieved but with many being overtreated(71). In India, the use of telemedicine as quality assurance for the use of colposcopy, showed no significant difference between the use of static images and live image assessment(72). If widely implemented in an Ethiopian screening setting, the treatment would have to occur at the same time as the triage. This to not loose women for follow-up.

Table. 3 Swedescore scoring model
Credit: Adapted from Strander et al 2005

Assesment	Score (0)	Score (1)	Score (2)
Acetouptake	None	Shady milk	Stearin
Margins and surface	None or diffuse	Sharp but irregular, satellites	Sharp and even, cuffing
Vessels	Fine, regular	Absent	Coarse, atypical
Lesion's size	<5mm	5-15mm or 2 quadrants	>15mm or 3-4 quadrants or endocervically undefinded
Iodine uptake	Brown	Faintly yellow	Distinct yellow (canary)

Methylation

In a cervical cancer screening setting, what we know more of is DNA methylation of host-cell(73). The increasing hypermethylation (the addition of metylgroups to cytosine) of genes coding for tumor-suppressor proteins is indicative of tumor progression(74). This analysis can be done on both cervical and self-samples. Moreover, detecting methylation has the advantage of being used even with an inconclusive or transformation zone 3, which is an issue of all subjective methods (VIA, cytology, colposcopy)(74, 75).

Most techniques first involve a bisulfite-conversion, which means the addition of sodium-bisulfite solution. This causes all un-methylated cytosines in the genetic material collected, to turn into uracil(76). Methylated cytosines remain unaffected and can be targeted with specific primers emitting fluorescence that can be quantified using PCR. Another inexpensive quantification method is pyrosequencing. Usually, a combination of methylation targets is tested to improve the sensitivity of the method, such as FAM19A4/miR124-2(77). The more hypermethylation is detected, the greater the risk that the women's dysplasia will progress to cancer and not regress(73).

Table 4. Diagnostic accuracy of triage methods for HPV positive women
S-VIA: Smartphone VIA

Triage methods	Sensitivity (%)	Specificty (%)	References	Threshold
VIA	25-65.6	74.2 - 87	**(78-80)**	CIN2+
Co-testing(ABCD)	77.1-80.8	31.2 - 43.8-	**(81, 82)**	CIN2+
S-VIA	74.6	61.8	**(83)**	CIN2+
Cytology	51.9,90.0	75.0- 85.2	**(78, 84)**	CIN2+, CIN3+
Colposcopy	76.9-91.2	37.0 - 50.1	**(71, 72)**	CIN2+, CIN3+
Dual stain	74.9	74.1	**(84)**	CIN3+
Genotyping	93.2	35.0	**(66)**	CIN2+
DNA Methylation (Self and cervical samples)	69.4-94.7	41.8-78.9	**(73)**	CIN3+

Table 5. Advantage and disadvantage of all primary screening and triage methods

Test	Advantage	Disadvantage
Visual inspection and treat	Single visit, material is inexpensive. Can be done using a smartphone. Can be performed by midwife, nurse	Low sensitvity, demands large pool of human resources and continous training. Subjective method. Cannot be used with Tz 3.
Cytology	Less invasive than biopsy	Subjective, demands complicated infrastructure and continous quality assurance
Dual stain	Makes the assessment more objective. Can be standardised in settings with few cytotechnicians or pathologists	Expensive reagents. Demands a complicated infratstructure.
Colposcopy	Guides to better histopathology. Possible with telemedicine.	Cannot be used with Tz 3. Subjective method.
DNA methylation	Gives information of progression not only of risk for disease. Objective method. Can be performed on self-samples.	Need for laboratory equipment and disposibles.

Treatment of pre-cancerous lesion

The third goal of WHO is treating 90% of those found to have pre-cancer. Essentially there have been four methods, which depending on resources and availability, have been used.

Cryotherapy or cryosurgery, is a controlled technique using freezing to destroy tissue(85). It has been used for over 100 years in several medical fields, with different chemicals. Industrial or medical carbon dioxide (CO_2) and liquid nitrogen (N_2O) are common gases utilised(86). Liquid nitrogen has the benefit of being able

to reach -89C before freezing. Industrial carbon dioxide is more easily available but freezes at -78C(85). To destroy malignant cells, a temperature of less than -50C is required but the lower temperature, the better. A probe suitable to treat cervical neoplasia was created in 1964(87). The probe is applied to the transformation zone and causes tissue necrosis by crystallisation of intracellular fluids(88). The recommended method involves freezing for three minutes, waiting to defrost for five minutes and then a repeat of freezing for three minutes(86). The depth of necrosis is important for cure rates(89). Liquid nitrogen achieves a mean depth of necrosis that is higher than carbon dioxide (5.3 vs. 3.4 $p<0.000$)(90) and is thus preferred for cervical pre-cancer treatment. A systematic review demonstrated cure rates that varied between 77-93%(88), this was however not reported with subgroups for different gases. There are few complications such as larger bleeding or infections with cryotherapy in comparison to excisional treatment techniques discussed below(89). However, finding high quality gas is an issue for many LMIC, where this method is primarily used. Industrial carbon dioxide, frequently used for beverages, is common and may include impurities and thus not reach the desired freezing temperature(89).

Thermal ablation is a method that gained favour as a contender to cryotherapy because of the lightweight handheld equipment that can be transported easily(91). The technique works by electricity generating heat up to 100C when the re-usable tip is touching the transformation zone(92). The treatment is over in 20-30 seconds, which is meant to lead to the destruction of the cervical lesion. Pooled treatment success for CIN2+ is 93.8%(91). The complication rates are like cryotherapy, low.

An excisional treatment is referred to as a conisation based on how it removes cervical neoplastic tissue in a cone-form. Their advantage lies in that there is material that can be sent for histopathology and thus be used for diagnostics(93). There are three main excisional procedures. The first one, Cold knife conisation (CKC), is done in an operating theatre under regional or general anaesthesia(94). A scalpel incises the cervix circumferentially including the Tz and dysplasia. This technique is preferred when a high risk of cancer exists and margins are important for the pathologist(93). CKC has the lowest rate (1.4%) of recurrence from both LEEP and cryotherapy(95).
Loop electrosurgical excision procedure (LEEP) is the treatment technique of choice for most high-income settings(89). For LEEP, a wire loop electrode is used to excise the transformation zone at a desired depth chosen by the operator(96). It coagulates the margins at the same time it cuts. The technique needs an experienced provider but can be done in local anaesthetic in an outpatient setting, which allows for wider use. In a large Swedish study, eight months after conisation, almost 70% had a negative HPV and cytology test(97). Two years after treatment the number was 84.4%. The treated women had a lower risk of HSIL compared to the general

screening population for the first three years. Longer follow-up studies are however needed to say anything about the long-term recurrence rate.

Another technique, laser, can be used for ablation of the transformation zone through photocoagulation(89). Moreover, laser can be used to cut a 1cm deep incision around the transformation zone. Both methods are not as commonly used as they are more expensive than other techniques, but previous studies report success rates of over 90%(88). Like CKC, margins are not burnt with laser, which makes it easier for the pathologist to evaluate the specimen(94).

The main disadvantage of conizations lies in its risk of bleeding, which has made it difficult to scale up in low-resource settings(89). It is therefore used as a second-line treatment for those that are not suitable for ablative treatment i.e large lesion, Tz type three. Complications related to the depth of the cone include cervical stenosis, which makes follow-up difficult for the women, and the increased risk of premature birth in the future (98, 99)

Psychological and societal factors to consider.

Eastern Africa bares the highest burden of cervical cancer in Africa(3). Not surprisingly, studies coming out from different countries in Eastern Africa show a low accurate knowledge of cervical cancer screening(100-102). In Ethiopia, radio and television including knowing someone who had cervical cancer, seem to be the route people gain knowledge(103). However, knowledge of cervical cancer screening has not been associated high screening attendance(103-105). Even with knowledge, screening attendance is under 10% in Ethiopian studies. There are psychological and actual barriers for the women to overcome. The scarcity of medical services make women and their families question why they should be screened if the treatment is not available where they live(106). There are also misconceptions of cervical screening, such as being asymptomatic does not warrant screening(103). Moreover, as screening currently needs a gynaecological exam for VIA, previous disrespect and abuse in conjunction with childbirth, may discourage women to attend(107).

Aims

The overall aim of this thesis was to evaluate a screening algorithm based on HPV self-sampling, and how this can be implemented in Ethiopia.

Paper I

To synthesize studies evaluating the effect of HPV self-sampling versus healthcare-provider screening methods for cervical cancer screening uptake in lower- and middle-income countries. To analyse available cost data to describe the difference in resource consumption between the two types of screening services.

Paper II

To evaluate the participation rate of a free of charge vaginal self-sample and determine the prevalence of HPV mRNA in an Ethiopian urban cohort.

Paper III

To examine the sensitivity and specificity of VIA with and without Lugol's Iodine as a triage test for women who tested positive in a self-sample for high-risk HPV.

Paper IV

To assess the feasibility and discuss the outcome and lessons learned of including pregnant women in this screening program.

Methods

Paper I

Before paper 1, a great number of studies on HPV self-sampling were being conducted but no systematic review with a meta-analysis that specifically focused on self-sampling's acceptance in LMIC. According to Cochrane methodology, a protocol was created using the PRISMA-P guidelines and then posted on Prospero's website for transparency. The article selection was based on PICO-S criteria: Population, Intervention, Comparator, Outcome, Studytype(108).

A librarian did the literature search based on criteria given by thr research team. The criteria: P=Patient living in LMIC eligible for cervical cancer screening, Intervention = HPV self-sampling, Comparator = any health-provider taken sample, Outcome = proportion of patients in self-sampling arm, Study type = Randomised controlled trials. This generated a large amount of titles that were uploaded to Covidence, a software for managing data for systematic reviews(109). Two persons went through all titles, accepting the articles that fulfilled the PICO-S criteria. Then two persons read through the abstract of the selected titles, and accepted the abstracts based on the same criteria. Finally, the remaining full-text articles were read and the ones that fulfilled all criteria were included in the systematic review. If there were any disagreements about including an article, it was discussed between the two reviewers.

Pre-defined variable data were extracted using an excel sheet. Attendance data for HPV self-sampling and health provider-taken sample was extracted as proportions and entered into Review manager 5 software(110). Pooled relative risks (RR) including their confidence intervals were calculated using the inverse variance method. To avoid over-estimating the effect of cluster-RCTs, we calculated an effective sample size using an intra-cluster coefficient of 0.098, if the study itself did not report one on their own. Statistical heterogeneity was assessed using I^2. Sensitivity analysis was performed using the inverse variance method. We performed subgroup analysis comparing low with middle income countries and low-risk bias with high-risk bias trials. Three authors assessed the risk of bias for all included studies using the Cochrane Risk-of-Bias tool 2.0 (for single and cluster randomized trials). The studies were deemed as having low, unclear or high-risk of bias. For the subgroup analysis, low-risk was defined as low risk of selection bias,

detection bias and reporting bias. The meta-analysis was presented using forest plots for visual representation of the results.

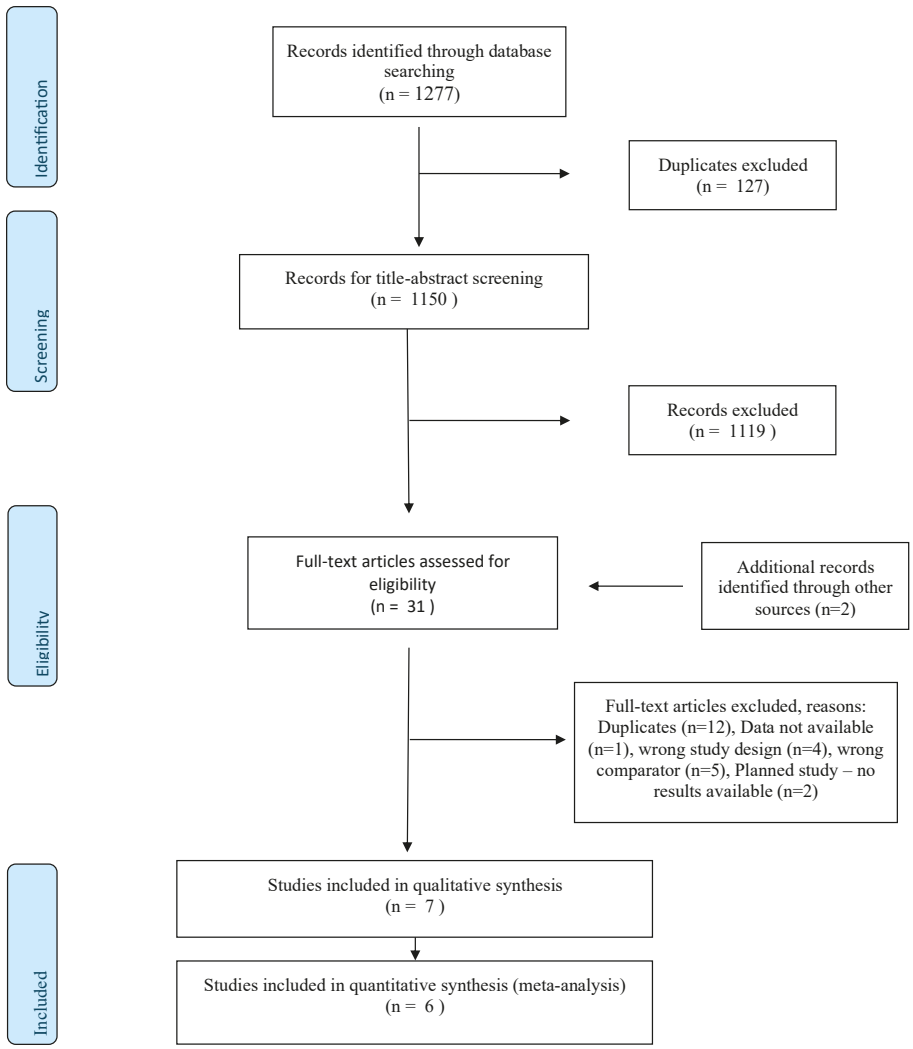


Figure 8. PRISMA Flow diagram

Discussion

The decision of conducting a systematic review was to get an overview of the field and to know if the clinical question, is HPV self-sampling better for screening uptake than a health-care professional taken sample, was answered. There had been a review in 2019 that included studies from the entire world, where only three studies came from LMIC. We chose to add the new studies that had been published and focus specifically on LMIC, as the discussion regarding uptake is different for the studies taking place in the global north. In three of our studies, most women had never been screened prior to enrolling. Thus, self-sampling is not just replacing a previous screening method, it is introducing screening to a new population.

We chose the most rigorous approach of doing a systematic review after I had completed my Cochrane methodology training. Another strength was the addition of two persons to the review team who had previous knowledge of how to conduct a meta-analysis and how to include cost-data. One potential limitation was the decision to include cluster-RCTs, which made up half of the studies. Commonly those allocated to a specific cluster answer similarly as the group and this may increase the effect of the assigned intervention. However, we took this into consideration in our methodology by adjusting the effect size. Another limitation to this review may be the few studies (six) we included, as there were not many randomised studies to find. We could have included all types of research studies reporting proportions of screening attendance. However, the reason we chose to only include RCTs was that they provide the highest quality of evidence according to GRADE(111). Thus, allowing a stronger recommendation on implementation or further research.

The low number of studies may limit the generalisability of our results and affect the quality of evidence. The same goes for the high heterogeneity reported. This was the main reason why we decided to conduct a sensitivity analysis. We removed one study that reported proportions, but where the primary outcome was not actually acceptance of screening method. This led to the sensitivity analysis not only revealing a greater relative risk favouring HPV self-sampling, but also made the results more homogenous (I^2 went from 97% to 42%). Thus, we received a higher certainty in the results.

Paper II

This was a cross-sectional study where we wanted to evaluate participation rate with HPV self-sampling as the screening method of choice. We also wanted to evaluate the prevalence of HPV mRNA in an Ethiopian urban cohort.

The study was done in collaboration with the Ethiopian airline's medical office. Women working for the airline had a week prior to the study commencement received information of the study and the possibility of being screened for HPV. There was then a health-event where both me and the medical director spoke about cervical cancer, screening, and the importance of prevention. Women were right after invited to participate in the study by filling out a health-questionnaire, consent-form and finally they received a self-sample kit. A nurse explained how to use the vaginal swab and then transfer it into the APTIMA media. The study was open for inclusion for three days in total. The samples were then sent to Sweden for analyses.

The HPV mRNA assay detects the mRNA sequence of the oncogenic proteins E6 and E7 from 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). A positive or negative result was read based on the sample containing any of these genotypes. HPV positive results underwent extended genotyping using the MGP-PCR Luminex HPV DNA

Figure 9. Women receiving information about the study by an Ethiopian Airline's nurse
Credit: Selamawit Mekuria's photo

All women received their results regardless of HPV status. Those that tested HPV positive were asked to schedule a follow-up date with the airline clinic's gynaecologist for triage with cervical LBC. The cytological specimens were analysed at a local pathology lab (ICL: International Clinical Laboratories).
Descriptive analyses were used for participation rate, follow-up rate, characteristics HPV status, cytology results.

Discussion

When this study was planned, published research about HPV prevalence or screening acceptance in Ethiopia was scarce. It was also the first time the initial research team had conducted research in Ethiopia. The methodology, cross-sectional, allowed us to hypothesize about future studies, and resulted in data on prevalence of HPV. The main limitation was the short three days inclusion time. This mainly had to do because of the study investigator's allowed time to be away from clinical work. More time would have allowed more women, especially those working as pilots and hostesses, to be able to take time to come for screening. The participation rate was low considering the total number of employees, but it was still more than previous screening efforts from the three previous years. We thus hypothesized that the short inclusion-time was contributing to the low participation rate. We couldn't confirm this because occupation was not included in the questionnaire.

Regarding HPV prevalence, we were aware that this was a small subsection of an urban population, and not representing Ethiopia.

Moreover, the choice to ship the self-sample kits to Sweden was done as we used the Hologic system for analysing HPV positivity. This is the laboratory method of choice for analysing HPV in Region Skåne where the initial team had its workplace. We could have used a different kit and system but since this was our first study in Ethiopia, we saw it as a chance to gain future collaborators. This cross-sectional study led us for paper 3 and 4, to collaborate with local researchers and do all molecular and pathology investigations in Ethiopia.

The decision to use LBC as triage was not optimal because the diagnosis changed for three samples. Furthermore, it meant an extra visit for result and possible treatment. This was only possible because the medical center at Ethiopian Airlines had the logistics in place. But it is not an algorithm that is possible to generalise for the whole of Ethiopia because of obvious logistic reasons.

Paper III

This was a two-armed randomised controlled trial (RCT) based on a previously established prospective cohort in Adama, Ethiopia. We wanted to evaluate the difference in diagnostic accuracy of VIA with or without Iodine.

The women were recruited at two health centres after first watching a film introducing them the topic of cervical cancer screenin. If the women fulfilled the inclusion criteria, not exlcuding pregnant women, they were asked questions by a research nurse. If they agreed to give a vaginal self-sample, the signed a consent form and took the kit with them to the bathroom.

The self-samples were analysed at Black Lion Hospital microbiology department in Addis Abeba. DNA was extracted from the vaginal material and using Anyplex™ II HPV HR Detection kits (Seegene, South Korea) with quantitative PCR, 14 high-risk genotypes were analysed. Results were communicated to the research-team in Adama. Negative results were given over phone. Women with positive HPV test-results were appointed to the VIA clinic at Adama Regional Hospital. At triage, all women were stratified according to age and pregnancy status and then randomised using the RedCap software to either VIA with or without Iodine.

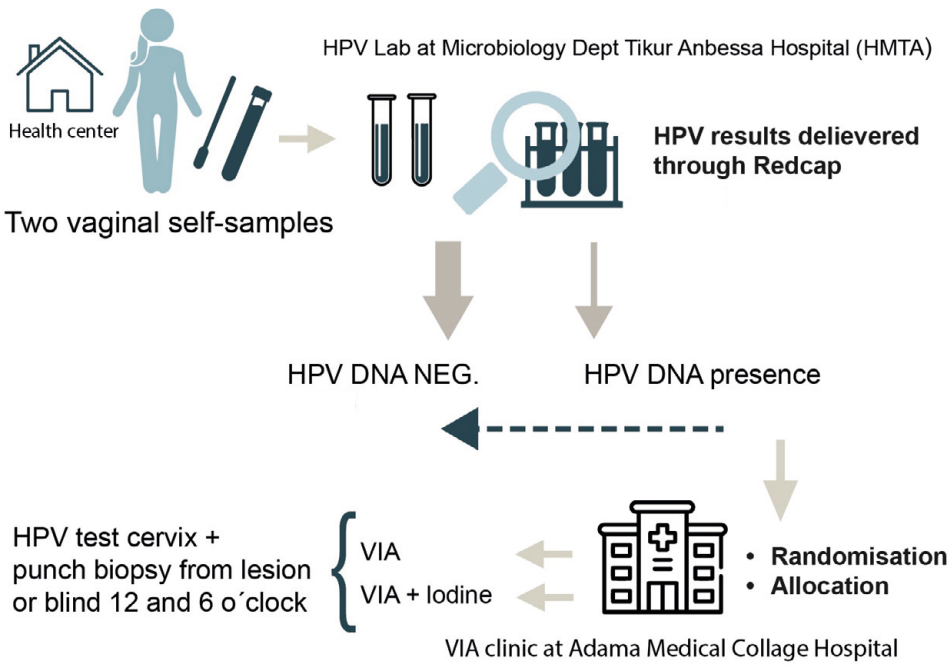


Figure 10. Workflow Paper 3 and 4
Credit: With help from Katarina Jandér, Lund University

One experienced midwife saw all the women at triage, gave them the results and explained the examination and potential need for treatment. The examination included examining the woman in lithotomy position with a metal speculum. A cervical swab was taken for HPV diagnostics before acetic acid was sprayed on the cervical surface and a waiting time of one-minute followed. If the woman was randomised to iodine, this was added before the assessment of the ectocervix. IARC criteria was used to diagnose the women as negative, positive, inconclusive or suspicion of cancer at the triage. Cervical biopsy was taken by the gynaecologist on-call, either from the lesion or from 12 and 6 O'clock. Treatment in the form of cryotherapy or thermal ablation was done for the women who tested positive in triage. If the woman was pregnant, biopsy was only taken if there was a suspicion of cancer. All cervical biopsy samples were sent to ONCO Pathology clinic in Addis Abeba.

Statistics: Descriptive statistics were used for all participant characteristics (proportions, percentages, median) and to compare the two arms against each other, chi2 test was used. Before the commencement of the study, the study was registered on clinical trials.gov and a power calculation was done. We hypothesized that to see a difference with 80% power and a type 1 error of α=0.05, we would need to recruit 82 participants in each arm. We assumed an increase in sensitivity of VIA from 60% to 80% with the addition of Iodine.
Sensitivity and specificity were calculated using cross-tabulation, where the biopsy result was gold standard. Area under the curve (AUC) and receiver operating curve was calculated for each arm and compared. Logistic regression was used for odds ratio. STATA statistics software was used for all statistical work(112)

Discussion

We knew from the work of the meta-analysis that HPV self-sampling was clearly preferable as a primary screening method. However, the world is still debating what is the best way to take care of the HPV positive women. The infrastructure of VIA-clinics is widening in Ethiopia and thus we wanted to evaluate if we could use an existing framework for follow-up. Iodine exists in some settings, particularly if they have the option of loop surgical excision (LEEP). By choosing to do a randomised interventional study we would clearly know if we by simple means could improve the sensitivity of the triage method.

We calculated based on previous studies that if we recruited 1000 women, roughly 220 women would be HPV positive. Even if we lost some women at follow-up, we would most likely have 165 women come for follow-up, which was needed according to our power calculation. However, this was not achieved mainly because it took time between primary screening and then finding the women, as well appointing them to a clinic. There was no person permanently based in Adama to

make sure that the project-flow was optimised. The gynaecologist that would take the biopsies, changed twice from the initial person we planned the project with.
As time passed, many of the women were lost to follow-up because of new phone numbers, moving away etc. Most of the follow-up clinic events thus occurred when the doctoral student was present in Adama.

A major strength in this work was that cervical biopsies was used as gold standard, and not cytology nor colposcopy. Probably this contributed to the low disease prevalence, which is sometimes over-estimated as they are seen as proxies to the histopathological diagnosis(96). With regards to prevalence, the long-time between primary screening and follow-up of HPV positive women, also most likely contributed to the low disease prevalence. We know that at least half of the women with CIN 2 will regress. Thus, even though the long time between part one and two of our study contributed to the loss of participants, it demonstrated the low prevalence of actual disease if we give the dysplasia time to regress.

In this study the laboratory work was done locally in Addis Abeba, which was an important part of the methodology if we wanted this to be implementation science(113). Challenges related to wide implementation of HPV diagnostics is a discussion topic for our colleagues working in the laboratory. However, as described in the background of this thesis, POC instruments are becoming more efficient and available.

Statistically we chose to not do more calculations because of the low prevalence of disease. Moreover, we cannot externally validate our results as they did not fulfil power. Instead, we had to look at other interventional studies evaluating different visual inspection techniques for suggestions of implementation. We believe that future studies instead of doing larger RCTs comparing visual inspection techniques, should focus on low-cost molecular triage for this group of HPV positive women.

Paper IV

This was an observational feasibility study based on the RCT conducted in Paper 3. We wanted to describe the pregnant subgroup participating in screening as this is not the norm in LMIC. The pregnant women underwent the same intervention as the non-pregnant participants, with the exception that no biopsy was taken, nor treatment given for a positive triage test. The aim of the triage for the pregnant women was to visually exclude any cancer before delivery. If they still had HPV in the cervix and were assessed as triage positive, they were re-booked 2 months after delivery for a new evaluation.

Descriptive statistics were used to describe characteristics between pregnant and non-pregnant women. Contingency tables and chi2 test were used to evaluate any correlation between background, behaviour, HPV prevalence among pregnant and non-pregnant women. Logistic regression was used to calculate the odds ratio of being pregnant and participating in screening.

Discussion

We wanted to specifically look at this subgroup in our study, because they are often excluded from interventional studies particularly in LMIC. By including women regardless of pregnancy status, we were able to compare if there were any demographic or behavioural differences between pregnant and non-pregnant women. Because this was an observational study nested into a study with a different primary outcome, we could not evaluate how including pregnant women would be best implemented. Even though experience from this study demonstrates it is possible to include this historically excluded group. For implemental purposes, a prospective study that integrates cervical cancer screening with antenatal care would have to be evaluated first.

Ethical considerations

Ethical approval was applied and approved for three of the studies in Ethiopia. Paper 1 did not need ethical approval. Paper 2 involved collecting self-samples in Ethiopia and transporting them to Lund, Sweden for HPV analysing. This meant that we also applied for ethics approval in Sweden.

Paper 2-4 involved women collecting biological material using swabs that gives minimal discomfort, like placing a tampon. We made sure the women understood the task before asking them to use the swab. Moreover, for women testing positive for HPV, it was important that they received follow-up. This meant that it was not enough for women to not answer their phone once or twice. We tried several times to reach them. In the workplace study, we also used their email accounts. For paper 3 and 4, we offered to pay transport and medical expenses related to further treatment. Not having funds is a common reason for patients to not follow-up on treatment(33).

There were no risks associated with being screened with a self-sample. However, many women who test positive may worry about potential disease. Despite education before screening, anything related to cancer tends to cause worry. For most women, this worry will be unwarranted. That is the downside of the HPV test, which captures many women who would heal on their own.

For triage we did take into consideration that when you are pregnant, to not take any biopsies unless there was a suspicion of cancer. This was decided because

pregnant women are more prone to bleeding, and even though it is possible to biopsy them, and we do this for example in Sweden at colposcopy. Paper 4 is one of the very few studies that includes pregnant women in screening in a low-resource setting. Thus, there are no previous guidelines or recommendations from WHO and we did not wish to take any un-necessary risks at this stage.

Taking blind biopsies on all un-pregnant women coming for follow-up was gold standard for detecting disease. We knew most would be benign, but as we wanted to evaluate the triage test, and cytology is inferior to histopathology, this was a choice made. A cytology test would need to be followed up with biopsy if there is suspicion of pathology. I believe this gave more reliable results, and decreased time from diagnosis to treatment.

Significant results

Paper I

HPV self-sampling improves participation in cervical cancer screening compared to health-provider dependent screening LMIC (RR: 1.82 (95% CI: 1.67–1.99; I^2 = 42%; 5 trials; 9590 participants). There is little cost data comparing screening strategies in LMIC, and they are not usually incorporated into the planning of the clinical study.

Paper II

One out of 5 women harbour HPV when self-sampling from the vagina. HPV self-sampling improved participation at this workplace, when compared to historically low screening attendees. Cytology needs great logistics and is not suitable for screening or triage purpose in Ethiopia.

Paper III

VIA with Iodine was numerically slightly better than simply VIA for triage of hrHPV positive women in self-samples. But because of low prevalence of disease at follow-up we could not say that this difference is not because of chance. Sensitivity for VIA alone 25.0% (95% CI 0.6-80.0%). Sensitivity for VIA and Iodine 50.0% (95% CI 0.7-93.2%). The odds of detecting CIN2+ if HIV positive was 6.4 times higher than if she was HIV negative. Thus, being HIV positive was better at predicting CIN2+ than both triage tests.

Paper IV

Pregnant women accepted screening in this study similarly to non-pregnant women. They had the same knowledge about cervical cancer but had a lower history of previous participation in screening (p=0.07). Women were triaged only to exclude cancer, which was successful.

Discussion and future perspectives

The use of HPV self-sampling is the way forward for cervical cancer screening around the world. However maybe more so in LMIC, because of the low sensitivity of VIA and the need to reach women who live in rural areas that are missing out on screening(114). Of course, there is the issue of cost, but VIA may be cheaper only because most women in countries using visual inspection as screening have a low participation rate. If everyone did decide to get screened, the costs for the increased need of health professionals because higher participation but also the narrower interval needed between screening, are likely to be high. It is thus important to compare the costs of the different strategies before commencement of HPV based screening. This would encourage stakeholders to invest in the initial logistics and laboratory equipment needed to scale up screening with HPV testing. Our research group is initiating a cost study based on the trial described in paper 3 and 4.

Furthermore, if LMIC would begin to screen for HPV, it would finally resulting comprehensive nationwide data on the circulating genotypes that cause cancer and which ones simply are passing infections or part of the local flora. It will be important not only for arguing regarding vaccine selection, where Africa is the only continent that does not have its fourth most common genotype present in the available 9-valent vaccine. We need to be able to continue monitor the genotypes to design a continued evolving effective screening program for the population(115). It would therefore be of interest in the future to initiate an online register for HPV genotypes found in screening and cervical cancer cases that are diagnosed.

The issue of triage is a potential roadblock for the implementation of HPV-based screening. Most HPV infections spontaneously regress(10). Dysplasia has long been seen as disease, but we now know that a majority of CIN 2 and about 30% of CIN 3 regress on their own(62), especially in young women. It is therefore important to realise the high treatment burden for pre-cancerous lesions that might heal spontaneously without healthcare involvement. Moreover, even if being HIV positive increases the odds of CIN2+ when HPV positive in comparison to HIV negative (paper 3). HPV infection can also regress on its own in this population. Progression from HPV positive to HSIL occur three times faster in HIV positive women, whereas regression in this group seems to take twice the time (hazard ratio 0.56) and has a lower probability than if you are HIV negative(116).

All the subjective triage tests i.e cytology, VIA, VILI, colposcopy do not discriminate between the lesions potential to regress on their own. Only methylation of certain genes, as discussed, seem to have this potential. Currently, there are no low-cost methylation kits that could be used in Ethiopia. However, the way forward to minimise unnecessary treatment, which itself is a major cost, is to have a complete molecular screening. This would mean fewer women who test positive for HPV, would need to be seen by a health professional. Future studies should focus on this.

As HIV is common and acts synergistic with HPV, it seems from available evidence as well as paper 3, that being HPV positive could be enough for treatment. If possible, the HPV test that should be recommended would use extended genotyping to target the genotypes commonly found in Ethiopian cervical cancer cases. Extended genotyping as triage has benefits if we know which ones cause cancer in our region(61). This is however not completely clear. It seems as if 16,18, 45 and 35 are the four most common ones. Therefore, when it comes to HIV positive women, HPV screening and potential genotyping then direct treatment, is definitely feasible and possible for Ethiopia and countries alike.

As discussed in paper 4, pregnant women have historically been excluded from screening in LMIC because the single visit approach with treatment the same day has not been feasible. This excludes many women from screening during their fertile years. In Paper 4 there was a numerical trend indicating that being pregnant was associated with a history of not participating in cervical cancer screening ($p=0.07$). Antenatal care coverage is increasing around the world(117), which lends itself to the opportunity of screening women who normally do not seek healthcare. Integrating cervical cancer screening into family planning service and HIV-clinics has been done in Sub-Saharan Africa(118, 119). It increased uptake for both services being offered. In Ethiopia, where HIV-clinics were the first to offer VIA as a single visit approach, health-system challenges became roadblocks to up-scaling(120). This can be mitigated by HPV self-sampling as it will only need an examination room for the minority that need follow-up. For implementational research, it seems that integrating cervical cancer screening is not only feasible, but it also removes the need for a new service-clinic to be initiated (121-123). It would instead mean educating staff who give antenatal care, family planning or HIV-care, to include cervical screening in the visit.

Treatment of pre-cancerous lesions was done for those that were VIA or co-testing positive at triage or later found to have CIN2+ in the biopsy. It is not a subject we have researched further. However, as the issue of loss to follow-up is another roadblock to HPV-based screening, treatment needs to be efficient. In HIV positive women, there are varying result regarding what method has the least recurrence rate. As described in the background section, cryotherapy success is dependent on quality gas. In Ethiopia, like many other low resource-settings, industrial carbon dioxide is

used because it is readily available, despite issues of quality. However, no studies have evaluated the type of gas and its success in treatment. The depth of freezing or heating, in the case of thermal ablation, is important to reach the basal layers where HPV is harboured. HPV persistence is the main predictor of future recurrence(97, 124). A cofounder for this in WLHIV, is a CD4 count less than 200cells/mm3, which gave a recurrence rate of 87% in a small study(125). An integrated HIV/cervical screening clinic would have the knowledge to make sure the women are well-treated for their HIV. This would hopefully lead to better cure rates.

A meta-analysis from 2019 on treatment success for thermal ablation in LMIC, pooled data from five studies, three of which included WLHIV (N=155)(91). Treatment success was seen in 84% but this was assessed mainly by VIA, a proxy for dysplasia. None of the studies used HPV, cytology, or pathology tests as a test of cure. In one of the studies, the recurrence rate of VIA in HIV positive women, was almost 20% but this was only six months after treatment(126). Hence for future studies, it would be important to evaluate recurrence rate for HIV positive women and different treating strategies using HPV as a test of cure. This would lessen the number of follow-up visits that otherwise would be necessary after treatment of dysplasia.

Conclusion

HPV self-sampling has the potential to transform the national screening by increasing attendance and finding more disease in a wider population than before. Including pregnant women and integrating cervical screening into other healthcare services needs further implementation research.

By taking these measures, hopefully cervical cancer prevalence will decrease in Ethiopia and stop being a disease we find at a late stage.

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