Assoziation von sexuell übertragenen Erkrankungen und stattgehabter Malaria-Infektion mit der HPV-Serologie bei Frauen in Äthiopien

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von Johanna Maria Angela Klein (geb. Komp)

Betreuerin: Prof. Dr. med. Eva J. Kantelhardt

Gutachter*innen:
Prof. Dr. Claudia Wickenhauser, Halle (Saale)
Prof. Dr. Jürgen Wacker, Bruchsal

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Die vorliegende Dissertation entstand im Rahmen einer Querschnittsstudie in Zusammenarbeit mit der Universität Addis Abeba (2013 – 2014) in sieben Zentren der Schwangerschaftsvorsorge und Familienplanung in Äthiopien, zur Evaluation von regionalen Einfluss- und Risikofaktoren des Zervixkarzinoms bei gesunden, mehrheitlich schwangeren Studienteilnehmerinnen im Alter von 18 bis 45 Jahren. Die Daten der Studienpopulation wurde dazu in Subgruppen erhoben und analysiert. Bestimmt wurde die Prävalenz von humanem Papillomavirus (HPV) sowie die HPV-Genotypverteilung aus Serum- und Vaginal-Lavage-Proben (n = 783). Die Vaginal-Lavage Proben wurden zusätzlich auf sexuell übertragbare Erkrankungen (STI) und bakterielle Vaginose untersucht (n=779). Als regionaler Einflussfaktor wurde die Malaria-Seroprävalenz (n=284) sowie die anamnestische Historie einer stattgehabten Malaria-Infektion (n=415) analysiert. In den Vaginal-Lavage-Proben konnte in 33,1% HPV-DNA nachgewiesen werden. In 22,1% war DNA von mindestens einem hochrisiko-HPV-Genotyp detektierbar. Die häufigsten HPV-Genotypen waren HPV 16 (6,6%), HPV 52 (4,3%), HPV 51 (2,9%) und HPV 39 (2,9%). Die Prävalenz der STIs lag bei 0,6% für *Chlamydia trachomatis*, 0,6% für *Neisseria gonorrhoeae*, 2,3% für *Trichomonas vaginalis* und 1,5% für HSV-2. Eine Korrelation mit HPV zeigte sich für *C. trachomatis* und HSV-2. Auch einige, mit bakterieller Vaginose (BV) assoziierte Bakterien zeigten eine Korrelation mit HPV, wohingegen ein positiver BV-Score nicht assoziiert war. Die HPV-Seroprävalenz lag bei 56,7%/44,3%. Eine Malaria-Seropositivität (gepoolt-positiv) fand sich in 50% der Serumproben. 40,2% der Studienteilnehmerinnen gaben eine positive Malaria-Historie an. Es zeigte sich eine Assoziation zwischen HPV-Seropositivität und einer gepoolt-positiven Malaria-Serologie (RR 2,23 (95%-KI: 1,34-3,71); p=0,002) sowie Polygamie (RR 2,40 (95%-KI: 0,98-5,86); p=0,05). Eine Subgruppen-Analyse erbrachte die Assoziation zwischen hr-HPV-Seropositivität und Malaria-Seropositivität (gepoolt-positiv: RR 2,01 (95%-KI: 1,11-3,65); p=0,02). Keine Korrelation zeigt sich zwischen einer positiven Malaria-Serologie und dem HPV-DNA-Nachweis aus Vaginal-Lavage-Proben. Zwischen einer anamnestisch stattgehabten Malaria-Infektion und HPV-Seropositivität, als auch soziodemographischen Subgrupppen fand sich kein statistisch signifikanter Zusammenhang, es ließ sich jedoch bei einigen ein positiver Trend darstellen. Eine Assoziation zeigte sich zwischen positivem HPV-DNA-Nachweis und anamnestisch stattgehabter Malaria-Infektion (RR 1,61 (95%-KI: 1,03-2,51); p=0,04) sowie städtischer Herkunft (RR 2,59 (95%-KI: 1,45-4,63); p=0,001). Für hr-HPV-DNA zeigte sich dieser Zusammenhang nicht. Die Daten zeigten eine hohe HPV-Prävalenz, passend zu regionalen Daten aus Ostafrika. Die positiven Befunde der STIs waren im Vergleich zu regionalen Prävalenzen jedoch niedrig. Die Malaria-Seroprävalenz war konkordant mit Ergebnissen anderer Studien. Anhand unserer Daten konnte erstmals eine Assoziation zwischen einer HPV-Seropositivität und positiver Malaria-Serologie gezeigt werden. Die vielfältigen Einfluss- und Risikofaktoren stellen Äthiopien mit seinen begrenzten medizinischen und finanziellen Ressourcen vor eine große Herausforderung im Kampf gegen das Zervixkarzinom. Auf Basis der bekannten Daten könnten die Einfluss- und Risikofaktoren durch ein multimodales Konzept reduziert und die Frauengesundheit in Äthiopien deutlich verbessert werden.

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Abkürzungsverzeichnis

DBD	Dried Blood Spots
DKFZ	Deutsches Krebs-Forschungszentrum
EDTA	Ethylendiamintetraacetat
ELISA	Enzym-Linked Immunosorbent Assay
HHV-8	Humanes Herpes Virus 8
HIV	Humanes Immundefizienz Virus
HSV	Herpes Simplex Virus
HPV	Humanes Papillomavirus
KSHV	Kaposi-Sarkom assoziiertes Herpes Virus
MFI	Mediane Fluoreszenz-Intensität
OD	Optische Dichte
SARS-CoV	Severe Acute Respiratory Syndrome-Coronavirus
STI	Sexually transmitted infection
STPH	Schweizerisches Tropen- und Public Health-Institut
WHO	World Health Organisation = Weltgesundheitsorganisation

1. Einleitung und Zielstellung

Das Zervixkarzinom ist in der weltweiten Statistik an vierter Stelle der häufigsten Malignome bei Frauen (1). Die Inzidenz (altersstandardisierte Inzidenzrate pro 100000 Personenjahre) differiert je nach Region von Raten zwischen 6,4 bis 15,7 in Europa bis zu 40,4 in Ostafrika (2–4). Deutschland hat mit 7,1 eine vergleichsweise niedrige Inzidenzrate (5). Innerhalb des afrikanischen Kontinents sind die höchsten Inzidenzen in Ostafrika zu finden, wo das Zervixkarzinom die Statistik der Malignome bei Frauen anführt (4). In Äthiopien lag das Zervixkarzinom 2022 mit 8168 Neuerkrankungen und einer Inzidenzrate von 22,3 sowie 5975 Todesfällen an zweiter Stelle der Malignome bei Frauen (6). In den 1970er Jahren wurde erstmals ein Zusammenhang zwischen Infektionen durch humane Papillomaviren und der Entstehung des Zervixkarzinoms geäußert, und die These durch experimentelle und epidemiologische Studien bestätigt (7). Die genauen Mechanismen sind weiterhin Bestandteil der Forschung (8). Weltweit sind über 200 HPV-Genotypen bekannt, die nach ihrem karzinogenen Risiko eingruppiert werden. Mit Hochrisiko-HPV-Genotypen (high risk HPV (hr-HPV) 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68; Genotypen mit karzinogenem Potential (probably-hr-HPV (prhr-HPV) 26, 53, 66, 67, 70, 73, 82) sowie Niedrigrisiko-HPV-Genotypen (lowrisk HPV (lr-HPV)) für die bisher kein stastisch signifikanter Zusammenhang nachgewiesen werden konnte (9–12). Die höchsten Inzidenzen von HPV-Infektionen finden sich in den Altesgruppen jünger als 25 Jahre (13). Hauptrisikofaktoren für eine HPV-Infektion sind junges Alter beim ersten Sexualkontakt, Anzahl der Sexualpartner, orale Kontrazeptiva, Rauchen, Multiparität und Alterationen des Immunsystems (14–16). In den meisten Fällen handelt es sich um transiente Infektionen. Kommt es zu einer Persistenz der HPV-Infektion steigt das Risiko der Transformation und Onkogenese (17, 18). Immunsuppression, Koinfektion mit mehreren HPV-Typen oder dem Humanen Immundefizienz Virus (HIV) erhöhen das Risiko einer Persistenz und stellen somit Risikofaktoren der Entwicklung eines Zervixkarzinoms dar (16, 19). Der Zusammenhang zwischen HPV-Infektionen und weiteren sexuell übertragbaren Erkrankungen wurde in unterschiedlichen Studien untersucht und wird kontrovers diskutiert. Signifikante Ergebnisse konnten zu Koinfektionen mit *Chlamydia trachomatis*, HSV-2 und HIV gezeigt werden, ließen sich jedoch je nach Studienpopulation nicht einheitlich bestätigen (16,

20–24). Gerade bei schwangeren Frauen stellen sexuell-übertragbare Infektionen ein erhöhtes Risiko für Komplikationen dar. Infektionen mit *C. trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, Herpes simplex-Virus-2 (HSV-2) sowie HIV, als auch eine Dysbalance der Vaginalflora im Sinne einer bakteriellen Vaginose bergen das Risiko von Schwangerschaftskomplikationen wie vorzeitigem Blasensprung, Frühgeburtsbestreben, Fehlgeburt, als auch Infektionen des Neugeboren oder postpartum Infektionen des Genitaltraktes bei Frauen mit dem Risiko einer sekundären Infertilität (25–32). Mit Blick auf die regionale Morbidität in Subsahara-Afrika insbesondere auch der Frauen und Kinder unter 5 Jahren, spielen Malaria-Infektionen eine bedeutende Rolle. Laut dem aktuellen Malaria-Bericht traten 2022 weltweit schätzungsweise 249 Millionen Malaria-Infektionen mit einer Fallinzidenz von 58 auf 1000 Personen unter Risiko einer Transmission auf (33). Afrika ist mit 94% der Fälle die am stärksten betroffene Region, in der schätzungsweise 36% der Schwangeren im Rahmen der Schwangerschaft einer Malaria-Infektion ausgesetzt sind (33). Durch das globale Malaria-Programm der WHO, das sich zum Ziel gesetzt hat Malaria zu kontrollieren und eliminieren, ist es in den vergangenen Jahren zu einem Rückgang der weltweiten Malaria-Infektionen sowie Reduktion der regionalen Transmissionsrisiken gekommen (33). Im Rahmen der SARS-CoV-2-Pandemie kam es zu einer Stagnation dieser Entwicklung und einem Anstieg der Fallzahlen, was auf die Einschränkungen in der Umsetzung der Präventionsprogramme zurückgeführt wird (33). Auch 2022 wurde das Ziel der „Global technical strategy for malaria 2016–2030“ verfehlt. Maßgeblich verantwortlich waren hierfür steigende Malaria-Fallzahlen in verschiedenen Ländern, unter anderem auch Äthiopien (33). Sowohl die politische Konfliktsituation als auch Änderungen in der Vektorpopulation (Zunahme der *Anopheles stephensi*-Population mit Resistenzen gegenüber Insektiziden) in Äthiopien, werden bei dieser Entwicklung als ursächliche Faktoren angesehen (33). Geographisch gesehen gibt es in Äthiopien regionale Unterschiede in Bezug auf das Risiko einer Malaria-Transmission. Insgesamt leben 68% der Bevölkerung in Malaria-Endemiegebieten (33). Durch Einbeziehung der verschiedenen Höhenlagen, die saisonalen Klimaänderungen sowie den jährlichen Malaria-Parasiten-Index kann das Land in Regionen mit unterschiedlichem Transmissionsrisiko (hoch/moderat/niedrig, risikofrei) eingeteilt werden (34). Für 2022 lag die geschätzte Fallinzidenz für Äthiopien bei 5.1 Millionen mit einer geschätzten

Anzahl an Todesfällen zwischen 3860 bis 20600, wobei die gemeldeten Todesfälle mit 180 stark davon abweichen und höchstwahrscheinlich deutlich unter der tatsächlichen Fallzahl liegen (33). Malaria ist eine, durch den Stich der weiblichen *Anopheles*-Mücke übertragene, parasitäre Erkrankung verursacht durch *Plasmodium spp*.. Humanpathogen gelten die fünf Arten *Pl. falciparum*, *Pl. vivax*, *Pl. ovale*, *Pl. malariae* und *Pl. knowlesi*. Sie unterscheiden sich im regionalen Vorkommen sowie im klinischen Erscheinungsbild und in der Schwere der Erkrankung. Ausschlaggebend für den Verlauf ist die frühzeitige Diagnosestellung und Therapie. Der Pathogenese liegt ein komplexer Lebenszyklus zugrunde. Nach Injektion der Sporozoiten durch die weibliche Anopheles-Mücke kommt es in den Leberzellen zu einer Vermehrung und letztendlich Freisetzung von Merozoiten in die Blutbahn, welche die Erythrozyten befallen und dort über die Trophozoiten zu Schizonten heranreifen, die nach Lyse des befallenen Erythrozyten weitere Erythrozyten infizieren. Manche der Trophozoiten reifen jedoch auch zu sexuellen Stadien (Gametozyten) heran, welche die Ausgangsstadien für die Aufnahme in der weiblichen *Anopheles*-Mücke darstellen. Über Zwischenstadien entwickelt sich aus den Gametozyten eine Oozyste, welche Sporozoiten freilässt, die in die Speicheldrüsen der Mücke migrieren und dort erneut in den Zyklus entlassen werden. Die Infektion durch *Plasmodium spp*. führt durch die verschiedenen zyklusabhängigen Stadien zu einer hochkomplexen Antwort des Immunsystems mit Aktivierung sowohl der unspezifischen Immunantwort (Makrophagen, Zytokine, etc.) als auch der Bildung von Antikörpern gegen multiple Antigene der verschiedenen Stadien (35). Zahlreiche Mechanismen erhöhen die Komplexität und Vielfalt der Immunantworten mit unterschiedlichen Auswirkungen auf den Verlauf und die Schwere der Erkrankung (36). In verschiedenen Studien konnte gezeigt werden, dass die immunmodulatorischen Prozesse im Rahmen von Malaria-Infektionen das Risiko für andere Infektionserkrankungen erhöhen und auch Reaktivierung insbesondere onkogener viraler Infektionen begünstigen (37, 38).

Das Ziel des Projektes war die Evaluation von Risikofaktoren für HPV-Infektionen bei Frauen zwischen 18 bis 45 Jahren in Äthiopien. Eingeschlossen wurden Studienteilnehmerinnen aus sieben verschiedenen Zentren der Schwangerschaftsvorsorge und Familienplanung. Neben einem Fragebogen wurden

mittels eines Selfsampling-Device Vaginal-Lavage-Proben zur weiteren molekulargenetischen Analyse sowie Blutproben für die serologische Diagnostik gewonnen.

Die Forschungsfragestellung des Projektes zielte auf die Evaluation möglicher Risikofaktoren von HPV-Infektionen in Äthiopien ab und wurde im Rahmen mehrerer Kleinprojekte erörtert.

- Wie hoch ist die HPV-Prävalenz bei Frauen zwischen 18 bis 45 Jahren in Äthiopien?
- Wie ist die HPV-Genotypenverteilung in der Studienpopulation?
- Welche soziodemographischen Faktoren zeigen eine Assoziation mit HPV-Positivität?
- Welche sexuell-übertragbaren Erreger lassen sich detektieren?
- Gibt es eine Assoziation nachgewiesener sexuell-übertragbarer Erreger mit HPV-Typen?
- Welche soziodemographischen Faktoren zeigen eine Assoziation mit dem Nachweis von sexuell-übertragbaren Erregern?

Forschungsschwerpunkt der vorliegenden Dissertation war die Evaluation einer möglichen Assoziation von positiver Malaria-Serologie mit HPV. Dabei wurden folgende Fragestellungen beantwortet:

- Wie hoch ist die Malaria-Seroprävalenz in der Studienpopulation?
- Besteht eine Assoziation zwischen einer Malaria-Seropositivität und HPV-Seropositivität?
- Besteht eine Assoziation zwischen Malaria-Seropositivität und dem Nachweis von HPV-DNA aus den Vaginal-Lavage-Proben?

2. Diskussion

Das Zervixkarzinom ist nach dem Mamma-Karzinom das zweithäufigste Malignom bei Frauen in Äthiopien (1). Die Früherkennung kann besonders auf dem Land meist nicht gewährleistet werden, da die flächendeckende Einrichtung von Screening-Maßnahmen aufgrund der fehlenden Infrastruktur (Gynäkologische Fachärzt_innen, Patholog_innen, entsprechend ausgestattete Labore) nicht umsetzbar ist. Entsprechend hoch ist auch die Mortalität. Aus diesem Grund stellen präventiven Maßnahmen eine wichtige Schlüsselposition dar. In >99% sind HPV-Infektionen ursächlich für die Entstehung eines Zervixkarzinoms (39). Studiendaten aus Äthiopien zeigen eine HPV-Positivität in Vaginal-Lavage-Proben von 33%, mit 22% hr-HPV-Typen sowie 7,8% prhr-HPV- und 18,5% lr-HPV-Typen (40). Die am häufigsten detektierten hr-HPV-Typen waren HPV 16 (6,6%), HPV 52 (4,3%), HPV 51 und HPV 39 (jeweils 2,9%) sowie HPV 53 (3,6%) aus der Gruppe der prhr-HPV-Typen (40). Nur wenige zeigten einen positiven Nachweis der hr-HPV-Typen HPV 18 (0,8%) und HPV 45 (0,5%) (40). In anderen Studien lag die HPV-Prävalenz in verschiedenen Regionen Afrikas zwischen 17,3 % bis 24% und zeigte eine andere Genotypverteilung, wobei sich die Studienpopulationen in wesentlichen Aspekten unterschieden (13, 41–43). Da die verschiedenen HPV-Genotypen ein unterschiedliches karzinogenes Potential haben, kann die Häufigkeit eines Genotypes nicht mit dem Karzinomrisiko gleichgesetzt werden. Daten zu einem vergleichbaren Studienkollektiv von asymptomatischen Frauen im Alter von 18 bis 45 Jahren in Äthiopien wurden bisher nicht publiziert, so dass die Ergebnisse der vorliegenden Arbeit als Ergänzung der Datenlage zu sehen sind. Impfungen gegen häufige hr-HPV-Typen ist eine der Säulen der globalen WHO Strategie zur Eliminierung des Zervixkarzinoms (44). Aktuell stehen weltweit 6 lizenzierte Impfstoffe zur Verfügung (44). Bivalente Impfstoffe enthalten Partikel gegen hr-HPV-Typ 16 und 18 wobei quadrivalente Impfstoffe zusätzlich mit Partikeln gegen die Anogenitalwarzen verursachenden Typen HPV 6 und 11 versehen sind. Der nonavalente Impfstoff enthält Partikel gegen HPV-Typ 6, 11 sowie die hr-HPV-Typ 16, 18, 31, 33, 45, 52 und 58 (44). Derzeit wird in dem staatlichen Impfprogramm in Äthiopien ein quadrivalenter Impfstoff (HPV 6, 11, 16, 18) eingesetzt. Dadurch wird nur ein unzureichender Anteil der in Äthiopien maßgeblich vorkommenden hr-HPV-Genotypen abgedeckt. Mit Blick auf die HPV-Genotyp-Verteilung in unserer

Studienpopulation wäre eine Impfung mit einem nonavalenten Impfstoff zu empfehlen, da durch ihn die zwei am häufigsten vorkommenden hr-HPV-Genotypen sowie weitere weniger häufige HPV-Genotypen abgedeckt werden. Auch andere Studien haben vergleichbare Daten gezeigt und Empfehlungen für einen Wechsel auf einen nonavalenten Impfstoff ausgesprochen, um den Schutz für die Bevölkerung zu verbessern (45, 46).

Neben HPV spielen weitere sexuell-übertragbare Erkrankungen bei Frauen im gebärfähigen Alter eine Rolle und erhöhen insbesondere in Ländern mit limitierter medizinischer Infrastruktur die Morbidität in dieser Bevölkerungsgruppe. Die Daten unserer Studienpopulation haben gezeigt, dass auch bei jungen asymptomatischen Frauen Infektionen durch sexuell-übertragbare Erreger mit 6,2% prävalent sind (47). *Trichomonas vaginalis* war mit 2,3% am häufigsten nachweisbar, gefolgt von HSV-2 (1,5%), *Mycoplasma genitalium* (1%), *Chlamydia trachomatis* (0,6%) und *Neisseria gonorrhoeae* (0,6%). Die Prävalenzen sind dabei im Vergleich zu Daten aus anderen Studien der Region, welche deutlich höhere Prävalenzen zeigten, niedrig (48–51). Eine mögliche Erklärung liegt in unserer Studienpopulation gesunder, asymptomatischer, mehrheitlich verheirateter Frauen. Methodische Einflussfaktoren sind jedoch aufgrund der deutlichen Differenz zu Daten anderer Studien als limitierender Faktor nicht auszuschließen. In unserer mehrheitlich schwangeren Studienpopulation zeigte sich in 24,3% eine Dysbalance der Vaginalflora im Sinne einer bakteriellen Vaginose (BV-Score ≥ 2) sowie eine hohe Anzahl an positiven Nachweisen der häufig mit BV assoziierten Erreger *Atopobium vaginae*, *Gardnerella vaginalis* und *Mycoplasma hominis*. Dabei liegt die Prävalenz im Bereich vergleichbarer Ergebnisse mit einer BV-Prävalenz von 25% in Subsahara-Afrika (52). Verschiedene Studien haben gezeigt, dass eine bakterielle Vaginose ein Risikofaktor für Schwangerschaftskomplikationen darstellt (32).

In verschiedenen Studien wurden Assoziationen von sexuell-übertragbaren Erregern mit HPV-Infektionen gezeigt und als mögliche Risikofaktoren für ein erhöhtes Zervixkarzinomrisiko postuliert (23). In unserer Studienpopulation konnte eine Korrelation zwischen HPV und *C. trachomatis* sowie HPV und HSV-2 gezeigt werden (47). Dieser Assoziation könnten sowohl Störungen der Barrierefunktion der Schleimhaut, als auch immunmodulatorische Effekte durch *C. trachomatis*-Infektionen zugrunde liegen (20). Ähnliche Mechanismen könnten für eine Infektion mit HSV-2 postuliert werden, da

bei dieser ebenfalls durch Untergang der infizierten Zellen eine Störung der Schleimhautbarriere entsteht. In weiteren Studien vermutete Korrelationen zwischen HPV und BV oder weiteren sexuell-übertragbaren Erregern konnte in unserer Population nicht bestätigt werden. Es zeigte sich jedoch eine Korrelation zwischen HPV und *Ureaplasma parvum*, welche erst in der letzten Zeit auch in der Literatur zunehmend diskutiert wird (53). Genauere Mechanismen sind bisher nicht bekannt.

Ein weiterer wichtiger morbiditäts- und mortalitätsrelevanter Faktor in Subsahara-Afrika ist die Vektor-übertragene Malaria-Infektion. Besonders gefährdet ist die vulnerable Population der Schwangeren und Kinder unter 5 Jahren. Im Jahr 2022 waren schätzungsweise 36% der Schwangeren in Afrika von einer Malaria-Infektion betroffen (33). Damit verbunden ist ein hohes Risiko für Schwangerschaftskomplikationen sowie eine erhöhte Morbidität und Mortalität sowohl der Frau, als auch des Fetus, des Neugeborenen und des Kleinkindes (33). In Äthiopien ist das Malaria-Risiko aufgrund seiner geographischen Lage mit Höhenlagen bis über 4000 m über n. N. sehr heterogen, mit Gebieten eines hohen Malaria-Transmissionsrisikos bis hin zu Malaria-freien Höhenlagen. 68% der Bevölkerung sind einem Malaria-Transmissionsrisiko ausgesetzt (33). Durch das geographisch bedingt unterschiedliche Transmissionsrisiko zeigen sich in Äthiopien regional deutliche Unterschiede in der Malaria-Seroprävalenz (54–56). Die Anamnese von stattgehabten Malaria-Infektionen als auch die Durchführung einer serologischen Malaria-Diagnostik stellen zwei mögliche Erhebungswerkzeuge dar. Studien haben gezeigt, dass Antikörper gegen *Plasmodium spp*. auch Jahre nach einer Infektion noch nachweisbar sind und als Marker einer zurückliegenden Exposition herangezogen werden können (54). Im Rahmen unserer Studie wurde auf Basis eines etablierten serologischen Verfahrens (inhouse-ELISA) ein Protokoll zur Bestimmung von Anti-*Plasmodium*-Antikörpern aus konservierten EDTA-Blutproben (Dried Blood Spots, DBS) entwickelt, validiert und als Diagnostikmethode eingesetzt. Als Antigen fungierte ein, aus einem *Plasmodium falciparum* NF-54 Stamm gewonnenes *P. falciparum*-Antigen, welches durch Kreuzreaktivität auch andere *Plasmodium spp*. miterfasst. Die Sensitivität des ELISA liegt bei 87-100% für *P. falciparum*, mit Kreuzreaktivität gegenüber anderen *Plasmodium spp*., und die Spezifität bei 98%. Die Ergebnisse wurden entsprechend den vorher, anhand von Ergebnissen gesunder Blutspender und Malaria-Patienten, festgelegten Grenzwerten interpretiert. Neben positiven (optische Dichte

(OD) ≥ 0.30) und negativen (OD < 0.15) Werten gab es einen reaktiven Graubereich (equivocal OD 0.15-0.29). Auf weitere Konfirmationstestungen wurde im Rahmen dieser Studie verzichtet. Die positiven und equivocal Befunde wurden als gepoolt-positiv gewertet. In Kohorte A zeigte die Hälfte der Teilnehmerinnen eine Seropositivität (gepoolt-positiv 50% [positiv 11,27%, equivocal 38,73%])) für Malaria (siehe Abbildung 1). Vergleichbare Werte zeigte eine Studie aus Äthiopien mit einer *P. falciparum* Seroprävalenz von 11 bis 65%, abhängig von dem entsprechenden regionalen Transmissionsrisiko (55). Die anamnestische Erhebung einer stattgehabten Malaria-Infektion in Kohorte B erbrachte in 40,2% der Studienpopulation ein positives Ergebnis. Der Entwicklungszyklus der Plasmodien im Rahmen einer Malaria-Infektion führt zur Aktivierung verschiedener immunologischer Prozesse. Die dadurch ausgelöste komplexe Immunreaktion kann über verschiedene Wege zu Immunpathologien führen und immunmodulatorische Auswirkung auf unterschiedlichen Ebenen des Immunsystems auslösen (36). Diese veränderte Immunitätslage kann auch bei viralen Koinfektionen zu veränderten Immunantworten führen. Dies konnte in epidemiologischen Studien gezeigt werden, in denen ein Zusammenhang zwischen Malaria-Endemizität und dem Auftreten des Epstein-Barr-Virus (EBV) assoziierten Burkitt-Lymphoms nachgewiesen wurde (37). Dabei kommt es bei Koinfektionen von Malaria und EBV zu einer Reaktivierung bzw. Virämie und klonaler Veränderung der B-Zellen mit dem Risiko zur Entwicklung eines Burkitt-Lymphoms (37, 57). Die genauen Mechanismen auf zellulärer und immunologischer Ebene sind weiter Bestandteil der Forschung. Andere Daten weisen auf einen möglichen Zusammenhang zwischen Malaria-Infektionen und Seropositivität für das Humane Herpes Virus 8 (HHV 8) oder auch Kaposi-Sarkom-assoziiertes Herpes-Virus (KSHV) genannt, mit einem erhöhten Risiko der Entwicklung eines Kaposi-Sarkoms, hin (38, 58). EBV, KSHV als auch HPV gehören zur Gruppe der onkogenen Viren. Zu einem möglichen Zusammenhang von Malaria und HPV sind bisher keine Daten verfügbar. Vor diesem Hintergrund war die Assoziation von HPV und Malaria der Forschungsschwerpunkt dieser Arbeit. Die serologische Diagnostik zur Ermittlung der HPV-Seroprävalenz wurde mittels einer Multiplex-Methode durchgeführt, welche es ermöglicht Antikörper gegen Antigene mehrerer HPV-Genotypen zeitgleich zu ermitteln (59). Dafür werden virale Antigene über Gluthation-Casein Moleküle an Diagnostik-Beads gekoppelt (59). Die

Quantifizierung erfolgte über einen zweiten biotinylierten, mit Streptavidin-R-phycoerythrin markierten Antikörper unter Messung der medianen Fluoreszenz-Intensität (MFI) (59). Zur Erhebung der HPV-Seroprävalenz wurden Antikörper gegen 14-HPV-Genotypen bestimmt (HPV 1, 2, 4, 8, 6, 11, 16, 18, 31, 33, 35, 45, 52, 58). Die HPV-Seroprävalenz unserer Studienpopulation lag bei 57%/44% (Kohorte A/Kohorte B) (siehe Abbildung 1). Eine HPV-Genotyp-spezifische Unterscheidung der Ergebnisse wurde im Rahmen der Fragestellung nicht durchgeführt. Serologische Daten anderer Studien zeigen ähnliche Ergebnisse mit HPV-Seroprävalenzen von 40,5% bis 65,7%, wobei sich bei differenzierterer Betrachtung Genotyp-spezifische Seroprävalenzen je nach Region unterscheiden (60–62).

Grundlage unserer Forschungsarbeit waren die HPV-Seroprävalenz sowie die Malaria-Seroprävalenz in Kohorte A und die anamnestisch erhobenen Daten zur stattgehabten Malaria-Infektion der Kohorte B. Die Ergebnisse zeigten eine deutliche Assoziation zwischen HPV-Seropositivität und einer positiven Malaria-Serologie.

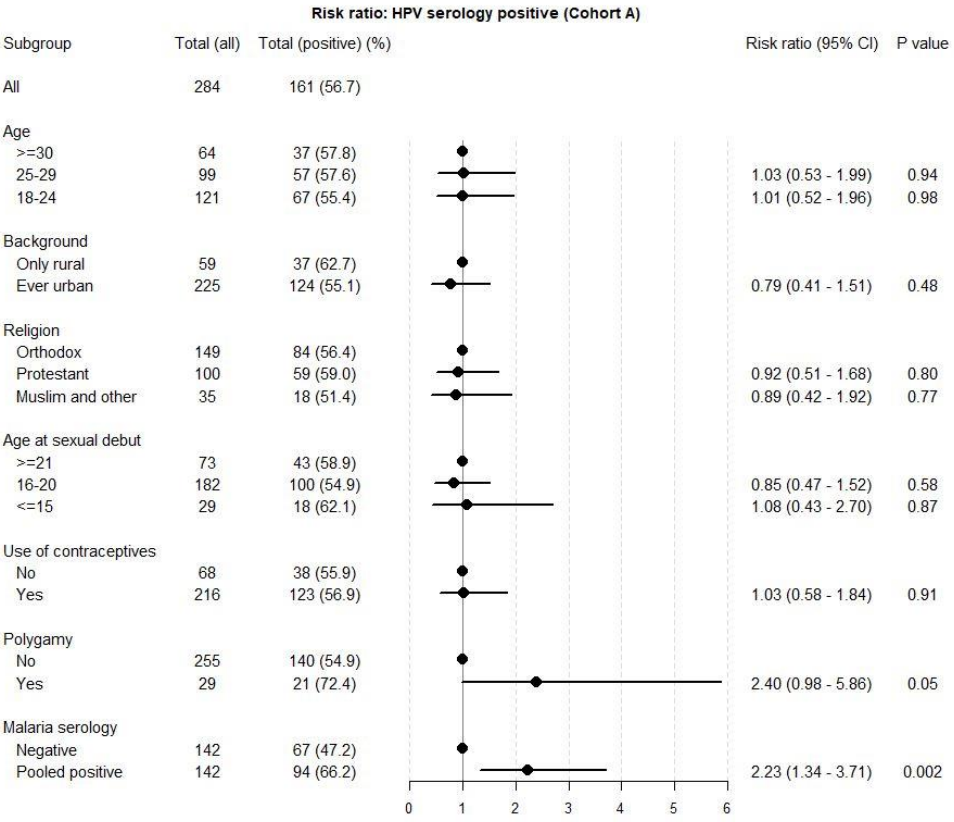

Abbildung 1: Zusammenhang von soziodemographischen Charakteristika sowie Malaria-Serologie-Ergebnissen und Vorliegen einer HPV-Seropositivität (Kohorte A)

Demnach bleibt die Frage ob, Malaria als immunmodulatorische Erkrankung einen Einfluss auf eine HPV-Infektion ggf. auch auf die Dauer bis zur Eliminierung hat, eine weiter zu erforschende Thematik, welcher durch spezifizierte Studienprotokolle nachgegangen werden sollte. Neben dem Einfluss einer Malaria-Infektion auf die HPV-Seropositivität wären auch Daten zu möglicher Persistenz von HPV-Infektionen im Rahmen von Malaria-Koinfektionen, als Risikofaktor für die Entwicklung eines Zervixkarzinoms von Interesse.

Mit Blick auf die vielfältigen regionalen Einflussfaktoren in Äthiopien ist die Herausforderung für die Kontrolle und Eliminierung des Zervixkarzinoms sowie die Verbesserung der Frauengesundheit sehr groß.

Ziel der WHO ist es bis 2030 weltweit eine 90% HPV-Impfquote bei Mädchen ≤ 15 Jahren zu erreichen, 70% der Frauen mittels eines „high-performance test“ im Alter von 35 und 40 Jahren zu screenen und 90% der Frauen mit präkanzerösen Veränderungen des Zervix oder Zervixkarzinom zu behandeln (44). Bisher hat die HPV-Impfung in Äthiopien noch keine flächendeckende Akzeptanz finden können. Studien in unterschiedlichen Studienpopulationen und Regionen Äthiopiens haben Impfraten von 42,05% bis 66,5% gezeigt (63–66). Das staatliche Impfprogramm sieht den Einsatz eines quadrivalenten HPV-Impstoffen (HPV 6, 11, 16, 18) vor. Sowohl unsere Daten als auch Ergebnisse anderer Studien haben gezeigt, dass in Äthiopien aufgrund der am häufigsten vorkommenden Serotypen ein Wechsel der quadrivalenten HPV-Vakzine zu einer nonavalenten HPV-Vakzine sinnvoll wäre, um langfristig einen größeren Teil der Infektionen präventiv abzudecken (40, 43, 46). Die Einführung von Testangeboten in Bezug auf weitere sexuell-übertragbare Erkrankungen könnten zusätzliche Risikofaktoren für HPV-Infektionen verringern und zu einer Verbesserung der maternalen als auch kindlichen Gesundheit durch die Reduktion von Schwangerschaftskomplikationen, Entwicklungsverzögerungen und postpartalen Infektionen führen. Der Strategieplan der WHO sieht eine Verbesserung der Laborkapazitäten sowie der Testinfrastruktur vor, um die Surveillance und das Management von sexuell-übertragbaren Erkrankungen zu verbessern (67). Als sensitivstes Verfahren gelten molekulargenetische Diagnostikmethoden, durch die zeitgleich mehrere sexuell-übertragbare Erreger getestet werden können. Nachteilig ist,

dass sie in vielen Ländern aufgrund der fehlenden Infrastruktur und der hohen Kosten nicht verfügbar oder einsetzbar sind. Dadurch können die empfohlen Screening-Programme auf nationaler Ebene in vielen Ländern nicht flächendeckend umgesetzt werden. Diese Problematik betrifft auch Äthiopien. Eine Verbesserung der Infrastruktur, der Laborkapazitäten und des entsprechend ausgebildeten Personals sowie die Verfügbarkeit kosteneffizienterer vergleichbarer Systeme, würden das Erreichen der gesetzten Ziele deutlich beschleunigen. Voraussetzung einer Erweiterung der Diagnostik ist jedoch das Vorhandensein von Therapieangeboten, um entsprechend kurative Maßnahmen ergreifen zu können. Neben der frühzeitigen Diagnostik und adäquaten Therapie, spielen präventive Maßnahmen wie Impfungen (HPV, Hepatitis B), sexuelle Früherziehung und Aufklärung sowie Verwendung von Präservativen eine bedeutende Rolle im Kampf gegen sexuell-übertragbare Erkrankungen. In Subsahara Afrika ist die Bekämpfung der Malaria ein zusätzlicher wichtiger Faktor zur Reduktion der maternalen und kindlichen Morbidität und Mortalität. Gemäß dem nationalen Malaria-Eliminierungsprogrammes von Äthiopien ist es das Ziel, durch verbesserte Prävention, Diagnostik und Therapie bis 2025 die Morbidität und Mortalität im Vergleich zu 2020 zu halbieren, sowie bis 2025 Malaria in Gebieten mit einer jährlichen Parasiten-Inzidenz von <10 zu eliminieren und eine Ausbreitung in bisher Malaria freie Gebiete zu verhindern (68).

Auf Grundlage der vorgestellten Daten und Hintergrundinformationen ist durch die Kombination der verschiedenen Maßnahmen und Programme zur Reduktion der HPV- sowie der STI-Prävalenz und möglicherweise auch durch die Reduktion der Endemizität und Inzidenz von Malaria ein präventiver Effekt in Bezug auf das Zervixkarzinom in Äthiopien möglich. Dies sollte in weiteren Studien und unter Zusammenführung der verschiedenen Programm-Endpunkte evaluiert werden.

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4. Thesen

1) Die Prävalenz von humaner Papillomavirus (HPV)-DNA (Hochrisiko-/Niedrigrisiko-HPV-Genotypen, HPV-Genotypen mit karzinogenem Potential) in Vaginal-Lavage-Proben von Frauen aus sieben Zentren der Schwangerschaftsvorsorge und Familienplanung verschiedener Gegenden in Äthiopien, ist mit 33,1% im regionalen und weltweiten Vergleich hoch; in 22,1% der Vaginal-Lavage-Proben wurde hochrisiko-HPV-DNA nachgewiesen.

2) Die Prävalenz sexuell-übertragbarer Erreger wie *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Trichomonas vaginalis*, Herpes simplex-2 sowie *Mycoplasma genitalium* unserer Studienpopulation gesunder mehrheitlich schwangerer Frauen in Äthiopien, ist im Vergleich zu Daten anderer regionaler Studien niedrig (6,2%).

3) Zwischen HPV-DNA-Nachweis (alle HPV-Genotypen, als auch hr-HPV) und Herpes simplex Virus-2 besteht eine relevante Assoziation.

4) Zwischen DNA-Nachweis von HPV und den bakterielle Vaginose (BV)-assoziierten Bakterien *Atopobium vaginae*, *Gardnerella vaginalis* oder *Mycoplasma hominis* zeigte sich eine relevante Korrelation; das Vorliegen einer Bakterielle Vaginose selber zeigt keine Korrelation mit dem Nachweis von HPV-DNA.

5) Die HPV-Seropositivität liegt mit 56,7% in Äthiopien im Bereich vergleichbarer Daten anderer Studien.

6) Eine serologische Diagnostik zum Nachweis von Malaria-Antikörpern aus „Dried Blood Spots“ ist möglich und valide; Malaria-Antikörper sind in 50% im Blut der 284 Frauen nachweisbar (gepoolt-positiv (11,3% seropositiv; 38,7% equivocal));

7) Es besteht eine Assoziation zwischen HPV (alle HPV-Genotypen) und Malaria-Seropositivität (gepoolt-positiv (RR 2,23 (95%-KI: 1,34-3,71); p=0,002). Dabei könnte die Immunmodulation im Rahmen der Malaria-Infektion eine HPV-Koinfektion begünstigen und möglicherweise die Elimination der HPV beeinflussen.

8) Eine Hochrisiko-HPV-Seropositivität zeigt eine Assoziation mit Malaria-Seropositivität (gepoolt-positiv (RR 2,01 (95%-KI: 1,11-3,65); p=0,02) wohingegen

HPV-DNA-Nachweis in der vaginalen Lavage als Zeichen einer aktuellen Infektion keine Assoziation mit einer positiven Malaria-Serologie zeigte.

9) Eine anamnestisch positive Historie einer stattgehabten Malaria-Infektion zeigt keine signifikante Assoziation mit einer positiven HPV-Serologie.

Publikationsteil

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Prevalence of human papillomaviruses in self-collected samples among women attending antenatal care in Ethiopia: a cross-sectional study

Isabel Runge[1,2], Johanna M A Klein[1,2], Ann-Katrin Pannen[1,2], Semaw Abera[2,3,4,5], Tariku Wakuma[2,6], Yirgu Gebrehiwot[7], Susanne Unverzagt[8], Andreas Wienke[2], Christoph Thomssen[1,2], Andreas M Kaufmann[9], Ahmedin Jemal[10], Tamrat Abebe[2,11], Dana Holzinger[12], Tim Waterboer[12], Daniela Höfler[12], Adamu Addissie[2,13] and Eva Johanna Kantelhardt[1,2]

[1]Department of Gynaecology, Martin-Luther-University Halle-Wittenberg, 06108 Halle, Germany
[2]Global Health Working Group, Institute of Medical Epidemiology, Biometrics and Informatics, Martin-Luther-University Halle-Wittenberg, 06108 Halle, Germany
[3]Department of Radiation Oncology, Faculty of Medicine, Martin-Luther-University Halle-Wittenberg, 06108 Halle (Saale), Germany
[4]School of Public Health, College of Health Sciences, Mekelle University, Mekele 0231, Ethiopia
[5]Kilte Awlaelo – Health and Demographic Surveillance Site, College of Health Sciences, Mekelle University, Mekele 0231, Ethiopia
[6]Aira Hospital, Aira, Ethiopia
[7]Department of Obstetrics and Gynaecology, College of Health Sciences, Addis Ababa University, Addis Ababa 1000, Ethiopia
[8]Institute of General Practice and Family Medicine, Center of Health Sciences, Martin-Luther-University Halle-Wittenberg, 06097 Halle (Saale), Germany
[9]Clinic for Gynecology, Charité – Universitätsmedizin Berlin, Corporate Member of Freie Universität Berlin, Berlin Institute of Health, Campus Virchow Klinikum, Humboldt-Universität zu Berlin, 10117 Berlin, Germany
[10]American Cancer Society, Atlanta, GA 30303, USA
[11]Department of Microbiology, Immunology & Parasitology, School of Medicine, College of Health Sciences, Addis Ababa University, Addis Ababa 1000, Ethiopia
[12]Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), 69120 Heidelberg, Germany
[13]School of Public Health, College of Health Sciences, Addis Ababa University, Addis Ababa 1000, Ethiopia

Research

Abstract

Cervical cancer is the second most commonly diagnosed cancer in women in Ethiopia. However, data are limited on the prevalence of human papillomavirus (HPV) genotypes. Self-sampled vaginal lavages were obtained consecutively from 783 women attending 7 health facilities across Ethiopia. Genotype prevalence was assessed by Multiplex-Papillomavirus-Genotyping which detects and individually identifies 51 genotypes and 3 subtypes. Genotype-specific prevalence was described and associations with known risk factors were analysed. The overall HPV prevalence (age range 18–45) was 33.1% (95% confidence interval (CI) 29.8–36.4). The prevalence of HPV was different in the rural and urban population with 17.6% (95%CI 11.6–23.7) and 36.8% (95%CI 33.1–40.6) ($p < 0.001$ chi-square test), respectively. The most common high-risk types were HPV 16 (6.6%), followed by HPV 52 (4.3%), 51 and 39 (both 2.9%). Urban women compared to rural women had a higher risk of being HPV positive (odds ratio 2.36 (95% CI 1.47–3.79; $p < 0.001$). Age at sexual debut ≤15 years and polygamous husband (in urban women) also increased the risk of being HPV positive nearly two-fold. The high prevalence of hr-HPV in Ethiopian women in the reproductive age group shows the need for screening programs. The nonavalent HPV vaccine covers the most prevalent hr-HPV genotypes as found in this study and can therefore be used effectively. Since antenatal care is the best-utilised health service, implementing self-sampled vaginal lavage could be an opportunity for screening in this age group. Screening algorithms and triage still need to be defined to avoid over-treatment in these women.

Key words: *cervical cancer screening*, *HPV test*, *Ethiopia*, *pregnancy*, *population-based*, *cervical cancer prevention*, *Africa*

Correspondence to: Eva Johanna Kantelhardt
Email: eva.kantelhardt@uk-halle.de

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Background

Importance of cervical cancer

Cervical cancer was responsible for an estimated 311,365 deaths worldwide in 2018 [4]; of those, the majority occurred in less developed regions. It is one of the diseases with high disparity in survival between the different countries. Cancer death rates are higher for women in low- and middle-income countries. East Africa has one of the highest age-standardised mortality rates with 30 per 100,000 women [4, 34]. A reason is the lack of adequate screening facilities and therefore low screening uptake, correct diagnosis and treatment; especially in sub-Saharan Africa despite the known effectiveness of prevention by screening programs [40].

Risk factors and preventive measures

The main risk factor for developing cervical cancer is persistent infection with high-risk (hr) human papillomavirus (HPV) [49]. The prevalence of HPV is age-dependent since women are infected after cohabitarche and for the majority clearance of the infection is achieved after some years [26]. There are risk factors for HPV acquisition such as sexual intercourse at an early age and multiple sexual partners [43]. Risk factors for high-risk (hr) HPV acquisition are sexual intercourse, young age at sexual debut, multiple partners, oral contraceptive use and smoking [9]. Changes in risk factors and especially the availability of screening can drastically change the incidence of cervical cancer. Screening by cytology has led to a drastic reduction in cervical cancer incidence in high-income countries [41]. This method is not feasible in low-resource settings due to a lack of health service structures, gynecologists and pathology services. It has been shown that screening by visual inspection with acetic acid can also reduce cervical cancer incidence and mortality [29] and is feasible in low-resource settings. Also, vaccination is available to reduce the population-based prevalence of HPV infection by most carcinogenic genotypes and consequently low-grade and high-grade cervical abnormalities [14]. Two recent systematic reviews on cervical cancer screening uptake in Ethiopia found 5% and 13% of women accessing screening, respectively [2, 20]. An important determinant factor for uptake was information provided by health service staff [15, 27]. In Ethiopia, HPV vaccination was introduced in 2018 for school girls in an organised program, and first studies showed an uptake of 80% [1].

HPV prevalence

More than 206 genotypes of HPV have been discovered of which 14 are considered hr and 6 possible-hr (phr) oncogenic types [8, 44]. A meta-analysis including more than 1 million women with normal cytological findings revealed a global HPV average prevalence of 11.7% for women of all ages. Higher proportions were found in sub-Saharan Africa (adjusted rate of 24.0% for ages 15 and above) [5].

Young population in rural areas with poor economy and low HIV prevalence

Ethiopia is a country with 115 million habitants and an annual population growth rate of 2.6%. More than half the population is below the age of 15. In Ethiopia, 78% of women (15–49 years) live in rural areas and 48% of all women do not have formal education. The total fertility rate is 4.6 for women aged 15–49; for rural areas, it is 5.2 compared to urban 2.3 [6]. The HIV prevalence is 1.9% in women aged 15-49 years, with 3.7% in urban and 0.6% in rural areas [7]. The economy is fast-growing by annually 10.2% whereas the country's per capita income in 2019 is still $740 [39]. The population-based registry in Addis Ababa estimates 7,095 cervical cancer patients annually in 2015 [13].

Cancer control plan and vaccination initiatives at pilot sites in Ethiopia

The Ethiopian Federal Ministry of Health has presented a cancer control plan for 2016–2020. As cervical cancer is one of the top cancers, prevention through vaccination and screening are the main activities [12]. In Ethiopia, there have already been efforts to start cervical cancer screening programs for HIV patients and since 2015 for all women above 30 years. Studies showed that only 10% of HIV-positive women had received screening and only 17% were informed by health workers about screening [37]. Between 2010 and 2014, a number of about 16,000

HIV-positive women were counselled and 99% of those were screened. The service was highly accepted and set the ground for upcoming nationwide activities [35, 36, 48]. Ethiopia introduced visual inspection with acetic acid in the National Cancer Control Plan in 2015. Uptake in a population-based study amounted to 50%, whereas innovative HPV-based self-sampling achieved up to 84% [16, 17].

Prevalence and types of HPV studied in pregnant women before vaccination initiative started

Vaccination is one of the most cost-effective strategies to prevent HPV-driven cancer [19]. This study aimed to provide information on HPV prevalence in young Ethiopian women attending antenatal care in 2015 before screening was initiated in the country. For logistic reasons and easy collection of vaginal samples, we attempted to include healthy women who visited health centers (avoiding the problems of long-distance travel, lack of privacy, problems with hygiene and so on, in a village setting). Therefore, healthy women attending antenatal care/reproductive services at the health center level were chosen as the study population. We anticipated a younger age structure, more primigravida and a lack of women who do not access formal health services compared to all pregnant women.

Methods

Study population

This cross-sectional study on HPV prevalence in vaginal lavage from non-vaccinated, healthy, pregnant women was conducted in 2013 and 2014 in seven centers across Ethiopia.

To obtain comprehensive data, sites were chosen conveniently from diverse regions (according to common ethnic groups, major religions and urban and rural background). Institutions ranged from university hospitals (n = 1), state and private hospitals (n = 5) to regional health centers (n = 1). Pregnant women from antenatal care (few women attending with unknown pregnancy status) (n = 34) were included. Initially, 1,239 women were invited to participate of which 1,041 were enrolled in the study with 783 valid results (Figure 1).

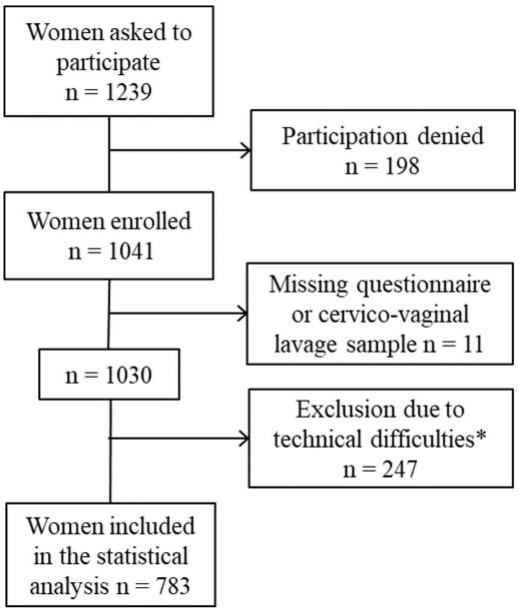

Figure 1. Inclusion of women into the study; * inadequate sample storage n = 245; inadequate DNA quality n = 2.

The main inclusion criteria were age 18–45 years, provision of cervicovaginal lavage self-sample and questionnaire. The main exclusion criteria were vaginal symptoms and obstetric conditions such as placenta previa, preeclampsia or recurrent miscarriages. Due to technical problems and operational difficulties, 247 samples were invalid for analysis. A total of 783 women were included in the final population: Addis Ababa *n* = 209, Mekelle *n* = 78, Bahir Dar *n* = 215, Harar *n* = 29, Aira *n* = 149, Wukro *n* = 65 and Ginir *n* = 38. Women who had never lived in an urban setting were considered 'rural' assuming that having ever lived in a rural setting could increase the risk of acquiring a persistent HPV infection.

Questionnaire

The questionnaire used was a modified version that originated from the International Agency for Research on Cancer [11]. Minor adaptations were made to suit the Ethiopian and pregnant populations. It included questions on socio-demographic background, reproductive issues, sexual behaviour, contraceptive methods and medical history. The questionnaire was translated and re-translated from the original English version into Amharic, Oromo and Tigrinya local languages by native speakers of both languages. A brief pre-testing of the questionnaire was done at the first site by ten women who confirmed understanding of the questions (Appendix 1 Questionnaire). The questionnaire was administered and explained through local nurses who were well-known to the clients.

Sample collection

Samples were collected from April to July 2013 and April to September 2014 during routine antenatal care or family planning clinics. Local nurses obtained informed consent, applied the questionnaire and helped to take the lavage samples. The cervicovaginal lavage sample was collected with assistance through a second-generation self-sampling device according to manufacturer´s instructions (Delphi Bioscience, Scherpenzeel, Netherlands) [10]. Cervicovaginal cells in the lavage obtained with buffered saline were immediately transferred into 3 mL buffered methanol. The samples were stored at room temperature (20°C–30°C) for a maximum of 3 months until transferred to Germany. The samples were stored in Germany at −20°C for a maximum of 6 months.

HPV DNA testing

HPV DNA testing was performed at the German Cancer Research Center (DKFZ) in Heidelberg, Germany. DNA was extracted from 500 µL lavage fluid using the MagNA Pure 96 DNA and Viral Na Large Volume Kit according to the manufacturer's recommendations (Roche Diagnostics, Switzerland). To amplify 51 HPV types, a multiplex polymerase chain reaction (PCR) with broad spectrum GP5+/6+ primers was performed followed by bead-based multiplex-papillomavirus-genotyping using the Luminex-reader (Luminex Corp., Austin, TX) as previously described [32]. The detected 51 HPV types included 14 hr- (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68b), six phr- (HPV 26, 53, 67, 70, 73 and 82) and 31 low-risk (lr) genotypes (HPV 6, 7, 11, 13, 30, 32, 34, 40, 42, 43, 44, 54, 61, 62, 69, 71, 72, 74, 81, 83, 84, 85, 86, 87, 89, 90, 91, 97, 102, 106 and 114). Additionally, three subtypes were integrated (HPV 55, 64 and 68a). Results were expressed as median fluorescence intensities (MFI) of >100 beads per HPV genotype-specific bead class per sample. The cutoff value (5 net MFI) to define DNA positivity was applied as described previously [32]. Determination of β-globin confirmed the presence of DNA. Samples were considered valid if they were positive for β-globin and/or HPV DNA. Viral loads were assessed for all HPV types [24, 30]. Quantification of HPV signals was accomplished by computing the relative HPV MFI signal (%) for each positive reaction by dividing the measured HPV MFI value with the maximum value detected for this HPV type using colony PCR products. Finally, the relative MFI value (%) was divided by the measured β-globin MFI value to form a non-descriptive viral load value (% HPV MFI / β-globin MFI). The high viral load was assessed by a pre-defined HPV type-independent high viral load cut-off of 0.0007 units as described [31].

Sample size

By attempting to detect a minimum 10% difference between the proportion of HPV-positive women in rural (assumed 14% prevalence and 20% of participants) and urban (assumed 24% prevalence and 80% of participants) areas, a total of 845 women had to be analysed (845 total = 169 rural + 676 urban) alpha 5%, power 80%, χ^2 test. The ratio of rural and urban was derived from a short inquiry into the sites.

Statistical analysis

SPSS software version 23 (SPSS, Inc., an IBM Company) was used for statistical analysis. Odds ratios with corresponding 95%-confidence intervals (CIs) and *p*-values were calculated by logistic regression analysis for risk factors associated with HPV infection.

Ethical clearance

Ethical clearance was given by Addis Ababa University in February 2011 (124/10/IM;032/2011), April 2013 (050/2013) and at Martin-Luther-University Halle-Wittenberg, Germany 23.8.2010.

Results

Study population

There was a high acceptance among screened women for using the self-sampled cervico-vaginal lavage device (with some assistance by the nurse because of the large abdominal circumference) at the health center during antenatal care visits. During sample collection, 258 women were asked about their experience (samples from the first 3 sites Aira, Wukro and Ginir); 74% mentioned no problem, 20% mentioned discomfort and 6% said it was a bit painful. Key characteristics of the women included with valid samples (783) are presented in Table 1 stratified for whether ever lived in an urban area or always lived in a rural surrounding. Among the 1,025 samples included in this study, 242 (23.6%) had invalid results due to inadequate sample storage.

Table 1. Socio-demographic and reproductive characteristics of the rural, urban and total population, Ethiopia, 2013-2014, *n* = 783.

	Rural	Urban	Total population
N[1]	153	630	783
Age $\bar{x}$ (range)	25.2 (18–40)	26.1 (18–45)	25.9 (18–45)
Ethnic group *n* (%)			
Amhara	15 (9.8)	262 (41.7)	277 (35.4)
Oromo	107 (69.9)	106 (16.9)	213 (27.2)
Tigre	26 (17.0)	118 (18.8)	144 (18.4)
Gurage	2 (1.3)	98 (15.6)	100 (12.8)
Other	3 (2.0)	45 (7.2)	48 (6.1)
Religion *n* (%)			
Orthodox	53 (34.6)	438 (69.5)	491 (62.7)
Protestant	91 (59.5)	78 (12.4)	169 (21.6)
Muslim	9 (5.9)	111 (17.6)	120 (15.3)
Other	0	3 (0.5)	3 (0.4)
Education *n* (%)			
Illiterate	52 (34.0)	97 (15.4)	149 (19.1)
Read and write only	25 (16.3)	31 (4.9)	56 (7.2)
Formal education	76 (49.7)	501 (79.7)	577 (73.8)
Menarche $\bar{x}$ (range)	14.2 (11–19)	14.8 (7–20)	14.7 (7–20)

(Continued)

Table 1. Socio-demographic and reproductive characteristics of the rural, urban and total population, Ethiopia, 2013-2014, *n* = 783. *(Continued)*

Age at sexual debut $\bar{x}$ (range)	18.7 (9–27)	19.2 (5–33)[2]	19.1 (5–33)[2]
Marital status *n* (%)			
Single	2 (1.3)	20 (3.2)	22 (2.8)
Married	147 (96.1)	596 (94.6)	743 (94.9)
Widowed/divorced	4 (2.6)	14 (2.2)	18 (2.3)
Parity *n* (%)			
Nulliparous	57 (37.3)	314 (49.8)	371 (47.4)
Primiparous	46 (30.1)	168 (26.7)	214 (27.3)
Multiparous	50 (32.7)	148 (23.5)	198 (25.3)
Female genital mutilation *n* (%)	108 (71.1)	330 (53.0)	438 (56.5)
Age at circ. $\bar{x}$ (range)	13 (0–20)	5.5 (0–19)	7.4 (0–20)
Formal Polygamy *n* (%)	15 (9.8)	90 (14.3)	105 (13.4)

[1] N refers to total number of women in this category. It varies due to missing answers in the questionnaire. Maximum were eight missing answers in one category (circ. circumcision)
[2] One woman reported about rape at the age of five

The age ranged from 18 to 45 years with a mean of 25.9. Most women were reported to be housewives (60%); 15.2% and 13.0% were government employees and merchants, respectively. All Ethiopian major religions and ethnic groups were present in the study population. Educational levels showed only 15.4% and 34.0% illiterate women from urban and rural backgrounds, respectively. The vast majority of women were pregnant (95.7%) of which 10.5%, 35.5% and 54.0% were in first, second and third trimesters, respectively. Almost all women reported to be married (94.9%); nearly half came during their first pregnancy (47.4%). The prevalence of ethnic groups and religions as well as female genital mutilation and polygamy varied between the rural and urban populations. However, age at menarche and sexual debut, parity and marital status were not considerably different in the two groups. Only 31 out of 783 women were ever screened at least once (PAP smear).

Overall HPV DNA prevalence

Nearly all samples were positive for β-globin and/or HPV DNA regardless of the amount of fluid collected in the lavage except for 2. The overall prevalence for any HPV genotype was 33.1% (95%CI 29.8–36.4). The prevalence of HPV was considerably different in the rural and urban population with 17.6% (95%CI 11.6–23.7) and 36.8% (95%CI 33.1–40.6) ($p < 0.001$ chi-square test), respectively. Of all, a total of 22.0% were infected with a hr-HPV type of which 62.2% showed a high viral load in at least one hr-HPV infection (13.7% of the total population). Phr- and lr-HPV infections were observed in 7.8% and 18.5% of the women, respectively. Among the targeted HPV types (51) by the assay, 14 hr- and all 6 phr-HPV as well as twenty-two of 31 lr-HPV types were found in one or more samples. Single infections with only one genotype were found in 17.4% (95%CI 14.7–20.0) of women while multiple infections were observed in 15.7% (95%CI 13.2–18.3). In 62 women, simultaneous presence of two HPV types was identified, in 19 women three types, in 23 four types, in twelve five types and in seven women six or more types were found as shown in Figure 2.

HPV prevalence did not differ substantially between pregnancy trimesters. In the first and second trimesters 35.1% and 36.7% of women were positive for any HPV genotype, in the third trimester HPV was a little less frequent (29.7%). The number of women who had been sexually active within the last 3 days was decreasing with increasing trimesters: only 12.7% of women in the third trimester compared to 28.6% and 24.7% in the first and second trimester.

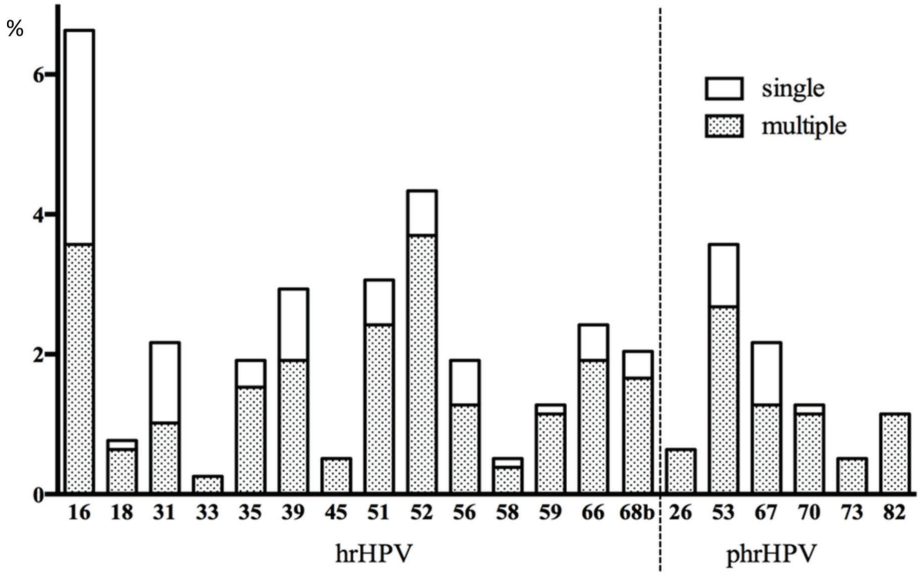

Figure 2. Overview of hr- and phr-HPV infection prevalence (%, *y*-axis) and presence of single or multiple infections in the total population (*x*-axis). Only the white part of the bar chart presents single infections, and the additional shaded part presents multiple infections.

Multiple infections were more common among women with lr- or phrHPV infections (65 of all lrHPV and 75% of all phrHPV positives) than women with hr-HPV infections (59% of all hr-HPV positives). Half of the HPV 16 and 31 infections presented as single infections. The prevalence of HPV types ranged from 6.6% (HPV 16) to 0.1% (HPV 69). The most commonly detected hr-HPV types were HPV 16 (6.6%), 52 (4.3%), 51 and 39 (both 2.9%). HPV 53 as a phr-HPV type was also common (3.6%). Only a few women were positive for the highly oncogenic HPV types 18 and 45 (0.8% and 0.5%). Common lr-HPV types were 81 (2.9%), 62 (2.4%), 42 (2.0%) and HPV 6 (2.0%) (Figure 2).

More than half of the patients presented with a high viral load of hr-HPV and phr-HPV (Figure 3).

Rural and urban HPV DNA prevalence

Of the 15 hr-HPV-positive (single or multiple) women with rural backgrounds 12 had a high viral load (80.0%) while only 95 of the 157 hr-HPV-positive women with urban backgrounds showed high viral loads (60.5%). The distribution of single versus multiple infections did not differ much between the different populations (Table 2).

Risk factor analysis

Risk factors were chosen according to previous studies: age, educational level, age at first sexual intercourse, use of contraceptives, parity, female genital mutilation and polygamy [43] and origin (rural versus urban) [23]. An exploratory analysis was done for women with urban backgrounds. Smoking is not common among Ethiopian women (n = 3) and was not included (Table 3).

The multivariable analysis indicated that women who ever lived in urban areas had a higher risk of being HPV positive with an OR of 2.36 (95%CI 1.47–3.79; p < 0.001) (Table 3). Young age at sexual debut (≤15 years of age) also increased the risk of being HPV positive (OR = 1.86 (95%CI 1.02–3.41; p = 0.043). This correlation was stronger after stratifying for urban background (OR = 2.18 (95%CI 1.14–4.17; p = 0.019). Women reporting that their husbands had more than one wife (polygamy) showed a moderate association with HPV positivity that became apparent when stratified by origin (OR = 1.62 (95%CI 1.00–2.63; p = 0.05). Other factors included were not statistically significantly associated with HPV.

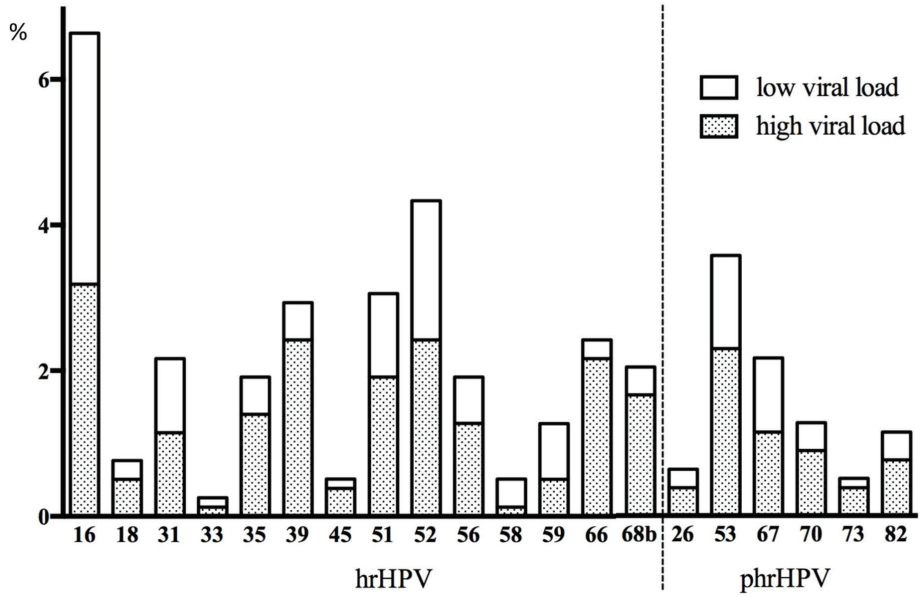

Figure 3. Overview of hr- and phr-HPV infection prevalence (*x*-axis) with low and high viral load in the total population (%, *y*-axis). The high viral load was assessed by a pre-defined HPV type-independent high viral load cut-off of 0.0007 units as described [31]. Only the white part of the bar chart presents a low viral load, the additional shaded part presents a high viral load.

Table 2. Prevalence of HPV and HPV genotypes in the rural, urban and total population.

	Rural (*n* = 153)			Urban (*n* = 630)			Total population (*n* = 783)		
	Single HPV	Multiple HPV	Total (%)	Single	Multiple	Total (%)	Single	Multiple	Total (%)
HPV negative	-	-	126 (82.4)	-	-	398 (63.2)	-	-	524 (66.9)
HPV positive	16	11	27 (17.6)	120	112	232 (36.8)	136	123	259 (33.1)
High-risk	5	10	15 (9.8)	65	92	157 (24.9)	70	102	172 (21.9)
Probable high-risk	2	5	7 (4.6)	13	41	54 (8.6)	15	46	61 (7.8)
Low-risk	9	7	16 (10.5)	42	87	129 (20.5)	51	94	145 (18.5)
any HPV with high viral load[1]	10	6	16 (10.5)	82	68	150 (23.8)	92	74	166 (21.2)
hrHPV with high viral load[1]	2	10	12 (7.8)	65	30	95 (15.1)	32	75	107 (13.7)
Proportion of hr HPV with high viral load within hrHPV			12/15 (80)			95/157 (60.5)			107/172 (62.2)
High-risk positive									
16	2	3	5 (3.3)	22	25	47 (7.5)	24	28	52 (6.6)
18	1	0	1 (0.7)	0	5	5 (0.8)	1	5	6 (0.8)
31	0	1	1 (0.7)	9	7	16 (2.5)	9	8	17 (2.2)
33	0	0	0	0	2	2 (0.3)	0	2	2 (0.3)

(Continued)

Table 2. Prevalence of HPV and HPV genotypes in the rural, urban and total population. ***(Continued)***

35	0	1	1 (0.7)	3	11	14 (2.2)	3	12	15 (1.9)
39	0	1	1 (0.7)	8	14	22 (3.5)	8	15	23 (2.9)
45	0	0	0	0	4	4 (0.6)	0	4	4 (0.5)
51	0	1	1 (0.7)	4	18	22 (3.5)	4	19	23 (2.9)
52	0	5	5 (3.3)	5	24	29 (4.6)	5	29	34 (4.3)
56	0	1	1 (0.7)	5	9	14 (2.2)	5	10	15 (1.9)
58	0	0	0	1	3	4 (0.6)	1	3	4 (0.5)
59	0	0	0	1	9	10 (1.6)	1	9	10 (1.3)
66	2	0	2 (1.3)	2	15	17 (2.7)	4	15	19 (2.4)
68b	0	2	2 (1.3)	3	11	14 (2.2)	3	13	16 (2.0)
Prob. High-risk									
26	0	1	1 (0.7)	0	4	4 (0.6)	0	5	5 (0.6)
53	1	0	1 (0.7)	6	21	27 (4.3)	7	21	28 (3.6)
67	1	1	2 (1.3)	6	9	15 (2.4)	7	10	17 (2.2)
70	0	1	1 (0.7)	1	8	9 (1.4)	1	9	10 (1.3)
73	0	0	0	0	4	4 (0.6)	0	4	4 (0.5)
82	0	2	2 (1.3)	0	7	7 (1.1)	0	9	9 (1.1)
Low-risk									
6	0	1	1 (0.7)	1	14	15 (2.4)	1	15	16 (2.0)
11	0	1	1 (0.7)	3	1	4 (0.6)	3	2	5 (0.6)
30	0	2	2 (1.3)	1	10	11 (1.7)	1	12	13 (1.7)
32	0	0	0	1	4	5 (0.8)	1	4	5 (0.6)
40	0	0	0	2	7	9 (1.4)	2	7	9 (1.1)
42	0	2	2 (1.3)	2	12	14 (2.2)	2	14	16 (2.0)
43	0	1	1 (0.7)	0	4	4 (0.6)	0	5	5 (0.6)
44	0	0	0	2	13	15 (2.4)	2	13	15 (1.9)
54	1	0	1 (0.7)	1	9	10 (1.6)	2	9	11 (1.4)
61	0	0	0	1	4	5 (0.8)	1	4	5 (0.6)
62	2	2	4 (2.6)	3	12	15 (2.4)	5	14	19 (2.4)
69	0	0	0	0	1	1 (0.2)	0	1	1 (0.1)
74	0	0	0	3	5	8 (1.3)	3	5	8 (1.0)
81	4	2	6 (3.9)	9	8	17 (2.7)	13	10	23 (2.9)
83	0	0	0	0	3	3 (0.5)	0	3	3 (0.4)
84	1	0	1 (0.7)	1	0	1 (0.2)	2	0	2 (0.3)
86	0	0	0	1	4	5 (0.8)	1	4	5 (0.6)
87	0	0	0	3	2	5 (0.8)	3	2	5 (0.6)
89	0	0	0	2	2	4 (0.6)	2	2	4 (0.5)

(Continued)

Table 2. Prevalence of HPV and HPV genotypes in the rural, urban and total population. ***(Continued)***

90	0	0	0	0	9	9 (1.4)	0	9	9 (1.1)
91	0	0	0	3	5	8 (1.3)	3	5	8 (1.0)
114	1	1	2 (1.3)	3	7	10 (1.6)	4	8	12 (1.5)

[1] High viral load: The high viral load was assessed by a pre-defined HPV type-independent high viral load cut-off of 0.0007 units as described [31]

Table 3. Risk factors for HPV positivity with odds ratio (OR) and *p*-values using multiple logistic regression.

	All women *n* = 752*				Women from urban areas *n* = 601			
	***n* Total**	***n* Positive (%)**	**Odds ratio**	***p*-value**	***n* Total**	***n* Positive (%)**	**Odds ratio**	***p*-value**
	752	246			601	220		
Background								
Rural	151	26 (17.2)	1 (-)					
Urban	601	220 (36.6)	**2.36 (1.47–3.79)**	**<0.001**				
Age								
≥ 30	176	60 (34.1)	1 (-)		144	56 (38.9)	1 (-)	
25–29	268	84 (31.3)	0.83 (0.52–1.31)	0.418	212	76 (35.8)	0.79 (0.48–1.29)	0.350
18–24	308	102 (33.1)	0.78 (0.47–1.3)	0.339	245	88 (35.9)	0.69 (0.4–1.18)	0.172
Education								
No formal education	194	52 (26.8)	1 (-)		120	43 (35.8)	1 (-)	
1–8 years.	220	72 (32.7)	1.24 (0.79–1.95)	0.344	181	66 (36.5)	1.21 (0.74–2.0)	0.451
≥ 9 years.	338	122 (36.1)	1.26 (0.82–1.95)	0.292	300	111 (37.0)	1.14 (0.71–1.83)	0.593
Age at sexual debut								
≥ 21	201	57 (28.4)	1 (-)		160	48 (30.0)	1 (-)	
16–20	471	158 (33.5)	1.35 (0.9–2.01)	0.149	376	143 (38)	1.52 (0.98-2.35)	0.06
≤ 15	80	31 (38.8)	**1.86 (1.02–3.41)**	**0.043**	65	29 (44.6)	**2.18 (1.14–4.17)**	**0.019**
Contraceptives								
No	220	72 (32.7)	1 (-)		173	61 (35.3)	1 (-)	
Hormone contraceptives[1]	532	174 (32.7)	1.02 (0.72–1.45)	0.928	428	159 (37.1)	1.1 (0.75–1.61)	0.622
Parity								
Nulli-/Primiparous	561	191 (34.0)	1 (-)		460	171 (37.2)	1 (-)	
Multiparous	191	55 (28.8)	0.69 (0.42–1.12)	0.128	141	49 (34.8)	0.65 (0.39–1.09)	0.103
Female genital mutilation								
Yes	432	123 (28.5)	1 (-)		324	108 (33.3)	1 (-)	
No	320	123 (38.4)	1.33 (0.96–1.84)	0.086	277	112 (40.4)	1.27 (0.89–1.8)	0.190
Polygamy								
No	654	205 (31.3)	1 (-)		517	180 (34.8)	1 (-)	
Yes	98	41 (41.8)	1.44 (0.91–2.27)	0.117	84	40 (47.6)	**1.62 (1.0–2.63)**	**0.05**

[1] Hormone contraceptives include pill, injectable and implant methods; All factors were fitted into a model as nominal and ordinal categories and a reference group was chosen indicated by 1(-). * Due to missing information 31 subjects of the total cohort were excluded leaving 752 subjects for analysis

Discussion

In this study, 783 women attending antenatal care in seven sites throughout Ethiopia were included and HPV was detected in one-third of self-sampled cervicovaginal lavages. Participants had a median age of 26 years, one in five had hr-HPV types and one in eight had a high viral load. The most common HPV types were hr-HPV 16, 52 and 51. The prevalence of HPV was 2.3 times higher in women with urban compared to rural backgrounds. Notably, in rural women, the proportion of hr-HPV with high viral load out of all women with hr-HPV was 80% compared to urban women with a proportion of 61%.

A large review summarized publications about women with normal and abnormal cytological findings in Africa (using different detection kits). The prevalence of any HPV type was estimated at 42.2% for East Africa and 12.8% for North Africa [25]. The overall prevalence of HPV in this study is high but not higher than the estimate for East Africa.

However, the prevalence of HPV in this study is higher than other reports from Ethiopia, 15.9% [28], 17.5% [23] and 23.2% [38]. A number of factors account for the difference in prevalence among which the technology used to test HPV and the coverage of the HPV test used are important. The first two studies used the Digene HPV test which tests fewer HPV genotypes compared to ours that targets about 51 different HPV genotypes whereas Teka *et al* [38] used almost similar technology. Here, the screened population was different including older age groups. Our finding is consistent with a report from Ghana [33] that employed a similar technology to test HPV and targeted a similar population of pregnant women. Another Ghanean study showed a prevalence of 32% hr-HPV using the same methodology as this study [22].

In terms of HPV genotypes detected, the top 10 hr-HPV detected from our study participants did not include HPV 18, 58, 33 and 45 reported to be detected from high-grade intraepithelial lesion cases in Africa [25]. Similarly, among the top 10 hr-HPV detected in this study six (HPV16, 35, 52, 31, 56 and 53) were also the top 10 in a population-based study [38]. However, it is always difficult to come into consensus about genotypes circulating in a given area by comparing studies that were made using different techniques among different populations and at different time points.

We compared our results to 3 studies from Ethiopia: the detailed study from rural Attat hospital found HPV type 16 most prevalent followed by 52, 56, 31 and 51 [23]. Wolday *et al* [47] investigated patients at a tertiary referral hospital and found 16, 35, 56, 58 and 18 as the five most common types among cytologically normal women and 16, 45, 31, 35 and 59 among those with cytologic abnormality [47]. Teka *et al* [38] recent population-based study found 16, 35, 52, 31 and 45 as the most common types [38]. HPV 56, 31 and 51 were also among the top 10 prevalent types in our study. In our study, we found 119 of 246 (48,4%) infections covered by the nonavalent vaccine (16, 18, 31, 33, 39, 52 and 58). We conclude that the HPV vaccination program in Ethiopia will effectively reduce the number of women with cervical cancer cases. First pilot sites have been established and school-based vaccination has been proven feasible [45, 46].

We found some individual factors increasing the risk for HPV infection. Women who ever lived in the urban setting had a higher risk of HPV infection with OR 2.36 (1.47–3.79) compared to women who never lived in urban areas. This is similar to the study of Leyh-Bannurah *et al* [23] revealing lower HPV prevalence in women from houses with a traditional roof. In Ethiopia, the urban setting is probably a surrogate marker for a modern lifestyle with more sexual contact of both partners and a known higher HIV prevalence (the number of partners and HIV status were not assessed in our study). A study analysed HIV prevalence in Ethiopia (2005 and 2011 Ethiopian Demographic and Health Surveys). The main risk factors were pre-marital sex, number of life-time sexual partners and high-risk sexual behaviour. These varied considerably between the regions and between ethnic groups explaining regional variation [21]. We postulate that similarly to sexual behaviour and HIV, also HPV prevalence may vary between regions. Our sample size allowed comparison between rural and urban areas but not between regions.

High viral load has been found associated with high-grade cervical intraepithelial neoplasia [42]. In our study, the proportion of women with any HPV type high viral load was 21.2% and women with hr-HPV types high viral load was 13.7%. These women definitely need a follow-up and probably treatment that is usually done only after delivery. Interestingly, in rural areas, the proportion of women with hr-HPV infection with high viral load (12/15) was higher than in women of urban areas (95/157). This could indicate that the rural population had a higher number of high-grade intraepithelial neoplasia and consequently a more advanced HPV infection than the urban population. This definitely shows a need for adequate health service and follow-up.

Early age at sexual debut was associated with a higher prevalence of HPV similar to other studies [43]. Notably early marriage is still rather common in Ethiopia with a median age of 17.1 years among women (age 25–49) in 2011 [6]. This puts a considerable number of women at risk.

The strength of this study is showing HPV prevalence in a representative sample of healthy women in the antenatal care setting as opposed to patients in the hospital setting who do have some kind of health complaint. Self-sampling was well accepted, similar to a study from Ghana [3, 22]. Second, a large number of 783 participants were included from different regions of the country including rural and urban areas. This reflects the diversity in the country to a certain extent considering higher proportions of rural habitants in this study compared to the country.

The limitation is some selection bias since our sample had a small proportion of multi-gravid mothers and women with lower socio-economic backgrounds since they less often attend antenatal care. Therefore, our study missed those women who have more risk factors, leading to possible underestimation in this study. Second, our age-range was narrow, therefore age-standardisation for general comparison was not possible. Of note, our study does not include never-pregnant women. Possibly the group of infertile women (with infertility caused by sexually transmitted infections) could be associated with an even higher proportion of positive HPV status.

Conclusion

Seeing the high prevalence of HPV in rural and urban populations, there is a clear need for suitable screening and treatment programs. Self- or health worker-based sampling during antenatal care seems feasible to determine HPV and could be a possible, well-accepted option to increase screening uptake using this window of opportunity for screening-eligible women between 30 and 50 years [18]. Linking cervical cancer screening to antenatal care may optimize resources within the health system – although logistic challenges have to be considered since positive women need to be further investigated after delivery. Spontaneous regression after delivery is also possible [2]. Primary prevention through vaccination has started in Ethiopia. The most prevalent HPV16 is covered by the vaccine currently available; the nonavalent vaccine will additionally target HPV 52 as the second most common hr-HPV type in our study. As there was an even higher prevalence in women with urban backgrounds, current trends in migration to cities will probably lead to an increase in the problem. Changes over time will be evaluated.

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Conflicts of interest

The authors have no conflict of interest.

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Informed consent

Ethical approval was obtained from Addis Ababa University Medical Faculty Institutional Review Board (Meeting 050/2013; Protocol 124/10/IM). Written informed consent was obtained from each study participant. All study participants consented orally to being included in the study.

Author contributions

IR, JMAK, AA and EJK designed the research; JMAK, IR, DHoe, AA SU, CT and EJK analysed the data; JMAK, DHoe and EJK were responsible for drafting the manuscript; JMAK, IR, AKP, TW, SFA, AA, MS, TW and DH provided the original samples, laboratory results and information on the respective populations, and advice on the study design, analysis and interpretation of the findings; all authors provided critical interpretation of the results and review of the first draft; all authors read and approved the final manuscript.

Data availability

Study data is available on reasonable request from the corresponding author.

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Appendix 1. Questionnaire for HPV prevalence survey

Risk factor questionnaire

1. Study ID number: |__||__||__||__|
2. Date of interview: *yyyy-mm-dd* - |__||__| -|__||__|
3. Name of interviewer: ..... |__|
4. Type of work |__|

Demographic data

5. Age (years): |__| |__| *(Woman should be of age 21-40 to participate in study)*
6. Birthplace? Town.....|__|
7. Resident now |__||__||__||__|
8. Marital Status: *Single=0, Married=1, Widowed=2, Separated/Divorced=3* |__|
9. How many times have you been married? *(never married=0)* |__|
10. How many years of schooling you have completed? |__|

Reproductive History

11. At what age did you have your first menstrual period? |__||__|
12. Are you pregnant now? *Yes=1, No=0* |__|
13. How many full-term births did you have before? |__||__|
14. Delivery place: *1=Province clinic, 2=Maternal house, 3=Public hospital, 4=at home* |__|
15. Did you ever have a spontaneous abortion? *Yes=1, No=0* |__|
16. If yes, how many |__||__|
17. Did you ever have a voluntary abortion?*Yes=1, No=0* |__|
18. If yes, how many |__||__|
19. How old is your youngest child? |__||__|
20. How old is your oldest child? |__||__|

Contraceptive History

21. What method(s) of contraception have you or your partner ever used? Please check all that apply

None	|_0_|	Birth control Pill	|_4_|
Calendar	|_1_|	Injectable contraceptive	|_5_|
Condom	|_2_|	Tubal ligation	|_6_|
Intrauterine device	|_3_|	Other	|_7_|, specify: .....

For users of the birth control pill or injectable contraceptive only:

22. At what age did you start using the contraceptive? |__||__|
23. At what age did you stop using the birth control pill or injectable contraceptive? |__||__| (current age for current users)
24. Are you currently using the contraceptive? *Yes=1, No=0* |__|

Research

Medical History

25. If women pregnant, how many months since your last period? |__||__|
26. As far as you know, have you ever had a PAP smear? *Yes=1, No=0* |__|
27. Do you have a previous history of cervical cancer or precancer (dysplasia)? *Yes=1, No=0* |__|
28. Have you ever been diagnosed with Tuberculosis? *Yes=1, No=0* |__|

Smoking History

29. Have you ever smoked regularly? *Yes, currently=1, Yes, but only in the past=2, No, never=0* |__|

If Yes

30. At what age did you start smoking |__||__|
31. How many cigarettes did/do you smoke per day |__||__|

Sexual History

32. How old were you when you first had sexual intercourse with a man? Age |__||__|
33. Throughout your life, how many men have you had sexual intercourse with? |__||__|

To your knowledge, did your husband ever have sexual intercourse with another woman

34. before becoming your partner/husband? *1=Yes, 0=No, 2=Don't know* |__|
35. while being your partner/husband? *1=Yes, 0=No, 2=Don't know* |__|
36. Does your husband have another wife? How many? |__|

Blood sampling

37. Blood sample taken: *Yes=1, No=0* specify reason: ..... |__|

Gynecological examination

38. Sample taken with self sampler : *Yes=1, No=0* specify reason: .....|__|

Number of self-sample specimen .....|__|

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Prevalence of bacterial vaginosis, sexually transmitted infections and their association with HPV infections in asymptomatic women attending antenatal care in Ethiopia

Johanna M. A. Klein[1,2], Isabel Runge[1,2], Ann-Katrin Pannen[1,2], Tariku Wakuma[2,3], Semaw Ferede Abera[4,5,6], Adamu Adissie[2,7], Susanne Unverzagt[8], Markus Schmitt[9], Tim Waterboer[9], Daniela Höfler[9], Christoph Thomssen[1,2] and Eva Johanna Kantelhardt[1,2]

[1]Department of Gynaecology, Martin Luther University Halle-Wittenberg, Halle (Saale) 06097, Germany
[2]Global Health Working Group, Martin Luther University Halle-Wittenberg, Halle (Saale) 06097, Germany
[3]Aira Hospital, Aira, Ethiopia
[4]Department of Radiation Oncology, Faculty of Medicine, Martin Luther University Halle-Wittenberg, Halle (Saale) 06097, Germany
[5]School of Public Health, College of Health Sciences, Mekelle University, Mekelle, Ethiopia
[6]Kilte Awlaelo - Health and Demographic Surveillance Site, College of Health Sciences, Mekelle University, Mekelle, Ethiopia
[7]School of Public Health, College of Health Sciences, Addis Ababa University, Addis Ababa 1000, Ethiopia
[8]Institute of General Practice and Family Medicine, Center of Health Sciences, Martin Luther University Halle-Wittenberg, Halle (Saale) 06097, Germany
[9]Infections and Cancer Epidemiology, German Cancer Research Center (DKFZ), Heidelberg 69120, Germany

Research

Abstract

Sexually transmitted infections (STIs) and human papillomavirus (HPV) infections are common among women of reproductive age and can lead to infertility, adverse pregnancy outcomes, neonatal infections and cervical cancer. In countries with limited medical coverage, untreated infections contribute to high morbidity. This study aimed to expand the current knowledge on the prevalence of bacterial vaginosis (BV) and STIs in pregnant Ethiopian women and assess the association of these conditions with HPV infections. Socio-demographic data and vaginal lavage samples were collected from 779 asymptomatic women aged 18 to 45 years (median age, 25.9 years) attending antenatal care in seven centres across Ethiopia. Multiplex polymerase chain reaction was used to test for BV, *Chlamydia trachomatis*, *Trichomonas vaginalis*, *Neisseria gonorrhoeae*, herpes simplex virus types 1 and 2 (HSV-1/2), *Mycoplasma*, *Ureaplasma*, *Candida species* and HPV. Overall, 26.8% (95% confidence interval (CI): 23.7–29.9) of women tested positive for BV or one of the following STIs: *C. trachomatis*, *T. vaginalis*, *N. gonorrhoeae*, *Mycoplasma genitalium*, HSV-1/2 or *Ureaplasma urealyticum*. Additionally, 22.1% tested positive for at least one high-risk HPV type. *Chlamydia trachomatis* and HSV-2 were significantly more common among women who were positive for HPV and high-risk HPV. This study reveals a high prevalence of asymptomatic pregnant women who are positive for BV, STIs or HPV, putting them at risk of adverse pregnancy outcomes, secondary infertility or cervical cancer in a country with limited medical coverage. Screening and treating these women could be crucial in reducing morbidity.

Keywords: *sexually transmitted diseases*, *bacterial vaginosis*, *Ethiopia*, *pregnancy*, *vaginal lavage*, *human papillomavirus*

Correspondence to: Eva Johanna Kantelhardt
Email: eva.kantelhardt@uk-halle.de

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Background

Bacterial vaginosis (BV) and sexually transmitted infections (STIs) are common among women of reproductive age. The association between BV and adverse pregnancy outcomes is a subject of ongoing debate [1]. Infections with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, *Treponema pallidum*, herpes simplex virus type 2 (HSV-2) and *Ureaplasma urealyticum* are strongly linked to adverse pregnancy outcomes, including miscarriages, premature rupture of membranes, preterm labour and delivery, neonatal infections, low birth weight, puerperal endomyometritis and secondary infertility [2–8]. Delayed diagnosis and treatment, especially in countries with limited medical resources, can lead to serious complications.

BV is characterised by a reduction in lactobacilli and an overgrowth of anaerobic bacteria such as *Gardnerella vaginalis*, *Atopobium vaginae* and *Mycoplasma hominis*, and it is a known risk factor for preterm labour [1]. While these bacteria may not be highly pathogenic on their own, they may facilitate infection by more pathogenic bacteria because the protective role of the physiological lactobacilli vaginal flora is compromised in BV. Consequently, it has been suggested that BV may also facilitate infections with high-risk human papillomavirus (HPV) [9]. High-risk HPV is a necessary, but not sufficient, cause of cervical cancer; however, most infections are transient and cause no or only mild cervical changes [10]. Associations between infections with *C. trachomatis* or HSV-2 and the development of HPV or cervical cancer suggest that these infections may act as cofactors in the development of cervical cancer and precancerous lesions [11–13].

Screening and treating pregnant women is challenging but crucial. Many women are asymptomatic and, therefore, remain untreated, resulting in serious medical complications for both mother and child. Early detection and treatment of BV and STIs may prevent preterm labour and vertical transmission of infections, while in women with high-risk HPV, it can reduce morbidity and mortality due to cervical cancer [12, 14].

A feasible screening method for BV and STIs in low-resource countries is STI profiling (STIP). STIP is a multiplex polymerase chain reaction (PCR) technique that can detect multiple infectious agents simultaneously, requiring only a single vaginal or cervical swab or a lavage sample for testing [15].

Limited data are available on BV, STIs and HPV infection in Ethiopia. The aim of this study was to expand the current knowledge on the prevalence of BV and STIs among asymptomatic young pregnant women in Ethiopia and to evaluate the association of these conditions with HPV infections. Such information will help estimate the number of women at risk for adverse pregnancy outcomes.

Methods

Study population

In 2013 and 2014, a cross-sectional study was conducted to test asymptomatic pregnant women for STIs and HPV across seven centres in Ethiopia [16]. To ensure comprehensive data collection, samples were obtained from antenatal care and family planning units at university hospitals, state and private hospitals, and regional health centres throughout the country.

Women aged 18 to 45 years were eligible to participate if they had no signs of placenta praevia, severe pre-eclampsia or other pregnancy-related complications. Women visiting the facility because of vaginal bleeding or discharge were also excluded, although some women showed signs of discharge upon examination (Supplementary Table 1). Of the 1,239 women attending antenatal and family planning units on the enrolment days, 1,041 agreed to participate and completed all study procedures. Due to missing samples and invalid analyses, 262 samples were excluded from the final analysis, leaving 779 valid samples from antenatal care (n = 747) and family planning (n = 32). Women from seven geographical areas were included in the study: Addis Ababa (n = 209), Mekelle (n = 74), Bahir Dar (n = 215), Harar (n = 29), Ginir (n = 38), Wukro (n = 65) and Aira (n = 149).

Patient involvement

The patients were involved in the design and conduct of this research. During the feasibility stage, the research question, choice of outcome measures and recruitment methods were informed by discussions with the patients through a rapid ethnographic assessment session conducted by the research team.

Sample size

To detect a minimum 10% difference between the proportion of STI-positive women in rural areas (assumed 14% prevalence and 20% of participants) and urban areas (assumed 24% prevalence and 80% of participants), 845 women were required for analysis (845 total = 169 rural + 676 urban), with an alpha of 5% and a power of 80%, using the chi-squared test. The ratio of rural to urban participants was derived from a preliminary inquiry at the sites.

Questionnaire

To obtain socio-demographic and reproductive data, local nurses administered a questionnaire that had been previously used in other studies [17]. The questionnaire was slightly modified to suit the study population. It was translated into Amharic, Oromo and Tigrinya and validated by retranslation conducted by native speakers to ensure accuracy and avoid any loss or misinterpretation of information.

Sample collection

Samples were collected during routine antenatal care and family planning visits. Local nurses provided a brief introduction to STIs, their transmission and their symptoms. The study procedures were explained, informed consent was obtained and codes were assigned to ensure confidentiality.

Vaginal samples were collected using a syringe-like self-sampling device (Delphi Bioscience, Scherpenzeel, Netherlands). The device was inserted into the vagina, releasing 3 mL of buffered saline by plunging the handle. The fluid was then retracted back into the device by releasing the handle. The lavage fluid was transferred into transportation tubes containing 3 mL of buffered methanol solution, where it was preserved for up to 3 months at room temperature (20°C–30°C). Medical staff assisted with the sample collection. The samples were analysed at the German Cancer Research Centre (DKFZ) in Heidelberg, Germany.

HPV DNA testing

For the detection of HPV DNA in the vaginal lavage samples, a multiplex papillomavirus genotyping assay was performed as previously described [18]. This method analyses 51 HPV types, of which 14 are considered high-risk: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68a/b [19]. Results were expressed as median fluorescence intensity values. Samples were considered positive if they contained β-globin as a human DNA marker and/or HPV DNA.

STIP

STIP, is a validated multiplex PCR that simultaneously analyses *Atopobium vaginae*, *Candida albicans*, *Candida glabrata*, *Candida krusei*, *Chlamydia trachomatis*, *Gardnerella vaginalis*, HSV-1/2, *Mycoplasma genitalium*, *Mycoplasma hominis*, *Mycoplasma pneumoniae*, *Mycoplasma spermatophilum*, *Neisseria gonorrhoeae*, *Treponema pallidum*, *Trichomonas vaginalis*, *Ureaplasma urealyticum*, *Ureaplasma parvum and Lactobacillus iners* [15]. All other *Lactobacillus* and *Candida* species were detected using universal probes. To confirm the presence of human DNA, the PolA sequence was analysed. Samples positive for STIs and/or PolA were considered valid. Results are reported in median fluorescence intensity values.

A score for BV (BV-score) was calculated as previously described, based on the ratios of *G. vaginalis* and *A. vaginae* to *Lactobacillus* [15]. The presence of *Mycoplasma hominis* was also taken into account.

Statistical analysis

All descriptive and statistical analyses were conducted using SPSS software version 23 (IBM Corp., Armonk, NY, USA). The prevalences of STIs and HPV, along with the corresponding 95% CIs, were calculated. Fisher's exact test was used to determine statistical significance, with *p*-values of <0.05 considered statistically significant.

Ethical clearance

Ethical clearance was obtained from Addis Ababa University and Martin Luther University Halle in February 2011 and April 2013, respectively.

Results

Study population

The participants ranged in age from 18 to 45 years, with a mean age of 25.9 years. All major ethnic and religious groups were represented. Table 1 provides an overview of the demographic and reproductive characteristics of the participants.

One fifth of the women had never lived in a city or town and were classified as rural for this study. The remaining women had lived in urban areas or under urban influence and were therefore classified as urban.

More than half of the women (59.7%) identified as housewives, while 15.2% were government employees and 13.2% were merchants. The remaining women were students, employed in other professions or unemployed.

Of the 747 pregnant women, 10.6% were in the first trimester, 35.3% in the second trimester and 54.1% in the third trimester of pregnancy. Most women (94.9%) were married, and 13.6% reported that their husbands had up to three other wives (polygamy). Before becoming pregnant, many women had used hormonal contraceptive methods (70.6%), but condom use was low (1.5%).

One quarter of the women had previously given birth to between one and eight children. Up to four past spontaneous abortions were reported by 18.5% of the women, while 7.8% had undergone up to three induced abortions. No data on the number of sexual partners were obtained to avoid the risk of response bias due to cultural and confidentiality reasons.

Prevalence of STIs and HPV

One third of the women (33.0%) tested positive for at least one mucosal HPV type, and 22.1% were positive for at least one high-risk HPV type. Table 2 presents the prevalence of STIs, organisms of the vaginal flora and HPV.

Infections with *C. trachomatis*, *T. vaginalis*, *N. gonorrhoeae*, *M. genitalium* and HSV-1/2 were categorised as STIs. The overall prevalence of these STIs in this population was low.

Only a small number of women tested positive for cervical infections with *C. trachomatis* (0.6%) and *N. gonorrhoeae* (0.6%). Infections with *T. vaginalis* (2.3%) and HSV-2 (1.5%) were detected more frequently but remained relatively low. A total of 6.2% of the women were diagnosed with at least one STI. However, only three women had two concurrent STIs: one woman had *C. trachomatis* and *T. vaginalis*, one had *C. trachomatis* and *M. genitalium*, and one had *M. genitalium* and HSV-2. No woman was positive for more than two STIs.

Among the 145 women who tested positive for BV, 126 had a BV-score of ≥2, indicating an unbalanced vaginal flora.

In total, 209 (26.8%) women were positive for BV (score of ≥2), any STI or *U. urealyticum*.

Women from rural backgrounds were more likely to have a BV-score of >2 (36.5% [95% CI: 26.2–46.7]) than women from urban backgrounds (22.0% [95% CI: 18.1–25.9]). No differences were found in the prevalence of STIs between rural and urban areas. Additionally, no significant differences were observed in the BV or STI prevalence based on age, trimester of pregnancy, educational level, age at sexual debut or circumcision status.

Table 1. Socio-demographic and reproductive data of the study population.

	Total population
	n = 779[1]
Age, years	25.9 (18–45)
Background	
Rural	152 (19.5)
Urban	626 (80.5)
Ethnic group	
Amhara	276 (35.5)
Oromo	213 (27.4)
Tigre	141 (18.1)
Gurage	100 (12.9)
Other	48 (6.2)
Religion	
Orthodox	488 (62.6)
Protestant	169 (21.7)
Muslim	119 (15.3)
Other	3 (0.4)
Education	
Illiterate	149 (19.3)
Read and write only	56 (7.2)
Formal education	572 (73.4)
Menarche, years	14.7 (7–20)
Age at sexual debut, years	19.1 (5–33)[2]
Marital status	
Single	22 (2.8)
Married	739 (94.9)
Widowed/divorced	18 (2.3)
Parity	
Nulliparous	370 (47.5)
Primiparous	214 (27.5)
Multiparous	195 (25.0)
Female circumcision - Age at circumcision, years	436 (56.5) 7.4 (0–20)
Polygamy	105 (13.6)

Data are presented as median (range) or *n* (%).
[1]*n* refers to total number of women in this category. It varies from line to line because of missing answers in the questionnaire. The maximum was eight missing answers in one category (circumcision). All listed percentages take this into account.
[2]One woman reported rape at the age of 5 years.

Table 2. Prevalence of STIs and HPV.

Organism	*n*	% (95% CI)	Total *n*
HPV			
Any HPV	257	33.0 (29.7–36.3)	779
High-risk HPV[1]	172	22.1 (19.2–25.0)	779
STI			
C. trachomatis	5	0.6 (0.1–1.2)	779
T. vaginalis	18	2.3 (1.3–3.4)	779
N. gonorrhoeae	5	0.6 (0.1–1.2)	779
M. genitalium	8	1.0 (0.3–1.7)	779
HSV-2	12	1.5 (0.7–2.4)	779
HSV-1	3	0.4 (0.1–1.2)	779
STI-positive[2]	48	6.2 (4.5–7.9)	779
BV			
A. vaginae	218	28.0 (24.8–31.1)	779
G. vaginalis	301	38.6 (35.2–42.1)	779
M. hominis	78	10.0 (7.9–12.1)	779
L. iners	489	62.8 (59.4–66.2)	779
Any lactobacillus	741	95.1 (93.6–96.6)	779
BV-score 1	19	3.7 (2.0–5.3)	518[4]
BV-score 2	49	9.5 (6.9–12.0)	518[4]
BV-score 3	7	1.4 (0.4–2.3)	518[4]
BV-score 4	44	8.5 (6.1–10.9)	518[4]
BV-score 5	26	5.0 (3.1–6.9)	518[4]
BV-score ≥ 2[3]	126	24.3 (20.6–28.0)	518[4]
Candida			
C. albicans	222	28.5 (25.3–31.7)	779
C. glabrata	55	7.1 (5.3–8.9)	779
C. krusei	36	4.6 (3.1–6.1)	779
Candida species	301	38.6 (35.2–42.1)	779
Other *Mycoplasma* and *Ureaplasma*			
M. spermatophilum	1	0.1 (0.01–0.8)	779
U. urealyticum	152	19.5 (16.7–22.3)	779
U. parvum	427	54.8 (51.3–58.3)	779

This table shows all detected organisms. No cases of *T. pallidum* or *M. pneumoniae* were found.

[1]High-risk HPV types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68a/b

[2]STI-positives include women positive for *M. genitalium*, *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, *T. pallidum* or HSV-1/2

[3]BV-score takes low levels of lactobacillus and positivity for *G. vaginalis*, *A. vaginae* and *M. hominis* into account

[4]BV-scores could only be calculated for samples with quantifiable *Lactobacillus*

Correlation between STIs and HPV

The observed correlations between STIs and HPV are shown in Table 3.

Chlamydia trachomatis and HSV-2 were significantly more common among women who tested positive for HPV and high-risk HPV, while *M. genitalium* was more frequent in women with HPV infections but not in those with high-risk HPV infections.

Although all three bacteria associated with BV (*A. vaginae*, *G. vaginalis* and *M. hominis*) were significantly associated with both HPV and high-risk HPV, having a positive BV-score (BV-score ≥ 2) was not associated with these infections. Candidiasis was also not associated with HPV. Among other bacteria, only *U. parvum* was found to be associated with HPV infection.

Table 3. Correlations between HPV and STI.

	HPV-positive (*n* = 257)			High-risk HPV-positive (*n* = 172)		
Organism	***n***	**% (95% CI)**	***p*-value**	***n***	**% (95% CI)**	***p*-value**
STI						
C. trachomatis	**5**	**1.9 (0.3–3.6)**	**0.004**	**4**	**2.3 (0.8–6.2)**	**0.010**
T. vaginalis	6	2.3 (0.5–4.2)	1.000	5	2.9 (0.4–5.4)	0.567
N. gonorrhoeae	3	1.2 (0.3–3.7)	0.338	1	0.6 (0.03–3.7)	1.000
M. genitalium	**6**	**2.3 (0.5–4.2)**	**0.018**	2	1.2 (0.2–4.6)	0.692
HSV-2	**10**	**3.9 (1.5–6.3)**	**<0.001**	**8**	**4.7 (1.5–7.8)**	**0.001**
HSV-1	1	0.4 (0.02–12.5)	1.000	1	0.6 (0.03–3.7)	0.527
STI-positive[1]	**28**	**10.9 (7.1–14.7)**	**<0.001**	**18**	**10.5 (5.9–15.0)**	**0.011**
BV						
A. vaginae	**85**	**33.1 (27.3–38.8)**	**0.028**	**60**	**34.9 (27.8–42.0)**	**0.027**
G. vaginalis	**114**	**44.4 (38.3–50.4)**	**0.023**	**83**	**48.3 (40.8–55.7)**	**0.004**
M. hominis	**48**	**18.7 (13.9–23.4)**	**<0.001**	**39**	**22.7 (16.4–28.9)**	**<0.001**
Any lactobacillus	242	94.2 (91.3–97.0)	0.381	160	93.0 (89.2–96.8)	0.161
BV-score ≥ 2[2,3]	45	27.3 (20.5–34.1)	0.323	33	30.6 (21.9–39.2)	0.101
Candida species[4]	110	42.8 (36.8–48.9)	0.101	77	44.8 (37.3–52.2)	0.063
Other *Mycoplasma* and *Ureaplasma*						
M. spermatophilum	1	0.4 (0.02–12.5)	0.330	1	0.6 (0.03–3.7)	0.221
U. urealyticum	60	23.3 (18.2–28.5)	0.680	40	23.3 (16.9–29.6)	0.191
U. parvum	**172**	**66.9 (61.2–72.7)**	**<0.001**	**116**	**67.4 (60.4–74.4)**	**<0.001**

This table shows the correlations between STIs and HPV and high-risk HPV positivity. All *p*-values were calculated by Fisher's exact test and are in reference to HPV-negative women.

[1]STI-positives include women positive for *M. genitalium*, *C. trachomatis*, *N. gonorrhoeae*, *T. vaginalis*, *T. pallidum* or HSV-1/2

[2]BV-score takes low level of lactobacillus and positivity for *G. vaginalis*, *A. vaginae* and *M. hominis* into account

[3]BV-scores could only be calculated for samples with quantifiable *Lactobacillus*

[4]None of the *Candida* subtypes showed a significant correlation with HPV and are therefore not listed separately

Discussion

Summary

This is the first multicentre survey to provide data on a broad spectrum of STIs in pregnant Ethiopian women attending antenatal care. This study included only women who considered themselves asymptomatic and were therefore not included in the syndromic approach to STI treatment, as recommended by the Ethiopian national plan. Among these asymptomatic women, 26.8% tested positive for BV, STIs or *U. urealyticum*, and 33.0% tested positive for HPV.

Background

According to Molla et al. [20], health-seeking behaviour regarding STIs is poor among young Ethiopians because of a lack of knowledge about STIs and the stigma associated with premarital sexual activities. Poor medical coverage and limited knowledge of treatment options among physicians further complicate the situation [21]. Apart from HIV and haemoglobin testing, no routine tests are performed on pregnant women in Ethiopia.

BV

BV during pregnancy can lead to premature rupture of membranes and preterm labour but is often asymptomatic, remaining undetected and untreated [1]. In this study, 24.3% of asymptomatic women tested positive for BV, which is consistent with results from different regions in Ethiopia showing BV prevalences of 19.4%–29.4% [22–25]. Although the benefits of treatment during pregnancy are debated, there is evidence supporting early treatment [26]. Unexpectedly, rural women were more likely to test positive for BV. The influence of hygienic conditions and nutritional factors should be explored in these settings.

HPV

High-risk HPV is a necessary but not sufficient cause of cervical cancer [27]. HPV can be sexually transmitted, and although most people will be infected at some point in their lives, the majority of infections are transient and resolve within months [10]. Young and sexually active individuals are particularly at risk. In this study, 33.0% of the population tested positive for any HPV and 22.1% tested positive for high-risk HPV. These findings are consistent with other studies that reported a 33.6% prevalence of HPV in East Africa and Ethiopia [28, 29].

Chlamydia trachomatis and *N. gonorrhoeae*

Cervical infections with *C. trachomatis* and *N. gonorrhoeae* during pregnancy may lead to miscarriages, preterm labour, neonatal infections, postpartum upper genital tract infection and secondary infertility [2–5, 30]. Adequate antimicrobial therapy can prevent these outcomes [30]. In this study, only 0.6% of participants tested positive for *C. trachomatis* and *N. gonorrhoeae*. Studies from around the world have shown higher prevalence rates in the female adult population, ranging from 5.0% to 14.7% for *C. trachomatis* and 0.4% to 6.7% for *N. gonorrhoeae*, with prevalences of 7.8% to 9.8% for *C. trachomatis* and 2.4%–4.3% for *N. gonorrhoeae* in Eastern Africa and Ethiopia [5, 31, 32, 33–35]. Our asymptomatic, fertile and married population might be expected to have a lower prevalence of cervical STIs in general. However, the high prevalence of other vaginal STIs and HPV suggests that the low detection of cervical infections may have been due to limitations in the detection method used, rather than reflecting the actual prevalence.

Other vaginal infections

The pregnant women in this study also demonstrated various types of vaginal infections, such as *T. vaginalis* (2.3%) and *U. urealyticum* (19.5%), which are suspected to cause preterm labour and placental inflammation [14, 36, 8]. However, treatment appears to be beneficial only for *U.*

urealyticum infections, not for trichomoniasis [37, 38]. The prevalence of *M. genitalium* infections (1.0%) in this population is consistent with other studies [31].

Primary infections with HSV-2 during pregnancy are known to cause severe neonatal infections and continue to be a concern [39]. In this study, 1.5% of the women had active HSV-2 infections, although it is unclear whether these were primary or recurrent infections. Fortunately, no cases of active syphilis were detected in this population.

Candida infections are common among pregnant women and were detected in 38.6% of women in this study. While the impact of *Candida* infections on pregnancy outcomes is debated, the morbidity associated with recurring *Candida* infections is undisputed [40].

Correlations of BV and STIs with HPV

Studies have suggested a possible correlation between HPV and BV, which was not observed in this study, although *G. vaginalis*, *A. vaginae* and *M. hominis* were significantly associated with both HPV and high-risk HPV [9]. A significant correlation was found between HPV and *C. trachomatis* and HSV-2, as noted in previous studies [11, 41]. While this correlation could be partly explained by the number of sexual partners, the lack of association between HPV and other STIs makes this an unlikely sole explanation.

In this study, *U. parvum* was associated with HPV (odds ratio, 2.12 [1.55–2.89]). This finding has not been reported in the literature, and further studies are needed to determine whether this is a coincidence.

Strengths and limitations

The main strength of this study lies in its large sample size of pregnant women from seven urban and rural centres across Ethiopia, capturing the country's diversity. Additionally, a broad spectrum of bacterial, viral and parasitic STIs was detected using a single sample analysed by PCR, an effective detection method. This study provides a comprehensive picture of the prevalence of BV, STIs and HPV in this pregnant population.

However, this study has certain limitations. The main limitation is the lack of information on recent antibiotic use, which may have reduced the prevalence of vaginal infections. Because we described a routine cohort, we assume this represents a normal population, including a proportion of women who have recently used antibiotics, which is common in the Ethiopian context. Another limitation is the disproportionate overrepresentation of urban women due to the location of the included health centres and possible misperception of the term 'urban' by participants from villages.

Although the vaginal lavage and STIP methods are validated, the combination has not been previously used in pregnant women. We were unable to compare this method with routine clinical tests for STIs in the Ethiopian setting. It is possible that due to technical issues, the lavage may not have reached the cervix as intended, potentially leading to an underestimation of cervical infections such as *C. trachomatis* and *N. gonorrhoeae*. Further studies should evaluate this combined method. Additionally, no data on pregnancy outcomes in this population could be obtained because of organisational limitations.

Conclusion

This study has demonstrated that many asymptomatic pregnant women aged 18 to 45 years have BV or curable STIs, putting them at risk for adverse pregnancy outcomes and secondary infertility. A relatively high proportion of women with potentially pathogenic infections was detected. In settings with poor medical coverage, preterm labour or infection of the unborn child can result in high morbidity or even mortality. Screening and treatment for BV and STIs may prevent these adverse outcomes.

As previous studies have shown, knowledge about STIs in Ethiopia is low [20]. Efforts should be made to educate young people about the transmission, symptoms, consequences and prevention of STIs. Adolescents should be educated at a younger age, preferably before sexual debut. Antenatal care presents a valuable opportunity to educate, screen and treat women for STIs and HPV and should be included in antenatal care protocols.

The self-sampling method is well accepted and could be suitable for low-resource countries. However, the multiplex methodology requires a well-equipped laboratory with expensive technology and specialised laboratory technicians.

Finding adequate screening methods and funding remains a challenge, but it is essential to protect pregnant women and their unborn children.

Conflicts of interest

The authors declare no conflict of interest.

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Ethical approval

Ethical clearance was obtained from Addis Ababa University, Ethiopia, in February 2011 (124/10/IM;032/2011) and April 2013 (050/2013), and from Martin Luther University Halle-Wittenberg, Germany, on 23 August 2010.

Informed consent

Verbal informed consent for publication was obtained from the patients, as suggested by the ethics committee due to low literacy rates and since a written consent is only provided concerning marriage or business contracts.

Authors' contributions

IR, JMAK, AA and EJK designed the research. JMAK, IR, DHoe, AA, SU, CT and EJK analysed the data. IR, JMAK, DHoe and EJK drafted the manuscript. JMAK, IR, AKP, TW, SFA, AA, MS, TW and DH provided the original samples, laboratory results, information on the respective populations and advice on study design, analysis and interpretation of the findings. All authors provided critical interpretation of the results and reviewed the first draft. All authors read and approved the final manuscript.

Availability of data and materials

Data are available on reasonable request and can be requested by email.

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Supplementary information

Supplementary Table 1. Inclusion and exclusion criteria.

Inclusion criteria	Exclusion criteria
• Women visiting the antenatal care/family planning units of the study centers	• Pregnancy-related complications: ◦ Placenta praevia ◦ Severe preeclampsia ◦ Vaginal bleeding ◦ Contractions ◦ Premature rupture of membranes
• Age 18–45 years	
• Able to understand the study	
• Consent given	
• Completion of the study procedures	• Multiple abortions in the past

Erklärungen

(1) Ich erkläre, dass ich mich an keiner anderen Hochschule einem Promotionsverfahren unterzogen bzw. eine Promotion begonnen habe.

(2) Ich erkläre, die Angaben wahrheitsgemäß gemacht und die wissenschaftliche Arbeit an keiner anderen wissenschaftlichen Einrichtung zur Erlangung eines akademischen Grades eingereicht zu haben.

(3) Ich erkläre an Eides statt, dass ich die Arbeit selbstständig und ohne fremde Hilfe verfasst habe. Alle Regeln der guten wissenschaftlichen Praxis wurden eingehalten; es wurden keine anderen als die von mir angegebenen Quellen und Hilfsmittel benutzt und die den benutzten Werken wörtlich oder inhaltlich entnommenen Stellen als solche kenntlich gemacht.

Datum, Unterschrift

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