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Detection of HPV mRNA in Self-collected Vaginal Samples Among Urban Ethiopian Women

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Abstract. *Background/Aim: Cervical cancer is the most common cancer among women in Ethiopia. The objective was to evaluate the participation rate of a free of charge vaginal self-sample (Aptima multitest swab, Hologic) for the detection of human papillomavirus (HPV) in an Ethiopian cohort. Patients and Methods: Specimens were collected from women employed by Ethiopian Airlines in Addis Abeba (N=5950). Samples were analysed for the presence of high-risk (HR) HPV mRNA by the Aptima HPV assay (Hologic) and HPV positive women were referred for cytology. Identification of HPV types among HPV positive samples was performed by Modified general primer-PCR and Luminex assay. Results: Participation rate was 3.1% and the prevalence of HPV mRNA was 20.6% (37/180). Conclusion: Primary HPV mRNA screening with vaginal self-sampling may be an acceptable approach in Ethiopia. One out of five women harbor HPV in their vaginal self-sample in agreement with other similar studies from the region.*

Human papillomavirus (HPV) is the main cause of cervical cancer (1). In Ethiopia, cervical cancer is considered the most common cancer among women (2), whereas in Europe it ranks as number seven (3) and worldwide as number four (4). The lower incidence in high income countries has been related to the implementation of nationwide screening programs for the detection of precancerous lesions (5).

There are about 40 HPV types which can infect the genital tract (6), but only 14 are considered high risk (HR) HPV types and are associated with almost all cases of severe cervical neoplasia and cancer (7). Some HPV types are referred to as low risk (LR) and cause mainly genital warts or manifest a low risk for the development of cervical cancer (8).

The HR HPV types, particularly 16 and 18, are responsible for 70% of all cervical cancers worldwide (9). Most HPV infections and pre-cancerous lesions are asymptomatic and are cleared spontaneously, however, a persisting infection can lead to cervical dysplasia and the development of cancer (1).

Ethiopia, a country with about 90 million inhabitants where approximately 29 million are women above 15 years (4). The majority of these women live in the countryside, and thus, a nationwide cytological cervical screening program would face severe organizational challenges in order to be implemented. The gold standard for cervical cancer screening in countries with a well-organized screening culture is cytological analysis (10), the Papanicolaou smear, which has been updated recently in many countries. It involves a primary HPV screening followed up by colposcopy in the case of abnormal cytology. However, in low- and middle-income countries, the recommendation from the World Health Organization (WHO) has been a "screen and treat" program, usually with visual inspection and acetic acid (VIA) (11). The WHO has revised the recommendation due to the importance of HPV detection. If an HPV assay is available, screening for HPV, followed-up by cytology or visual inspection, is the current recommendation in order to minimize over-treatment of lesions that are HPV negative (11). The introduction of HPV self-sampling has the potential to increase coverage in different settings (12). Furthermore, the approach of using self-sampling for HPV-analyses, means that each woman receives a self-sampling kit to be used in her own private setting, which should be both cheaper and reduce workload for health care workers involved in cervical screening.

The objective of this study was to evaluate the participation rate of a free of charge vaginal self-sample and determine the prevalence of HPV mRNA in an Ethiopian urban cohort.

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Key Words: HPV, self-sampling, Ethiopia, screening, cervical cancer.

Patients and Methods

The study commenced with an awareness event for cervical cancer that was open for all Ethiopian Airlines employees. One week prior to the event, invitations and information regarding the self-sampling study were sent out through newsletters to all employees. The women were invited to participate in the study as an alternative to booking an appointment with a gynecologist. The self-sampling test was free of charge and there was no financial compensation for the participants.

Women who had never been sexually active, who had had cervical cancer, or who had undergone total hysterectomies were excluded from the study. Eligible participants were women between 18-70 years old, with no gynecological symptoms.

They were given both oral and written information in Amharic, before signing the consent form. They were also asked to fill out a health questionnaire consisting of 14 questions.

Procedure. Each participant was given two Aptima Multitest Swabs and two tubes, each pre-filled with 2.9 ml Aptima Multitest Swap Transport Media (STM) (Hologic Inc, Marlborough, MA, USA). The test tubes were marked with a barcode number, which was linked to the participant's name, birthdate and telephone number.
A self-testing swab was shown to each participant and they were informed of how to insert the swab 2-3 cm into the vagina and turn it 360 degrees in the canal for 2-3 times, using drawings explained by a study nurse. The women were then instructed to transfer the swab into the test tube. The women performed the procedure in the private setting of a toilet located in the health office or at home. The test tubes were kept at room temperature and one test tube was transported to Sweden, where they were analysed at the Microbiology department at Region Skåne using HPV mRNA analysis (Aptima Hologic) within 2 months.

The HPV mRNA assay detects the mRNA sequence of the oncogenic proteins E6 and E7 from 14 HR-HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68). Before inserting the self-samples to the Panther instrument (Hologic) a preheating step at 90˚C for 1 h was performed in a heating chamber (Termaks AS. Bergen, Norway) (13).

The HPV mRNA positive samples were then further processed for identification of the HPV type by the MGP-PCR Luminex HPV DNA assay, which detects several HPV types simultaneously (9-11). Initially, sample DNA was purified by MagnaPure LC (Roche). Subsequently, HPV DNA was amplified by PCR with modified GP5+/6+ primers (MGP) (14). After amplification, the Luminex-based HPV genotyping enabled identification of the following 40 HPV types: 6, 11, 16, 18, 26, 30, 31, 33, 35, 39, 40, 42, 43, 45, 51, 52, 53, 54, 56, 58, 59, 61, 62, 66, 67, 68 (a and b), 69, 70, 73, 74, 81, 82, 83, 85, 86, 87, 89, 90, 91 and 114. The following HPV types were classified as HR types 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and probable HR type 68 (A and B) as well as possibly HR types 26, 30, 53, 66, 67, 70 69, 73 and 82 as described in the IARC classification of the year 2012 (15). In the present study, probable and possible HR-HPV types were classified as potential HR HPV types, and HPV6, 11, 40, 42, 43, 54, 61, 62, 74, 81, 83, 85, 86, 87, 90, 91 and 114 as LR types.

The Aptima HPV results were announced by sending out a general email to inform the women that the results were ready to collect. The participants with a negative Aptima HPV test result were informed that they did not need follow-up evaluation but that a regular screening every three years was recommended. The women with a positive Aptima HPV test result were asked to book an appointment for further evaluation by a gynecologist. Cervical specimen was then collected for liquid based cytology (Thin Prep). The cytological specimens were analyzed at the International clinical laboratories in Addis Abeba.

Ethical approval was obtained from Lund University EPN Dnr 2018/07, Armauer Hansen Research Institute protocol number P02/18, and the National Ethics committee in Ethiopia.

Results

All women (N=5950) from the Ethiopian Airlines were invited to participate in the cervical cancer screening information event. In total, approximately 400 women attended. In three days, a participation rate of 3.1% (183/5950) was observed. Two subjects (2/183) were excluded due to empty test tubes and one sample was rejected because of an invalid HPV assay result.

The self-reported characteristics of the women are presented in Table I.

The age varied from 18 to 59 years, with a median age of 35.

The HPV mRNA prevalence was 20.6% (37/180) (95 %CI=14.9-27.2%) (Table II).

A wide range of HPV types was detected in 92% (34/37) of the HPV-mRNA positive cases. Overall, 12.6% (23/183), 3.82% (7/183) and 2.18% (4/183) of the women harbored HR-HPV types, PHR-HPV types and LR-HPV types (without HR/PHR HPV types), respectively (Table III).

Among the 37 HPV-mRNA positive cases, 10 different HR HPV types were detected (Table III). Other than HR HPV types, we also identified HPV67 (2 samples) and HPV70 (one sample). Among six samples only LR HPV types were identified, including HPV42 (2 samples), HPV42 and HPV114 (1 sample), HPV81 (1 sample), HPV87 (1 sample) and HPV114 (1 sample) (Table III). Furthermore, one sample had both HPV81 and the PHR HPV53 (not detectable by Aptima assay) (Table III).

The Aptima HPV positive assay results were given over the phone and 97% (36/37) of HPV positive women were reached.

After six months and three reminders later, 69% (25/36) of the HPV positive women had underwent follow up examination with liquid based cytology. In this group, one presented with low squamous intraepithelial lesion (LSIL), one with atypical squamous cells of undetermined origin (ASCUS) and one with high grade squamous epithelial lesion (HSIL). These women were hereafter treated according to existing protocols at the health clinic as determined by the consulting gynecologists.

Discussion

Among the invited urban Ethiopian women, the participation rate was 3.1% for taking vaginal self-samples. Among these women, the Aptima assay demonstrated an HPV prevalence of 20.6% and 10 different HR HPV types were detected.

Table I. *Women characteristics*.

	Number of women	Percentage %
Have you ever been treated for a sexual transmitted disease?	24	13.7
Have you ever heard about cervical cancer screening?	112	63.2
Have you ever been screened for cervical cancer?	52	29.3
Married	129	72.9
Single	39	22.0
Divorced	6	3.3
Widow	2	1.1
More than 1 children	49	32.7
Tested for HIV	118	67.0
Self-reported HIV positive	1	0.1

Table II. *HPV prevalence within different age groups*.

Age categories	Number of women	HPV positive women	HPV positive %
Age: 19-24	20	7	35.0%
Age: 25-29	32	8	25.0%
Age: 30-35	43	5	11.6%
Age: 36-40	28	5	17.9%
Age: 41-50	37	7	18.9%
Age: 51-60	19	5	26.3%
Unreported	1	0	0.0%
Total	180	37	20.6%

Table III. *HPV type distribution among 34 vaginal self-collected samples from Ethiopia, originally HPV positive with Aptima HPV assay.*

HPV type	16	30	31	35	39	42	45	51	52	53	56	58	59	66	67	68A	68B	70	81	87	90	114
HR,PHR,LR*	HR	PHR	HR	HR	HR	LR	HR	HR	HR	PHR	HR	HR	HR	PHR	PHR	PHR	PHR	PHR	LR	LR	LR	LR
Total No	4a	1[i]	1	4[b]	4[b,e,f]	3[c]	1[d]	3[f]	3[c]	1[g]	3[e,h]	2[i]	2[d]	2[j]	2[k]	1[k]	1[l]	1	2[l]	1[g]	3[a,h,j]	2[c]

*Classification of HPV type as HR: High risk, PHR: Potential high risk, LR: Low risk HPV type. [a, b, c, d, e, g, h, I, j, k, l]Single sample, double HPV positive for marked HPV types. aFor example, one sample had both HPV16 and HPV90, whereas 3 samples had only HPV16. HPV68B is a subtype of HPV68A.

Among the Aptima HPV positive women, 69% attended follow-up examination, where one case manifested HSIL. Ethiopian Airlines is a company with over 16,000 employees worldwide. The company has an interest in preventive medicine and has women employed in different age groups and at various educational levels.

A limitation of our study was the short period of three days that the self-sampling option was available, which may have caused the low participation rate (3.1%), when all women employed at Ethiopian airlines are taken into account. Another limitation was that the women had to be off duty to participate, which for a majority could have been difficult due to their occupations such as pilots and hostesses, whom can be on duty for a couple of days at a time. It was a shortcoming that information regarding occupation was not included in the questionnaire.

Noteworthy is that the participation for cervical cancer screening was low before this study commenced; only 132 cervical samples had been collected during the last three years, despite the opportunistic screening that was offered to the employees. In comparison, the offered method of vaginal self-sampling seems to have had a greater acceptability amongst the women at Ethiopian Airlines. Furthermore, self-sampling, in comparison to VIA as the primary screening method, has been shown to improve participation as demonstrated in a recent Ethiopian study (16).

This is the first study in Ethiopia to use HR HPV mRNA analysis. The HR HPV mRNA prevalence (20.6%) is in agreement with other self-sample studies from East Africa (17, 18). However, the HPV mRNA prevalence in this Ethiopian Airline cohort represents a city population and further studies in the other parts of Ethiopia need to be performed to explore the prevalence of HPV mRNA in the general Ethiopian population. Nevertheless, self-sampling and detection of HPV mRNA could function as a primary screening method with a "triage and treat" option for women that manifest lesions after VIA inspection. The triage system based on VIA would also allow for cervical collection of HPVs, which could be the basis for a national population-based study on the HPV genotypes present in Ethiopia.

Interestingly, the sensitivity of the HPV mRNA analysis regarding self-samples is a subject for discussion, as most studies are performed based on the use of clinically acquired cervical samples. One meta-analysis, with inclusion of only one APTIMA mRNA self-sample study concluded that the sensitivity of detecting HSIL was generally lower than clinical cervical based samples (19). However, another study demonstrated a sensitivity of 85.5% (95%CI=75.0-92.8) for the detection of HSIL with the self-sampling method (20), which was increased to 95% by the use of a preheating step that was also performed in our study (13).

In a recent cross-sectional study of cervical samples from Addis Abeba involving 366 participants, the majority of HR HPV types were not 16 and 18 (21). In our study of self-samples, we observed an absence of HPV18, and a low frequency of HPV16, which was similar to other HR HPV types.

Concerning detection of 14 HR HPV types as specified by the Aptima assay, we observed additional HPV types such as HPV67 and HPV70, which is most likely attributed to known cross-reactions of the Aptima assay (Kit insert, APTIMA HPV Assay, nr 503744). In addition, in six samples only LR HPV types such as HPV42, 81, 87 and 114 (and one sample had both HPV81 and the PHR HPV53, not detectable by Aptima assay) were detected in six samples only. These findings suggest that HPV42, 81, 87, and 114 types could cross react with the HPV assay, perhaps if present with high viral loads. In accordance, we recently detected the LR HPV42 and HPV90 in an Aptima positive cervical sample (13). Moreover, for three of the Aptima positive samples no HPV DNA was type identified (one had HSIL at follow-up), which may be due to a lower analytical sensitivity of the typing method compared to the Aptima assay (22).

The relatively low follow-up rate (69%) in our study may be attributed to two major factors. The women were themselves responsible for booking their appointment with the gynecologist and the follow-up did not occur in conjunction with the delivery of the HPV mRNA result. Furthermore, it has been reported that logistics and fear are barriers to follow-up (23). However, women need to know that there is a system that takes care of them in a consistent way when they are HPV positive by the use of self-sampling (23). The fact that all women could be easily reached because they are employees of Ethiopian Airlines, should be ideal for a follow-up system based on cytology. However, this study showed that even in this setting, the follow-up of women was incomplete. There is also a lack of capacity for cytological analyses and the cytology quality may vary, which will delay proper treatment. In Ethiopia, cervical cytology laboratories exist at the main University hospital, Black Lion, and at a few private institutions. Hence, to rely on cervical cytology as a triage system for nationwide implementation becomes cumbersome since the requested human resources and infrastructure are lacking. Furthermore, in this study, the cervical cytology results were secondarily reviewed by a senior pathologist, which led to one LSIL turning into HSIL and two LSILs changing into normal.

Conclusion

In conclusion, the study results indicate that self-sampling may be acceptable as a primary screening method for Ethiopian women and that one out of five women harbor HPV in their vaginal self-sample, in agreement with other self-sample studies from this region.

Conflicts of Interest

Hologic Inc. supplied the vaginal swabs used for the study. They did not have any influence on the study design, statistical analyses or manuscript writing.

Authors' Contributions

SM, MJ, OF, CB designed the study. SM and SF gathered the data. SM performed the statistical analysis. SM, MJ, OF, CB wrote the manuscript. SM, MJ, OF, CB and SF reviewed data and provided critical input during manuscript writing.

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References

1 Facts sheet: Details: Human papillomavirus (HPV) and cervical cancer. World Health, 2019. organisation. Available at: https://www.who.int/news-room/fact-sheets/detail/human-papillomavirus-(HPV)-and-cervical-cancer [Last accessed on February 11, 2020]

2 Schonfeld A, Feldt T, Tufa TB, Orth HM, Fuchs A, Mesfun MG, Pfafflin F, Nordmann T, Breuer M, Hampl M and Haussinger D: Prevalence and impact of sexually transmitted infections in pregnant women in central Ethiopia. Int J STD AIDS *29(3)*: 251-258, 2018. PMID: 28776463. DOI: 10.1177/0956462417723545
3 Ferlay J, Steliarova-Foucher E, Lortet-Tieulent J, Rosso S, Coebergh JW, Comber H, Forman D and Bray F: Cancer incidence and mortality patterns in Europe: Estimates for 40 countries in 2012. Eur J Cancer *49(6)*: 1374-1403, 2013. PMID: 23485231. DOI: 10.1016/j.ejca.2012.12.027
4 Bruni L, Alberto G, Serrano B, Mena M, Gómez D, Muñoz J, Bosch FX and de Sanjosé S: Human papillomavirus and related diseases in Ethiopia. Summary report. ICO Information Centre on HPV and Cancer (HPV Information Centre), 2019. Available at: https://www.HPVcentre.net/statistics/reports/ETH.pdf [Last accessed on February 11, 2020]
5 Arbyn M, Ronco G, Anttila A, Meijer CJ, Poljak M, Ogilvie G, Koliopoulos G, Naucler P, Sankaranarayanan R and Peto J: Evidence regarding human papillomavirus testing in secondary prevention of cervical cancer. Vaccine *30(Suppl 5)*: F88-99, 2012. PMID: 23199969. DOI: 10.1016/j.vaccine.2012.06.095
6 de Villiers EM, Fauquet C, Broker TR, Bernard HU and zur Hausen H: Classification of papillomaviruses. Virology *324(1)*: 17-27, 2004. PMID: 15183049. DOI: 10.1016/j.virol.2004.03.033
7 IARC Working Group on the Evaluation of Carcinogenic Risks to Humans: Biological agents. Volume 100 b. A review of human carcinogens. IARC Monogr Eval Carcinog Risks Hum *100(Pt B)*: 1-441, 2012. PMID: 23189750.
8 Lowy DR, Solomon D, Hildesheim A, Schiller JT and Schiffman M: Human papillomavirus infection and the primary and secondary prevention of cervical cancer. Cancer *113(7 Suppl)*: 1980-1993, 2008. PMID: 18798536. DOI: 10.1002/cncr.23704
9 World Health Organization: Guidelines for screening and treatment of precancerous lesions for cervical cancer prevention, 2013. Available at: https://apps.who.int/iris/bitstream/handle/10665/94830/9789241548694_eng.pdf;jsessionid=D78F5D939BFA85408E883B2189D878C5?sequence=1 [Last accessed on February 11, 2020]
10 Boone JD, Erickson BK and Huh WK: New insights into cervical cancer screening. J Gynecol Oncol *23(4)*: 282-287, 2012. PMID: 23094132. DOI: 10.3802/jgo.2012.23.4.282
11 De Vuyst H, Alemany L, Lacey C, Chibwesha CJ, Sahasrabuddhe V, Banura C, Denny L and Parham GP: The burden of human papillomavirus infections and related diseases in sub-Saharan Africa. Vaccine *31*: F32-46, 2013. PMID: 2433174. DOI: 10.1016/j.vaccine.2012.07.092
12 Yeh PT, Kennedy CE, de Vuyst H and Narasimhan M: Self-sampling for human papillomavirus (HPV) testing: A systematic review and meta-analysis. BMJ Glob Health *4(3)*: e001351, 2019. PMID: 31179035. DOI: 10.1136/bmjgh-2018-001351
13 Borgfeldt C and Forslund O: Increased HPV detection by the use of a pre-heating step on vaginal self-samples analysed by aptima HPV assay. J Virol Methods *270*: 18-20, 2019. PMID: 31002831. DOI: 10.1016/j.jviromet.2019.04.015
14 Soderlund-Strand A, Carlson J and Dillner J: Modified general primer pcr system for sensitive detection of multiple types of oncogenic human papillomavirus. J Clin Microbiol *47(3)*: 541-546, 2009. PMID: 1914481. DOI: 10.1128/JCM.02007-08
15 Muñoz N, Bosch FX, de Sanjosé S, Herrero R, Castellsagué X, Shah KV, Snijders PJ, Meijer CJ and International Agency for Research on Cancer Multicenter Cervical Cancer Study Group: Epidemiologic classification of human papillomavirus types associated with cervical cancer. N Engl J Med *348(6)*: 518-527, 2003. PMID: 12571259. DOI: 10.1056/NEJMoa021641
16 Gizaw M, Teka B, Ruddies F, Abebe T, Kaufmann AM, Worku A, Wienke A, Jemal A, Addissie A and Kantelhardt EJ: Uptake of cervical cancer screening in Ethiopia by self-sampling HPV DNA compared to visual inspection with acetic acid: A cluster randomized trial. Cancer Prev Res (Phila) *12(9)*: 609-616, 2019. PMID: 31337647. DOI: 10.1158/1940-6207.CAPR-19-0156
17 Haile EL, Cindy S, Ina B, Belay G, Jean-Pierre VG, Sharon R, Lisbeth LR and Paul BJ: HPV testing on vaginal/cervical nurse-assisted self-samples *versus* clinician-taken specimens and the HPV prevalence, in Adama town, Ethiopia. Medicine (Baltimore) *98(35)*: e16970, 2019. PMID: 31464941. DOI: 10.1097/MD.0000000000016970
18 Nakalembe M, Makanga P, Mubiru F, Swanson M, Martin J and Huchko M: Prevalence, correlates, and predictive value of high-risk human papillomavirus mRNA detection in a community-based cervical cancer screening program in western Uganda. Infect Agent Cancer *14*: 14, 2019. PMID: 31114629. DOI: 10.1186/s13027-019-0230-0
19 Arbyn M, Verdoodt F, Snijders PJ, Verhoef VM, Suonio E, Dillner L, Minozzi S, Bellisario C, Banzi R, Zhao FH, Hillemanns P and Anttila A: Accuracy of human papillomavirus testing on self-collected *versus* clinician-collected samples: A meta-analysis. Lancet Oncol *15(2)*: 172-183, 2014. PMID: 24433684. DOI: 10.1016/S1470-2045(13)70570-9
20 Asciutto KC, Ernstson A, Forslund O and Borgfeldt C: Self-sampling with HPV mRNA analyses from vagina and urine compared with cervical samples. J Clin Virol *101*: 69-73, 2018. PMID: 29433016. DOI: 10.1016/j.jcv.2018.02.002
21 Ali KE, Mohammed IA, Difabachew MN, Demeke DS, Haile T, Ten Hove RJ, Kumssa TH, Woldu ZL, Haile EL and Tullu KD: Burden and genotype distribution of high-risk human papillomavirus infection and cervical cytology abnormalities at selected obstetrics and gynecology clinics of Addis Ababa, Ethiopia. BMC Cancer *19(1)*: 768, 2019. PMID: 31382907. DOI: 10.1186/s12885-019-5953-1
22 Haedicke J and Iftner T: A review of the clinical performance of the aptima HPV assay. J Clin Virol *76(Suppl 1)*: S40-S48, 2016. PMID: 6614686. DOI: 10.1016/j.jcv.2015.10.027
23 Brandt T, Wubneh SB, Handebo S, Debalkie G, Ayanaw Y, Alemu K, Jede F, von Knebel Doeberitz M and Bussmann H: Genital self-sampling for HPV-based cervical cancer screening: A qualitative study of preferences and barriers in rural Ethiopia. BMC Public Health *19(1)*: 1026, 2019. PMID: 31366402. DOI: 10.1186/s12889-019-7354-4

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