ADDIS ABABA UNIVERSITY COLLEGE OF HEALTH SCIENCES, SCHOOL OF ALLIED HEALTH SCIENCE, DEPARTMENT OF MEDICAL LABORATORY SCIENCE

Prevalence and Genotype distribution of High Risk Human Papilloma Virus and Cervical cytology abnormalities at Selected Obstetrics and Gynecology Clinics, in Addis Ababa, Ethiopia

By:

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A RESEARCH THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES, SCHOOL OF ALLIED HEALTH SCIENCES, COLLEGE OF HEALTH SCIENCES, ADDIS ABABAUNIVERSITY IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF MASTER OF SCIENCE IN CLINICAL LABORATORY SCIENCE (DIAGNOSTIC AND PUBLIC HEALTH MICROBIOLOGY)

October 2015

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Full Title of the Project	Prevalence and Genotype Distribution of High Risk Human Papilloma Virus and Cervical cytology abnormalities at Selected Obstetrics and Gynecology Clinics, Addis Ababa, Ethiopia
Duration of the project	20th March to 15th October 2015
Study Area	Hemen Maternal and Child Hospital, Senamokshe Women Health Center, Family Guidance Reproductive health clinic
Total cost of the project	**201,816.63Birr**
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Acknowledgment

First, and foremost I would like to thank the almighty God. My heart full gratitude will go to Dr. Ibrahim Ali, Assistant professor, Dean of the School of Allied Health Sciences, Addis Ababa University and academic advisor, Mr. Kassu Desta, Assistant professor at department of Medical Laboratory science, Addis Ababa University, Dr. Mesfin Nigussie, Medical Director of International Clinical Laboratories, Addis Ababa, main advisors and Dr. Tasew Haile; Director of Senamokshe Women Health Center. Dr. Dawit Solomon; International Clinical Laboratories; Pathologist Dr. Zufan Lakew; Director of Hemen Maternal and Child Health Hospital, Dr. Robert Ten Hove, Molecular Lab Advisor, international clinical laboratories, Mr. Tsegaye Hailu, Statistician, AHRI collaborative advisors of this project for their very helpful technical advices and unreserved support and follow-up of this research project work. I would also like to give my acknowledgment to Addis Ababa University College of Health Science, School of allied Health Sciences, Department of Medical Laboratory Science. My deepest gratitude to Abbott Molecular diagnostics especially Mr. Daan J Potgieter, for sponsoring the HR HPV reagents and kits, International Clinical Laboratories in particular Mr. Tamrat Bekele and Mr. Wudneh Yitayew for the facilitation of reagents donation from Abbott molecular diagnostics, Pap kits and reagents support, and permission for the molecular and cytology laboratory facility to conduct this research and made the project work in reality. My sincere gratitude will also extend to Mr. Tadele Getachew, Mrs. Azeb Adamu, Mr. Giorgis Okabegzi, Mrs. Ruth Getachew, Mr. Melaku Tesfaye for their generous technical and coordination support of the entire laboratory work. All the study participants deserve special thanks for their very kind willingness and participation in this study. At last, all selected health facilities management and data / sample collectors and coordinators (Sr. Hamelmal, Sr. Husniya, Sr. Shibre, and Sr. Etetu) for their dedication, commitment, and willingness throughout this project work.

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Definition of Terms

Cervical Intraepithelial Neoplasia (CIN) / Squamous Intraepithelial Lesions (SIL)

SIL and CIN are two commonly used terms to describe precancerous lesions or the abnormal growth of squamous cells observed in the cervix. SIL is an abnormal result derived from cervical cytological screening or Pap smear testing. CIN is a histological diagnosis made upon analysis of cervical tissue obtained by biopsy or surgical excision. The condition is graded as CIN 1, 2 or 3, according to the thickness of the abnormal epithelium (1/3, 2/3 or the entire thickness).

Low-grade cervical lesions (LSIL/CIN-1)

Low-grade cervical lesions are defined by early changes in size, shape, and number of abnormal cells formed on the surface of the cervix and may be referred to as mild dysplasia, LSIL, or CIN1.

High-grade cervical lesions (HSIL/ CIN-2 / CIN-3 / CIS)

High-grade cervical lesions are defined by a large number of precancerous cells on the surface of the cervix that are distinctly different from normal cells. They have the potential to become cancerous cells and invade deeper tissues of the cervix. These lesions may be referred to as moderate or severe dysplasia, HSIL, CIN-2, CIN-3 or cervical carcinoma in situ (CIS).

Invasive cervical cancer (ICC) / cervical cancer

If the high-grade precancerous cells invade the basement membrane is called ICC. ICC stages range from stage I (cancer is in the cervix or uterus only) to stage IV (the cancer has spread to distant organs, such as the liver).

Invasive squamous cell carcinoma

Invasive carcinoma composed of cells resembling those of squamous epithelium.

Adenocarcinoma

Invasive tumor with glandular and squamous elements intermingled.

HR HPV Prevalence

HR HPV prevalence is the proportion of subjects infected by the high-risk human papillomavirus (HPV) according to an HR HPV DNA test at a specific time point.

Type-specific HPV prevalence

HPV-type prevalence is the proportion of subjects infected by a specific HPV genotype according to a type-specific HPV DNA test at a given time point.

Parity

is the number of times a woman has given birth. High parity has been associated with an increased risk of invasive cervical cancer.

List of abbreviations

ASCUS	Atypical Squamous Cells of Undetermined Significance
ASC-H	Atypical Squamous Cells- cannot exclude HSIL
CIN	Cervical Intraepithelial Neoplasia
CKC	Cold knife conization
DNA	Deoxyribonucleic Acid
FDA	Federal Drug Administration and Control Authority
GP	General Primer
HIV	Human Immunodeficiency virus
HLA	Human Leukocyte Antigen
HPV	Human Papilloma virus
HR HPV	High-Risk Human Papilloma virus
HSIL	High Grade Squamous Intraepithelial Lesion
HSV	Herpes Simplex virus
IARC	International Agency for Research on Cancer
ICC	Invasive Cervical Cancer
LCR	Long control Region
LEEP	Loop Electrical Excision Procedure
LLETZ	Large Loop Excision of the Transformation Zone
LSIL	Low-grade Squamous Intraepithelial Lesion
NILM	Negative for Intraepithelial Lesion or Malignancy
OC	Oral Contraceptive
PAP	Papanicolau
PCR	Polymerase Chain Reaction
SCC	Squamous Cell Carcinoma
SSA	Sub-Saharan Africa
STD	Sexually Transmitted Disease
VIA	Visual Inspection with Acetic acid
WHO	World Health Organization

ABSTRACT

Background: Cervical cancer is a preventable disease affecting an estimated 530,000 women each year and leading to nearly 275,000 deaths. Human papillomavirus (HPV) has been recognized as an important cause of cervical cancer and it is implicated in 99.7% of cervical squamous cell cancer cases in the world. It is recognized as the third most common type of cancer in women worldwide and the second most prevalent cancer type and cause of cancer-related mortality in women in developing countries. In Ethiopia, every year 7095 women diagnosed with cervical cancer and 4732 die from the disease. Very low screening practice and inadequate screening coverage in the country makes cervical cancer as one of the major public health concern in Ethiopia. There is also insufficient data on the prevalence of HR-HPV and cervical cytology abnormalities as a nationwide basis.

Objective: To assess the prevalence and genotype distribution of High Risk Human Papilloma Virus and Cervical Cytology abnormalities among women attending selected Obstetrics and Gynecology clinics, of Addis Ababa, Ethiopia

Method: Institutional based cross sectional study design was used in three selected Obstetrics and Gynecology Clinics, Addis Ababa; from 15th June to 10th October 2015. Cervical samples were collected from the os of the cervix using Abbott cervi-cyt collection material for HR HPV DNA and cyto-brush for Pap smear screening. A Structured Interview based questionnaire was administered to assess the associated risk factors. A total of 366 participants were enrolled based on the set inclusion criteria. High Risk HPV DNA was analyzed using Abbott Real Time PCR and cervical cytology screening using conventional Pap smear techniques. Data entry was performed using Epi-data version 3.1 and data analysis was performed by using STATA version 11.0.

Result: The overall HR HPV prevalence was 13.7% (50/366), with 76% (38/50) of Other HR HPV genotypes. Abnormal cytology was observed in 13.1% (48/366) with 81.3%, 12.5%, and 6.3%, are LSIL, ASCUS and HSIL respectively. HR HPV DNA PCR and Conventional Pap smear cytology screening methods showed overall agreement of 78% with kappa value of 0.12, 95% CI (0.00-0.243).

Conclusion and Recommendation

In this study, Non-16/18 genotypes contributed the largest proportion of the overall HR HPV. The highest frequency of HR HPV positives was women without cervical cytology abnormality. The HR HPV with Pap smear co-screening in women whose age is >30 shall be in place. Further evaluation between the two screening methods against a perfect reference method shall be performed.

Key Words

High Risk Human Papilloma Virus, Cervical Cytology, Obstetrics and Gynecology, Prevalence, Genotype distribution, PCR, LSIL, ASCUS, HSIL

CHAPTER ONE

1.0 INTRODUCTION

The World Health Organization estimates that yearly, about 530,000 women worldwide are identified with cervical cancer and 275,000 women die from the disease. Cervical cancer is heralded as being the third most common cause of cancer among women in the world and the second most common form of cancer in women in the developing world. Cervical cancer is responsible for the largest cause of mortality in women due to cancer in most developing countries (1).

Cervical cancer is the second most common cancer among women in the developing world, and the largest cancer killer among women in most developing countries. Each year, over 500,000 women develop cervical cancer and about 275,000 women die from the disease. The vast majority of these unnecessary deaths occur in developing countries, or in disadvantaged communities within wealthy countries (2).

Cervical cancer has been recognized as a rare outcome of a common, sexually transmitted infection whose etiologic association is restricted to a few human papillomavirus (HPV) types. The association between HPV and cervical cancer is the universal fact and the variability among the different types is geographically limited. With optimal testing systems HPV DNA can be identified in nearly all specimens of invasive cervical cancer, and it is claimed that infection of the cervix with HPV is a necessary cause of cervical cancer. (3).

Historically, in early 1980s cervical cells were known to contain HPV DNA. Women infected persistently with High Risk oncogenic HPV types are more likely to develop cervical neoplasia due to ineffective cell-mediated immunity to eliminate HPV infected cells. High grade abnormalities are usually at risk of progressing to cervical cancer being as pre-cursors. Most HPV infections are asymptomatic and may not show clinical pictures earlier (4).

VIROLOGY OF HPV

The classification in virology for HPV is, they are members of the Papillomaviridae family, non-enveloped, small, and circular, double stranded DNA viruses measuring about 55nm. It has a double chain and contains approximately 8000 base pairs. Its genome has three major areas; the long control region (LCR), the early region (E=early), and the late region (L=late) (5).

The HPV virus has 8 overlapping reading frames, or genes, categorized as either early or late, depending on when they are expressed. Early genes, E1 and E2, participate in genome replication and transcription while the E4 gene promotes the productive phase of the viral life cycle (29). The oncoproteins, E6 and E7, form complexes with tumor suppressors, and thus can lead to host cell transformation and progression to cervical cancer. The L1 and L2 late genes encode viral capsid proteins. Most HPV assays target the L1 region for classification of HPV genotypes, although the E1 gene is also used. The discovery of DNA viruses as a major factor in causing most of the cervical squamous cancers has led to new hopes to use a virology test for cervical screening of precancerous lesions and to use an HPV vaccine for cancer prevention (6, 7, and 8).

Over 200 types of HPV are predicted to exist, including 30 types that are sexually transmitted and result in cervical infections. Based on the frequency of detection in cervical cancer, HPV genotypes are sub-divided into high-risk HPV types (16, 18, 31 and 45), intermediate-risk types (33, 35, 39, 51, 52, 56, 58, 59, and 68) and low-risk types (6, 11, 42 and 44) (9).

More than 40 HPV genotypes have been detected in the anogenital mucosa and are usually transmitted through sexual activity. The HPV genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and 68 are associated with cervical cancer and have been classified as High Risk (HR) group (10). The major steps in cervical carcinogenesis include infection of the metaplastic epithelium of the cervical transformation zone with one or more of the 12–18 carcinogenic types of human papillomavirus (HPV) infection, viral persistence, clonal progression of the persistently infected epithelium to cervical pre-cancer, and invasion. (11).

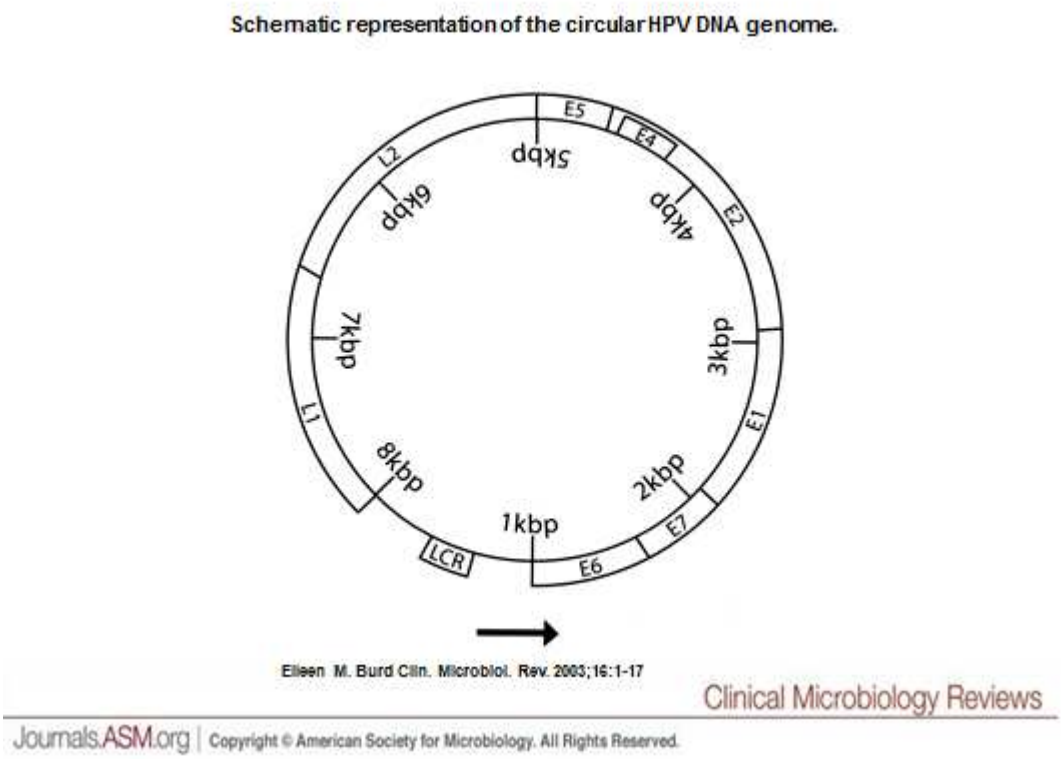


Figure 1.1: Shematic representation of the circular HPV genome

DIAGNOSTIC AND SCREENING METHODS

Since persistent infection with high-risk HPV types can result in cervical intraepithelial neoplasia (CIN) of different grades and invasive cancer within several years, and progression to cancer can be prevented by early detection of abnormalities and subsequent treatment, it is important to establish cost effective, sensitive, and accurate cervical screening protocols within routine clinical practice (8).

At the present time, detection of persistent high-risk HPV infection and cytologic monitoring of the precancerous changes, if present, are the two screening tests which the practitioners rely on for patient management in cervical cancer prevention. The U.S. FDA has approved using a human papillomavirus test for primary cervical cancer screening for women of 25 years or older (9).

The 2012 updated consensus guideline for management of abnormal cervical cancer screening tests describes that Human papillomavirus-negative atypical squamous cells of undetermined significance (ASCUS) results are followed with co-testing at 3 years before return to routine screening and are not sufficient for exiting women from screening at age

65 years. Women aged 21-24 years need less invasive management, especially for minor abnormalities; post colposcopy management strategies incorporate co-testing; endocervical sampling reported as CIN 1 should be managed as CIN 1; unsatisfactory cytology should be repeated in most circumstances, even when HPV results from co-testing are known, while most cases of negative cytology with absent or insufficient endocervical cells or transformation zone component can be managed without intensive follow-up (12).

Cervical intraepithelial neoplasia (CIN) is a premalignant lesion that may exist at any one of three stages: CIN1, CIN2, or CIN3. If left untreated, CIN2 or CIN3 (collectively referred to as CIN2+) can progress to cervical cancer. Instead of screening and diagnosis by the standard sequence of cytology, colposcopy, biopsy, and histological confirmation of CIN, an alternative method is to use a 'screen-and-treat' approach in which the treatment decision is based on a screening test and treatment or, ideally, immediately after a positive screening test. Available screening tests include a human papillomavirus (HPV) test, visual inspection with acetic acid (VIA), and cytology (Pap test). Available treatments include cryotherapy, large loop excision of the transformation zone (LEEP/LLETZ), and cold knife conization (CKC) (13).

The major diagnostic techniques for HPV detection and genotyping are target amplification, signal amplification, and probe amplification. Target amplification duplicates fragments of DNA from a targeted gene sequence. The most well-known example of this is polymerase chain reaction (PCR). Signal amplification uses branched DNA technology or hybrid capture to increase the DNA-proportional signal to detectable levels. Probe amplification includes technologies such as ligase chain reaction, which amplify the probe (6).

Polymerase chain reaction (PCR) is the most commonly used tool in the detection of HPV DNA. In theory, PCR can take a single double-stranded piece of DNA and amplify it to 1billion copies after 30 cycles. Typically, PCR procedures for HPV detection use primers targeted to the viral capsid L1 gene, which can detect numerous HPV types. Commonly used L1 consensus primer sets include PGMY09/11, GP5+/6+, and SPF10, along with a number of proprietary primers having the ability to identify a large range of HPV types with 1 amplification. Real-time PCR is highly sensitive target amplification technique available for HPV-DNA detection. Real-time PCR combines fluorescent

probes with PCR primers, allowing for accurate quantification of virus present in a sample. Viral load estimation of HPV is a particular advantage of real-time PCR, using the nuclear genome to control for cellular content of the sample (6, 7).

Papanicolau (or Pap) smear is a cervical screening test used to detect premalignant and malignant (cancerous) processes in the ectocervix. A medical professional uses a swab or stick to wipe cells off from the cervix, the opening lining of the womb (uterus) and these cells are then evaluated to determine presence or absence of abnormalities. Screening is not undertaken to diagnose cervical cancer disease, but to identify individuals with a high probability of having or developing cervical cancer (by detecting precancerous changes in the cervix uteri, which untreated, may lead to cancer). Histological diagnosis is the "gold standard" for identifying precancerous and cancerous lesions (14).

There are two forms of Pap smears, conventional and liquid-based cytology. In the conventional method cells are obtained from the neck of the cervix and then the cells are spread on a glass slide. In the liquid-based cytology method, the cells are obtained from the neck of the cervix, but instead of being spread on a glass slide, they are placed in a small glass vial that contains preserving fluid. At the present time HPV DNA testing has the highest sensitivity, which can additionally be used with Pap smears (co-testing) for optimizing diagnosis of high grade cervical intraepithelial neoplasia. (15).

Due to the higher sensitivity of HPV testing compared to conventional cytology, the rate of colposcopy referral with HPV testing alone is higher than the rate with conventional cytology (16).

RISK FACTORS FOR CERVICAL CANCER

There are multiple risk factors that have been connected with the acquisition of HPV infection and cervical cancer. This prominent risk increases with higher number of sexual partners of a woman or her partner. Cofactors that modify the risk for HPV DNA-positive women include the use of oral contraceptives (OCs) for 5 or more years, high parity (5 or more full-term pregnancies), and previous exposure to other sexually transmitted diseases such as *Chlamydia trachomatis* and herpes simplex virus type 2 (HSV-2). Women exposed to the human immunodeficiency virus (HIV) are at high risk for HPV infection, HPV DNA persistence, and progression of HPV lesions to cervical cancer (3, 17).

In addition to those listed above, other sexual and reproductive risk factors associated with HPV infection and cervical cancer include: initiation of sexual activity at an early age (= 18 years), earlier age at first full-term pregnancy (< 18 years), the use of tobacco, both current and past, increases the risk of squamous cell cervical carcinoma, and the risk rises with quantity of cigarettes smoked per day and number of years smoked. Infection with HIV is strongly associated with incidence and persistence of HPV infection, and advancement to invasive cervical cancer from squamous intraepithelial lesions. The vast majority of women infected with human immunodeficiency virus (HIV) will be co-infected with human papillomavirus (HPV). The interaction between the two sexually transmitted infections appears to be related to the alteration in cell-mediated immunity in HIV infected persons, increased susceptibility, and possibly reactivation of latent HPV infection (18, 19, and 20).

1.1 STATEMENT OF THE PROBLEM

In the year 2012, estimates of the worldwide number of cervical cancer cases and deaths accounted to 528 000 new cases every year, cervical cancer is the fourth most common cancer affecting women worldwide, after breast, colorectal, and lung cancers; it is most notable in the lower resource countries of sub-Saharan Africa. It is also the fourth most common cause of cancer death (266,000 deaths in 2012) in women worldwide. Almost 70% of the global burden falls in the developing countries (21).

Despite the scarcity of high quality cancer registries and lack of reliable mortality data, it is clear that human papillomavirus (HPV)-associated diseases, particularly cervical cancer, are major causes of morbidity and mortality in sub-Saharan Africa (SSA). Cervical cancer incidence rates in SSA are the highest in the world and the disease is the most common cause of cancer death among women in the region. The high incidence of cervical cancer is a consequence of the inability of most countries to either initiate or sustain cervical cancer prevention services. Cervical cancer in 2012 estimated age standardized incidence and mortality rates were 26.4 and 18.4 per 100,000 Ethiopian women, respectively, corresponding to a 4- and 9-times higher incidence and mortality rate than in Western Europe (22).

In Ethiopia, women ages 15 years and older who are at risk of developing cervical cancer are around 27.9 million. Current estimates indicate that every year 7095 women are diagnosed with cervical cancer and 4732 die from the disease. Cervical cancer ranks as the second most frequent cancer among women and the second most frequent cancer among women between 15 and 44 years of age in Ethiopia. The screening practice is underdeveloped, since, only 0.6% among the total women population whose age is 18-69 years screened every 3 years, and 1.6% urban women and 0.4% rural women. In Tikur Anbessa Specialized Hospital, the cancer referral facility in Ethiopia, of all cancer types diagnosed around 30.3% accounted for cervical cancer. Sufficient data is not available on the HPV burden in the general population of Ethiopia. However, in Eastern Africa, about 35.8% of women in the general population are estimated to harbor cervical HPV infection at a given time and 76.5% of invasive cervical cancers are attributed to HPVs 16 or 18 (23,24).

Apart from the direct health impact cervical cancer also creates an immense economic burden on patients. Outpatient treatment cost was found to be dependent on patient residence distance from the hospital, number of employed household members, number of facility visited and occupation.

Longer duration of inpatient hospital stay and existence of co-morbidity were associated with higher inpatient cost (25).

There is lack of awareness and weak health seeking behavior for cervical cancer in Ethiopia due to misconceptions about the cause of the disease. Profound social consequences and exclusion are also common. Access to services for diagnosis and treatment were poor for a variety of psycho-social, and health system reasons (26).

However, many developing countries that tried cytology-based cervical cancer screening programs did not get such success in terms of reducing the mortality and incidence of cervical cancer. Low coverage of cervical cancer screening is a serious problem and a major barrier in reducing the mortality and morbidity in the developing countries. Specifically in Sub-Saharan Africa very few women are ever screened for cervical cancer. In Ethiopia coverage of screening for cervical cancer is very poor (27).

However, previous studies conducted in Ethiopia; Gurage Zone, Attat Hospital, Jimma Zone, and Tikur Anbessa Specialized Hospital, Addis Ababa (41, 42, 43, and 44) which are incorporated in the literature review part; each of the studies has their own limitations described briefly by the investigators. For instance the study conducted in Gurage Zone showed that participants were recruited from a single setting of Attat Hospital and morbidity might be higher than in the general population, and in addition to this the study had no cytological evaluation that would have been allowed further sample stratification. The other study which was conducted in Tikur Anbessa Hospital, Addis Ababa, shows that inability to detect presence of multiple HPV infection; Simple cotton tipped wooden applicator stick was used to take the cervical samples which reduced the quality of the sample that might also miss the right anatomical site; the transformation zone. The one which was conducted in Jimma zone also uses participants with known cervical dysplasia but in our case they are not predefined to be cervical dysplasia to be included in the study group. Therefore, this study has considered the previous studies limitations and scarcity of public health information on HR HPV and cervical cytology abnormalities.

1.2 SIGNIFICANCE OF THE STUDY

Although cervical cancer pause significant and devastating public health burden in developing countries in particular sub-Saharan African Countries like Ethiopia, little is known about the prevalence of High Risk HPV, the associated risk factors, and the screening methods. In Ethiopia, nationwide cervical cancer screening is rarely established and HPV vaccination programs expected to be an alternative and cost-effective option in these settings. However, there is great heterogeneity with regard to the country-specific HPV prevalence, type-specific distribution and associated risk factors which must be considered prior to introducing HPV vaccination. And this study will try to answer these questions. In addition to this, in the long past Pap smear was the only available screening method having less sensitivity and inaccessible to many of the health facilities. In contrast, HPV-DNA is known to be more sensitive supposed to identify High Risk Genotypes which are mainly responsible for pre-cancerous cervical lesion and cervical cancer development. Nonetheless, these screening methods are not still sufficiently supported by evaluation studies in Ethiopia. Taking all the above facts in to consideration, this study has produced substantial inputs and supplementary and very relevant figures for HR HPV genotypes and cervical cytology abnormalities.

1.3 LITRATURE REVIEW

A global meta-analysis shows that the prevalence of HPV in 157,879 women with normal cytology was 10.4%. The prevalence estimates by the region were Africa 22.1%, Central America and Mexico 20.4%, Northern America 11.3%, Europe 8.1%, and Asia 8.0%. Of all high-risk human papillomavirus (HR HPV) genotypes, HPV 16 has been regarded as the most prevalent genotypes (28). Cervical cancer is the most common cancer among women in 45 countries of the world, and it kills more women than any other form of cancer in 55 countries. These include many countries in sub-Saharan Africa, many in Asia (including India), and some Central and South American countries (29).

In the United States, approximately one-half of the cervical cancers diagnosed are in women who were never screened, and an additional 10% of cancers occur among women not screened within the past 5 years. The current opportunistic approach to cervical cancer screening in the United States fails to reach subpopulations of women mainly living in low-resource, medically underserved regions, and thus invasive cervical cancer is one among a complex of diseases strongly linked to socioeconomic, geographic, and/or racial disparities. Annual rates of cervical cancer incidence and mortality in these populations are several-fold higher than the rates in the general US population and are similar to the rates observed in some lower income countries (30).

Cross-sectional studies have shown that the overall prevalence of any HPV type in the general populations of sub-Saharan Africa for women with normal cytology is 21.8%. The prevalence of HPV types 16 and 18 among ICC cases ranges from43.7% in Senegal to 90.2% in Ethiopia (31).

An independent, prospective, multi-centered, hospital-based cross-sectional studies involving Malaysia, Vietnam, Singapore, South Korea and the Philippines evaluate the prevalence of human papillomavirus (HPV) in women older than 21 years old with invasive cervical cancer (ICC) and high-grade precancerous lesions. Out of 500 women confirmed with ICC, the HPV types detected were HPV 16 (36.8%-61.3%), HPV 18 (12.9%-35.4%), HPV 52 (5.4%-10.3%), and HPV 45 (1.5%17.2%), whereas among the CIN 2/3/AIS cases, HPV 16 (29.7%-46.6%) was the most commonly observed type followed by HPV 52 (17.0%-66.7%) and HPV 58 (8.6%-16.0%) (32).

A study conducted in Trinidad, India on the relative prevalence of HPV genotypes shows that HPV infections were identified in 126 of 310 (40.6%) women. Of them, 83 (65.8%) were infected with high-risk HPV, 16 (12.7%) with low-risk HPV, and 27 (21.4%) with HPV types of unknown risk. HPV 52 (12.7%) was the most frequently occurring high risk type, followed by HPV 66 (10.3%), HPV 16 (9.5%), and HPV 18 (8.6%). High-risk types HPV 16 and HPV 66 were each found in 3 (20.0%) and HPV 18 was found in 1 (6.6%) of the 15 women with abnormal cytology (33).

In Southern Israel a study conducted to determine the prevalence of HPV infection and cervical cytology abnormalities, and to assess the possible influence of HIV infection on HPV carriage in HIV positive. The result shows that Forty-nine (58.3%) of the study participants were HPV-positive; 34 of them had oncogenic genotypes. Young age (<16 years) at first sexual intercourse was the only variable significantly associated with HPV infection (P <0.05). Abnormal cervical cytology was present in 17 women (20.3%); 21 women were referred to colposcopy, which was abnormal in 9 (10.7%) (34).

A study conducted in Nigeria, a total of nine different HR-HPV types were identified with an HPV prevalence of 21.6% overall and 22.7% among women with cervical lesions. The predominant HR-HPV types were HPV 16, 53, 18 and 52. In all, 41.7% of the infections involved more than one HPV type. Unlike in most populations studied so far, HPV prevalence was high not only among young women, but also in middle and old age. It was also observed that the prevalence of HR-HPV increases with parity. This study shows that HPV 53 is the second most common type after HPV 16 in our environment (35).

Another study conducted in Lagos, Nigeria shows that the prevalence of HPV among HIV positive women was 44.9% while the prevalence of high risk types was 37.5%. The commonest high risk types seen were types 31, 52, 53 and 35. The prevalence of HPV among the HIV negative women was 11%. The commonest high risk types seen were types 18, 16, 52 and 56 (**36**).

Among a total of 2,964 women, 1,289 HIV-infected (HIV [+]) and 1,675 HIV-uninfected (HIV [-]), aged 30-60 years and living in Rwanda were enrolled in 2010. HR HPV prevalence was higher in HIV [+] (31.8%, 95% CI = 29.2-34.4%) than HIV [-] women (8.2%, 95% CI = 6.7-9.8%; P < 0.0001). In multivariate analysis of HIV [+] women, testing HR HPV positive was positively associated CD4 count of <200 cells/µL, history

of 3 or more sexual partners, and history of using hormonal contraception, and negatively associated with older age. In HIV [-] women, testing HR HPV positive was negatively associated only with older age groups of 45-49 and 50-60 years and surprisingly was not associated with lifetime number of sexual partners (37).

One hundred and sixty-four ICC cases from Mali and Senegal were tested and from which 138 were positive (adjusted prevalence = 86.8%; 95% CI = 79.7–91.7%). HPV16 and HPV18 accounted for 57.2% of infections and HPV45 for 16.7%. In SSA countries, HPV16 was less frequent than in the rest of the world (49.4% vs. 62.6%; $P < 0.0001$) but HPV18 and HPV45 were two times more frequent (19.3% vs. 9.4%; $P < 0.0001$ and 10.3% vs. 5.6%; $P < 0.0001$, respectively). There was an ecological correlation between HIV prevalence and the increase of HPV18 and the decrease of HPV45 in ICC in SSA ($P = 0.037$ for both) (38).

A study conducted in South Africa shows that, of 1 472 women with valid cytology results, abnormalities were detected in 17.3% (n=255), of which 9.1% (n=134) were high-grade squamous intraepithelial lesions, and 0.5% (n=8) suggestive of squamous carcinoma. Of the 1 445 women with complete data, the overall and high-risk HPV DNA prevalence were 74.6% (n=1 078) and 54.3% (n=784), respectively. HPV type 16 and/or 18 were detected in 19.5% (n=282) of women. Age-specific prevalence of HPV showed a plateau-shaped curve (39).

A study conducted in Sudan on detection of HPV in Cervical Squamous cell carcinoma produced that GP primers detected mucosal HPVs in 75% (n, 47) of the patients, and none of the controls. HPV16 and HPV18 types were identified from 70% (n, 33) and 9% (n, 4) of the GP positives. HPV16 was identified from all tumor differentiations degrees especially moderately differentiated tumors, while HPV18 was identified from moderate and well differentiated tumors. HPV16 was isolated from all ages but mostly isolated from older patients, while HPV18 was isolated from younger patients. Ten unknown mucosal HPV types were detected. HPV16 and HPV18 were mostly detected in house wives ethnically from Central Sudan (40).

Another study conducted in Ethiopia, Gurage zone displayed that the age-standardized prevalence of HPV, HPV HR and HPV LR infection was 17.3% (95% CI 14.1-20.5), 15.8% (95% CI 12.7-18.9) and 3.9% (95% CI 2.3-5.6), respectively. Among HC2 HPV HR positive infections (n = 86), the most common genotype was HPV 16 (24.4%),

followed by 52 (11.6%), 56 (10.5%) and 31 (10.5%). Non-married relationship and widowhood, increasing number of lifetime sexual partners, human immunodeficiency virus infection and non-traditional housing type, but not age, were significantly associated with HR HPV infection (41).

Outpatients attending Attat hospital in rural Ethiopia were examined for the presence of HPV DNA using the Digene HPV test. 15.9% of patients were found to be HPV positive. The proportion of HPV high risk types was 13.2% [age-standardized rates: HPV: 14.4% (95% CI: 8.5–20.2); HPV high risk: 11.6% (95% CI: 6.3–16.9)]. Compared to other countries HPV prevalence is high, especially of high risk types. Until vaccination programs take effect, screening programs should not be based on HPV testing alone as this will lead to significant overtreatment of healthy women (42).

Over the period 1998–2001 women attending Jimma hospital (southwest Ethiopia) with cervical dysplasia were screened for human papillomavirus (HPV), identifying a prevalence of 67.1% in this population. High-risk HPV types 16 (55.7%), 18 (8.2%), 56 (8.2%), 45 (4.1%), 39 (2.5%), 52 (1.6%), 31 (1.6%), 35 (1.6%), 58 (0.8%), 33 (0.8%), 59 (0.8%) caused severe pathology as single / multiple infection. Strategies need to be envisioned for vaccinating children, young women prior to first sexual contact and preventive screening of HPV high-risk types (43).

A study conducted in 2010 in Tikur Anbessa Hospital, Addis Ababa, Ethiopia on the HPV Infection and genotype distribution in relation to abnormal cytology shows that over all Human Papillomavirus was detected in 64.4% (232/360) of the study participants. A broad diversity of HPV genotypes were detected on the direct sequencing assay, 33 HPV genotypes was identified among the 232 HPV positive specimens. The mean age of women with HPV infection was 45.5yrs. Women without HPV infection accounted for 35.6% (128/360), their mean age was 34.8yrs. Overall, HR HPV accounted for 78% (181/232), and the most abundant HR-HPV types were HPV 16, HPV 35, HPV 56, HPV 45 and HPV 18 in descending order. HPV-16 was detected in 46.4% (84/181) of the HR HPV positive specimens. In contrast, HPV types 31, 33, 39, 51, 52, 58, 59 and 70 were detected in smaller proportions of samples, ranging from 1% to 2% of the all HR HPVs (44).

1.4 HYPOTHESIS

First Hypothesis

Null Hypothesis

The overall HR HPV Prevalence and genotype distribution in this study group is the same as found in the previous studies

Second Hypothesis

Null Hypothesis

There is statistically significant percent agreement between HR HPV DNA PCR and Pap smear cytology screening methods

CHAPTER TWO

2.0 OBJECTIVE OF THE STUDY

2.1 GENERAL OBJECTIVE:

To determine the prevalence and genotype distribution of High Risk Human Papilloma Virus and Cervical Cytology abnormalities at selected Obstetrics and Gynecology clinics, Addis Ababa, Ethiopia

2.2 SPECIFIC OBJECTIVES:

- To determine the overall prevalence of High Risk Human Papilloma Virus in the study population
- To determine the genotype distribution of High Risk Human Papilloma Virus among the study group
- To assess the risk factors associated with HPV infection and abnormal cervical cytology abnormalities
- To assess the percent agreement between High Risk Human Papilloma Virus DNA PCR and Pap smear screening methods

CHAPTER THREE

3.0 MATERIALS, METHODS, AND PROCEDURES

3.1 STUDY DESIGN AND PERIOD

Institutional based cross-sectional study was conducted in three selected Obstetrics and Gynecology clinics, Addis Ababa, Ethiopia from 15th June to 10th October, 2015.

3.2 STUDY SITE

Family Guidance Association of Ethiopia, Addis Ababa Area Reproductive Health Clinic: is one of the reproductive health clinics found in Addis Ababa around "Saris area". Among its service areas sexual and reproductive health, family planning, anti-natal care and follow-up, cervical cancer screening program, and health information promotion are included. **Hemen Maternal and Children Health Center:** is one of the MCH center found in Addis Ababa Its main areas of service are Anti-natal care, Delivery services, cervical cancer screening program, and other Gynecological and obstetrics services. **SinamokshEthio Women's Health Special Clinic:** It is priority of establishment is standing for women health. It provides outpatient services for obstetrics and gynecology cases, and mainly it works for cervical cancer screening program, treatment of cervical precancerous lesions and different trainings for health professionals on cervical cancer screening and other sexually transmitted diseases managements.

3.3 SOURCE POPULATION

The source population was women who visited the selected Gynecology and Obstetrics clinics during the study period.

3.4 STUDY POPULATION

The study population was women who came for any gynecological visit including cervical cancer screening plan in the Gynecology units of the clinics that fulfilled the inclusion criteria was included.

3.5 SAMPLING PROCEDURE AND SAMPLE SIZE

3.5.1 SAMPLING PROCEDURE

Non-probability convenient sampling technique was used to select the study sites. Those sites are selected because of their scope of service that is highly related with our study objective. They have remarkable number of client visits for cervical cancer screening which enabled them to be potential selected sites for this study. All women who were visiting each clinic during the study period and eligible for this study were taken consecutively until the number of clients reached the calculated minimum sample size.

3.5.2 SAMPLE SIZE DETERMINATION

Sample size calculation was performed using single population proportion formula;

$$\mathbf{n = z^2 P(1\text{-}P) / d^2}$$

Where n = sample size,

Z = Z statistic for a level of confidence (95% level of confidence; z=1.96),

P = expected prevalence or proportion

(P= 0.358 taken from ICO information center on HPV-Ethiopia, 2014) and

d = precision (in proportion of one; if 5%, d = 0.05).

$n = 1.96^2\ 0.358(1\text{-}0.358)/0.05^2$

n= 353

Taking 10% contingency; the total sample size will be 353+35=388

3.6 INCLUSION CRITERIA

- Non-pregnant women
- Age >=18
- Women who has sexual exposure
- Willing to give sample for both Pap smear and HR HPV DNA PCR

3.7 EXCLUSION CRITERIA

- Total Hysterctomized
- Prior surgical procedures of cervix
- Gross tumor on cervix
- Women on menstrual cycle (included when the bleeding stopped)
- Virgin women
- Sexual intercourse within 24 hours
- Vaginal douching within 48 hours
- Unwilling to participate in the study

3.8 STUDY VARIABLES

3.8.1 DEPENDENT VARIABLES

- High Risk Human Papilloma Virus and Genotype distribution
- Abnormal Cytology

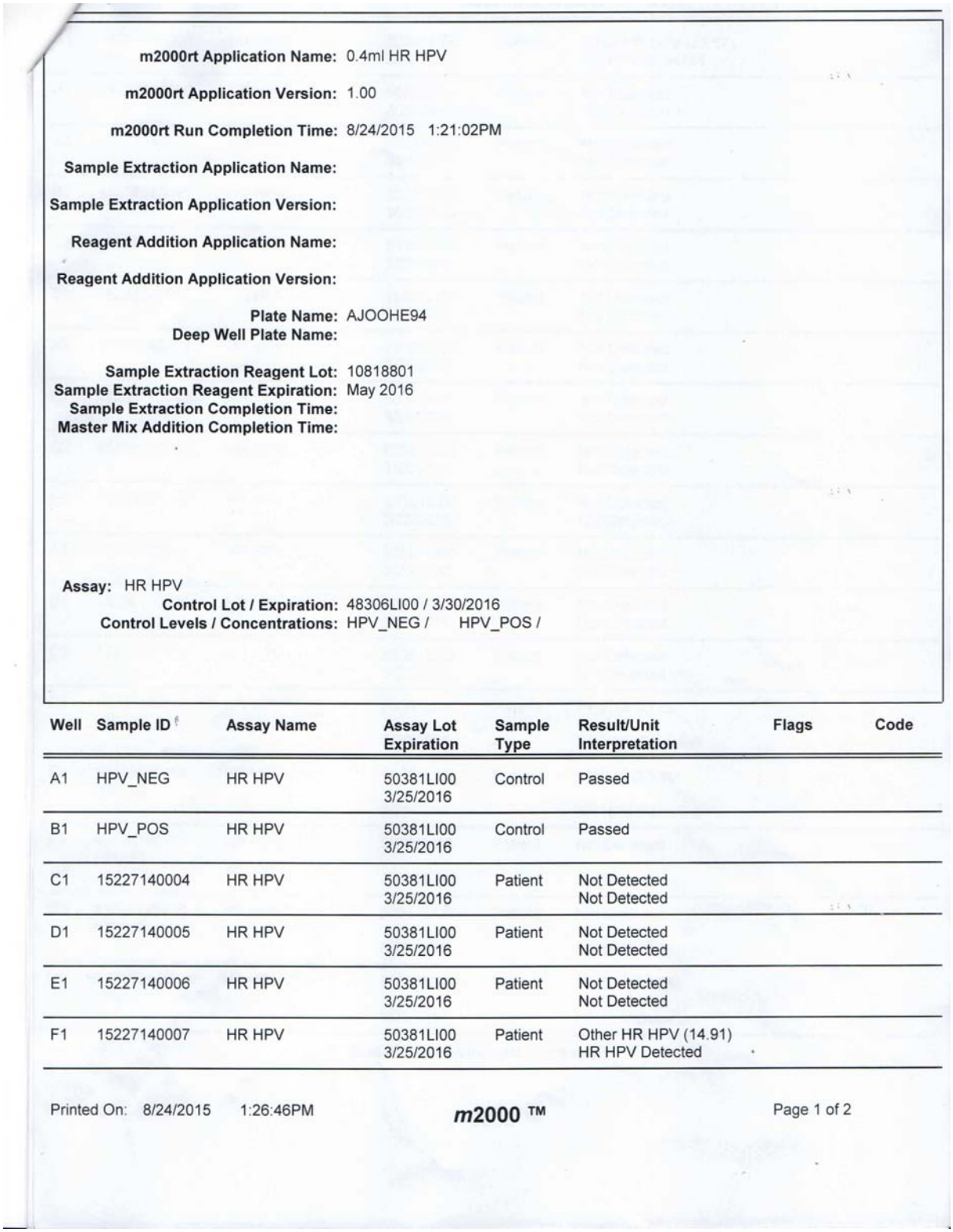
3.8.2 INDEPENDENT VARIABLES

- Age
- Marital Status
- Age at first marriage
- Parity
- Contraceptive use
- Life time number of sexual partners
- History of condom use
- History of sexually transmitted infections
- Family History of Cervical cancer
- HIV Status
- Cigarette smoking
- Alcohol consumption

3.9 MEASUREMENT AND DATA COLLECTION

Cervical specimens were collected for HR HPV testing with the Abbott Cervi-Collect Specimen Collection Kit. Before directly move on specimen collection, first the patient prepared in lithotomy position, if excess mucus or abnormal exudates from the cervical os and surrounding ectocervix observed, removed by using cotton or Darcon swab. Next to this the cervical brush was removed from the kit and inserted in to the os of the cervix until only the bottom-most bristles are exposed. The brush was slowly rotated 3 full turns in one direction and withdrawn carefully. The transport tube cap unscrewed and the cervical brush placed and immersed in to the buffer of the tube. The cervical brush was rinsed in the buffer by rotating 10 times while pushing it against the wall of the transport tube and the cervical brush discarded in the separated waste container. The transport tubes recapped and sealed tightly. The tubes labeled with the unique ID, date, and time of collection and sent to the laboratory with the copy of sample registry log. During specimen transportation, the specimens were transported at 2°C to 30°C and stored until 14 days and when more storage was needed they were stored at -20^{o}C (*Detailed Instruction followed displayed under Annex VII*).

The HR HPV DNA PCR was measured through a primer mix consisting of three forward primers and two reverse primers targeting a conserved L1 region is used to amplify HPV targets. Signal for fourteen HR HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) is generated with the use of fluorescent labeled probes. Three HPV signals corresponding to HPV 16, HPV 18 and Other HR HPV are evaluated for each sample. Each signal is either determined as “Detected” if the CN is less than a fixed assay cutoff cycle or is determined as “Not Detected” if the CN is not generated or the CN is greater than or equal to the assay cutoff cycle. All the detected signals (HPV 16, HPV 18 or Other HR HPV) are reported in the sample result with the respective CN values (in parenthesis after the target result). Samples with any of the three HR HPV signals detected will have an interpretation of “HR HPV Detected”. Samples with all three HR HPV signals not detected will have an interpretation of “Not Detected”. The Abbott Real Time HR HPV assay detects the endogenous human beta globin sequence as sample validity control for the cell adequacy, sample extraction and amplification efficiency (Annex VIII).



m2000rt Application Name: 0.4ml HR HPV

m2000rt Application Version: 1.00

m2000rt Run Completion Time: 8/24/2015 1:21:02PM

Sample Extraction Application Name:

Sample Extraction Application Version:

Reagent Addition Application Name:

Reagent Addition Application Version:

Plate Name: AJOOHE94
Deep Well Plate Name:

Sample Extraction Reagent Lot: 10818801
Sample Extraction Reagent Expiration: May 2016
Sample Extraction Completion Time:
Master Mix Addition Completion Time:

Assay: HR HPV
Control Lot / Expiration: 48306LI00 / 3/30/2016
Control Levels / Concentrations: HPV_NEG / HPV_POS /

Well	Sample ID	Assay Name	Assay Lot Expiration	Sample Type	Result/Unit Interpretation	Flags	Code
A1	HPV_NEG	HR HPV	50381LI00 3/25/2016	Control	Passed		
B1	HPV_POS	HR HPV	50381LI00 3/25/2016	Control	Passed		
C1	15227140004	HR HPV	50381LI00 3/25/2016	Patient	Not Detected Not Detected		
D1	15227140005	HR HPV	50381LI00 3/25/2016	Patient	Not Detected Not Detected		
E1	15227140006	HR HPV	50381LI00 3/25/2016	Patient	Not Detected Not Detected		
F1	15227140007	HR HPV	50381LI00 3/25/2016	Patient	Other HR HPV (14.91) HR HPV Detected		

Printed On: 8/24/2015 1:26:46PM

m2000 ™

Page 1 of 2

Figure 3.1 HR HPV detection result out put from Abbott Real Time PCR, 24th July 2015

For Pap smear, endocervical specimens were collected with a cyto-brush using a 360 degree rotation within the canal and ectocervical specimens were collected with a spatula using a 360 degree rotation just inside the cervix, and sampling both the transformation zone and ectocervix. Endocervical and ectocervical specimens are smeared on to the glass slide immediately after collection and fixed with ethanol and stained for visualization under a microscope.

The Pap smear results were reported based on the "Bethsida current grading system" which are NILM, ASCUS, LSIL, and HSIL respective to the nature of the abnormality.*(Detailed Instruction with diagram displayed under Annex X)*

All laboratory raw data was collected using separate excel spreadsheet and transcribed on different dummy tables prepared for the variables and ready for analysis.

Interview based Structured questionnaire was administered to assess the associated risk factors with the necessary clinical information by the trained health care personnel (Gynecologists or Midwives or Nurses).

3.10 DATAENTRY AND ANALYSES

Responses which were gathered from the structured questionnaire, and the laboratory (HR HPV DNA PCR results, and cervical cytology (Pap) results) were entered into **Epi data software Version 13.0** and data was analyzed using **STATA Software Version - 11.0.** Descriptive statistics; proportions and actual number of cases were used to describe frequency outputs for categorical variables and arithmetic mean to describe the average age of the participants. Cross-tabulations were used to explore and display the relationship between two categorical variables. Chi-square statistics was used to identify the existence of association between two categorical variables in the cross-tabulation. Multi-variate logistic regression analysis (adjusted odds ratio) was used to identify the strength of association of the various potential risk factors with presence of HR HPV infection and cervical cytology abnormality. Positive and Negative percentage agreement and overall percentage agreement was assessed for HR HPV DNA PCR and Pap smear screening methods. P-value of less than 0.05 was considered to be statistically significant.

3.11 QUALITY ASSURANCE

Specimen collection, transportation protocol, and special safety precautions were provided with the necessary job aids for the facilities under study to monitor their process quality. Cervical specimens collected with the Abbott Cervi-Collect Specimen Collection Kit instruction (54) was followed for transportation at 2°C to 30°C and stored in the appropriate temperature until analysis. Specimens were also checked for not undergo more than four freeze/thaw cycles and thawing temperature was within 2°C to 30°C. Samples' data entry was cross checked by other lab personnel after the one who entered all the data before running the samples on RT-PCR. The Abbott RealTime HR HPV assay detects the endogenous human beta globin sequence as Internal Control (IC) signal to evaluate cell adequacy, sample extraction and amplification efficiency.

A Negative Control and a Positive Control were used for every run to verify that the sample processing, the amplification, and the detection steps are performed correctly. To remove contamination, the sample preparation (m*2000sp*) and amplification (m*2000rt*) instruments were cleaned based on the decontamination and cleaning protocol found within the operator manual. The proficiency panel runs together with our samples for more verification of our result.

Quality Control in cytology - Specimen adequacy was assessed using Bethesda 2001 system designates specimen adequacy as "satisfactory" or "unsatisfactory." Specimen quality indicators such as the presence or absence of a transformation zone component, or of obscuring inflammation or blood, are reported after the adequacy designation. Images depicting low-power (~4×) microscope fields with a low number of cells should be used as a comparison for adequacy. Pap Smears which were reported as abnormal were cross-checked by two pathologists and verified. The data from the structured questionnaire was checked for its completeness and accuracy. And Data cleaning and Double-data entry were applied in order to assure quality of the data.

3.12 . ETHICAL CONSIDERATIONS

The study proposal was reviewed and approved by the departmental research and ethics review committee (DRERC) of the medical laboratory sciences, school of allied health sciences, College of Health Sciences; Addis Ababa University (**Letter Ref Number: MLS/388/15**) on the date of **08/04/2015**. The participants were ensured to be free from any coercion, undue influence, inducement or intimidation, and their right to withdraw from the study whenever they feel inconveniences was ensured. A formal individual written consent was taken from each participant. Consent was obtained by interpretation in their local dialect and/or using proxy consent. The privacy and confidentiality of each individual participant was ensured. The participants were not identified by their name or other personal identifier; rather appropriate coding system was used. All of the client results were reported with standard result format to their clinician with a specified turn-around time and utilized for the clinician judgment.

3.13 RESULT DISSIMINATION

The result produced from this study will be disseminated to each of the facilities management, Addis Ababa City Administration Health Bureau, Federal Ministry of Health, and other stake holders working in the public health intervention related with this issue according to the university's and other Ethical Regulations.

CHAPTER FOUR

4.0 RESULTS

4.1 SOCIO-DEMOGRAHIC CHARACTERISTICS

A total of 366 participants were enrolled in this study. The participants were between 18 to 68 years of age. The mean age was found to be 42.7 with+/- 10.7 SD. Most study subjects 87.7. % (321/366) were found within 30-65 years of age range. In terms of residence, 352 (96.2%) of them visited the clinics from Addis Ababa. Out of the total participants 287 (78.4%) of them were married. Seventy one (19.4%) of the study group was married for the first time before 18 years of age. From the predefined number of parity 29 (7.9%) of the participants has > 5 complete pregnancy record and delivery and 281 (76.8%) from 1 to 5 parity.

Overall, 248 (67.8%) of the study participants were self-employed, 147/332 (44.3%) were house wives and 109/332 (32.8%) were business woman. The highest proportion 158/366(43.4%) has Diploma/Degree and above educational qualification, and only 39 (10.7%) were unable to read and write. The overall socio demographic characteristic of the participants is shown under Table 4. 1.

Table 4.1: Socio-demographic characteristics of study participants, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Variable	Number	%
Age		
18-21	2	0.55
22-29	38	10.38
30-65	321	87.7
>65	5	1.37
Address		
Addis Ababa	352	96.2
Outside Addis Ababa	14	3.8
Marital Status		
Single	32	8.7
Married	287	78.4
Widowed	28	7.7
Divorced	19	5.2
Age at first marriage		
<15	39	10.66
15-17	32	8.74
>=18	295	80.6
Parity		
0	56	15.3
1 to 5	281	76.8
>5	29	7.9
Occupation		
Employed(Government/Private/NGO)	108	29.5
Self employed	248	67.8
Unemployed	10	2.7
Education		
Unable to read and write	39	10.7
Elementary	64	17.5
High school	105	28.7
Diploma/Degree and above	158	43.2

4.2 PREVALENCE of *High Risk Human Papilloma* VIRUS AND ITS GENOTYPES

Overall High Risk Human papilloma virus (HR HPV) prevalence was 50/366 (13.7%). Among the HR HPV positive cases 8 (16%) of them were HR HPV 16; 38(76%) "other HR HPV"(HR HPV genotypes of 31,33,35,39,45,51,52,56,58,59,66,or 68; 2 (4%) genotype 16 together with "other HR HPV" type;1 (2%) genotype 18 together with "other HR HPV" types, and 1(2%) genotype 18 were identified. The HR HPV genotype distribution shows that "Other HR HPV types" were predominated over genotype 16 and 18 as shown in Figure 4.1.

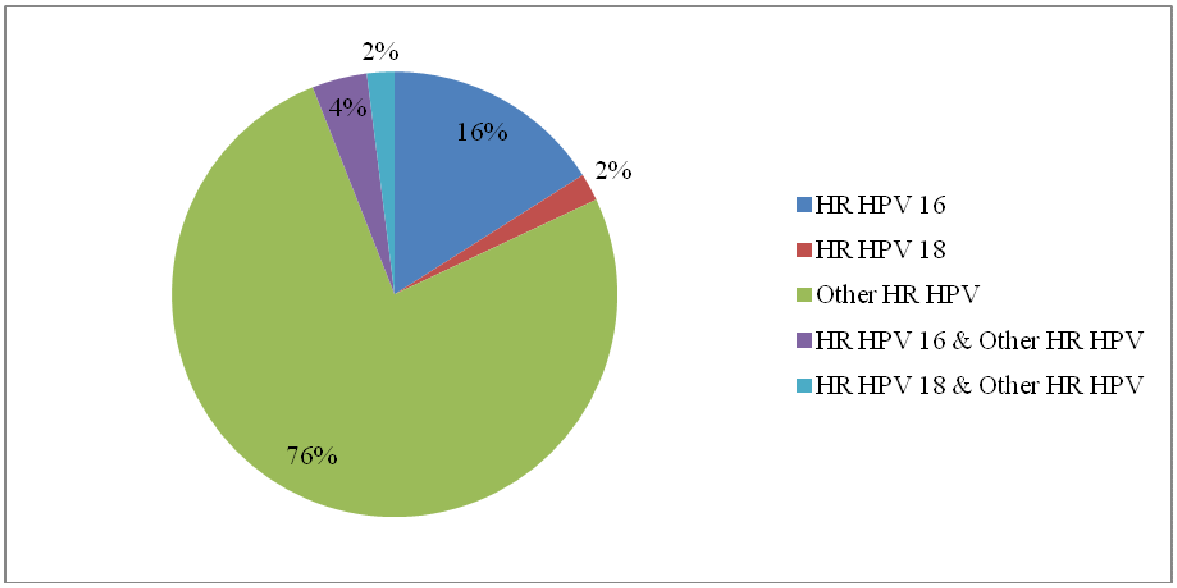


Figure 4.1: HR HPV genotypes proportional distribution among the total HR HPV positive clients, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Despite low proportion, multiple infections were identified for HR HPV 16 and Other HR HPV and HR HPV 18 and Other HR HPV, 4% and 2% respectively. The proportion of HR HPV positives are 2%,20%,76%, and 2% in the age ranges 19-21, 22-29,30-65, and >65 respectively. Age range which is 30-65 was found to be the highest proportion of positivity as shown in Figure 2 below.

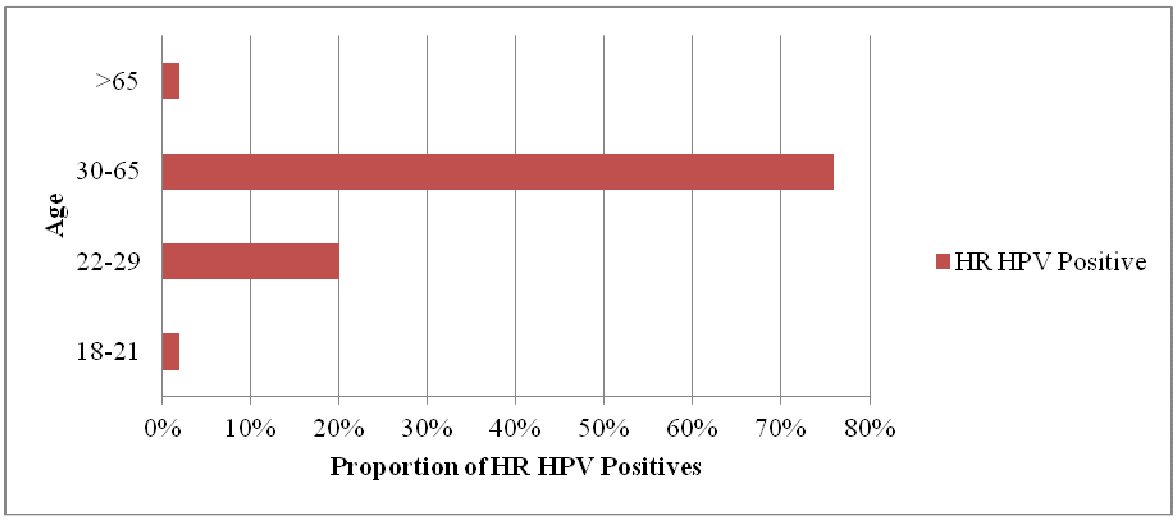


Figure 4.2: Proportion of HR HPV positives by different age categories, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Association between High Risk human papilloma virus prevalence with the socio-demographic and reproductive health, sexual behavior, and other risk factors was analyzed through bi-variate analysis using chi-square test. Age (P=0.000), Parity(P=0.017), age at first marriage (P=0.027), and education (P=0.003), condom use during sexual intercourse (0.011), cigarette smoking (0.000), and family history of cervical cancer (0.003) have statistically significant association (Table 4.2), but ever use of any type of contraceptive, age at first sexual intercourse, more than one life time sexual partnership , history of STD, alcohol consumption, , and HIV-sero status with P-values; 0.106, 0.266, 0.334, 0.824, 0.227, and 0.688 have no statistical significant association(Table 4.2 and 4.3).

Table 4.2: Socio-demographic and reproductive health variables association with prevalence of HR HPV, bi-variate analysis, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Socio-demographic and reproductive health variables	Variables Response Category	HR HPV Positive No (%)	Chi-square	P-value
Age	**18-21**	1(2.0)	41.14	0.000**
	22-29	10(20)		
	30-65	38(76)		
	>65	1(2.0)		
Address	**Addis Ababa**	47(94.0)	2.24	0.815
	Outside Addis Ababa	3(6.0)		
Marital Status	**Married**	37(74.0)	23.78	0.069
	Unmarried	10(20.0)		
	Widowed	3(6.0)		
	Divorced	0(0.00)		
Age at first marriage	**<15**	5(10.0)	20.2	0.027**
	15-17	14(28.0)		
	>=18	31(62.0)		
Parity	**0**	12(24.0)	21.57	0.017**
	1 to 5	33(66.0)		
	>5	5(10.0)		
Occupation	**Employed(GO/Private/NGO)**	16(32.0)	13.92	0.177
	Self employed	30(60.0)		
	Unemployed	4(8.0)		
Education	**Unable to read and write**	3(6.0)	13.58	0.558
	Elementary	7(14.0)		
	High school	13(26.0)		
	Diploma/Degree and above	27(54.0)		
	Yes	7(14.0)	17.996	0.003**
	No	43(86.0)		

*** There is statistical significant association between the variables and HR HPV prevalence*

Table 4.3: Sexual behavior and other risk factors association with prevalence of HR HPV, a bi-variate analysis, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Sexual behavior and other risk factor variables	Response Category	HR HPV Positive No(%)	Chi-square	P-value
Ever use of contraceptive	**Yes**	26(52.0)	9.07	0.106
	No	24(48.0)		
Age at first sexual intercourse	**<15**	5(10.0)	129.2	0.266
	15-17	14(28.0)		
	>=18	31(62.0)		
More than one life time partnership	**Yes**	32(64.0)	5.72	0.334
	No	18(36.0)		
Condom use in sexual intercourse	**Yes**	20(40.0)	14.82	0.011
	No	30(60.0)		
History of STD	**Yes**	8(16.0)	2.18	0.824
	No	42((84.0)		
Cigarette smoking	**Yes**	4(8.0)	29.55	0.000
	No	46(92.0)		
Family history of cervical cancer	**Yes**	7(14.0)	17.99	0.003
	No	43(86.0)		
Alcohol consumption	**Usually**	3(6.0)	12.93	0.227
	Occasionally	24(48.0)		
	Never	23(46.0)		
HIV sero-status	**Negative**	32(64.0)	7.6	0.668
	Positive	4(8.0)		
	Status unknown	14(28.0)		

*** There is statistical significant association between the variables and HR HPV prevalence*

In multivariate analysis using logistic regression, only “other HR HPV” type has statistically significant association with address, occupation, and HIV sero-status having P-values; 0.031,0.009,and 0.028 respectively . Individuals who live outside Addis Ababa are 11.9 times more likely to have Other HR HPV type infection than who lives in Addis Ababa. The likelihood of having Other HR HPV type infection among unemployed individuals is 7.8 times higher than individuals who are employed ones. Compared to diploma/degree holder women, women who are not able to read and write are less likely to be infected with Other HR HPV type (Table 4.4). HR HPV 16, HR HPV 18, HR HPV 16 together with other HR HPV, and HR HPV 18 together with other HR HPV prevalence was tested in logistic regression for predefined independent variables but they were found to be statistically not significant.

Table 4.4: Socio-demographic factors association with prevalence of "other HR HPV" genotypes, multi-variate analysis, Addis Ababa, Ethiopia, 15th July to 10th October, 2015

Socio-demographic	Response Category	"other HR HPV" positives	COR(95% CI)	P-value	AOR(95%CI)	P-value
Age	**18-21**	**1(2.0)**	Ref*			
	22-29	**7(14.0)**	0.25(0.01,4.51)	0.348	0.92(0.03,27.9)	0.963
	30-65	**30(60.0)**	0.11(0.01,1.76)	0.117	0.25(0.01,6.76)	0.409
	>65	**0(0.0)**	0 (0 to infinity)	0.994	0(0 to infinity)	0.999
Address	**Addis Ababa**	**35(70.0)**	Ref*			
	Out of Addis Ababa	**3(6.0)**	2.35(0.62,8.81)	0.207	13.7(1.3,148.3)	0.031**
Marital Status	**Married**	**28(56.0)**	Ref*			
	Unmarried	**7(14.0)**	2.795(1.096,7.128)	0.031	2.44(0.55,10.76)	0.238
	Widowed	**3(6.0)**	1.05(0.299,3.716)	0.934	2.15(0.47,9.9)	0.324
	Divorced	**0(0.0)**	0(0 to infinity)	0.994	0.000000025(0 to infinity)	0.997
Age at first marriage	**<15**	**2(4.0)**	Ref*			
	15-17	**4(8.0)**	0.25(0.025,2.59)	0.248	5.18(0.12,219.4)	0.389
	>=18	**32(64.0)**	0.14(0.029,0.646)	0.012	17.9(0.55,584.2)	0.105
Parity	0	7(14.0)	Ref*			
	1 to 5	**28(56.0)**	0.72(0.3,1.8)	0.471	3.43(0.73,16.2))	0.119
	>5	**3(6.0)**	0.79(0.2,3.3)	0.743	11.98(0.97,148.7)	0.053
Occupation	**Employed(Government/Private/NGO)**	**12(24.0)**	Ref*			
	Self employed	**22(44.0)**	0.79(0.37,1.66)	0.531	1.41(0.52,3.84)	0.502
	Unemployed	**4(8.0)**	5.1(1.26,20.73)	0.023	13.5(1.89,95.9)	0.009**
Education	**Unable to read and write**	**1(2.0)**	Ref*			
	Elementary	**6(12.0)**	3.75(0.43,32.48)	0.23	3.15(0.17,57.14)	0.438
	High school	**11(22.0)**	4.33(0.54,34.77)	0.168	12.7(0.75,216.5))	0.08
	Diploma/Degree and above	**20(40.0)**	5.34(0.69,41.21)	0.108	14.7(0.8,271.3)	0.07

Reference* * There is statistical significant association between the variables and "other HR HPV" prevalence*

Table 4.5: Sexual behavior and other risk factor variables association with "other HR HPV" genotypes, multi-variate analysis, Addis Ababa Ethiopia, 15th June to 10th October, 2015

Sexual behavior and other risk factor variables	Response Category	"other HR HPV" positives	COR(95% CI)	P-value	AOR(95%CI)	P-value
Ever use of contraceptive	**Yes**	**24(48.0)**	Ref*			
	No	**14(28.0)**	0.01(0.3,1.2)	0.164	0.73(0.3,1.77)	0.484
Age at first sexual intercourse	**<15**	**3(6.0)**	Ref*			
	15-17	**10(20.0)**	1.39(0.35,5.53)	0.637	0.28(0.01,6.76)	0.431
	>=18	**25(50.0)**	0.82(0.23,2.9)	0.753	0.07(0.003,1.72)	0.105
More than one life time partnership	**Yes**	**25(50.0)**	Ref*			
	No	**13(26.0)**	0.65(0.32,1.31)	0.229	1.04(0.41,2.64)	0.941
Condom use during sexual intercourse	**Yes**	**14(28.0)**	Ref*			
	No	**24(48.0)**	0.53(0.3,0.9)	0.076	0.49(0.18,1.29)	0.148
History of STD	**Yes**	**6(12.0)**	Ref*			
	No	**32(64.0)**	1.24(0.5,3.1)	0.641	2.62(0.7,9.8)	0.151
Cigarette smoking	**Yes**	**2(4.0)**	Ref*			
	No	**36(72.0)**	0.53(0.1,2.6)	0.435	0.28(0.03,2.26)	0.231
Family history of cervical cancer	**Yes**	**4(8.0)**	Ref*			
	No	**34(68.0)**	0.01(0.2,1.9)	0.402	1.1(0.23,5.28)	0.895
Alcohol consumption	**Usually**	**3(6.0)**	Ref*			
	Occasionally	**16(32.0)**	1.32(0.4,4.9)	0.68	6.96(0.79,61.2)	0.08
	Never	**19(38.0)**	0.75(0.2,2.7)	0.67	3.83(0.44,33.6)	0.23
HIV sero-status	**Negative**	**25(50.0)**	Ref*			
	Positive	**3(6.0)**	2.67(0.7,10.3)	0.156	9.47(1.27,70.5)	0.028**
	Status unknown	**10(20.0)**	0.01(0.2,1.9)	0.816	1.05(0.39,2.87)	0.917

Reference* * There is statistical significant association between the variables and Other HR HPV genotypes*

4.3 PREVALENCE OF ABNORMAL PAP SMEAR CYTOLOGY

Overall Pap abnormality was observed in 13.1 % (48/366). Among the abnormalities 3(6.3%), 39(81.3%), 6(12.5%) were ASCUS, LSIL, and HSIL respectively. The low grade abnormality (LSIL) is the highest frequency among the abnormal cytology categories. The low grade squamous intraepithelial lesion (LSIL) and high grade intraepithelial lesion (HSIL) in the age category of 30-65 were 36(75%) and 6(12.5%) respectively, and found to be higher compared to the other age categories.

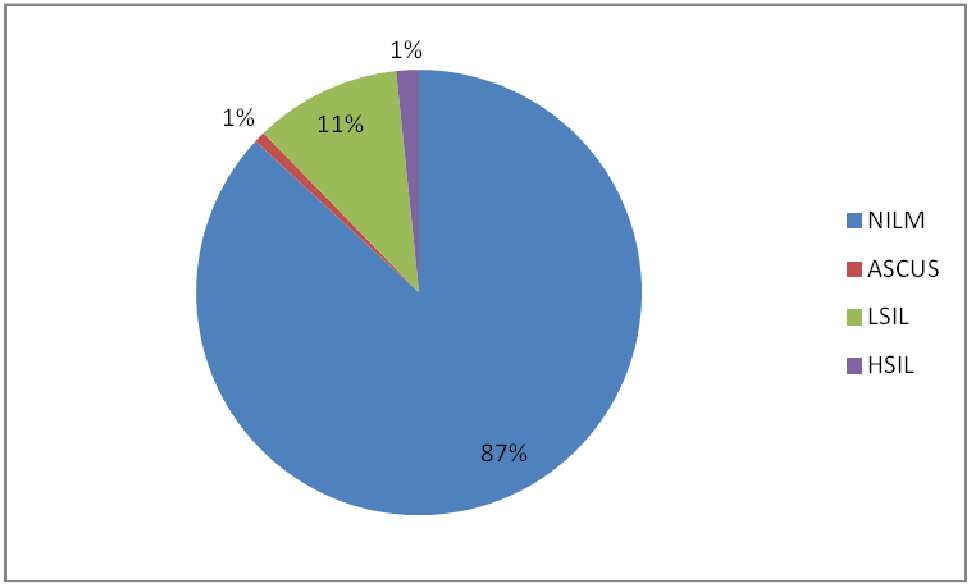


Figure 4.3: Proportion of Pap smear cytology results among the study population, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Table 4.6 Frequency of abnormal Pap smear cytology by age categories, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Abnormal Pap smear cytology	Age Categories				Total
	18-21	22-29	30-65	>65	
	No (%)	No (%)	No (%)	No (%)	
ASCUS	0(0.00)	0(0.00)	3(6.25)	0(0.00)	3(6.25)
LSIL	1(2.10)	2(4.20)	36(75.0))	0(0.00)	39(81.3)
HSIL	0(0.00)	0(0.00)	6(12.5)	0(0.00)	6(12.5)
Total	1(2.10)	2(4.20)	44 (91.7)	0(0.00)	48(100.0)

Table 4.7: Socio-demographic and other risk factors association with prevalence of abnormal Pap smear cytology, bi-variate analysis, Addis Ababa, Ethiopia, 15th June to 10th October 2015

Socio-demographic and Reproductive health variables	Response category	Abnormal No. (%)	Chi-square	P-value
Age	**18-21**	1(2.1)	6.57	0.682
	22-29	2(4.2)		
	30-65	45(93.8)		
	>65	0(0.0)		
Address	**Addis Ababa**	47(97.9)	8.53	0.481
	Outside Addis Ababa	1(2.1)		
Marital Status	**Married**	39(81.2)	3.54	0.939
	Unmarried	2(4.2)		
	Widowed	5(10.4)		
	Divorced	2(4.2)		
Age at first marriage	**<15**	4(8.3)	17.07	0.009**
	15-17	10(20.8)		
	>=18	34(70.8)		
Parity	**0**	6(12.5)	6.59	0.36
	1 - 5	37(77.1)		
	>5	5(10.1)		
Occupation	**Employed(Government/Private/NGO)**	18(37.5)	4.08	0.666
	Self employed	28(58.3)		
	Unemployed	2(4.2)		
Education	**Unable to read and write**	6(12.5)	15.15	0.087
	Elementary	4(8.3)		
	High school	16(33.3)		
	Diploma/Degree and above	27(56.3)		

*** There is statistical significant association between the variables and ASCUS, LSIL, and HSIL Pap smear abnormalities*

Table 4.8: Socio-demographic and other risk factors association with prevalence of abnormal Pap smear cytology, bi-variate analysis, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Sexual behavior and other risk factor variables	Response category	Pap Smear Abnormal No (%)	Chi-square	P-value
Ever use of contraceptive	**Yes**	24(50.0)	0.39	0.943
	No	24(50.0)		
Age at first sexual intercourse	**<15**	3(6.25)	66.08	0.674
	15-17	13(27.1)		
	>=18	32(66.7)		
More than one life time sexual partnership	**Yes**	25(52.1)	1.68	0.642
	No	23(47.9)		
Condom use during sexual intercourse	**Yes**	14(29.2)	0.89	0.828
	No	34(70.8)		
History of STD	**Yes**	9(18.8)	0.8	0.85
	No	39(81.2)		
Cigarette smoking	**Yes**	1(2.1)	0.51	0.918
	No	47(97.9)		
Family History of cervical cancer	**Yes**	2(4.2)	2.53	0.469
	No	46(95.8)		
Alcohol consumption	**Usually**	2(4.1)	9.71	0.138
	Occasionally	19(39.6)		
	Never	27(56.3)		
HIV Sero-status	**Negative**	36(75.0)	4.55	0.603
	Positive	3(6.0)		
	Status Unknown	9(18.8)		

In multivariate analysis using logistic regression, LSIL abnormality was the only one associated with age ($P<0.05$). The likelihood of being LSIL abnormality in the age category of 22-29 is 0.03 times higher than the age group of 18-21 years (Table 4.8).

Table 4.9: Association of age with prevalence of LSIL abnormal Pap smear cytology, multi-variate analysis, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

Variable	Response Category	LSIL No. (%)	COR(95% CI)	P-value	AOR(95%CI)	P-value
Age	**18-21**	1(2.1)	Ref*			
	22-29	2(4.2)	0.06(0.002,1.25)	0.069	0.03(0.001,0.92)	0.044**
	30-65	36(75.0)	0.13(0.008,2.16)	0.156	0.08(0.004,1.69)	0.104
	>65	0(0.0)	***	0.993	***	0.998

**Reference ** There is statistical significant association ***Too small number*

HSIL abnormal cytology was significantly associated with the variable "age at first marriage" and "education" (P<0.05). The likelihood of having HSIL among individuals whose educational background is high- school level 0.01 times higher than the elementary levels. Women whose age are 18 years and above are 0.16 times more likely to have HSIL Pap cytology abnormality (Table 4.9).

Table 4.10: Association of "Age at first marriage" and "Educational level" with the prevalence of HSIL abnormal Pap smear cytology, multi-variate analysis, Addis Ababa, Ethiopia, October 2015

Variables	Response Category	HSIL No. (%)	COR(95% CI)	P-value	AOR(95%CI)	P-value
Age at first marriage	**<15**	1(2.1)				
	15-17	3(6.3)	2.9(0.28,29.5)	0.215	0.34(0.008,14.4)	0.571
	>=18	2(4.2)	0.2(0.02,2.48)	0.488	0.0007(0.00000067,0.72)	0.04**
Education	**Unable to read and write**	3(6.3)				
	Elementary	0(0.0)	0	0.986	0	0.996
	High school	1(2.1)	7.97(0.013,1.25)	0.077	0.01(0.0001,0.92)	0.046**
	Diploma/Degree and above	2(4.2)	0.16(0.025,0.98)	0.047**	0.07(0.0007,6.54)	0.249

***There is statistical significant association between age >=18 and High school educational level, and HSIL Pap smear cytology abnormality*

Ever use of any type of contraceptive, Oral contraceptive use, age at first sexual intercourse, more than one life time partnership, frequency of condom use, frequency of cigarette smoking, history

of STD, alcohol consumption, with P-values; 0.943,0.751,0.674,0.642,0.842,0.165,0.850, and 0.138 have no statistical significant association with abnormal Pap cytology prevalence.

Prevalence of HR HPV by cytological abnormality was summarized in Table 8 below. The table describes only cytology results among the total HR HPV positives. When we see the overall prevalence of HR HPV among the total normal cytology results of the participants, it is 40/318(12.6%). As the table shows that 40(80%) of HR HPV positives has normal cytology (NILM),5(10%),4(8%),1(2%) were LSIL,HSIL, and ASCUS respectively. HR HPV 16 was found in 2 of the HSIL abnormalities and the remaining 2 were contributed by Other HR HPV genotypes. HR HPV 18 was only found in NILM findings but HR HPV 16 was found in NILM and HSIL cytology results. But other HR HPV genotypes were found across all cytology findings (NILM, ASCUS, LSIL, and HSIL).

Table 4.11: HR HPV genotypes against cytological findings, Addis Ababa, Ethiopia, 15th June to 10th October, 2015

HR HPV Genotypes	NILM	ASCUS	LSIL	HSIL
HR HPV 16	6(12%)	0(0%)	0(0%)	2(4%)
HR HPV 18	1(2%)	0(0%)	0(0%)	0(0%)
Other HR HPV	30(60%)	1(2%)	5(10%)	2(4%)
HR HPV 16 and Other HR HPV	2(4%)	0(0%)	0(0%)	0(0%)
HR HPV 18 and Other HR HPV	1(2%)	0(0%)	0(0%)	0(0%)
Total	40(100%)	1(100%)	5(100%)	4(100%)

4.3 PERCENT AGREEMENT BETWEEN HR HPV DNA PCR AND CONVENTIONAL PAP SMEAR CYTOLOGY

The agreement between HR HPV DNA PCR and Pap smear cervical cancer screening methods was analyzed using positive, negative, and overall percent agreement and Kappa measurement. The positive and negative percent agreement was found to be 87.7% and 22.4% respectively. But the overall percent agreement was 78.96% with Kappa value 0.12, 95% CI (0.00-0.243), P=0.01. The overall percent agreement shows that there is statistically significant agreement between HR HPV DNA PCR and conventional Pap smear cytology screening methods ($P<0.05$). But as the kappa value is less than 0.12, it is a weak agreement.

Table 4.12: HR HPV against Pap smear cytology findings, cross-tabulation, Addis Ababa, Ethiopia, October 2015

	Normal Pap	Abnormal Pap	Total
HR HPV Negative	278	38	316
HR HPV Positive	39	11	50
Total	317	49	366

In addition to the percent agreement the above table also shows that highest proportion (39/50) of high risk HPV women who have normal cytology.

CHAPTER FIVE

5.0 DISCUSSION

This study mainly aimed at assessing the prevalence of HR HPV and cervical cytology abnormalities along with the potential associated socio-demographic, sexual behavior, reproductive health variables and other risk factors at three Obstetrics and Gynecology, and reproductive health clinics in Addis Ababa, Ethiopia.

In this study the overall HR HPV prevalence is 13.7 %. is relatively similar with the one studied in in Gurage zone (41) and Attat Hospital (42), which reported prevalence rate of 13.2% and 15.8% respectively, but it is much lower than studies conducted in Jimma zone, south-west Ethiopia (43) and Tikur Anbessa Hospital(44), 67.1% and 64.4% respectively. This could be due to one, the participants in Jimma study were women with cervical complaints and all samples were having cervical dysplasia which can practically increases the prevalence but in our study cytological samples were taken from women who are not necessarily with cervical dysplasia or cervical complaints. The reason for Tikur Anbessa hospital might be one, it is the only specialized cancer center in Ethiopia providing referral services from all over the country the possibility of getting more positive HR HPV might be increased, and in addition to this among the study participants around 34% of them are HIV positive and due to this increased prevalence of HR HPV is expected (27, 36). Compared to the overall estimated prevalence for any HPV type in sub-Saharan African countries (21.8%) and with a study in Nigeria (21.6%), the finding with this study is relatively lower (31).

However, studies conducted in Ethiopia (42,43) reported HR HPV 16 is the predominant type in all cases except in a study conducted in Gurage zone and a study by Muluken at Tikur Anbessa having 72.3% and 48% was dominated by other HR HPV types followed by HR HPV 16 and 18 types (43,44), and the most frequent genotypes identified in this study Other HR HPV genotypes (31,33,35,39,45,51,52,56,58,59,66, or 68 types) with 76% of contribution followed by HR HPV 16 (16%). Our finding can also be comparable to the finding in worldwide meta-analysis review performed by Silivia *et al*., which depicts the predominant genotype in Eastern Africa is HR HPV 52 followed by 16 types (28,46,47). This difference in genotypes in various studies might be due to Geographic differences in the relative prevalence of HPV genotypes may be related to the complex interplay between different HPV genotypes and/or variants with host immunogenetic factors (e.g., HLA polymorphisms). Alternatively, a recent study showed that HPV16 appears less influenced by immune status than other HPV genotypes. This fact, coupled with impairment

in cellular immunity (e.g., through chronic cervical inflammation, parasitic infection, malnutrition, and / or more recently HIV), may be somehow contributing to the penetrance of HPV genotypes other than HPV16 in some populations (48).

Multiple HR HPV infection was found in 7.9% of the positive HR HPV individuals by Mohammed et al. in North-Eastern Nigeria (49), and it is similar with our finding, 6%. In contrast, a study conducted by Abate et al. in Ethiopia and Sudan women (50) 17.5% multiple infections was reported from gynecological referral population and it is comparatively higher than the present study which shows only 6% HR HPV multiple genotypes infection. This could be due to the nature of processed samples in Abate et al. study that which was tissue blocks with histology result showing cervical intraepithelial neoplasia or carcinoma increases the number of multiple HPV infections and also women with cervical pathology might have higher chance of being infected with more than one type of HR HPV genotypes (43). Since the higher proportion of HR HPV was found between age group 30-65 years, the possibility of getting multiple infections is lower (49).

Age specific Human Papilloma virus infection studied in South-Africa shows that the highest frequency (74.6%) of infection was found to be in women whose age is greater than 25 years (39). The study by Muluken et al., at Tikur Anbessa hospital also shows that 50.6% of HPV infected women were within 30-50 years of age range (44). Those studies are comparative with the finding in this study which is 76% of the women were within 30 to 65 years of age. However, there is statistically significant association between age group in the bi-variate analysis ($P<0.05$) but not in the multivariate analysis and it is similar with the study conducted by Sami-ramzi et al. in Gurage zone, Ethiopia (41). In contrast a study by Andall B in Trinidad (50) showed that the highest (63%) prevalence of HPV infection observed among women aged <30 years ($P<0.0001$), this might be due to the detection of Low-risk HPV in addition to HR HPV.

In this study, Address, Occupation, and HIV sero-status were found to be significantly associated with the prevalence of HR HPV in the multivariate analysis. This is comparable with a study by Quamrun et al. in Bangladish (55) which showed that occupation and Address have statistically significant association with HPV infection. However, a study by Muluken. et al. in Tikur Anbessa Specialized Hospital (44) reported that HIV and address were not having significant association with HR HPV prevalence, this could be due to the difference in sampling, type of the participants, and also the data collection method.

Ever use of any type of contraceptive, age at first sexual intercourse, and more than one life time, sexual partnership, not associated with HR HPV infection. This is comparable with a study by Mega AC et al., in Rural Nigeria (51). In the present study HIV-sero status was not significantly

associated with HR HPV infection, but different studies contrasted with our findings as stating HIV a potential cofactor for the development and progression of HR HPV infection (28, 31, and 37), this difference could be due to the number of HIV infected participants and partial record of secondary data our result might not be indicative to show HIV as a potential risk factor for HR HP infection.

The prevalence of abnormal cytology in the present study is 13.1%. The prevalence is lower when it is compared to the Muluken's study in Tikur Anbessa (44), Abel's study in southern Ethiopia (52), and another study in South Africa (39), which have a prevalence of 26.1%, 22.1%, and 17.3% respectively. This could be due to higher number of HIV infected individuals who are not easily able to resolve the infection and progress to the development of precancerous to cancerous lesion included in those studies (53). Furthermore, the sensitivity of conventional pap smear might also attribute to the lower prevalence. In the bi-variate analysis "Age at first marriage" has statistically significant association (P=0.009). But in multi variate analysis Age was associated with LSIL abnormality and HSIL abnormality was significantly associated with "Age at first marriage" and "Educational level" with p=0.04 and 0.046.A study by Abel et al. also supports this finding as "Age at first marriage" and "Educational status" having statistical significant association in their study (52).

In this study High Risk HPV genotypes were categorized in all types of cervical cytology results. HR HPV 16 was found in 50% of the HSIL reports and the remaining 50% was by other HR HPV genotypes. The highest frequency of HR HPV genotype in LSIL abnormal cytology was found to be Other HR HPV genotypes. In addition woman who had normal cervical cytology results, the most frequent genotypes are "other HR HPV" genotypes. Compared to a meta-analysis review by Gary C. et al. HPV type distribution in women with and without cervical neoplastic diseases, the most common HR HPV type in HSIL cytology abnormalities was HR HPV 16, which is similar with our findings. However, many other HR HPV genotypes detected in LSIL and the broad heterogeneity of HR HPV types noted in LSIL abnormalities, HR HPV 16 is still the predominant type in LSIL and NILM, but in our study all the LSIL and NILM results were attributed by Other HR HPV genotypes. In general, in our study other HR HPV genotypes were found across all grade levels of cytological findings. This is also noted by reviews, as HPV-positive women in sub-Saharan Africa were found to be significantly less likely to be infected with HR HPV 16 than their counterparts in Europe, although more likely to be infected with other high-risk HPV genotypes (47, 48, and 49).

5.1 CONCLUSION

The prevalence of HR HPV prevalence in this study in unselected women population is similar with previous studies and reviews in Ethiopia particularly in Gurage zone and Attat Hospital studies, and some-how lower against the estimated prevalence for sub-Saharan Africa. The age range of 30-65 years has the highest proportion of HR HPV positivity. Unlike the previous studies, other High-Risk Human Papilloma virus genotypes contributed the largest proportion of the HR HPV positive study population. More than single genotypes (multiple type infection) are found in sexually active women of the population. Address, occupation, and HIV sero-status are also found as potential risk factors for the prevalence of HR HPV, and age, age at first marriage, and education for cervical cytology abnormalities. The highest frequency of HR HPV positive population is the group of women without cervical cytology abnormality. “Other HR HPV” genotypes are found across all cervical cytology grade levels. HR HPV 16 is the major attributable genotype in HSIL abnormalities. There is a 78 % overall percent agreement between HR HPV DNA PCR and conventional Pap smear cytology. But the agreement is weak in terms of Kappa value, 0.12.

5.2 STRENGTH AND LIMITATION OF THE STUDY

STRENGTH

- The study used well trained and informative data collectors and coordinators throughout the study period.
- Every participant's results were formally reported for clinicians screening and judgment input in a timely manner.
- The study covered wider age range demography.
- Laboratory results peer-review before reporting was done for controversial and borderline results especially for Pap smear screening.

LIMITATIONS

- The study could not identify the specific genotypes within the 12 Other HR HPV genotypes and unable to quantify their frequency and proportion with type 16 and 18 due to the limitation of the Abbott Real Time PCR which is primarily intended for screening and except type 16 and 18 it can't separately identify the remaining 12 HR HPV genotypes.
- There was no reference method used to compare the HR HPV DNA PCR with the conventional Pap cytology, due to this percent agreement was used and it is usually affected by the case prevalence and also does not tell us the correctness or accuracy rather it tells us the agreement or correlation.
- Selective response bias for questions in the structured questionnaire regarding sexual and reproductive behaviors by considering that as taboo.
- However, HIV is one of the major risk factor for HR HPV prevalence, HIV-sero status results were taken from the secondary data and the data also not for all participants and might not representative for inference.
- Respondent recall bias of some of the risk factor related questions particularly time related questions.
- The cross-sectional nature of the study allowed us to see the prevalence of HR HPV at that point only and not be able to see the transformation, persistence, progression or resolution of the infection among the study group.
- Since it is institutional based cross-sectional study, it may not tell us the general population picture.

5.3 RECOMMENDATIONS

- ✓ The HR HPV with Pap smear cytology co-screening needs to be considered in particular for women whose age is greater than or equal to 30 years as a clinical triage tool complying with the national guideline for cervical cancer prevention and control.
- ✓ Those women whose age is greater than or equal to 30 years with HR HPV positive but cytology negative, ASCUS, or LSIL result shall be rechecked at least for Pap cytology after a year.
- ✓ The performance of Abbott Real Time HR HPV DNA PCR and Pap smear cytology screening methods performance shall further be evaluated against histology method as a gold standard method
- ✓ The screening program for early age sexually active women shall be promoted in more health facilities in Ethiopia particularly in Addis Ababa.
- ✓ Health Education and promotion as well as awareness appraisal shall be provided on the risk factors of HR HPV infection like that of other potentially transmitted infection in sexually active women.
- ✓ Considering that most of the “Other HR HPV” types are observed in our study beyond using the current bi-valent as well as quadri-valent prophylactic vaccine in Ethiopia the 9-valent vaccine is the promising vaccine of choice which can address more oncogenic “other HR HPV” types.
- ✓ Large scale community based cohort national study shall be designed and implemented by FMOH to identify the molecular epidemiology of HR HPV and its variants in different geographical settings of the country since there is a very wide geographical and socio-cultural diversity in Ethiopia.

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ANNEXES:

Annex –I. Participant information sheet and consent form

I- Participant Information Sheet and consent form (English Version)

Project Title: Prevalence and Genotype distribution of High Risk Human papillomavirus and Cervical Cytology Abnormalities

Introduction

Cervical cancer is the second most common cancer among women in the developing countries. The majority of cervical cancers are nowadays attributed to infection with human papilloma virus (HPV). Human papillomavirus (HPV) has been recognized as an important cause of cervical cancer and it is implicated in 99.7% of cervical squamous cell cancer cases in the world. Verylittle is known about the prevalence of High Risk HPV and its genotypes, the associated risk factors, and the screening methods in our country set-up.

Purpose

The objective of this study is to determine the prevalence and genotypic distribution and associated risk factors of High Risk Human Papilloma Virus (HR HPV) and abnormal cervical cytology among women attending Gynecology and Obstetrics clinics. You are being kindly invited to participate in this research. Your doctor will explain the details of the study to you. You should feel free to ask any questions.

Study Procedure

If you decide to participate in the study, you will be asked to provide a medical history and give Cervical samples for both High Risk Human Papilloma Virus and Pap smear screening. The amount of sample for both Pap smear and HR HPV DNA will be one time cervical scrapping sufficient as the exact anatomical site identified. The results will be reported using result reporting form to the clinician as soon as it is completed.

Possible Risks

There is only a minimal risks associated with cervical sampling such as bruising, minor bleeding, and discomfort. In most situations the additional cervical sample will be taken during a routine Pap smear screen as part of your normal diagnostic workup.

Benefits

When you participate in this study, you will know your HPV and Pap smear status which help you to prevent, or monitor and take actions based on the result you will find through your doctors consultation and instructions. Your participation will contribute to our country by providing essential information about the associated risk factors that will help using public health information, and help as an input for the vaccine program at large in the community level. There will not be any payment for your participation in this study. All extra laboratory tests performed for the study are for free.

Confidentiality

I would like to indicate that your name will not be written in the form and I assure you all the information you give will be kept strictly confidential. The researchers conducting this study will review your records and follow the progress of the research, but nothing that can be used to identify you will be used in reports of this study.

The study proposal will be reviewed and approved by the departmental research and ethics review committee(DRERC) of the medical laboratory sciences, school of allied health sciences, College of Health Sciences; Addis Ababa University, to make sure that participants are protected from any harm.

If you wish to find about more about the Ethics Review Committee, contact at telephone number +251-11-2-755170 or if you have any question about the study, contact the principal investigator, at telephone number +251-911-02-43-03 or send e-mail at kirub1625@gmail.com. Add telephone no of SMLS

II- Participant Consent Form (English Version)

Consent

Your participation is voluntary and you are not obliged to answer any question that you do not want to answer. If you are not comfortable with the study, please feel free to withdraw at any time you want. You have the right to not participate. Your choice will not affect your relationship with your doctor or your access to medical care. By signing this document, you do not give up any of your legal rights. A copy of this form will be kept in your medical records.

Thank you for your participation!

Consent Declaration:

I have read this Patient Information and Consent Form, or this form has been read to me. I havehad the opportunity to ask, and I have received answers to any questions I had regarding the study. I understand that if I have any additional questions, I may contact my treating clinician. I understand there is no serious invasive procedure at the beginning as well as at the end of the study. I agreed on the terms and the conditions indicated above. And I have received a copy of this Patient Information and Consent Form.

Participant's :	Clinician's Name:	For those who cannot Read and write Witness
Signature	Signature	Signature:
Date	Date	Date:

III- Participant Information and Consent Form (Amharic Version)

የጥናቱ ርዕስ፡-የሂዉማን ፓፒሎማ ቫይረስ ኢንፌክሽን ዘረ-መል ስርጭትና የማህጸን ጫፍ ካንሰር ሕመም ^ና ተዛማጅ ምክኒያቶች ጋር ያለዉን ግንኙነት ለማየት የሚደረግ ጥናት

መግቢያ

የሴቶች የማህፀን ጫፍ ካንሰር በታዳጊ አገሮች በሁለተኛ ደረጃ ላይ የሚገኝ የከንሰር ዓይነት ነዉ፡፡ ይህ በሽq በዋነኝነት በሂዉማን ፓፒሎማ ቫይረስ ምክንያት ^ንደሚከሰት በተለያዩ የዓለማችን ክፍሎች የተሰሩ ጥናቶች ያመለክታሉ፡፡ ይህም 99.7 በመቶ የሚሆነዉን ድርሻ ይይዛል፡፡ በሀገራችን ኢትዮጵያ ችግሩ በስፋት አንዳለ ቢተወቅም ስለ ሂዉማን ፓፒሎማ ቫይረስ ኢንፌክሽን ዘረ መል ስርጭት አና ከማህጸን አፍ ሕመም አና ሌሎች ተዘማጅ ምክኒያቶች ጋር ባለዉ ቁርኝት ዙሪያያሉት መረጃዎች አጅግ ዉስን ናቸዉ፡፡

ዋና ዓላማ

ይህ ጥናት የሂዉማን ፓፒሎማ ቫይረስን የዘረ-መል ስርጭት ^ና ከማህፀን ጫፍ ካንሰር ህመም ^ና ከሌሎች ተዛማጅ ምክኒያቶች ጋር ያለውን ቁርኝት ለማየት የሚደረግ ጥናት ነዉ፡፡

የጥናቱ ሂደት

በዚሁጥናት ለመሳተፍ ፍቃደኛ ከሆኑ ፣ የማህፀን ጫፍ ናሙና የሚወሰድልዎት ሲሆን በተጨማሪም ከበሽqዉ ጋር ተዛማጅነት አላቸዉ ተብለዉ ስለሚqወቁ ምክኒያቶች በባለሙያ ጥያቄዎችን ይቀርብለዎqል፡፡የናሙናዉ መጠን ደግሞ በናሙና መዉሰጃዉ መሳሪያ የማህጸን ጫፍ አከባቢ አንድ ጊዜ በመጥረግ የሚገኘዉን ያክል ይሆናል፡፡ የናሙናዎቹ የምርመራ ዉጤት ^ንደደረሰ በሀኪምዎ በኩል ዉጤቱ አንዲደርስዎት የሚደረግ ሲሆን ሀኪምዎ የደረሰዉን የምርመራ ዉጤት ለ^ርስዎ የጤና ምርመራ ክትትል የሚጠቀሙበት ይሆናል፡፡

በጥናቱ ከተሳተፉ የሚደርስ ጉዳት

በዚህ የምርምር ፕሮጀክት ዉስጥ ሲሳተፉ ናሙናዉ በሚወሰድበት ወቅት ከሚያጋጥም አነስተኛ መድማት በስተቀር ምንም አይነት ጉዳት አይደርስብዎትም፡፡ ለዚሁም ተገቢውን ህክምና ያገኛሉ፡፡

በጥናቱ ከተሳተፉ የሚያገኙት ጥቅም፣

በዚህ ጥናት በመሳተፍ ብቻ የሚያገኙት ቀጥተኛ ጥቅም ላይኖር ይችላል፡፡ ከዚህ ጥናት የሚገኘው መረጃ ለ^ርስዎና ለሌሎች ኢትዮጵያውያን የሚሰጠውን የህክምናና የሕብረተሰብ ጤና አገልግሎት ደረጃና ጥራት ለማሻሻል ^ንዲሁም የችግሩን መጠን ለሚመለከተቸዉ መንገስqዊም ይሁን መንግስqዊ

ያልሆኑ አከላት ለመጠቆም ይረዳል። ለዚህም ምርመራ የሚከፈሉት ምንም አይነት ክፍያ አይኖርም ፤የለም።

የጥናቱ ተሳታፊ መረጃ አያያዝ

የተሳተፊዉ ስም፤ አድራሻንና ሌሎች የተሳqፊዉን ማንነት ለመለየት ሊያገለግሉ የሚችሉ ነገሮች በሙሉ ከዚህ ክሊኒክ ዉጭ ከሚላኩት መረጃዎች ላይ ˆናጠፋለን። ተሳqፊዉን የሚመለከት መረጃ በሙሉ ተቆልፎ የሚቀመጥ ሲሆን በኮምፕዩተር የምንይዘው መረጃም ምስጢራዊነቱ የተጠበቀ ይሆናል። ይህንን መረጃ የማየት መብት ለክሊኒኩና በዚህ ጥናት ተሳታፊ ለሆኑ ባለሞያዎች ብቻ የተሰጠ ይሆናል፡ ይህም የሚሆነው የመረጃ አስባስብ ጥራትን ለመጠበቅና ለመከqተል ሲባል ብቻ ነው።

ይህ የጥናትና የምርምሩ ሰነድ በአዲስ አበባ ዩኒቨርስቲ በጤና ምርምርና የስነ-ምግባር አጣሪ ኮሚቴ ታይቶ የፀደቀ መሆኑንና፣ማንኛውንም ጥናቱን በተመለከተ ጥያቄ ወይም መረጃ ከፈለጉ በስ.ቁ. +251-11-2-755170 ወይንም የጥናቱን ዋና አስተባባሪ በስልክ ቁጥር +251-911-02-43-03 ወይም በkirub1625@gmail.comኢሜይልአድራሻ ቢልኩልን አስፈላጊውን መረጃ ሁሉ ˆንሰጠqለን።

በዚህ ጥናት ለመሳተፍ የሚደረግ ስምምነት

በዚህ ጥናት የሚሳተፉት በሙሉ ፈቃደኝነት ብቻ ነው። አልሳተፍም ቢሉ፣ ለመሳተፍ ከተስማሙ በኃላም ቢሆን ለመቀጠል ካልፈለጉ በማንኛውም ጊዜ ያለመቀጠል መብትዎ የተጠበቀ ሆኖ፣ ያለምንም ተጽዕኖ ˆንደበፊቱ ህክምና ማግኘትዎን ይቀጥላሉ።

IV- Participant Consent Form (Amharic Version)

የስምምነት ቅጽ

ከዚህ በላይ የተገለፀውን መረጃና የስምምነት ቅጽ አንብቤ ወይንም ተነቦልኝ በደንብ የተረዳሁ መሆኔን ˆገልጻለሁኝ፡፡ ስለ ጥናቱ ያልገባኝን ነገር ሁሉ የመጠየቅ ዕድል አግኝቼ ተገቢ መልስ ሁሉ አግኝቼአለሁኝ፡፡

ለወደፊትም ለመጠየቅ ከፈለግኩኝ ሐኪሞቼን ለመጠየቅ ˆንደምችል ተነግሮኛል፡፡ ከዚህ ጥናት የሚገኝ ውን ውጤት ማወቅ ከፈለግኩኝ በክሊኒኩ ባለሞያ ወይም በሀኪሜ በኩል ማግኘት ˆንደምችል ተነግሮኛ ል፡፡

በዚህ ጥናት በመሳqፍ ከማህፀን ጫፍ ከሚገኝ ፈሳሽ ለሂዉማን ፓፒሎማ ቫይረስ ˆና የፓፕ ስሚር ምርመራ አንዲደረግልኝ ˆንዲሁም በተዛማጅ ምክኒያቶች ዙሪያ ለሚቀርብልኝ ጥያቄዎች ምላሽ ለመስጠት ፈቅደኛነቴን አረጋግጣለሁ፡፡

የጥናቱ ተሳታፊ፡	**የአሳታፊ ስም፡**	**የስምምነት ቅጽን ማንበብ አና መጻፍ ለማይችሉ፡** **የምስክር ስም፡**
ፊርማ፡	**ፊርማ፡**	**ፊርማ፡**
ቀን፡	**ቀን፡**	**ቀን፡**

Annex II. Questionnaire (English Version)

QUESTIONAIRE			
Name of Health Facility:			
Participant Code:- **KHPV/SIN /000**		Date:	**Time:-**
Please make a#√; mark in the provided boxes.			
SNo.	**Variable**	**Response**	**Remark**
Part I-Socio-demographic characteristics			
1	Age:	_____Years	
2	Address:	☐ Addis Ababa	
		☐ Outside Addis Ababa	
5	Marital Status	☐ Married	
		☐ Unmarried	
		☐ Widowed	
		☐ Divorced	
6	Age at first marriage	☐ <15 years of age	
		☐ 15-17 years of age	
		☐ >=18 years of age	
7	Parity:	☐ ***0***	
		☐ ***1-5***	
		☐ ***>5***	
8	Occupation:	☐ Employed(GO/Private/NGO)	
		☐ Self-employed	
		☐ Unemployed	
10	Educational Status:	☐ Unable to read and write	
		☐ Elementary/Junior(**1-8**)	
		☐ High School(**9-12**)	
		☐ Diploma/Degree and Above	
Part-II Reproductive and Sexual Health risk factors characteristics			
1	Previous history of contraceptive use	☐ Yes	
		☐ No	
2	If "Yes" for question # 1, which type of contraceptive you used to have?	☐ Oral Contraceptive (OCP)	
		☐ IUCD	
		☐ Implant	
		☐ Depo (Injectable)	
		☐ Permanent (Tuba ligation)	
		Other, specify:	
3	If you used to have OCP, for how many year/s you stayed?	☐ <5 years	
		☐ 5 years	
		☐ >5 years	
4	Age at first sexual inter-course?	☐ <15	
		☐ 15-17	
		☐ >=18	

5	More than one life time sexual partnership	☐	Yes	
		☐	No	
6	History of condom use during sexual intercourse	☐	Some times	
		☐	Always	
		☐	No	
7	Do you smoke cigarette	☐	Some times	
		☐	Always	
		☐	No	
8	Do you have previous history of sexually transmitted disease	☐	Yes	
		☐	No	
9	Do you have previous family history of cervical cancer	☐	Yes	
		☐	No	
10	Do you consume alcohol	☐	Usually	
		☐	Occasionally	
		☐	Never	
11	HIV Sero-status	☐	Negative	From client's medical record
		☐	Positive	

Thank you very much for your willingness and kindly response!!!

II- Questionnaire (Amharic Version)

መጠይቅ			
የሕክምና መስጫ ተkሙስም:			
የተሳታፊዉ መለያቁጥር (ኮድ)፡- KHPV/SIN /000		ቀን፡- ወር/ቀን	ሰዓት፡-
በሰንጠረ ÿ ዉስጥ ለሚገኙት ጥያቄዎች ለሚሰጡት ምላሾች በአማራጭመልሶቹ ስር በሚገኙት ሳጥኖች ዉስጥ የ # √;ምልክት ያድርጉ፡፡			
ተ.ቁ	የመረጃዉ ዓይነት	አማራጭመልሶች	ማብራሪያ
ክፍል I- ማህበራዊና ስነ-ህዝባዊ መረጃዎች			
	ዕድሜ:	_____ዓመት	
1	የመኖሪያ አድራሻ:	☐ ከተማ	
		☐ ገጠር	
2	የጋብቻ ሁኔq	☐ ያገባች	
		☐ ያላገባች	
		☐ ባለቤቷ በህይወት የሌለ	
		☐ የፈqች	
3	ያገባች ከሆነ የመጀመሪያ ጋብቻ የፈጸመቡት ዕድሜ?	☐ <15	
		☐ 15-17	
		☐ >=18	
4	የወሊድ መጠን	☐ 0	
		☐ 1-5	
		☐ >5	
5	የሥራ ሁኔq፡	☐ ተቀጣሪ(መንግስqዊ/የግል/NGO)	
		☐ የግል ሥራ	
		☐ ሥራ ፈላጊ	
6	የትምህርት ደረጃ:	☐ ማንበብና መጻፍ የማይችል	
		☐ አንደኛ ደረጃ(1-8)	
		☐ ሁለተኛ ደረጃ(9-12)	
		☐ ዲፕሎማ/ ዲግሪና ከዚያ በላይ	
ክፍልII. የሥነ-ተዋልዶና ተዛማጅ ምክኒያቶች ዳሰሳ			
1	የወሊድ መቆጣጠሪያ ተጠቅመዉ ያዉቃሉ?	☐ አዎ	
		☐ አላዉቅም	

2	መልስዎ #አዎ; ከሆነ፤ ምን ዓይነት የወሊድ መቆጣጠሪያ ይጠቀሙ ነበር?	☐	ኦራልኮንተራሴፕቲቭ	
		☐	አይ ዩ ሲ ዲ	
		☐	ኢምፕላንት	
		☐	ዶፖ(Injectable)	
		☐	ፐርማነንት(ቲዩባል ሊጌሽን)	
		ሌላ(ይገለጽ)		
3	በአፍ የሚወሰድ የወሊድ መቆጣጠሪያ የሚጠቀሙ ከሆነ ለምን ያክል ጊዜ ሲጠቀሙ ቆዩ?	☐	ከ5 ዓመትበ q ች	
		☐	5 ዓመት	
		☐	ከ5 ዓመትበላይ	
4	የመጀመሪያ የፍቅር አጋር ግንኙነትዎ የጀመሩት በስንት ዕድሜዎ ነበር?	☐	ከ15 ዓመት ዕድሜ ቀድሞ	
		☐	ከ15-17 ዓመትባለዉ ዕድሜ	
		☐	18 ዓመትና ከዚያ በላይ	
5	ከአንድ በላይ የፍቅር አጋር ግንኙነት ነበረዎት?	☐	አዎ	
		☐	አይደለም	
6	በፍቅር ግንኙነ r ዎ ወ i ት ኮንዶም ይጠቀማሉ?	☐	አልፎ አልፎ	
		☐	ሁል ጊዜ	
		☐	አልጠቀምም	
7	ሲጋራ ያጨሳሉ?	☐	አልፎ አልፎ	
		☐	ሁል ጊዜ	
		☐	አላጨስም	
8	ከዚህ በፊት የአባለ ዘር በሽ q q መዉ ያዉቃሉ?	☐	አዎ	
		☐	የለም	
9	ከቤተሰብዎ መከከል የማህጸን lሃከንሰር ሕመምተኛ አለ/ነበረ	☐	አለ	
		☐	የለም	
10	የአልኮል መጠጥ ይወስዳሉ?	☐	ˆወስዳለሁ	
		☐	አልፎ አልፎ	
		☐	አልወስድም	
11	በደም ዉስጥ የኤች አይ ቪ ቫይረስ አለ።	☐	ኔጌቲቭ	ከሕክምናሪኮርድ(ፋይል) የሚወሰድ
		☐	ፖዘቲቭ	

ስለ መልክም ፈቃደኝነትዎና ስለሰጡን ምላሽˆጅግ በጣም ˆናመሰግናለን!!!

Annex III. Pap smear result work sheet

DATE_____

SAMPLE ID_____

PATIENT ID_____

AGE_____

SPECIMEN ADEQUECY

Adequate ☐ Inadequate ☐

CONCLUSION

NILM ☐

LSIL ☐

High SIL ☐

ASCUS ☐

ASC-H ☐

Cancerous ☐

COMMENT:

_____

_____

_____

_____

_____

Annex IV. Laboratory result recording form

SNo.	Patient ID	Sample ID	Age	HR HPV (Positive/Negative)	HR HPV Genotype	Pap Result	Remark

Annex V- Data collectors orientation attendance sheet

NAME OF HEALTH FACILITY:-_____

SNo.	NAME	TITLE	SIGNATURE

ORIENTATION GIVEN BY:

NAME:_____ TITLE_____

DATE _____SIGNATURE_____

Annex VI- Sample collection kit distribution and tracking sheet

NAME OF HEALTH FACILITY:-_____

SNo.	DATE	NUMBER OF KIT DISTRIBUTED		RECIEVER NAME AND SIGNATURE	REMARK
		HPV	Pap		
\					

Annex VII. Standard operating procedure (SOP) for HR HPV DNA PCR method

Intended use

The Abbott RealTime High Risk HPV is a qualitative in-vitro test for the detection of DNA from 14 high Risk Human Papilloma virus (HR HPV) genotypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68 in clinical specimens.

Principle

A primer mix consisting of three forward primers and two reverse primers targeting a conserved L1 region is used to amplify HPV targets. Signal for fourteen HR HPV genotypes (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) is generated with the use of fluorescent labeled probes. Internal Control (IC) amplicons are generated with a primer set targeting an endogenous human beta globin sequence and is detected with the IC specific probe. The Abbott RealTi*m*e HR HPV assay detects the endogenous human beta globin sequence as sample validity control for cell adequacy, sample extraction and amplification efficiency. Probes for HPV 16, HPV 18, non-HPV 16/18 genotypes (Other HR HPV) and IC are labeled with different fluorophores allowing their signals to be distinguishable in a single reaction.

Procedure for using the m2000sp instrument

1. Thaw control- and amplification reagents. In mean time:
2. Vortex each specimen for 15-20 seconds. Liquid has to be on bottom of vial. Specimens collected with the Abbott Cervi-Collect tubes, can be loaded without cap directly on the m2000sp.
3. When controls are thawed. Vortex each control 15-20 seconds. Ensure that contents are on bottom.
4. Place the controls and the patient specimens into the m2000sp sample rack. Bar codes on tube labels must face right for scanning.
5. Open the mSample preparation system$_{DNA}$ reagent pack(s). Prepare the mWash2$_{DNA}$ by adding 70mL of Ethanol (95% - 100%; do not use denaturants!) to the mWash2$_{DNA}$ bottle. Mix by gently inverting and check if there are no crystals.
6. Vigorously mix the mMicroparticles$_{DNA}$ and pour into the 200mL reagent vessel.
7. Initiate the sample extraction protocol.
8. While the m2000sp is performing the sample preparation, switch on and initialize the m2000rt for a 15-minute warm-up.
9. Load the amplification reagents and the master mix tube on the m2000sp worktable.
10. Initiate the m2000sp master mix addition protocol.
11. After the m2000sp has completed the addition of samples and amplification reagents, seal the reaction plate.
12. In PCR-3 room, place the reaction plate in the m2000rt and initiate the RealTime HR HPV assay protocol.
13. After the m2000rt has completed the amplification and detection protocol, remove the reaction plate and dispose.
14. Clean and decontaminate all working areas.

RESULT INTERPRETATION

Result	Reporting
HPV 16 (	HPV 16 with CN of 20.76 is detected but HPV 18 and other HR HPV are not detected.
HPV 18	HPV 18 with CN 21.20 is detected but HPV 16 and other HR HPV are not detected.
Other HR HPV	Other HR HPV with CN of 14.48 is detected but HPV 16 and HPV 18 are not detected.
HPV 16, Other HR HPV	HPV 16 and Other HR HPV with CN of 22.0 and 17.21 respectively are detected HPV 18 is not detected.
HPV 18, Other HR HPV	HPV 18 and Other HR HPV with CN of 18.67 and 15.88 respectively are detected HPV 16 is not detected.
HPV 16, HPV 18	HPV 16 and HPV 18 with CN of 24.51 and 23.11 respectively are detected Other HR HPV is not detected.
HPV 16, HPV 18, and Other HR HPV	HPV 16 and HPV 18 and Other HR HPV with CN of 21.35, 22.60, and 19.45 respectively are detected
Not Detected	HR HPV is not detected*

***"Not detected" does not mean that the patient is "Negative" for HPV infection. The assay should be interpreted in conjunction with other clinical and laboratory findings.*

QUALITY CONTROL

INTERNAL

Detection of inhibition and/or Cell Inadequacy:

The HR HPV assay detects the endogenous human beta globin sequence as Internal Control (IC) signal to evaluate cell adequacy, sample extraction and amplification efficiency.

Negative and Positive control

The controls need to be processed together with the samples prior to running the amplification portion of the assay.

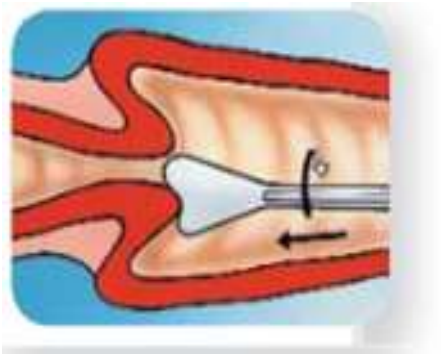
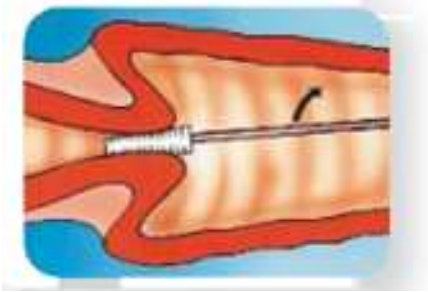
The negative control is formulated with DNA containing IC sequence. The only signal detected for negative control should be the IC signal in the Cy5 channel.

The positive control is formulated with DNA containing HPV 16 (VIC), HPV 18 (NED), HPV 58 (FAM) and IC (Cy5) sequences.

EXTERNAL

The performance of the HR HPV assay will be assessed 3 times in a year with a proficiency panel from College of American Pathologists (CAP).

Annex VIII. Specimen collection procedure diagrams for HPV analysis

	adequate sampling from the exocervix using a plastic spatula. If desired, use lukewarm water to warm and lubricate the speculum. Select contoured end of plastic spatula and rotate it 360 degrees around the entire exocervix while maintaining tight contact with exocervical surface.
	an adequate sampling from the endocervix using an Endocervical brush device. Insert the brush into the cervix until only the bottom-most fibers are exposed. Slowly rotate ¼ or ½ turn in one direction. DO NOT OVER -ROTATE.
	the spatula as quickly as possible into the vial by swirling the spatula vigorously in the vial 10 times. Discard the spatula.
	The brush as quickly as possible into the vial by swirling the spatula vigorously in the vial 10 times, while pushing against the vial wall. Discard the spatula.

	The cap so that the torque line on the cap passes the torque line on the vial.
	The patient's name and ID number on the vial and test requisition paper. Proper patient identification is a MUST

Sourece: Fremont Rideout Health Group Laboratory services Policy and Procedure.2009

Available at

http://www.frhg.org/documents/Lab_Manuals/Collection-of-Specimens-for-Conventional-and-Thin-Prep-Pap-Tests,-HPV-Tests,-and-GC-CT-Tests.pdf

Accessed date: 10th April, 2015

Annex IX. Standard operating procedure (SOP) for conventional Pap smear method

Principle

Pap test is a test done on cervical cells scarped from the surface as well as the exfoliated cells in order to look for morphological abnormalities that indicate premalignant conditions. The cells are smeared on a slide, fixed with ethanol and stained for visualization under a microscope.

Specimen

The optimal time for gynecologic cytology specimen collection is two weeks after the start of the patient's last menstrual period. She should avoid vaginal medications, spermicides, and douches for 48 hours and inter course for 24 hours before the day of her appointment.

Procedure

1. Complete out the Pap smear requisition completely.
2. Label the frosted end of the slide with the patient's name and date of birth in pencil, date and time of collection.
3. Do not remove the glass slide from the paper holder for safety and convenience.
4. The speculum is lubricated with warm water only, not lubricant jelly.
5. It is positioned to expose the cervix at the end of the speculum.
6. If large quantities of mucus or exudates are present, gently remove by patting with dry gauze without disturbing the epithelium.
7. Three specimen sources can be sampled: endocervix, ectocervix, and vaginal pool.
8. Endocervical specimens are collected with a cytobrush using a 360 degree rotation within the canal.
9. Ectocervical specimens are collected with a spatula using a 360 degree rotation just inside the cervix, and sampling both the transformation zone and ectocervix.
10. Endocervical and ectocervical specimens are smeared onto the glass slide *immediately* after collection and *immediately* fixed with cytology fixative. Best results are obtained with a uniform thin smear.
11. Smear the specimens thinly and evenly across the slide and fix *immediately* with cytology fixative.
12. Fold the card board container with patient's name and date of birth and date and time of collection with a ball point pen.
13. Deliver the sample and sample registry log to the laboratory.

Quality Control

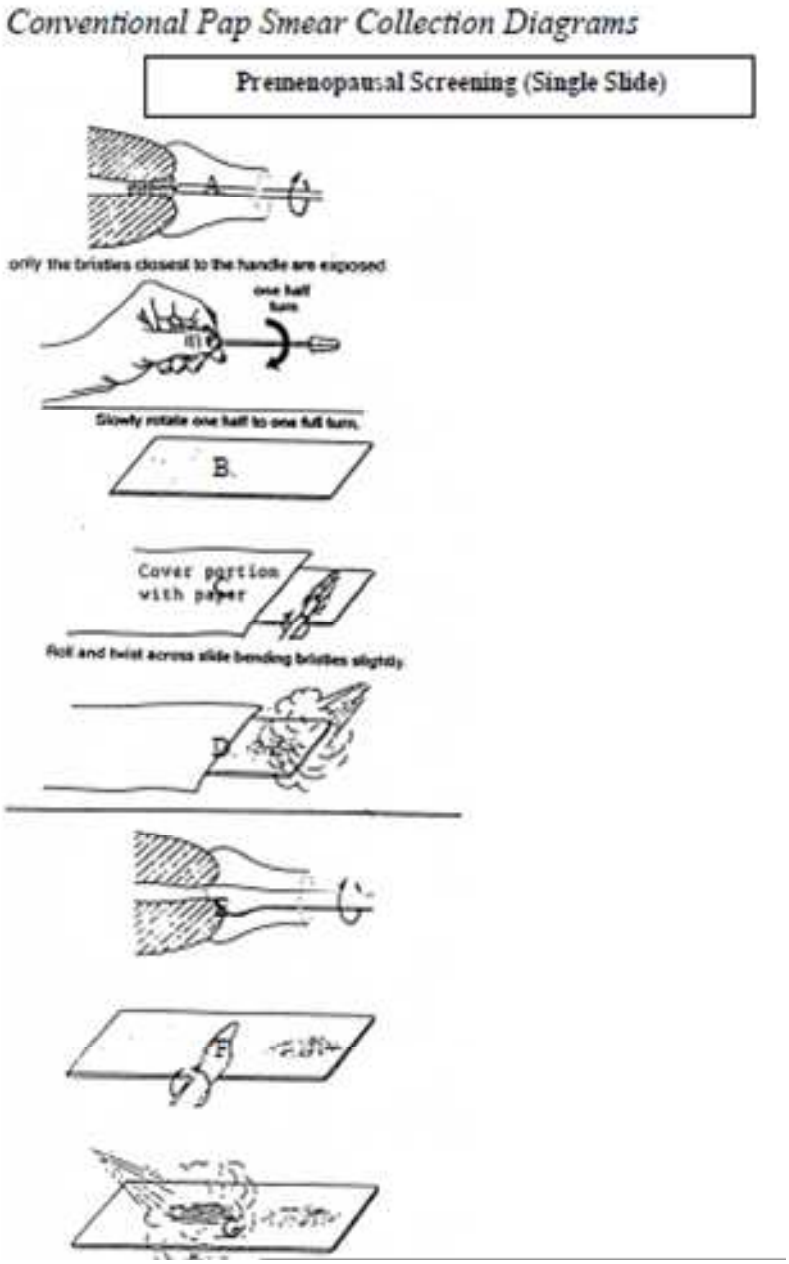
Specimen adequacy will be assessed using Bethesda 2001 system. Bethesda 2001 designates specimen adequacy as “satisfactory” or “unsatisfactory.” Specimen quality indicators such as the presence or absence of a transformation zone component, or of obscuring inflammation or blood, are reported after the adequacy designation. The criteria for the classification of adequacy as “satisfactory” or “unsatisfactory” are based on:

a) Cellular material covers at least 10 percent of the area under the coverslip ($125mm^2$) at a “normal” or “usual” cell density, or that 10 percent or $125mm^2$ of the slide is actually covered or obscured because of the presence of cells.

b) Smear contains between 8,000 and 12,000 well-preserved, well-visualized cells. This assessment is not done by counting cells manually. Images below depicting low-power (~4×) microscope fields with a low number of cells should be used as a comparison for adequacy.

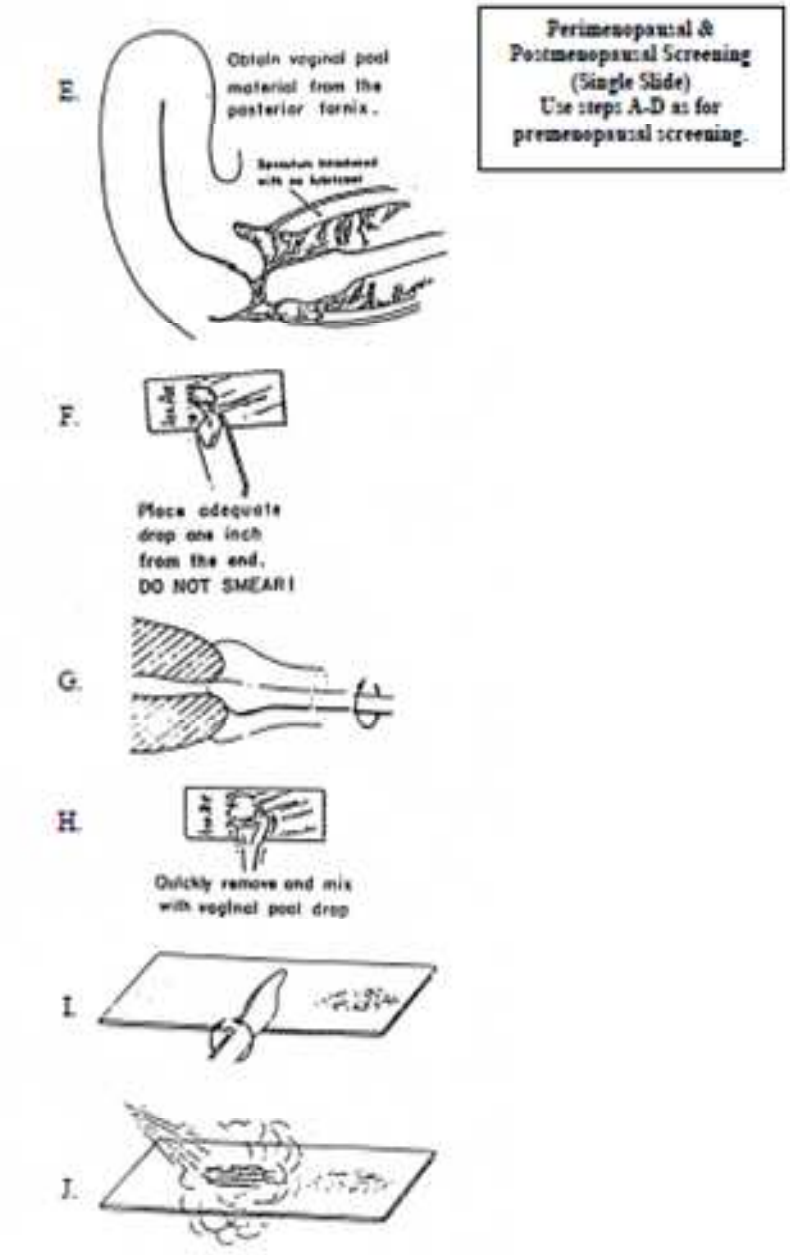
Annex X: Conventional collection procedure diagram for Pap smear

Diagram:



Diagram, continued

Conventional Pap Smear Collection Diagrams



Sourece: Fremont Rideout Health Group Laboratory services Policy and Procedure.2009

Available at

http://www.frhg.org/documents/Lab_Manuals/Collection-of-Specimens-for-Conventional-and-Thin-Prep-Pap-Tests,-HPV-Tests,-and-GC-CT-Tests.pdf

Accessed date: 10th April, 2015

Annex X. Institutional Ethical Clearance Letter

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Date: 03/04/15

Ref.No. MLS/388/15

Departmental Research and Ethics Review Committee (DRERC) decision

Meeting No: 010/2015

Protocol number: DRERC 151/15/MLS

Protocol title: Prevalence and genotype distribution of High risk Human Papilloma virus and cervical cytology abnormalities at selected obstetrics and Gynecology clinics, Addis Ababa, Ethiopia

Principal investigator: KIRUBEL ESHETU

Institute: AAU-MF CLS

Elements reviewed (AAUMF 01) [√] Attached [ ] Not attached

Review of revised application [√] Yes [ ] No

Date of previous review: _____

Decision of the meeting: [√] **Approved** [ ] Approved with recommendation

[ ] Approved on Condition (Major revision) [ ] Disapproved

Obligation of the PI-

1. Should comply with the standard international and national scientific and ethical guidelines
2. All the amendments and changes made in protocol and consent form needs DRERC approval
3. The PI should report DRERC within 10 days of the event.
4. End of the study, including manuscripts and thesis works should be reported to the DRERC

Departmental Research and Ethics Review Committee (DRERC) Approval period: from **April 08/ 2015 to April 07 /2017**

Follow up report expected in

3 months_____ 6 months__√__ 9 months_____ one year_____

Chairperson, DRERC: GEBRU MULUGETA

Signature: _____

Date: April 08/2015

School head: TEDLA MINDAYE

Signature: _____

Date: _____

ADDIS ABABA UNIVERSITY ... School of Medical Laboratory Technology