MOLECULAR EPIDEMIOLOGY OF HUMAN PAPILLOMAVIRUS IN NORTH AND CENTRAL PART OF ETHIOPIA

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LIST OF ABBREVIATIONS

ACS	…….……	American Cancer Society
AIN	……	Anal Intraepithelial Neoplasia
AP 1	……	Activating protein 1
CDK	……	Cycline dependent kinase
CIN	……	Cervical intraepithelial neoplasia
CSA	……	Central Statistics Agency
DNA	……	Deocyribonucleic Acid
E gene	……	Early gene
E.C	……	Ethiopian Calendar
E6AP	……	Early protein 6-associated protein
FGA	……	Family Guidance Association
FMOH	……..……	Federal Ministry of Health
GAVI	……	Global alliance for Vaccine & Immunization
HPV	……	Human Papillomavirus
HSDP	……	Health Sector Development Program
ICH	……	Immunohisto Chemistry
L gene	……	Late gene
LCR	……	Long Control Region
MDM2	……	Double minute 2 gene
NRERC	……	National Research ethics review committee
NF-I	……	Nuclear factor 1
ORF	……	Open reading frame
p21	……	21-kDa protein
PBS	……	Phosphate buffered bovine serum
pRb	……	Retinoblastoma protein
PV	……	Papillomavirus
RNA	……	Ribonucleic Acid
SNPs	……	Single nucleotide polymorphisms
SOP	……	Standard operating procedure
SPSS	……	Statistical Package for Social Sciences
STIP	……	Sexually transmitted infection profiling
UNDP	……	United Nations Development Program
VAIN	……	Vaginal Intraepithelial Neoplasia
VIA	……	Visual Inspection with Acetic acid
VILI	……	Visual Inspection with Lugol's Iodine
VIN	……	Vulvar intraepithelial neoplasia
VLP	……..……	Virus like particles

ABSTRACT

Human Papillomavirus (HPV), the sole causative agent of cervical cancer, is the most common sexually transmitted viral infection globally. Specifically, persistent infection with high-risk HPV types is the main risk factor for cervical cancer initiation and progression. However, the risk factors for its acquisition and induction of cervical cancer are widespread in resource limited countries like Ethiopia. In Ethiopia, cervical cancer is the second most frequent cancer, next to breast cancer, among the entire female population with estimated 7,095 and 4,732 annual cases and mortality, respectively. However, there are effective vaccines and many alternative intervention strategies that could reduce about two-third of its occurrence. At the same time ingredient for instituting HPV and cervical cancer intervention strategies, identifying circulating HPV genotypes, is lacking in Ethiopia. Therefore, the aim of this PhD project was to determine the molecular epidemiology of HPV among a population of women visiting gynecology clinics in the North and Central part of Ethiopia. A cross-sectional study was conducted from September/2015 to January/ 2016 in Addis Ababa, Dessie, Mekelle, Bahr Dar and Gondar. Cervical swabs were collected from 915 women attending gynecological clinics using PapCone® in PreservCyt buffer. Total DNA was extracted using Maxwell blood kit. HPV was genotyped by multiplex Polymerase Chain Reaction (PCR) using GP5+/6+ primers and readout by Luminex xMAP200 technology. The genotyping method was able to detect 14 high-risk HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 (a/ b), five putative high-risk HPV 26, 53, 70, 73 and HPV 82, and seven low risk HPV6, 11, 42, 43, 54, 72 and HPV 90 types. Moreover, STI and BV associated infections were typed using similar method, i.e. PCR and Luminex xMAP200 technology. The mean age of study participants was 37.7(±10.3) years. Analysis of HPV genotyping results confirmed that HPV DNA was detected in 33.9% (310/915) of samples. The five most prevalent high-risk HPV types identified were HPV16 (10.3%), 52(5.6%), 56 (4.2%), 59(4.1%), and HPV 35 (3.5%) in their decreasing order of prevalence. HPV 16 was exceedingly the most prevalent HPV type identified with prevalence of (94/915) and 30.3% (94/310) of all and HPV positive samples, respectively. Among HPV positive samples, high-risk HPV types were the most prevalent infections identified, 55.5% (172/310) followed by mixed HPV infections, 25.2% (78/310), putative high-risk 12.6% (39/310) and low risk 6.8%(21/310) HPV types. Overall, at least one high-risk HPV positivity was 80.3% (249/310) and 42.9% (133/310)

of the women had multiple HPV infections (2.84±1.5), with maximum of 12 HPV genotypes identified. Moreover, the prevalence and distribution of HPV genotypes was different among different sites. Among HPV associated sexually transmitted infections, *HSV-2, T. vaginalis* and *N. gonorrhea* were the most prevalent agents identified. Although, the prevalence of STI was lower than reported elsewhere, HPV infection was significantly (p=0.002) higher among STI positive women than their STI negative counterparts. Among them, HPV infection was significantly associated (OR:3.14; p<0.05) with HSV-2 than STI negative counterparts. Bacterial vaginosis associated infections were relatively high among our study participants. Besides, HPV infection was significantly associated to *G. vaginalis* and *A. vaginae* coinfection (OR: 1.64; p<0.05) and the three (p<0.05). Consequently, HPV infection was significantly associated with very strong BV score (p<0.05). Total cervical abnormality was 19.1% (175/915) of which 19.6% (59/301) and 18.9% (116/614) women were Pap smear and VIA positive, respectively. HPV infection was responsible for 30.5% (18/59) and 38.8% (45/116) of Pap smear and VIA positive women, respectively. Moreover, HPV 16 was the most important genotype associated with cervical abnormalities. The prevalence of both HPV and pathological finding showed that there is clear difference among our study sites. Therefore, the results indicated that high-risk and multiple HPV infection, among the investigated population, was high implying the risk of developing cervical cancer could be high in the future. Unlike previous reports the awareness level of cervical cancer is improving (66.7%) and 28.7% were screened at least once before this study. Screening strategies including HPV genotyping, vaccination, awareness creation employing health professionals especially extension health workers would be effective in preventing cervical cancer in Ethiopia.

Keywords: HPV, Genotypes, Prevalence, Epidemiology, BV, Cervical Cancer, STI, Ethiopia

1. INTRODUCTION

Human Papillomaviruses (HPV) are group of epitheliotropic, naked icosahedral (55nm) DNA viruses that belongs to the family of Papillomaviridae, PV (Doorbar *et al.*, 2015). Their classification is based on the capsid protein encoding *L1* gene nucleotide sequence. Accordingly, the family comprises 29 genera and 189 types encompassing PVs of human, other mammals, birds and reptiles (Bernard *et al.*, 2010). HPVs are grouped under five genera: α-, β-, γ-, μ- and ν PVs; where alpha and beta HPVs are responsible for most of mucosal and cutaneous infections (De Villiers *et al.*, 2004a) and each has its own tissue tropism (Stanley, 2012).

Epidemiological studies indicated that there are more than 40 HPV types adapted to infect the mucosal surfaces causing different types of benign and severe diseases including cervical cancer. Based on cervical cancer epidemiological studies, the mucosal HPV types are grouped into three categories high-risk, putative high-risk and low risk HPV types. The high-risk HPV types encompass HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and HPV 68 are associated with cervical cancer. The putative high-risk HPV types include HPV26, 53, 70, 73, and HPV 82 that are found in some cervical cancers cases; and low risk HPV types include HPV6, 11, 42, 43, 53, 90 etc are associated with benign epithelial proliferations, warts (Clifford, Howell-Jones and Franceschi, 2011).

High-risk HPV infection associated cancers include cancers of the cervix, vagina, vulva, penis, anus, a subset of head and neck cancers. Moreover, less sever HPV associated manifestations include ano-genital warts, recurrent respiratory Papillomatosis (RRP) and warts on different parts of the body. However, cervical cancer is almost exclusively caused due to persistent infection of high-risk HPV types; where 99.7% of all cases contain HPV DNA (Walboomers *et al.*, 1999). Among the high-risk HPV types, HPV 16 and 18 are the most prevalent high-risk HPV types covering 70% of all these cases (Doorbar *et al.*, 2015).

Globally cervical cancer is the fourth most important and common deadly cancer of women, next to breast cancer (Naghavi, 2015; Torre *et al.*, 2015). As per WHO/ICO statistics, globally 527,624 diagnosed with cervical cancer and 265,653 of them die annually (Bruni *et al.*, 2014). However, it is excessively affecting women living in developing countries of Sub-Saharan Africa and Latin America. Still Eastern African countries bear the highest prevalence of HPV infection and cervical cancer associated morbidity and mortality rates (Bruni *et al.*, 2010). This is attributable to lack of trained manpower, diagnostic and treatment facilities, abundance of risk factors, poor public awareness, poverty and instabilities. Ethiopia, located in this subcontinent, is expected to be endemic to HPV infection and cervical cancer.

In Ethiopia, the annual morbidity and mortality rate of cervical cancer is not clearly defined. WHO/ICO (2007) estimated that the annual incidence and mortality rates were 7,600 and 6,000, respectively. Moreover, in 2013 the same office estimated the annual incidence and mortality rates to be 7,095 and 4,732, respectively (HPV/ICO, 2016). However, the cause of this minor mortality rate reduction is not clear, as there was no corrective measure in place between 2007 and 2013 in Ethiopia.

Concerning HPV genotypic distribution, HPV -16 (91%), HPV-52, HPV-58 and HPV-18 were the most prevalent HPV types among Ethiopian cancer patients (Abate *et al.*, 2013). However, Bekele *et al* (2010) reported that HPV 16(55.7%), HPV18 (8.2%), 56 (8.2%) and HPV45 (4.1%) were the top four HPV types in cervical cancer biopsy blocks.

Public awareness about HPV and associated cancer is low in Ethiopia. According to Yifru Terefe and Ashebir Gaym (2008), 81.2% of women living in Addis Ababa did not have any information about HPV infection, cervical cancer or Pap smear test. Furthermore, the level of awareness at a rural area of Jimma (330Km West of Addis Ababa) regarding cervical cancer and its cause was very low as all of study participants were unaware of

cervical cancer and its cause (Birhanu *et al.*, 2012). However, public awareness is critical factor to plan and implement effective cervical cancer and HPV intervention strategies.

In spite of all these, to the best of our knowledge, there are no programs that implements and coordinates HPV and cervical cancer surveillance system, conduct epidemiological studies and recommend public health policy makers to initiate vaccination and screening programs in Ethiopia. Consequently, lack of base line epidemiological data is crippling efforts to initiate HPV vaccination and cervical cancer intervention strategies.

Cervical cancer, the major preventable but deadly cancer among women was not at priority in our country. Moreover, the sole causative agent of cervical cancer, Human Papillomavirus (HPV), is almost unknown by the public. However, starting from 2014 promoting cervical cancer created some level of public awareness and free intervention was in place but lack momentum towards establishing screening program. Nevertheless, there is only one cancer treatment center in Ethiopia, located at Addis Ababa Tikur Anbessa Specialized Hospital, where patients must travel hundreds to thousands of Kms to reach the center. Thus, women from countryside are disadvantaged due to unbearable living cost and extended time to get and follow treatment at Tikur Anbessa Hospital (Hailu and Mariam, 2013).

Therefore, we conducted this research to fill these gaps and equip the public health policy makers with up to date data to pass informed decision to implement intervention strategies towards controlling HPV infection and cervical cancer. Cervical swab samples were collected from five study sites. Pap smear (Addis Ababa) and VIA (other sites) were used to diagnose cervical abnormality. Total DNA was extracted using Levi blood kit and the *L1* gene was amplified. The PCR products were hybridized with HPV type specific beads. Then, the hybridization products were read using Lumunex xMAP200 technology to identify circulating HPV types in the study sites.

1.1. Statement of the problem

Human Papillomavirus infection is among the most frequent viral STIs and the pre-eminent etiologic agent of cervical cancer, a major global public health problem. Almost all cervical cancer cases contain HPV DNA of high-risk types. Globally, it is the second most common cancer with 527,624 cases and 265,653 deaths annually (Bruni *et al.*, 2014). However, HPV infection and cervical cancer are terribly devastating disease of women living in developing countries facing 88% and 85% of mortality and morbidity rates, respectively (Arbyn *et al.*, 2011).

Sub-Saharan, mainly eastern Africa, countries bear the highest burden of HPV infection and cervical cancer morbidity (42.7/100,000) and mortality (27.6/100,000) rates (Torre *et al.*, 2015). Ethiopia being in this high burden sub-continent is expected to be endemic to HPV infection. Indeed, previously conducted studies in Ethiopia indicated that the prevalence of HPV infection in otherwise healthy women at 'Atat' hospital, were 15.9% (Ruland *et al.*, 2006) and 17.3% (R. Leyh-Bannurah, Prugger *et al.*, 2014) of the hospital outpatient clients. Besides, WHO/ICO (2012) reported that Ethiopia incurred 7095 cases and 4732 deaths(HPV/ICO, 2016). However, the estimate is facility based but many die unnoticeably in the countryside.

As opposed to such high burden to this form of cancer, diagnostic and treatment facilities are scarce in Ethiopia. Only one, Tikur Anbessa, public referral hospital has cervical cancer treatment facility for >100 million people. This creates long waiting time, year or more, for getting treatment; and its cost is unbearable (Hailu and Mariam, 2013) especially for referral patients. On the other hand, there are plenty of opportunities to reduce the impact of HPV induced maladies in Ethiopia. Vaccination using either Cervarix® or Gardasil® is confirmed to reduce the oncogenic HPV 16 and 18 infection close to 100% (Cutts *et al.*, 2007) and thereby curb close to 70% of cervical cancer. Besides, well-planned screening programs are instrumental to reduce cervical cancer death. However, prior

knowledge of the epidemiology of circulating HPV genotypes and cervical cancer are primary resources to initiate any intervention strategy. But such data is missing and becoming an obstacle for the national plan. Hence, this project is designed to provide all the necessary epidemiological information for the government to initiate and implement an appropriate intervention strategy that fits our nation.

1.2. Significance of the study

The immediate outcome of this research project is uncovering the molecular epidemiology of HPV genotypes in the North and Central Part of Ethiopia. Many institutions of governmental, nongovernmental and other interested bodies may use the information mined from this study. These organizations could use it for public health planning including resource management and expanding research in the area.

Moreover, the scientific community may use it to advance research in the field. Defining the existing HPV genotypes could enable public health policy makers to choose the right intervention strategy that suits our conditions. Moreover, based on the geographic distribution of these HPV genotypes, health related resources; diagnostic reagents, treatment options etc, will be allocated to the region in need efficiently. Consequently, the intervention strategies will minimize the morbidity and mortality rates of cervical cancer. The information will enable clinicians to monitor treatment and vaccination strategies, patient management, and prediction of the course of cervical cancer treatment. Furthermore, it could be used to mobilize the society to fight cervical cancer.

The second most valuable outcome of this research project is capacity building and technology transfer. The research output will help us to determine the circulating HPV genotypes; evaluate if the country could benefit from the currently available expensive vaccine or screening strategy; to evaluate if molecular techniques or PCR based methods

could be used in the screening and/or diagnosis of cervical cancer and monitoring of prognosis of cervical cancer in the Ethiopian context. The results could be used to improve existing therapeutic, diagnostic, and screening strategies for cervical cancer, and establishment of an effective cervical cancer prevention programs.

2. LITERATURE REVIEW

2.1. Virology of Human Papillomavirus

Human Papillomavirus (HPV) virion is 50 to 60nm naked icosahedral DNA tumor virus (Doorbar, 2006). Its capsid is mainly composed of one type of protein called major capsid protein, L1, and small amount of minor protein, L2 (Doorbar *et al.*, 2015). The major capsid protein, L1, polypeptide has unique self-assembly property (Stanley, Lowy and Frazer, 2006) where 5 L1 peptides interact and stabilize via hydrogen bond to form individual pentameric capsomers (Doorbar *et al.*, 2015). When the five L1 peptides assembled into a capsomer, a groove is created at the center, a site where the L2 protein situates itself from inside. Moreover, the L1 protein at its C-terminal loops out and forms two contacts with adjacent capsomers at their bases via disulfide bonds (Wolf *et al.*, 2010). These contacts help firm assembly of the pentamers in the icosahedral capsid symmetry (De Villiers *et al.*, 2004a). Such 72 capsomers, 360 L1 molecules, form the viral icosahedral shell encapsidating a double stranded ~8kbp DNA (dsDNA) genome forming an intact HPV particle (Bienkowska-Haba, 2010).

Taxonomy of Papillomavirus

Evidences from evolutionary studies revealed that emergence of Papillomaviruses, PVs, begun with the evolution of reptiles, about 350 million years ago (Fig.1). The absence of PVs in amphibians and lower phyla supports this notion of evolutionary time. Then, PV evolved with their hosts in each speciation processes (Doorbar *et al.*, 2015). Hence, PVs are parasitizing human, mammals, birds and reptiles (de Villiers, 2013).

Co-evolution of PVs with their hosts restricted the viruses to be species specific and enables it to establish chronic infections (Doorbar *et al.*, 2015). Of all PVs, HPVs are the

most studied viruses due to their incidence malady mainly cervical cancer (Bernard *et al.*, 2010). Scientists classified HPV in five genera (Alpha-, Beta-, Gamma-, Mu- and Nu-PVs).

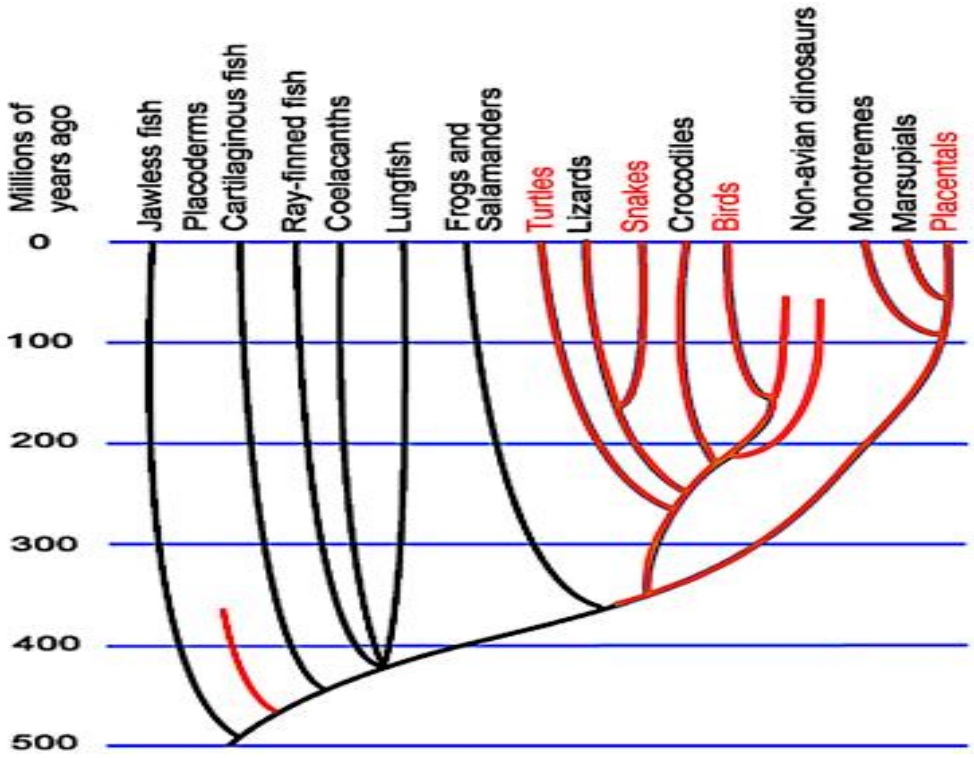

Figure 1: Evolutionary tree of Papillomavirus (Doorbar *et al.*, 2015)

Previous classification of Papillomavirus, PV, was under the family of Papovaviridae, together with the Polyomavirus. However, clear differences in genome size (8Kb Vs 5Kb), virion size (55 Vs 40nm), low DNA hybridization, and antigenic properties separate them into two families, Papillomaviridae and Polyomaviridae (Alba *et al.*, 2009).

Papillomaviridae are classified using the relatively conserved gene, *L1*, nucleotide sequence relatedness (De Villiers *et al.*, 2004a). Its sequence allows genome based scheme for classification, nomenclature and construction of phylogenetic trees. Thus, de Villiers *et*

al (2004b) proposed a classifications system to represent PV Family, Genus, Species, Type, Subtype, and Variants (De Villiers *et al.*, 2004b) (fig.2).

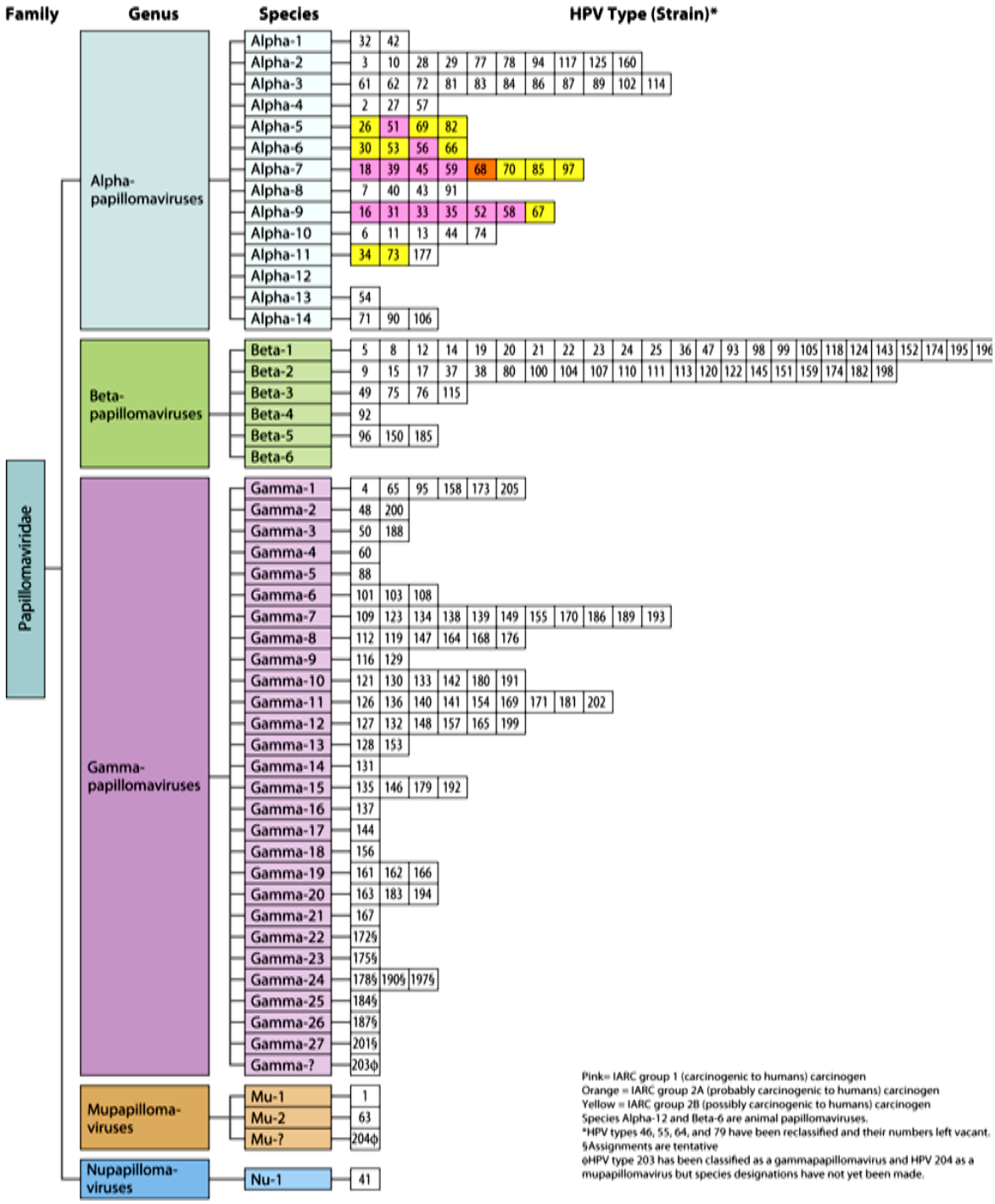

Figure 2: HPV classification and naming (Burd, 2016)

Moreover, they commenced nomenclature of HPV genera using Greek letters. And, they defined the topology of HPV phylogenetic trees, biologically distinguishing features (host, target tissue, pathogenicity, and genome organization), and set quantitative threshold in *L1* nucleotide sequence for classification. Consequently, the description of types, subtypes and variants is accepted to be a sequence difference of *L1* gene to be >10%, 2-10% and < 2%, respectively(De Villiers *et al.*, 2004b; Calleja-macias *et al.*, 2005).

However, many new HPVs are being discovered that create instability to the classification system (de Villiers, 2013). For instance, after 2004, 13 new distinct genera were discovered from different hosts (totally 29 genera) exceeding the 24 Greek letters; that demanded amendment of classification and nomenclature system (Bernard *et al.*, 2010).

Human Papillomavirus genome organization, transcription and expression patterns

Characteristically, all HPVs have single circular double stranded (ds) DNA about 8k base pair (bp) (Motoyama *et al.*, 2004) long with minor differences (Motoyama *et al.*, 2004; Burk, Chen and Van Doorslaer, 2009). It is organized into two; coding and a none coding regions (Lazarczyk *et al.*, 2009). The non-coding region is about 400 to 1kilo base pairs (Kbp) long. It contains a variety of *cis*-elements that regulate promoter regions like p97 (HPV 16) and gene expression patterns (early and late regions). The coding region in turn has two regions that encode functional (Early) and structural (late) proteins. The early region constituting ORFs of E1, E2, E4, E5, E6 and E7 encode proteins that are involved in HPV replication and pathogenesis. These proteins are responsible for its replication, transcription and controlling cellular functions (fig.3) (Graham, 2012; Johansson and Schwartz, 2013). The late region contains *L1* and *L2* ORFs encoding L1 and L2 structural proteins that are expressed at the end of HPV life cycle that are used for virion encapsidation (Villa, 2006).

Its gene replication, transcription and expression system is complex that employs many cellular and transcription factors. These mediators attach in the LCR and outside it leading to various transcription products. Early transcription produces polycistronic pre-mRNA which is alternatively spliced into several mRNAs (De and Fernandes, 2013).

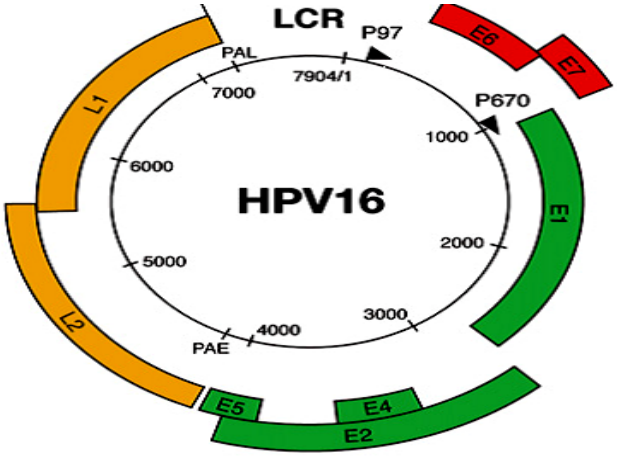

Figure 3: Schematic representation of HPV genome structure (Villa, 2006)

HPV gene expression patterns are systematically controlled and coordinated with the differentiation program of keratinocytes. At the basal cells, the genome is replicating slowly and maintained at low copy number via E2 mediated replication control mechanisms (Doorbar *et al.*, 2015). It attaches to *cis*-acting elements and tightly closes the expression of the oncogenes, E6 and E7, maintaining low genome copy number at the basal cells. However, gene expression is high at the stratum spinosum as the differentiation-dependent late promoter induces high-level viral gene expression. Consequently, the viral genome is amplified in hundreds (Stanley, Pett and Coleman, 2007).

Life cycle of Human Papillomavirus

The main target or host cells of genital HPV are the undifferentiated basal cells. These cells are exposed to HPV as a result of micro trauma created due to many activities including sexual intercourse. Thus, the virus, deposited on the epithelium from infected partner, may fall into the micro-trauma and gets access to the basal cells (Graham, 2012). Many researchers indicated that the first HPV particle to basal cell dialogue is via L1 capsid protein. In line with this, it is found that HPV 16 establishes infection after interacting with the mucosal epithelium mediated via conserved lysine residues on the capsid protein L1 located at positions K278, K356, and K361, constitute binding sites (Biryukov and Meyers, 2015).

These conserved L1 binding sites interact with cellular receptors, heparan sulfate proteoglycan (HSPG) molecules (Surviladze, Sterkand and Ozbun, 2015). This was confirmed using in vitro and mouse model experiments. An excellent experiment conducted using K562 wild (no HSPG expression) and transfected with HSPG gene cells showed that HPV 11 and HPV16 VLP attached effectively to K562 cells that express HSPG than wild K562 cells. This confirms the importance of HSPG for effective HPV adsorption and entry (Shafti-keramat *et al.*, 2003). Likewise, employing attachment inhibitors, carrageenan (Lowy *et al.*, 2006), structurally similar to HSPG, dispirotripiperazine (DSTP27), heparinase and neutralizing antibodies (Selinka *et al.*, 2007) that interact with HSPGs, effectively inhibits initial HPV attachment and subsequent entry (Lowy *et al.*, 2006). This confirms the importance of HSPGs for initial attachment of HPV.

The interaction between the specific L1 residues with HSPG destabilizes the capsid structure that exposes hidden L2 protein, *N*-terminus, which is subjected to furin cleavage (Richards *et al.*, 2005). Furin cleavage exposes two, 13 to 31 and 108 to 120 amino acid

residues, critical regions that mediate secondary attachment to unknown receptor (Kawana *et al.*, 2001; Yang *et al.*, 2003).

Many researchers confirmed that HPV is internalized via receptor, clathrin and caveolae, mediated conduits (fig 4). To confirm this, many researchers reported that HPV 16 and HPV 58 use clathrin-mediated endocytosis while HPV 31 uses caveolae mediated endocytosis to enter their host cells (Bousarghin *et al.*, 2003; Selinka *et al.*, 2003). Importantly, it is clearly indicated that HPV 31 is highly sensitive to caveolae inhibition (Smith, Campos and Ozbun, 2007).

Uncoating and Nuclear entry

Once the endocytic vesicle reached the cytoplasm, the L1 major coat protein starts to dissociate from its partner L2, the minor capsid protein. Low pH and cellular proteins, cyclophilins, mediate the dissociation process (Bienkowska-haba *et al.*, 2012; Sapp and Weiller, 2013). Then, the L2-DNA complex sorted out from L1 and escapes from the endocytic vesicle (fig 5) (Ka *et al.*, 2006). Then, it was found that the L2-DNA composite trafficking to the trans Golgi network (TGN) employs retromer subunits (Lipovsky *et al.*, 2013; Sapp and Weiller, 2013).

Then, once the complex is in the lumen of TGN, it remained there until the onset of early stages of cellular mitosis (Digiuseppe, Bienkowska-haba and Sapp, 2016). However, at a later stage of the mitosis, the complex leaves TGN in a transport vesicle associated with condensed mitotic chromosomes. Then, the HPV DNA become visible in the nucleus when the nuclear membrane reorganizes (Digiuseppe, Bienkowska-haba and Sapp, 2016).

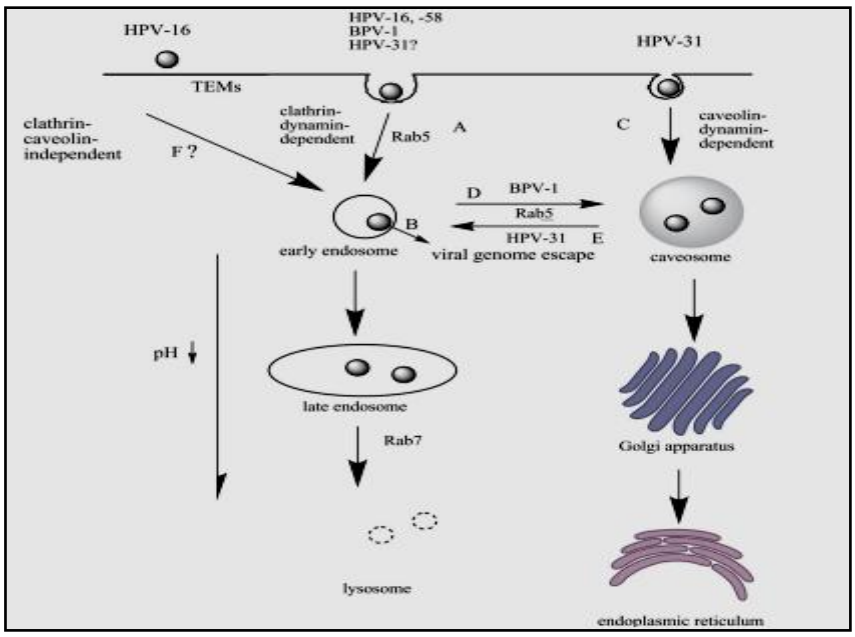

Figure 4: Endocytic pathways of HPVs adapted from (Letian and Tianyu, 2010)

Biological activities of HPV proteins in the cervical epithelium

The HPV genome once in the nucleus of the daughter cell, immediate transcription leads to the detection of early transcripts E1 and E2 proteins as early as 4hrs post nuclear entry while early replication is detectable after 8hrs (Ozbun, 2002).

Early proteins E1 and E2

E1 is the only HPV encoded protein that has enzymatic activity; helicase and ATPase (Bergvall, Melendy and Archambault, 2013) while E2 does not have enzymatic activity. But it has multitude of functions in the HPV life cycle that spans transcriptional regulation, initiating DNA replication, partitioning HPV DNA among daughter cells, virion packaging etc (McBride, 2013).

E1 has three regions specially designed for specific activities while E2 needs dimerization with E1 to exert its biological activities. Its N-terminus is important for exciting phosphorylation of CDK2 to induces cell cycle. Second the central part of the peptide has E2 binding site.

The E1:E2 dimer complex interacts with high affinity and specificity at the HPV origin (Ori) of replication (Bergvall, Melendy and Archambault, 2013). Furthermore, it recruits cellular replication mediators (polymerases) to begin DNA amplification and maintenance (Longworth and Laimins, 2004; Ammermann *et al.*, 2008). Thus, early amplified HPV genome copy number per infected basal cell reached between 50 and 100 copies (Doorbar *et al.*, 2015). Then, HPV DNA may replicate in an ordered manner once per S-phase (Hoffmann *et al.*, 2006) with the basal cell. That is, when the basal cell divides into two, similarly the HPV DNA doubles, segregate and shared among the daughter cells (Jr *et al.*, 2015). The third region, the C-terminus, is the region with helicase activity (Bergvall, Melendy and Archambault, 2013) that unwinds the HPV genome (Araldi *et al.*, 2017).

Besides, E2 has four binding sites that found two at proximal to the early promoter, one at the Ori and one at the enhancer regions of the LCR (Graham, 2016). First, it serves as replication factor that recruits E1 to the Ori of replication. Second it controls the expression level of E6 and E7 oncogenic proteins. At high concentration, E2 binds to a specific palindrome (5'-ACCG (N4) CGGT-3') sequence in the early promoter P97 that blocks E6 and E7 expression. Hence, it acts as transcription repressor. Third E2 distributes viral genome among daughter cells during mitosis (Nishimura *et al.*, 2000; McBride, 2013; Graham, 2016).

Therefore, HPV DNA established as episomes in the nucleus of undifferentiated basal cell. Then, genomic replication, expression, assembly and virion release are tightly synchronized with the different differentiation stages of the keratinocytes (Graham, 2012).

Ultimately, HPV needs getting its genome amplified and late proteins expressed for final viral assembly so as to release the progeny particles to the immediate environ while the keratinocyte is differentiating. However, differentiating keratinocyte has minimal replication related resources including nucleotides, polymerases etc. To crack this crisis and achieve its biological fitness, HPVs express viral *E6* and *E7* genes that produce oncoproteins E6 and E7, respectively.

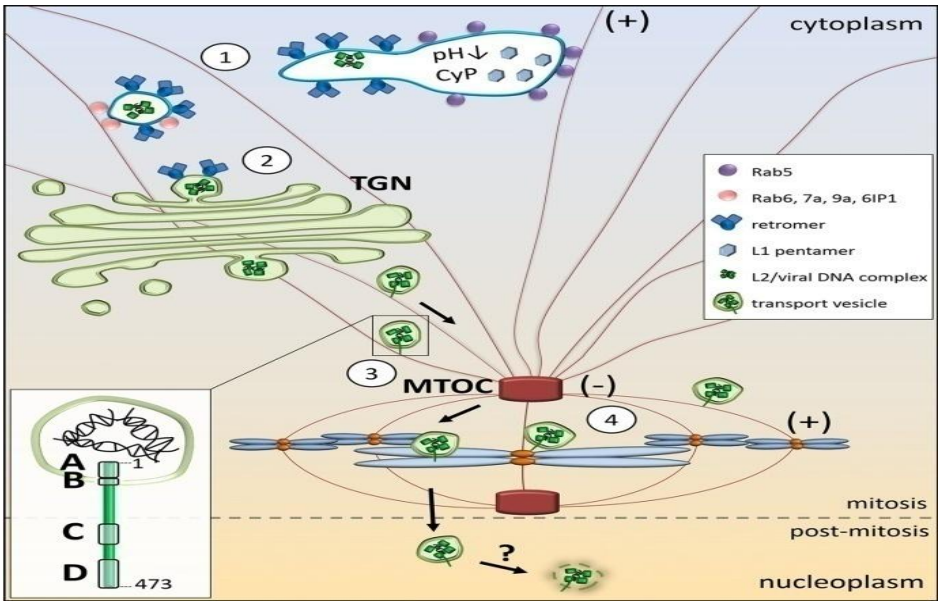

Figure 5: Schematic representation of HPV16 translocation (Digiuseppe, Bienkowska-haba and Sapp, 2016)

Early protein 6 is small, 150 amino acid long (HPV16) oncoprotein. Structurally, it contains four C-x-x-C motifs. These motifs are critical players of E6 function, such as transcription, transformation, immortalization and association with cellular proteins. The first protein to associate with E6 is E6 associated protein (E6AP) at its LXXLL sequences (Scott B. Vande Pol1, 2014). This association is critical for achieving E6's functional conformation and stability. In other words in the absence of wild type E6AP, E6 cannot associate, ubiquitinate and mediate degradation of p53 (Wade, Brimer and Pol, 2008; James D. Baleja, Jonathan J. Cherry, Zhiguo Liu, Hua Gao, Marc C. Nicklaus, Johannes H. Voigt, Jason J. Chen, 2009; Zanier *et al.*, 2013).

Finally, the formation of protein complexes (E6, E6AP, and others) induce change of many molecular processes affecting cell survival, transcription, differentiation process, DNA damage response, and cell cycle (Scott B. Vande Pol1, 2014). For instance, E6-E6AP complex attaches to NFX1-9 (cellular repressor of hTERT promoter) and induce its degradation that allows myc binding to the promoter and activates hTERT expression, extends telomere to avoid cellular senescence (Narisawa-Saito and Kiyono, 2007).

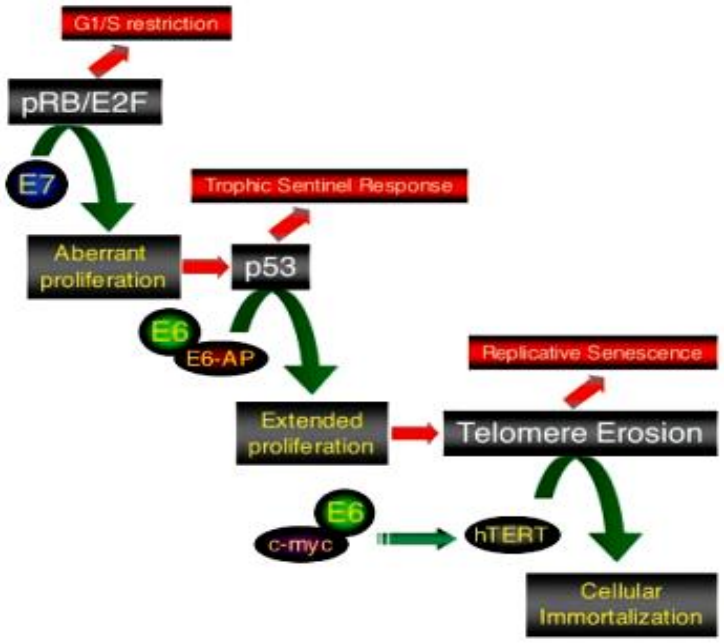

Figure 6: Schematic representation of E6 and E7 mediated transformation of cells (Münger *et al.*, 2004)

Early protein 7 (E7) is another oncogenic polypeptide that binds to protein retinoblastoma family at their conserved binding motif LXCXE. It hyper phosphorylates pRb to release E2F where free E2F induces synthesis of cycine E (Fig. 7). Cycline E in turn activates transcription of cycline dependent kinase 2 (CDK2) (Jo and Kim, 2005; Moody and Laimins, 2010). Then, cycline E and CDK2 induce cell cycle entry, S-phase (Bertoli, Skotheim and de Bruin, 2013). Such cells, in an artificial S-phase, create a resourceful and

rich modified cells suitable for HPV replication (Münger *et al.*, 2004; Narisawa-Saito and Kiyono, 2007; White *et al.*, 2012) (fig.6).

Assembly and release

The final step of HPV lifecycle is producing infectious virus particles. In productive HPV infection the host cells differentiate slowly as a result of E2 mediated early promoter (p97) suppression that arrest *E6* and *E7* genes (Burd, 2003). This phenomena activates the second promoter p670 (HPV 16) at the upper layer of the epithelium to synthesize *L1* and *L2*, capsid proteins (Conway and Meyers, 2009).

The L1 capsid protein has self assembly property to form capsomers. It has amino acid sequences that form intra- and inter peptide binding as well as hydrophobic sequences that bind to C-terminus of the minor capsid protein, L2. Besides, the L2 N-terminus has sequences that bind to the HPV genome (Hughes, 2013). Therefore, during encapsidation, E2 protein recruits the minor capsid protein, L2, to bind to the amplified HPV genome. Then, via its C-terminus, L2, recruits L1 creating L1-L2-DNA nucleation (Hughes, 2013) for virion assembly. Then, L1 forms pentamers and many such capsomers wrap up the viral DNA in the nucleus to form new HPV virion (Conway and Meyers, 2009; Hughes, 2013; Cerqueira *et al.*, 2015). At this point, the virions are inside the keratinocyte and need to be released to the external milieu to initiate new cycle of infection (Hoffmann *et al.*, 2006) in the same women or to infect exposed partner (Fig. 6 A and B). This escape mechanism is taught to be effected via early protein 4.

The E4 protein encoding gene is located within E2 ORF. Some researchers reported that E4 is the most expressed protein at the upper strata and it is multifaceted functions (Nakahara *et al.*, 2005). In a productive HPV infection, it is expressed before L1 and L2 proteins from a spliced mRNA of E1^E4 (Doorbar, 2013). Thus, many researchers claim

that E4 protein is important for viral escape mechanisms from the squame via deregulating cytokeratin filaments (Graham, 2012).

The other outcome of HPV infection is abortive infection. In the case of abortive HPV infection some of the differentiating cells contain integrated HPV DNA within the host genome. Integration of HPV DNA into the host cell genome mostly occurs at the E2 ORF that fragments the *E2* gene. Loss of *E2* gene leaves p97 open, boosting the expression of E6 and E7 oncoproteins. These proteins interact with their substrates, p53 and pRb, forcing the host cell enter cell cycle leading to tumor formation (Doorbar *et al.*, 2015).

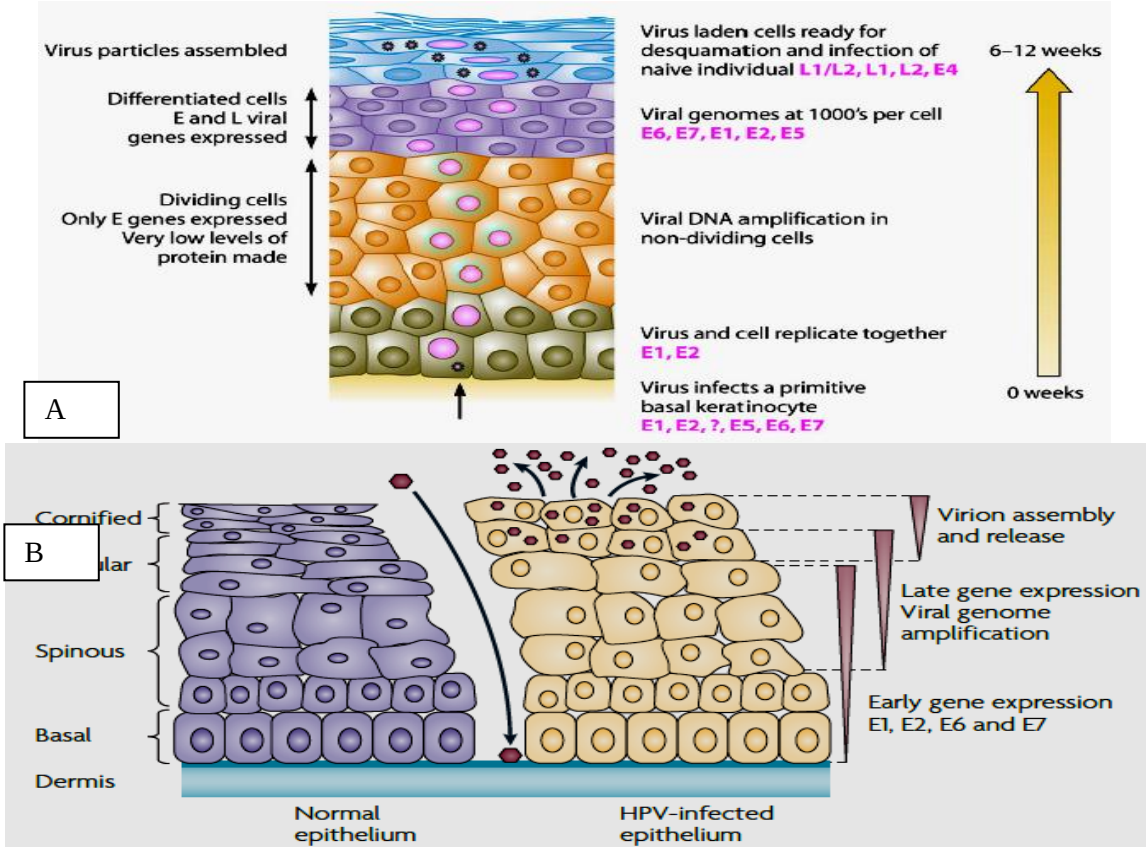

Figure 7: Comparison of normal and anbormal cervical epithelium (A) and life cycle of HPV(B) (Stanely, 2012)

2.2. HPV pathogenesis

HPV infection is one of the most prevalent sexually transmitted infectious agents. It establishes infection in the basal cells of the cervix. Then, after entry to the host cell's nucleus, it starts replicating in line with the natural biology of the epithelial cells with minor damage (Horvath *et al.*, 2010). However, deviation from the normal productive infection, HPV infection is usually associated with a number of sorrowful maladies. Of all, instigation of cervical cancer is the major one (Jo and Kim, 2005) others include cancers of the vagina, Vulva, penile, anal, a fraction of head and neck cancers, genital warts etc (Mclaughlin-Drubin and Münger, 2010).

Noble laureate Harald zur Hausen, in 1975, was the first to hypothesize the possible role of HPV infection in the development of cervical cancer. Consequently, HPV 16 and HPV 18 were isolated from cervical cancer specimens. Then, several molecular and epidemiological studies established that HPV is the prime etiologic agent of cervical cancer (Bosch *et al.*, 2002; Alba *et al.*, 2009). As a result, researchers identified 12 to 15 high-risk HPV types that are usually associated to cervical cancer (Munoz *et al.*, 2003).

These HPV types differently and continuously express copious amount of the oncogenic proteins, E6 and E7, to induce degradation of the tumor suppressor proteins, p53 and pRB, respectively (fig.6) (Villa, 2006). As a result of impaired p53 and pRB function some cells re-enter into cell cycle. Moreover; E6 and E7 increase genomic instability, accumulation of mutations, loss of apoptosis and cell cycle control, and ultimately induce development of cervical cancer (Kadaja *et al.*, 2009).

The other mechanism by which HPV induces cancer is by integrating its DNA into the host genome as confirmed via analysis of cervical specimens. The curse of HPV DNA integration is that the integration process disrupts E2 gene, leaving the early promoter, p97

(HPV 16) open (Mclaughlin-Drubin and Münger, 2010) leading to free and consistent expression of E6 and E7 oncoproteins. In turn, these proteins eliminate p53 and pRb from such cells. Then, the cells immersed into uncontrolled cellular proliferation that end up in cancer (Kadaja *et al.*, 2009). Thus, it was confirmed that cells with integrated HPV genome have a selective growth advantage over cells with episomal viral DNA (Moody and Laimins, 2010) and finally forming cervical cancer precursors.

To sum up the interactions between E7-pRb and E6-p53 force the victim cell to proliferate wildly and immortalized; and accumulate cellular mutations (fig.8). So, this abnormal and uncontrolled cell growth establishes pre-cursor lesions that may end up in malignancy (Doorbar *et al.*, 2015).

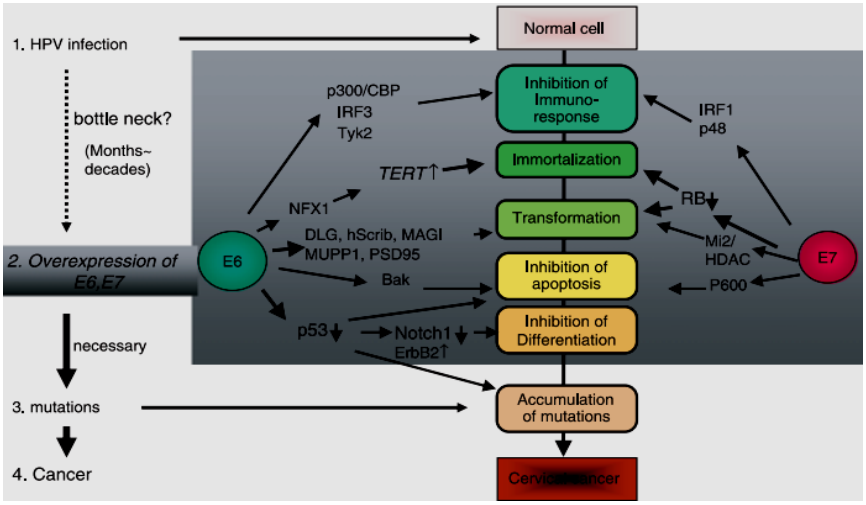

Figure 8: The effect of E6 and E7 mediated carcinogenesis (Narisawa-Saito and Kiyono, 2007)

Immune evasion mechanisms

One of the most important pathogenesis factors is immune avoidance or immune evasion mechanisms of pathogens. With this aspect, HPV is the most successful pathogen that employ strategies to blind or evade the host immune system.

Unique life cycle; HPV life cycle or its biological success is fully dependent on the biology of cervical epithelium. When the basal cells divide, the virus replicates its genome and shares its genome among daughter cells. Then, when one of the daughter basal cells moves upward and undergoes terminal differentiation process, the virus completes specific biological process at each stratum. Finally, the infected keratinocyte naturally desquamates, harboring hundreds of viral particles. Therefore, HPV completes its life cycle without the following common consequences of viral infection (Williams *et al.*, 2011):

- HPV never cause any cytopathic death on infected cells under normal conditions.
- No viremia in its life cycle that avoids interacting with immune cells and molecules.
- HPV never escape from infected cells via lysis or any form of virus induced cell death. Therefore, the immune system is blinded via well organized immune evading life cycle (Williams *et al.*, 2011).

Upon HPV infection, basal cells never or minimally release proinflammatory cytokines. At the lower strata, there is only transient protein synthesis, minimal replication, up to 100 copies, hence reduce escape of antigens. Thereby, immune surveillance and antigen presenting cells (APC, Langerhans cells) can't locate and induce immune responses. HPV infection is entirely intraepithelial. Therefore, the virus could stay for a long time without immune recognition. Moreover, HPV mediated immune suppression is indicated via HPV E5, a potent regulatory of MHC molecules (Ibeanu, 2017) to avoid antigen presentation.

2.3. Epidemiology of Human Papillomavirus and cervical cancer

Global epidemiology of HPV and Cervical cancer

Genital HPV infection represents the most common viral STIs and a major public health problem leading to significant morbidity and mortality rates worldwide. Moreover, it is estimated that up to 80% of women will be exposed to HPV by the age of 50 indicating

that HPV infection is common and could affect almost every individual at least once in life time. The main root of transmission for genital HPV is via unprotected genital contact (Kru *et al.*, 2001). Hence, the greater the number of sex partners, the greater the risk of HPV infection. Hence, sexually active young and young adults are the top risk group (Kru *et al.*, 2001) for HPV infection.

Table 2. 1: Cervical cancer incidence and mortality rate in Africa (Smith *et al.*, 2008)

Region	Annual incidence			Annul mortality rate		
	Cases	Crude rate	ASR	Cases	Crude rate	ASR
Africa	99,038	18.5	27.6	60,098	11.2	17.5
Eastern Africa	45, 707	25.8	42.7	28,197	15.9	27.6
Middle Africa	11,540	17.2	30.6	7,917	11.8	22.2
Northern Africa	5,813	5.6	6.6	2,717	2.6	3.2
Southern Africa	8,652	29.3	31.5	4,721	16.0	17.9
Western Africa	27,326	17.2	29.3	16,546	10.4	18.5

HPV infection may lead to genital warts or cancers (Ghittoni, 2015); where cervical cancer is the most important cancer among women. Therefore, based on their association with cervical cancer and their epidemiology mucosal HPV types were grouped into three categories (fig.2) as: High-risk HPV virus is changing from time to time and currently 12 (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59) being considered as high-risk HPV types. However, diagnostic methods include HPV 66 and HPV 68 to the high-risk group and many researchers also consider these two HPV types as high-risk HPV types (Wang *et al.*, 2015; Burd, 2016). Putative carcinogenic viruses HPV 26, 53, 70, 73, and HPV 82 associated with cervical cancer in a few case-control studies. Finally, low-risk HPVs (HPV 6, 11, 40, 42, 43, 44, 54, 61, 72, 81 and 89), were associated with benign warts (Smith *et al.*, 2007).

Cervical cancer molecular studies showed that HPV DNA could be isolated from ~99.7% of all cases (Walboomers *et al.*, 1999) confirming its importance in initiating cervical cancer and maintaining cancer phenotype (Mclaughlin-Drubin and Münger, 2010).

Although all women are at risk of developing HPV induced cervical cancer; few women develop cervical cancer (CDC, 2007). The incidence and mortality rates vary as a function of ethnicity, demography, geography, socioeconomic status, and access to healthcare facility (Bruni *et al.*, 2010). Thus, the highest incidence of morbidity and mortality rates occur in developing countries of mainly sub-Saharan Africa and South America constituting more than 85% of morbidity and 88% of mortality rates (Arbyn *et al.*, 2011).

The 2012 global cervical cancer incidence and mortality rate was 527,624 and 265,700, respectively (Torre *et al.*, 2015). While it is the most common women's cause of cancer death in Sub-Saharan Africa as about 60-75% of cervical cancer patients live in rural area with poor accessibility of health care facility go untreated. Eastern Africa is a region with the highest prevalence of HPV infection and cervical cancer. In line with this, poor socioeconomic and educational status, high HIV prevalence, war, drought, instability and rural residence have been fuelling it in the region (Table 1).

Although most HPV infections clear after certain period of time, some women develop cervical cancer. This difference is largely attributable to the HPV type involved and individual genetic difference (Brown and Trimble, 2012). Moreover, there are several risk factors thought to play a role in the development of cervical cancer; susceptibility, nutrition, immunosuppression, endogenous and exogenous hormones, prolonged use of oral contraceptive, age of sexual debut, number of sexual partners, smoking, parity etc (Castellsague 2002). Co-infection with HIV(Adler *et al.*, 2016) enhances immune suppression and pathogenesis, HSV-2 (Bosch, Qiao and Castellsague, 2006; Williams *et al.*, 2011), *C. trachomatis* (Verteramo *et al.*, 2009a) found to enhance inflammation that increases HPV infection and pathogenesis.

Genital HPV transmission and symptoms

The principal route of transmission of genital HPV is unprotected sexual contact (Winer *et al.*, 2008). This is confirmed by the fact that sexual partners found to be infected by identical genotype, the rarity of genital HPV infection in virgin women(Kru *et al.*, 2001), and increased risk of HPV acquisition following new and recent sex partners. Moreover, HPV acquisition is prevalent in adolescents and sexually active individuals. Rarely mother to child transmission occurs during delivery that results in recurrent respiratory papillomatosis (Kru *et al.*, 2001).

HPV infection and early stage cervical cancer don't show evident symptoms. A watery discharge may be noted which will eventually become abnormal vaginal bleeding, often post coital or intermenstrual or post menopausal time (WHO, 2013). Symptoms are usually associated with advanced stage disease progression that may include lower back pain, longer menstruation cycles and kidney distention due to obstruction of urethra, bladder invasion causes haematuria, and pain during sex.

Risk factors for HPV acquisition

Many researches confirmed that the main route of transmission for genital HPV types is unprotected sexual contact. Thus, the virus acquires access to a new host after an infected partner engaged in unprotected sexual intercourse. Therefore, a woman with many partners or a monogamous woman with polygamous husband is at greater risk of acquiring HPV infection. Many other risk factors contribute to HPV acquisition, persistence or clearance. To mention some; number of sexual partners, sex debut, smoking, oral contraceptive etc were proposed as important risk factors(Vaccarella *et al.*, 2006; Roura *et al.*, 2012; Castellsagué *et al.*, 2014).

Number of partners: it is repeatedly indicated that number of sexual partners is an important and significant factor for HPV acquisition. Increasing number of epidemiologic studies indicated that women who had more than one sexual partner are at increased risk of HPV infection. The IARC encompassing 9 countries from four continents found that number of life time sexual partners is a clear risk factor for HPV positivity (Vaccarella *et al.*, 2006). Educated reports from the randomized controlled Patricia trial researcher found more than one number of partners was associated with increased risk of HPV infection (Castellsagué *et al.*, 2014). In line with this, the Denmark cohort study indicated that having ≥3 life time sexual partner is associated with strong association of risk of HPV infection. Similar conclusions reached from USA a study that recruited and followed virgin participants (Kru *et al.*, 2001; Roura *et al.*, 2012) and their HPV acquisition dynamics. Moreover, the number of sexual affairs of the male partner is also highly associated with HPV acquisition. In a large study, strong association (OR, 1.4; 95%CI: 1.24, 1.70) of HPV infection among women whose male partners had extra sexual affairs was reported (Vaccarella *et al.*, 2006). Moreover, women whose first sexual partner with sexual experience were at higher risk of HPV infection at first sex debut (Vaccarella *et al.*, 2006; Winer *et al.*, 2008). Therefore, number of sexual partners of both partners is independently and strongly associated with HPV acquisition.

Sexual debut

Sexual debut at early age, before the age of 18 is a risk factor for HPV infection and development of cervical abnormal Pap smear test. In line with this, researchers clearly identified that early sexual debut or marriage (≤18 Years old) was strongly and significantly (P=0.001) associated with HPV infection among their study participants (Khalaf, Rasheed and Hussain, 2015; Niyazi *et al.*, 2015). However, many large cohort studies could not clearly confirm early marriage or sexual debut as the main risk factor for HPV acquisition (Roura *et al.*, 2012; Castellsagué *et al.*, 2014). Therefore, early sexual debut or marriage is debatable risk factor.

Infection with other sexually transmitted infectious agents

Sexually transmitted infections are bacterial, viral or protozoan infectious agents that are transmitted via sexual activity. These include *Chlamydia trachomatis, Neisseria gonorrhea, Trepanoma pallidum, Trichomonas vaginalis*, Herpes Simplex Virus (HSV-2), HIV etc. These infections impose health problems associated with sever morbidity, mortality and financial burden. Some of these infections cause life threatening diseases such as AIDS, ectopic pregnancy, infertility, PID etc(Mu, 2014).

Moreover, some of these infections were found to enhance HPV infectivity and its consequences directly or indirectly. These infectious agents increase vulnerability of women to cervical cancer via suppressing the immune system or inducing host DNA damage and deleterious mutations. These STIs include *C. trachomatis, N. gonorrhea*, HSV-2 etc (Verteramo *et al.*, 2009b).

Chlamydia trachomatis

Chlamydia trachomatis is one of the most common bacterial infectious agent all over the world. It causes asymptomatic infection with a lot of consequences. Its infection induces intense inflammation and disrupt the integrity of cervical epithelium that increases the chance of HPV infection. Moreover, persistent inflammation destabilizes host cell genome which in turn enhances HPV DNA integration favoring cervical cancer (Madeleine *et al.*, 2007; Verteramo *et al.*, 2009a). Consequently, it was clearly indicated that SCC increase in association to increased level of *C. trachomatis* antibodies. Thus, *C. trachomatis* positive women were 1.6 (95%CI: 1.1, 2.2) times more likely to develop SCC than *C. trachomatis* negative women (Madeleine *et al.*, 2007). Moreover, HPV co-infection with *C. trachomatis* increases the risk of SCC progression by 4.03 (95%CI: 3.15, 5.16) (Zhu *et al.*, 2016). Thus, it was forwarded that integrating *C. trachomatis* screening with HPV and cervical cancer screening programs will be effective in reducing the burden of cervical cancer (Zhu *et al.*, 2016).

Neisseria gonorrhea

Neisseria gonorrhea is gram-negative diplococcus bacterium and strict human pathogen. It is one of the most prevalent sexually transmitted infectious agents with an annual incidence of more than 100 million individuals (Sangita *et al.*, 2016). Infection with *N. gonorrhea* has many devastating consequences to the cervical epithelium. Researchers found that *N. gonorrhea* infected cells have high level of double strand DNA break and abolish p53 expression (Vielfort *et al.*, 2013). This trait of *N. gonorrhea* is an import factor that increases HPV infection and integration of its DNA into the host genome that lead to cervical cancer precursors.

Moreover, *N. gonorrhea* is becoming one of the most medically important bacteria in its drug resistance. Some isolates are becoming superbugs resisting to all drugs consequently researchers are calling for development of vaccine against this bacteria(Unemo and Dillon, 2011; Brunner *et al.*, 2014).

Herpes Simplex virus (HSV-2)

Herpes simplex viruses (HSV) are enveloped DNA viruses that infect epithelial and neural cells. HSV 2 is one of the most common STIs that cause latent and recurrent infections with important morbidity (Mu, 2014). Its replication is associated with lysis of epithelial cells and disrupts epithelial surface integrity causing ulcers that could expose the basal cells (Caldeira *et al.*, 2013). It encodes enzymes that could destabilize and mutate host DNA and impairs the immune system via suppresses antigen presentation. All these outcomes of HSV-2 create conducive environment for HPV infectivity and its persistence (Raju, 2015).

Human immunodeficiency virus (HIV)

It is known that HIV infection destroys CD_4T-cell mediated responses that impairs cell mediated immune response (Verma *et al.*, 2017) which is the major HPV fighting arm of our immune system. Hence, HIV infected women have high rate of HPV infection and persistence. Conversely, it was confirmed that an effective antiretroviral treatment was associated with HPV clearance due to restoration of CD4+ T cell population (Mooij *et al.*, 2014).

Bacterial vaginosis

Bacterial vaginosis (BV) is a common infection worldwide among women in the reproductive age (Bautista *et al.*, 2016) manifested with abnormal vaginal discharge with foul odor and replacement of the *Lactobacilli* dominated normal flora with *G. vaginalis* dominated abnormal polymicrobial growth (Ling *et al.*, 2010; Onderdonk, Delaney and Fichorova, 2016). This results in pH increment as a result of reduced lactic acid production and increased amine production from anaerobic bacteria which is associated with positive whiff test. Then all these associated with adverse outcomes of pregnancy including preterm birth, recurrent abortions etc. Its specific causative agent is unsettled issue.

The notion that BV is sexually transmitted infection remained controversial. Some researchers argue that BV is sexually transmitted infection taking into account isolation of same BV associated infection after sexual debut and presence of the same bacteria in male partners; its association with risky sexual behavior (Muzny and Schwebke, 2016). However, other researchers claim that BV could be considered as sexually enhanced disease but not sexually transmitted infection, as BV associated organisms exist among virgin women, with no experience of vaginal sex (Verstraelen *et al.*, 2010). However, the first concept, BV is a sexually transmitted infection, is getting momentum.

Epidemiologically, BV is more associated with blacks or hispanic and with low socioeconomic status. The difference may be due to difference in mucosal immunity (genetic difference), hygienic practices etc (Mitra *et al.*, 2016). It may be symptomatic or asymptomatic condition. Moreover, it is a risk factor for many STIs more importantly to HPV, HIV, *N. gonorrhea, C. trachomatis* etc (Schwebke *et al.*, 2016).

There are two reliable and less expensive diagnostic methods of BV, the Nugent score and Amsel's criteria that contributed a lot in the reduction of BV associated outcomes (Rao *et al.*, 2016). However, as more and more information about the composition of the polymicrobial growth becomes clear, BV is largely associated to some bacterial species that synergistically orchestrate its occurrence. Consequently, researchers developed PCR techniques to identify the composition of microbes in liquid based genital swabs. One of these PCR techniques is sexually transmitted infection profiling (STIP) developed by Schmitt *et al* (2014). STIP is a high throughput multiplex PCR technique that detects as many as 18 STI agents using a multiplex PCR amplification followed by bead-based hybridization, detection using agent specific probes using Luminex as described elsewhere (Schmitt *et al.*, 2014).

Consequently, Schmitt *et al* (2014) standardized a BV scoring system using the most common and strongly BV associated bacteria *A. vaginae, G. vaginalis* and *M. hominis*. The scoring techniques is based on the ratio of *G. vaginalis* to *Lactobacilli* (Gv/L), *A. vaginae/ Lactobacilli (Av/L)* and their coinfection as well as presence of *M. hominis.* The score ranges from 0 (negative) to 5 (very strong) BV (detail in section 4).

2.4. Outcomes of HPV infection

Cervical cancer

Cervical cancer is the second most common cancer among women globally. The uterine cervix is the lower portion of the uterus that has three portion; ectocervix is the visible

external part, transformation zone and the endocervix is largely invisible upper part of the cervix. The ectocervix is covered by pink stratified epithelium of squamous cells, thin and flat cells while the endocervix is covered by glandular cells (columnar). The junction between the two types of cells contains both types of cells, squamocolumunar cells, is called the transformation zone (IARC group, 2015) where cervical cancer starts (fig.9).

Cervical cancer is an insidious disease where the early precursor lesions do not show decipherable sign and symptoms. As a result, women do not seek medical intervention until it reached advanced stages at which treatment may not reverse the damage. Thus, diagnosing cervical cancer after appearance of sign and symptoms may be useless or costly. On the other hand, if cervical cancer is diagnosed at its early stage, it is a manageable cancer. Other than cervical cancers, HPV cause vulva, vagina, penile and anal cancers (Bosch, Broker and Forman, 2014). HPV induced cervical cancer caused due to many interrelated genetic processes and dysregulated expression of HPV oncoproteins and inability of the cell to maintain genome repair and stability.

2.4.1. Preconditions for cervical cancer

Inflammation

Inflammation is one of the main inducers of reactive Oxygen and Nitrogen species formation (Williams *et al.,* 2011). These reactive Oxygen and Nitrogen species induce DNA damage. Therefore, inflammatory reactions in the presence of high concentration of amplified HPV DNA increase the chance of HPV DNA integration. Likewise, infectious agents that inherently induce inflammation are risk factors for HPV DNA integration. In this notion, *C. trachomatis* and Herpes Simplex virus 2 (HSV 2) are important risk factors that induce intense inflammatory reaction(Bosch, Qiao and Castellsague, 2006).

Integration of HPV DNA to host genome

Integration of HPV DNA into host cell genome is possible at sites where there is double strand breaks. Therefore, HPV proteins could create these DNA breaks or due to reactive

oxygen mediated oxidative stresses (Williams *et al.*, 2011) and impaired DNA repair mechanism. The most important check point is the G1/S check point which is followed by p53 mediated repair mechanism. Any defect in this check point and failure to repair leads to genomic instability in turn this is associated with oncogenesis (Bartek and Lukas, 2001). Many researchers reported that the early genes E1 and E2 are the sites where HPV DNA integrates to host genome. Recently, researchers evaluated cervical cancer samples to identify the HPV DNA regions that commonly integrate to host cells. They found that the HPV DNAs were integrated at the E1 and E2 genes disrupting these genes (Williams *et al.*, 2011; Liu *et al.*, 2016). This leaves the HPV early promoter (p97) open followed by expression of copious amount of the oncoproteins, E6 and E7. In such cells, E6 and E7 eliminate pRb and p53. Therefore, the host DNA re-enters cell cycle without proper surveillance and DNA repair mechanisms (Senapati, Senapati and Dwibedi, 2016).

Accumulation of DNA mutations

The above two condition, HPV DNA integration and inflammatory reactions, lead to excess production of oncoproteins and mutations. As a result of these events and impaired cellular DNA check points and repair system, hyper mutated DNA pile up in these cells that lead to cervical cancer (Williams *et al.*, 2011). Besides, researchers frequently identified host genome instability; rearrangements (Liu *et al.*, 2016).

2.4.2. Other HPV associated cancers

Head and neck cancer

Head and neck cancers (HNCC) is oncogenic HPV induced oropharyngeal squamous cell carcinoma. The most common sites for HNCC include oral cavity and tonsillar crypts, nasal cavity, larynx, hypopahrynx, and the oropharynx (Whang, Filippova and Duerksen-hughes, 2015). However, the distribution of the disease around the globe varies due to difference in HPV infection and associated factors including smoking, alcohol consumption, and hygiene (Whang, Filippova and Duerksen-hughes, 2015).

The other cancer is recurrent respiratory papilomatous (RRP) develops in babies due to HPV infection during vaginal delivery to infected mother. Papillomas develop in the airways cause respiratory obstruction and mostly not invasive. The most applicable solution for RRP is clearing the respiratory route via surgery but its recurrence is challenging (Bosch, Broker and Forman, 2014).

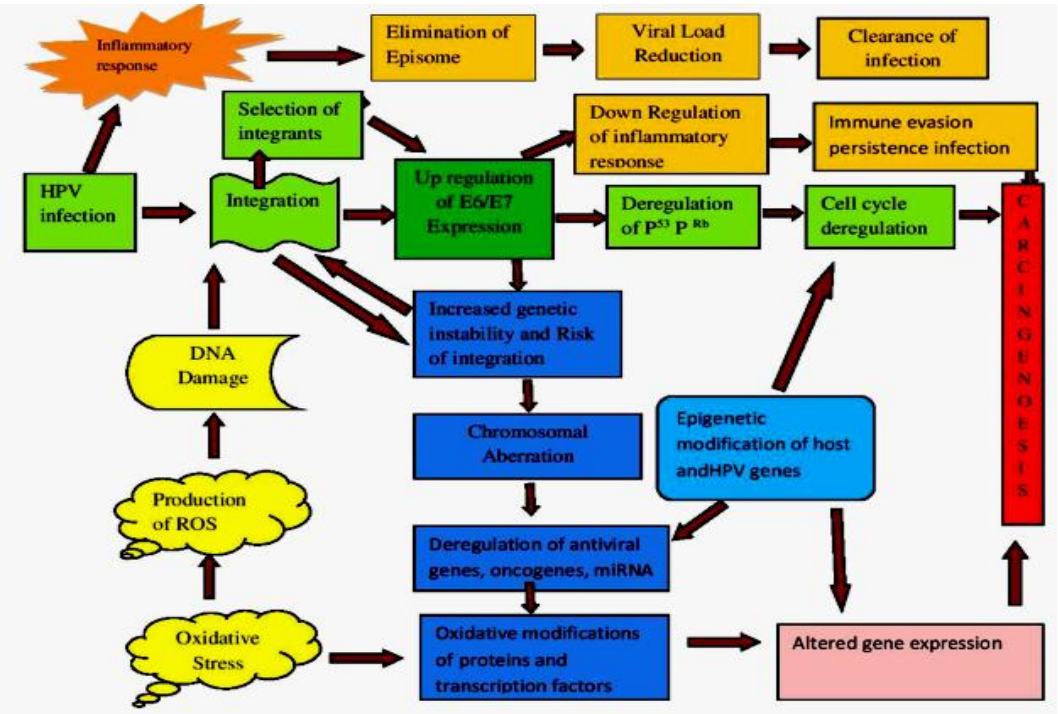

Figure 9: Description of HPV carcinogenesis (Senapati, Senapati and Dwibedi, 2016)

Moreover, less frequently, HPV causes penile, anal, vaginal etc cancers (Parkin and Bray, 2006). The anal epithelium has similar squamous cells to the uterine cervix. Specifically the anal canal has squamous cells while the rectum has columnar cells. Therefore, there is a junction between these two types of cells equivalent to transitional zone of the cervix. Infection of these squamous cells by HPV 16, 18, 6, 31, 52, 11, and 42 is leads to anal cancer. However, HPV 16 is the main risk factor for anal cancer as it is responsible for approximately 85% of anal cancer reviewed by Burd (2016).

2.5. Diagnosis

Early diagnosis is a milestone to avert the unacceptable women mortality due to cervical cancer. In a cohort study, Peirson and his colleagues reported that mortality rate was reduced by 35% among screened women than their unscreened counterparts (Peirson *et al.*, 2013). Therefore, to minimize unacceptable death of our mothers, instituting well organized screening program is critical. Moreover, scientists devised many alternative strategies to diagnose either the etiologic agent, HPV, or its protean morbidities, precancerous lesions or many stages of cervical cancer.

HPV and cervical cancer related diagnostic methods could be grouped as: direct and indirect methods. The direct methods are used to detect the virus, its products or antibodies mounted against it. Such methods are called molecular methods (Molijn *et al.*, 2005) that include: immunological, nucleic acid and protein detection methods. The indirect methods used to detect the outcome of HPV infection or clinical signs on cells or tissue but not the virus. They detect the presence of histological or cellular changes. Direct visual examination methods include VIA, Lugol's Iodine (VILI), colposcopy etc.

2.5.1. Nucleic acid based diagnostics

The main obstacle in HPV detection is the absence of cell culture method to cultivate and identify it. Thus, HPV detection depends mainly on molecular techniques including DNA and RNA identification methods. So far, different methods were developed and some of them were FDA approved. It is indicated that HPV based molecular diagnostic techniques have many possible clinical applications (Abreu *et al.*, 2012b) for follow up and triage of women diagnosed with cervical dysplasia and forecasting management outcomes.

Polymerase chain reaction (PCR)

Polymerase chain reaction (PCR) is used for rapid detection of HPV infection from clinical samples. This technique employs primers that are specific to certain sequences in the viral genome, mostly the *L1* gene. Hence, it is possible to detect the existence of HPV and typing it using either consensus or type specific primers, respectively (Schiffman *et al.*, 2011).

Hybrid capture II (HC2)

The HC2 technology combines nucleic acid hybridization and signal amplification assays in a microplate. It is designed to detect 13 high-risk HPV types; HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59 and HPV 68, 5 low risk HPV types; HPV-6, 11, 42, 43, and HPV44 as well. Detection starts when DNA extracted from specimens hybridizes with a specific HPV RNA probe cocktail. Then, if one of these specific HPV DNA is available it forms RNA-DNA hybrid. Then, the microwells precoated with specific antibodies recognize and bind to DNA-RNA hybrids. These Ab-RNA-DNA captured on microplate surface are detected by specific Abs conjugated with alkaline phosphatase. Then, addition of substrate to the microwell librates light when the enzyme cleaves the substrate. The emitted light is recorded at luminator as relative light units (RLU). Since, multiple conjugated Abs bind to each captured hybrid results in substantial signal amplification. The intensity of the emitted light denotes the presence or absence of target DNA in the specimen (Digene corporsation, 2007). This has no means to genotype the HPV types identified but indicates one of the high-risk types identified in the test. The sensitivity and specificity of the test are found to be between 84.5 to 100% and 65. 9 to 95.8% for CIN 2, respectively (Leiman, 2014). As a limitation there are cross reactions with other HPV types like HPV 26, 66, 70, 73, etc.

Cervista ® HPV (Hologene, USA)

It is a signal amplification test which is marketed as two types of products: Cervista HPV 16/18 and cervista HR. The cervista 16/18 detects the two HPV 16 and 18, while HPV HR

identifies 14 high-risk HPV types those 13 that are included in HC2 and additional HPV 66. As compared to HC2, Cervista shows 98% and 100% sensitivity in detecting CIN III and CIN II, respectively. The cervista 16/18 was recommended as helpful for risk stratification of women with cervical lesion of CIN II or above (Einstein *et al.*, 2011). Unlike the HC2 system, cervista has histone 2 gene (HIST2BE) as internal control to monitor the possibility of false negative results due to sample insufficiency which is clear advantage over HC2. Moreover, it needs half the volume of specimen needed for HC2. Although low in cross reaction, it also suffer cross reaction with low risk HPV types 67, 84, 91 etc (Burd, 2016).

Bead based test

Cobas 4800 HPV test (Roche Molecular Diagnostics, USA)

It combines PCR and NA hybridization with four different fluorescent reporter probes that concurrently detect the L1 gene of HPV 16 and HPV 18 separately and the rest 12 high-risk HPV types (HPV 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68) jointly detected. It is automated system except the sample loading into the microwell plate. Moreover, it uses ß-globin to control extraction and cellularity of specimen. Moreover, it has advantage of using samples collected in PreservCyt buffer stored for 6 months at 2 to 30^{o}C. However, as this and including other test that depend on *L1* gene amplification methods are unable to detect HPV from cancer cells with fully integrated HPV DNA (Abreu *et al.*, 2012a; Burd, 2016).

Multiplex Human Papillomavirus Genotyping (MPG)

The method developed by Schmitt and colleagues, couples PCR and hybridization techniques not only to identify HPV infection but also quantify and genotype multiple HPV genotypes. The test employs broad spectrum primes to amplify HPV DNA from samples and then hybridized with type specific oligonucleotides covalently coupled to fluorescent labeled polystyrene beads (Luminex technology). Then Luminex instrument is

used to read and quantify the products(Schmitt *et al.*, 2006). In this type of detection it is possible to detect as many HPV types as possible from a single specimen. Moreover, it has internal control ß-globin to control DNA quality; external positive and negative controls to control processes. Many improvements are undergoing to include more HPV types and discrimination power(Schmitt *et al.*, 2006, 2008).

RNA based tests

The main objective of designing HPV diagnostic tools is to identify early enough and prevent HPV induced cervical cancer death. Therefore, it is imperative to use genes expressed constitutively and differentially in any HPV infection and precancer/cancer cases, respectively. One important factor is the status of the HPV DNA that is whether it is present in the form of episome or in integrated form. If the HPV DNA is integrated some of the ORFs, of the virus are disrupted; commonly E1, E2, L1 thereby E5 and E4 are affected. Therefore, L1 or E2 based RNA detection may not give accurate diagnosis (Morris, 2006).

To meet such objectives, E6 and E7 genes are important as these genes are expressed in productive and abortive infection or cancer cells with different proportions. It is confirmed that over expression of E6/E7 mRNA is related to persistent infection and cancer cells (especially integrated HPV genome) (Burd, 2016).

Aptima HPV assay (Hologic Gen-Probe, Inc., USA)

In this system, the Aptima HPV test detects E6/E7 mRNA for 14 high-risk HPV types without discriminating them. The company produces Aptima cervical specimen collection and transport kit which contains a medium that lyses cells to release the mRNA and protect its degradation. The sample could be stored at -20^{o}C for about two years (Burd, 2016).

This media is important for countries like Ethiopia with poor infrastructure to take sample from field to central lab.

The detection procedure is using automated and oligonucleotides to isolate the HPV mRNA target onto magnetic microparticles. Then, it is amplified and tested by hybridization assay using chemiluminescence (Burd, 2016). There are other advantages of AHPV test that could improve cervical cancer management.

It is helpful for large scale diagnosis as it enables to test large number of samples in a day. The Aptima Panter and Tigris systems have the capacity of testing samples up to 275 and 450, respectively (Burd, 2016). No cross reactivity with other HPVs or microbes of the cervix, better than HC2 and has low limit of detection. Aptima detects 24 to 488 copies of mRNA with high specificity (Abreu *et al.*, 2012b; Burd, 2016).

In comparison with cytology and HC2, the Aptima HPV assay reduced colposcopy referrals and cost of management (Leiman, 2014) with improved sensitivity (specificity) of cytology, HC2 and Aptima assay for the detection of CIN2+ as 84.9% (66.3%), 91.3% (61.0%), 91.7%(75.0%) respectively. Furthermore, in detecting CIN3+ the assays showed sensitivity (specificity) 93.9% (54.4%), 95.7% (46.0%), and 98.2% (61.0%), respectively. Thus, Aptima assay has improved specificity and sensitivity as compared to cytology (Clad *et al.*, 2011).

Polymerase chain reaction (PCR)

The PCR based diagnostic methods use primers like PGMY09/11, GP5+/6+ etc that can amplify wide range of HPV types in a single reaction. The primers identify specific targets in the *L1* gene and amplify up to billions of copies from a single dsDNA copy (Villa,

2009). Then the PCR products can be identified to specific HPV types of interest using many methods including sequencing, or type specific RNA etc.

Many different PCR systems can be used including real time PCR (Romero-pastrana, 2012). Real time PCR helps to quantify viral load and specific HPV types at real time. Moreover, it can be used to identify mRNA of E6 or E7 (Morris, 2006). The real time PCR system is rapid, reliable and reproducible system that can be used to identify mRNA and small concentration of NAs.

2.5.2. The indirect methods of diagnosis

The indirect methods use cells or tissue level diagnosis of disease or agent induced molecular changes but not the causative agent. Cell or tissue transformations are detected using acetic acid (VIA), Lugol's solution (VILI), colposcopy, Pap-smear etc. Arbyn *et al* (2008) showed that VIA and VILI showed similar specificity (79% CIN 2+, 83% CIN3+) while VILI was 10 % more sensitive than VIA (85% CIN 2+, 84% CIN 3+). Pap smear showed sensitivity and specificity of 57% and 93%, respectively (Arbyn *et al.*, 2008).

Visual inspection using Acetic acid (VIA)

Visual inspection using acetic acid (VIA) is used to detect the presence of transformed tissue or lesions in the cervix. It needs no extensive facility and high level trained expert as well as very fast and cheap. One important advantage of VIA is coupling diagnosis and treatment in the same day, “see and treat”, reducing client dropout. Moreover, women acceptance of the test was found to be more than 80% (Tebeu *et al.*, 2017).

Increasing number of publications indicated that VIA significantly reduced cervical cancer mortality by 31% and efficiently performed by primary health workers (Shastri *et al.*, 2014). In another study, it was reported that the efficiency of VIA diagnosis by nurses and clinicians was not significantly different. Hence, careful training on interpretation and case definition to local health workers would be sufficient to reduce cervical cancer mortality significantly (Raifu *et al.*, 2017). On the contrary, some researchers warranted that VIA test may detect false positive in clients without cervical cancer due to inflammations (Vedantham *et al.*, 2011). Therefore, cautious monitoring is important to reduce false positive diagnosis and over treating them.

VIA is applied in such a way that the woman lie in the lithotomy and speculum is passed the vagina to visualize the uterine cervix. Then, the clinician applies dilute acetic acid (3 to 5%) to the cervix. Then, after a minute, using magnifying glass or naked eye the clinician examines the cervix for the presence of white blotches, if any. Aceto-white blotches are easily detectable from the normal cervix (fig.10). The sensitivity and specificity is between 55% - 96% and 49 - 98%, respectively (Shastri *et al.*, 2014). Weakness could arise from being subjective, over treatment and no standard quality assurance.

Figure 10: Comparison of VIA positive and negative cervix (Tebeu *et al.*, 2017)

Lugol's iodine

Normal cells of the cervix have carbohydrate reserves in the form of glycogen. On application of Iodine, the color of the cells, or the cervix, turns black. However, when

transformed cells present in the cervix, glycogen reserve depletes. On application of Iodine, such cells do not retain it; hence appear yellow with clear margins against the black normal tissue (Raifu *et al*., 2017).

Pap (Papanicolaou) Smear and liquid based cytology

Conventional Pap smear test has been the way-out to reduce cervical cancer. Its impact was immense on reducing more than three-fourth of mortality and morbidity rates (Mehta, Vasanth and Balachandran, 2009). Conventional Pap smear and liquid based cytology are under use to diagnose cancerous changes in cells scraped from uterine cervix. Nowadays, the conventional cytology is being replaced by liquid based cytology with advantages of contaminant free slid preparation and standardization of cells (Simion *et al.*, 2014).

Liquid based cytology (LBC) is a technique that involves preservative fluids before smear preparation. In this technique, the cervical cells are not directly used to prepare smear, rather placed in SurePath™ or ThinPrep™ collection fluids. Removal of mucus, pus, blood cells, inflammatory cells, and chance of preparation of representative smear cells are some of the advantages over classical Pap smear (Simion *et al.*, 2014).

Colposcopy

It involves microscopic examination of the cervix. A speculum is inserted into the vagina and the cervix is visualized and stained with acetic acid and Iodine to assess the size, shape, margin, and location of any neoplastic lesion. Biopsies could be taken from suspected sites or lesions for further evaluation (Saslow *et al.*, 2012).

2.6. Treatment and prevention methods of HPV infection and cervical cancer

Treatment

Cervical cancer is curable cancer if it is diagnosed at its early stage of development (Weyn *et al.*, 2013). There are many types of cervical cancer treatment methods including surgery, radiation and hysterectomy. These methods need extensive facility and highly trained professionals. These methods are not suitable or scarcely supplied in developing countries while cryotherapy needs minimal equipment, cheap and easily to operate (WHO, 2011, 2013; Bosch, Broker and Forman, 2014).

Prevention

Prevention methods to HPV infection and cervical cancer may be seen in three strategies; primary, secondary and tertiary prevention strategies (Finocchario-Kessler *et al.*, 2016).

Primary prevention strategy

The main objective of primary prevention strategy is to prevent HPV infection and cervical cancer initiation. This can be achieved using one of the two effective vaccines. Unlike most other cancers, HPV induced cervical cancer is largely vaccine preventable. Vaccination could reduce the half million annual cases and associated deaths dramatically (World Health Organization, 2013).

On principle the best vaccine is one that induces protection via mounting strong humoral and cell mediated immunity to reduce infection due to targeted HPV types and clear lesions (Stanley, Lowy and Frazer, 2006). To this effect, two effective HPV vaccines: Gardasil® (quadrivalent) and CervarixTM (bivalent) (Dillner *et al.*, 2010) are under use in many parts of the world. The bivalent, Cervarix, targets the two most prevalent and effective oncogenic HPV types; HPV 16 and HPV 18. Both HPV types roughly cause

about 70% of cervical cancer globally. While, the quadrivalent, Gardasil, on top of the HPV types targeted by cervarix, included HPV 6 and HPV 11that are responsible for 90% of genital warts (Dillner *et al.*, 2010). Thus, HPV vaccination will mount high titer neutralizing antibodies (Castellsagué *et al.*, 2011) to combat infection of these HPV types.

Three doses were approved in the course of six month. However, as of 2014, WHO recommended two doses in the age range of 9-13 and 9-14 years old for Gardasil and Cervarix, respectively. Currently most developed countries are using HPV vaccination programs to rollout HPV infection and its consequences. Some African countries like Rwanda and Uganda are instituting HPV vaccination programs.

Now, a new 9 valent HPV vaccine (V503) is ready for use. This vaccine included five extra high-risk types to the Gardasil® targeted HPV types. Therefore, V503 is effective against HPV types included in Gardasil and additional HPV 31, 33, 45, 52 and HPV 58 (Serrano *et al.*, 2014). The impact of V503 was studied in four different countries, India, Brazi, China and Mexico. Serrano *et al* (2014) confirmed that V503 has the potential to reduce 90% of HPV induced cancer. Therefore, instituting HPV vaccination program using either of the alternative vaccines will be crucial to rollout HPV induced cervical cancer in Africa, Ethiopia.

Secondary prevention

The secondary prevention strategies focus on identifying HPV induced diseases and their management. Development of cervical cancer takes long duration up to 20 years. After infection with high risk HPV, there is persistence of the virus, change of the epithelium and development of abnormal cells. Most important, screening will detect those precancerous lesions. Those lesion can then be treated and thus cervical cancer is actually prevented. Hence, cervical cancer is treatable cancer unless it is diagnosed at advanced stage. Therefore, this form of prevention encompasses screening and treatment methods.

Well organized and managed screening programs have the potential of reducing cervical cancer death up to 75%(Mehta, Vasanth and Balachandran, 2009). This has been achieved via Pap smear screening and improved treatment systems in the West. However, the story among developing countries, like Ethiopia, is disappointing as most of the women appear for medical help are found at advanced stage (PELZER *et al.*, 1992; Abate, 2015). Such differences between developed and developing countries are unacceptable; as all the means to reduce are cheaply available in developing countries too.

There are many screening methods that can be used to reduce the burden of cervical cancer in resource-limited countries like VIA, Lugol's Iodine and cryotherapy for point of care screening and treatment options. These screening methods are simple and require minimum facility best suits resource limited countries (Sankaranarayanan *et al.*, 2007). However, these cheap life saving screening methods are not widely in use in Sub-Saharan countries for unclear reasons.

Cryotherapy is liquid nitrogen using treatment option to kill the infected cells. Its advantage includes low facility requirement and nurses or midwives can use it at ease. Moreover, it induce low level tissue destruction and reported to be effective up to 90%(WHO, 2013) (fig.10).

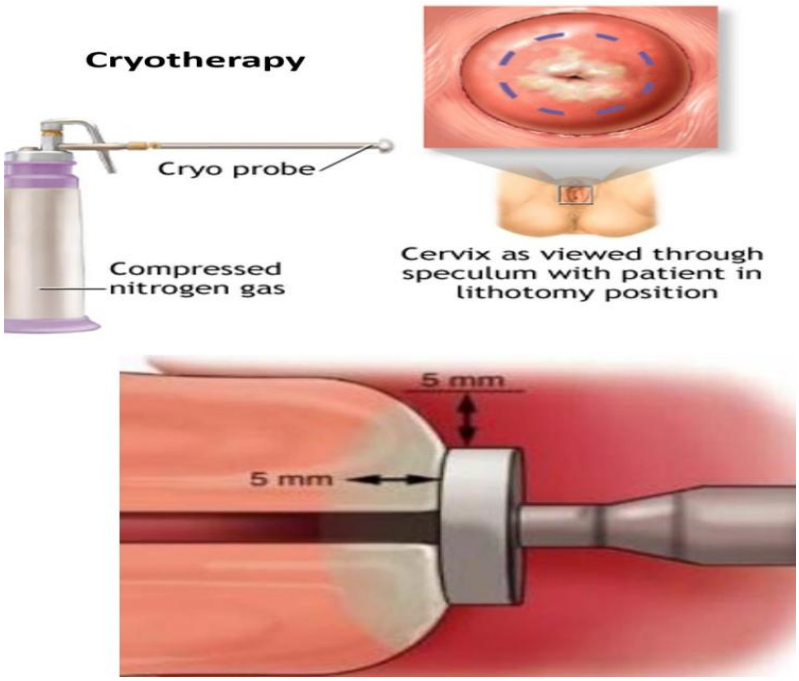

Figure 11: Cryotherapy equipment and its application

Tertiary prevention

Tertiary prevention is mainly focusing on treatment of women with sever cervical dysplasia. These women in this strategy have cervical lesion; the treatment may be initiated using many alternatives including hysterectomy, radiotherapy, chemotherapy and combined therapy(Abnormal *et al.*, 2014). However, these treatment methods need both instrumental facility and well trained manpower that are lacking in most developing countries. Consequently, only small proportion of those women diagnosed with advanced form of the cancer have access to these form of treatments. Consequently, women in developing countries bear the highest proportion of cervical cancer and associated burden, morbidity and mortality (Bruni *et al.*, 2010). Similar problem exists in Ethiopia. Currently Ethiopia has one cancer treatment facility for more than100milion people. Although the treatment was made free, the waiting time to get the facility is more than a year. Thus, women may die before they were called for treatment (personal observation). Overall WHO recommendation of the management considerations for cervical cancer is clearly shown in fig.12

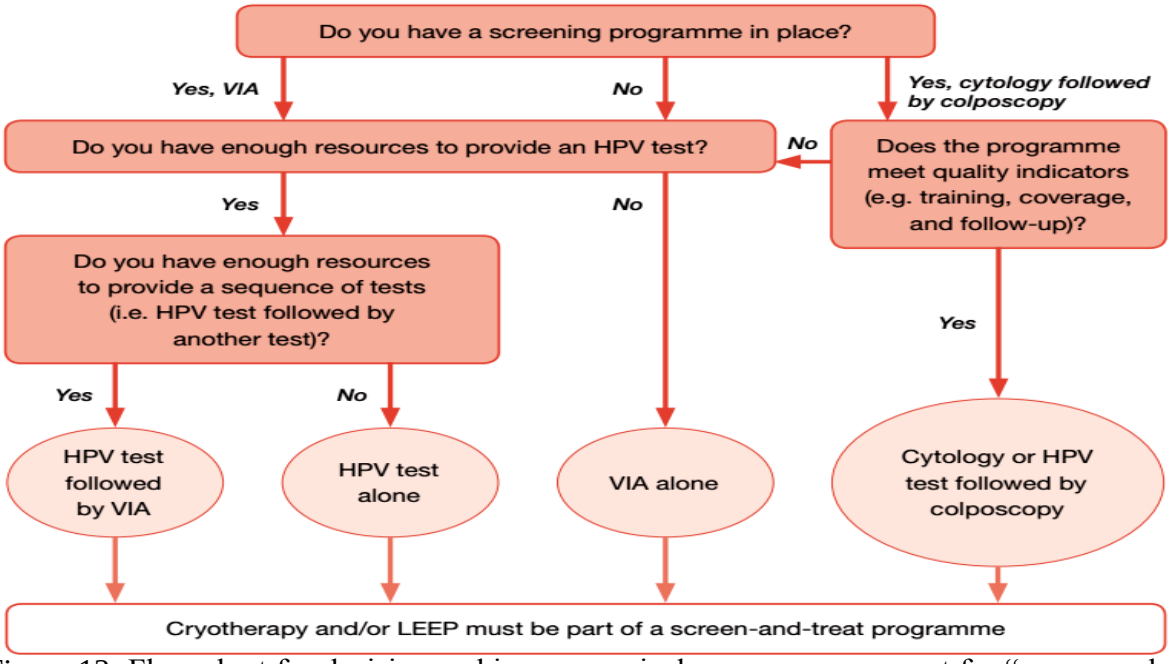

Figure 12: Flow chart for decision making on cervical cancer management for "screen and treat" strategies(WHO, 2013)

2.7. HPV infection and cervical cancer in Ethiopia

Ethiopia is the most populous country in East Africa, a region thought to be endemic to HPV infection and cervical cancer. In 2010 pathfinder (PATHfinder, 2010) reported that among 22 million women over the age of 15 were at risk of HPV infection and its consequences. The office reported that approximately 7,600 women were diagnosed with cervical cancer. Off which roughly 6000 women die of cervical cancer annually.

Although many, reports indicated that Ethiopia has high prevalence of HPV infection and cervical cancer; reports contradict each other, lack clarity and coherence. For instance, WHO/ICO (2007) reported that the annual cervical cancer incidence and mortality rate was 7,600 and 6,000, respectively; while recently WHO/ICO (HPV/ICO, 2016) reported that 7095 women diagnosed with cervical cancer and 4732 of them die annually. However, there is no explanation for the reduction of cervical cancer prevalence from 7,600 in 2007 to 7095 and the mortality rate from 6,000 to 4732 while there was no any intervention

strategy initiated between 2007 and 2013. On the other hand, the recent reported cervical cancer epidemiology by Bruni *et al* (HPV/ICO, 2016) clearly places Ethiopia as one of the countries with the highest number of cervical cancer cases.

In line with this, HPV type distribution is also a determinant factor in predicting its outcome and intervention strategy. In 2006 Ruland *et al* reported that the prevalence of HPV infection among out patients at Atat hospital was 15.9% and high-risk HPV types were prevalent. The most prevalent HPV types from cervical cancer biopsy samples were HPV 16 (71.8%), HPV 18 (18.4%), HPV 45 (1.8%) and HPV 58 (0.6%) (Fanta, 2005) and HPV 52 and 56 also reported (Bekele *et al.*, 2010). However, the genotypic distribution reported by these researchers is contradicting. This could be as a result of using different tests that detect different types of HPV. Thus, these sporadic studies conducted on HPV infection and its cancer are not only limited but also lack representativeness.

On the other hand, knowledge about HPV and cervical cancer among Ethiopian women was limited. At Addis Ababa (Terefe and Gaym, 2008), it was reported that 81.2% of their respondents never heard of HPV infection, its diseases and Pap smear. Among those respondents who had the information, only 6.5% had Pap smear test after they were consulted by their doctors. This lack of public awareness in Ethiopia confirms the report three decades ago that most cervical cancer patients were diagnosed at their advanced stages; stage 4 or above (PELZER *et al.*, 1992).

From this, it can be inferred that HPV infection and cervical cancer are common in Ethiopia. Therefore, robust epidemiological data from representative sites is vital to signify the status of HPV and cervical cancer in Ethiopia. The information will help policy makers pass educated decisions towards HPV and cervical cancer intervention strategies in the country.

3. Hypotheses

Ho_1: There is no difference in circulating HPV genotypes among the study sites and elsewhere

Ho_2: There is no difference in the prevalence of HPV infection in the study area and elsewhere

Ho_3: There are no other infectious agents associated to HPV infection

Ho_4: There is no difference in HPV associated factors among the study sites, Eastern Africa and elsewhere

4. Objectives of the study

4.1. General objective

To investigate the molecular epidemiology of Human Papillomavirus in North and Central part of Ethiopia

3.2. Specific objectives

- To elucidate HPV types circulating in the study area
- To identify the association of selected sexually transmitted infections to HPV infection
- To elucidate the prevalence of different HPV types among women with cervical dysplasia
- To identify HPV associated factors in the study area
- To determine the role of awareness level to HPV infection among study participants

5. MATERIALS AND METHODS

5.1. Study design and population

Study design: A cross-sectional study was conducted from September/ 2015 to January/2016 at five sites in the North and Central part of Ethiopia:

Tigray regional state

Northern region, Mekelle, Ayder referral hospital and Mekelle hospital have cervical cancer screening facility and collaborated in this research. Mekelle is located at 783Kms North of Addis Ababa, at $13^0$29'N$39^0$28E, and at 2,084m above sea level (asl).

Amhara Regional State

Gondar University Hospital, Gondar is a city located in the North-Western part of Ethiopia. Located at 661Kms far from Addis Ababa at 12^o36'N 37^o28'E, 2,133 meters asl.

South Wello, Dessie Hospital, Dessie is a city 400Kms far from Addis Ababa in the northern part of Ethiopia. It is situated at 11^o8'N and 39^o38'E2,470 meters asl.

Felege Hiwot Referral Hospital at Bahir Dar. Bahir Dar is the capital city of Amhara regional state, 549Kms far from Addis Ababa in North-Western part, located at 11^o36'N 37^o23'E & 1800m asl.

Addis Ababa City Administration

Family Guidance Association model clinic was used for sample collection from Addis Ababa (fig. 13).

Target population includes all women who attended gynecology clinics.

Source population: all women ≥18 years old who attended gynecological clinics.

Study population: The study participants were comprised of women ≥18 years old who visited gynecology clinics located at the study sites during the study period.

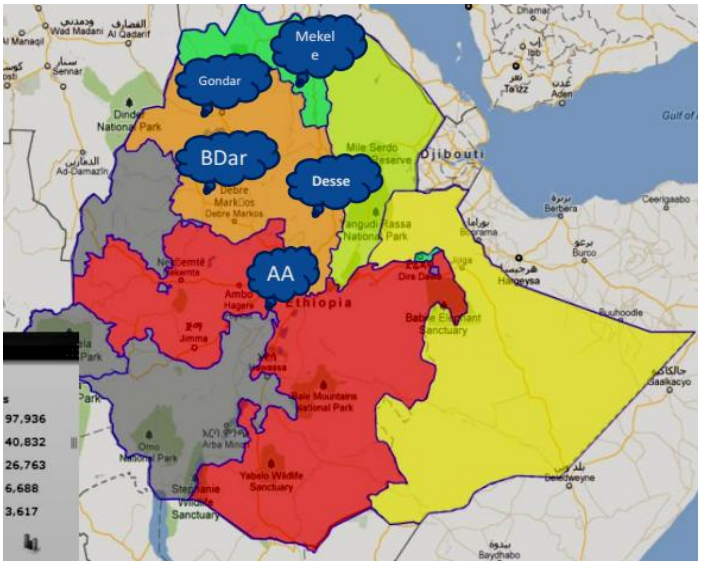

Figure 13: Current map of Ethiopia and the location of collaborating hospitals study

5.2. Sample size

Sample size was calculated using the East African estimate for HPV prevalence, 35.8% (Bruni *et al.,* 2014). Thus, the samples size was calculate as follows:

$N = (Z_{\alpha/2})^2 * P (1-P)/d^2$; Where: N=sample size; Zα/2 is the 95% confidence interval (1.96); d is degree of precision (3%); P is estimated prevalence (35.8%).

Thus, 981 cervical swab samples were collected from the study sites.

Table 2. 2 Population proportion and samples size of study sites

Zone/ cities	Population	Proportion	Sample size
Addis Ababa	1,890,707	31	304
West Gojam, Bahr Dar	741,705	12	119
South Wello, Dessie	932,842	15	149
Tigray, Mekelle	1,562,854	26	250
North Gondar, Gondar	1,009,519	16	161
Total	6,137,338	100	981

To each study site, the total samples size was calculated based on their proportion of ≥10 years old females in the 2007 census.

5.3. Inclusion and Exclusion criteria

Inclusion criteria:

- Women, age ≥18 years old
- Sexually experienced women

Exclusion criteria:

- Those who were on any form of cervical cancer treatment
- Pregnant woman

5.4. Sample collection and laboratory procedures

A Pilot test was conducted to insure the completeness of the research instruments and sample collection methods at Tikur Anbessa Specialized Hospital (TASH) gynecology clinic. The nurses participated in the pilot study came up with critical suggestions on the overall process and the client responses. Then, necessary correction was made on all the processes and data collection instruments. Thus, the questionnaire was restructured and the time needed to fill it was reduced from 25-30 to 18-20 minutes and standardized. Then cervical swab samples were sent to Charite University Medical Center, Berlin. The samples were found to contain enough cell pellet and adequate DNA concentration as well as cellularity (all positive for ß globulin) tests. Then, the cervical research team decided to start data and sample collection at all study sites.

Sample collection was started after clear understanding via discussion on the expected outcomes of the research project and demonstration of the data and sample collection procedures was reached with the collaborating gynecologists and participating midwives. Gynecologists from each collaborating institution trained the midwives theoretically and

practically using volunteer clients, on how to visualize the cervix properly using speculum, insert the cone, collect adequate cervical epithelial cell samples.

After that, all required sample collection materials, questionnaires, PapCone® sticks and 15mL falcon tubes filled with 2.5mL PreservCyt buffer were supplied to each study site. Then, the data and sample processes were carefully followed. All processes of sample collection, transportation and analysis were handled according to SOPs (Annexed). Then all the molecular tests were performed as per the manufacturer's instructions. For all molecular tests positive and negative controls were used to control the quality of all tests.

Socio-demographic data

Study participants were interviewed in a separate room using the standardized questionnaire to obtain basic information about demographic, socioeconomic and behavioral characteristics (Annexed).

Cervical swab and smear collection

The trained midwife who interviewed the study participant collected cervical swab using PapCone® (Otto Bock, Germany). Briefly, the woman lie on lithotomy position and speculum was used to view the cervix. Then, the PapCone is inserted until two-third of the cone is inside the cervical canal and rotating the cone, 360^{o}, twice collects sufficient cells and removed carefully. Then, immediately the cone was held against the edge of frosted end microscope slides (Fig 14). Applying slight pressure and rotating the PapCone across the slide transfers monolayer of cervical cells and fixed using 97% alcohol and kept at $4^{o}C$ until Pap smear test.

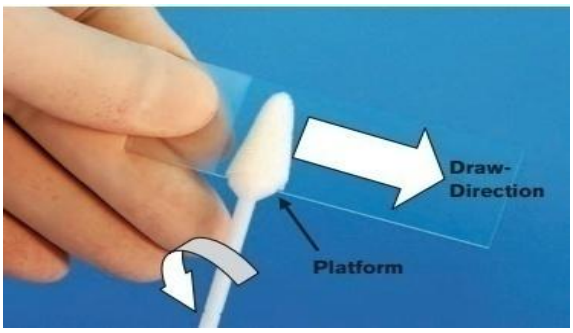

Figure 14: Smear preparation from PapCone swab (Wiley, 1987; Castle and Giuliano, 2003)

Then, the Cone was inserted into 15mL Falcon tubes with 2.5mL PreservCyt (in house formulated) buffer. Then carefully applying slight pressure against the edge of the tube, at its weak point, leaves the swab cone inside the tube and stored at 4^{o}C.

5.5. Visual inspection of cervix using acetic acid (VIA)

Trained midwives tested the study participants for the presence or absence of precancerous lesions using 4% acetic acid. Briefly, the women lie on lithotomy position and the trained midwives applied acetic acid (4%) to the cervix and kept for a minute to investigate if an acetowhite blotch exists. A woman was considered as VIA positive if both collaborating midwives were agreed on the presence of acetowhitening blotch(s). If both midwives could not agree on the VIA result, then the collaborating gynecologist will reinvestigate the client and give final decision but never happen during the study period. All VIA positive study participant were treated using Cryotherapy.

5.6. DNA extraction and genotyping

Molecular analysis of our samples took place at Charite University, Medical Campus, Benjamin Franklin, Berlin. Maxwell® 16 instrument and Levi blood kit (Promega, USA)

was used for total DNA extraction. To describe briefly, the swab samples were spun vigorously in vortex to dislodge maximum amount of cells from the cones. Subsequently, the suspension was transferred into labeled 2mL safe-lock tubes with filtered 2mL disposable pipettes and a control tube were centrifuged at 4000RPM, 23^{o}C for 5 minutes.

Figure 15 Maxwell® 16 instrument and Levi blood DNA purification kit (Promega)

The cell pellet was mixed well with lysis buffer, 300 µL PBS, 300µL lysis buffer and 30 µL proteinase K (Levi blood kit). Then, 630 µL well mixed cell suspension was pipetted into 1.5mL tubes and incubated at 600RPM shaker, 56^{o}C for 20m. In the mean time, 60 µL elution buffer containing tubes were labeled and placed in the Levi cartridge. The samples were transferred to the respective cartridge wells carefully and the Maxwell rack containing the Levi cartridge were placed inside Maxwell Nucleic Acid Extraction Instrument. When completed extracted DNA was stored at -20^{o}C until further use.

5.6.1. Polymerase Chan Reaction (PCR)

The methodology developed by Schmitt *et al*(2008) was used for all PCR and Luminex typing systems. Briefly, broad spectrum GP5+/6+ (GP5+: 5'-TTT GTT ACT GTG GTA GAT ACT AC-3' and GP6+-5'-GAA AAA TAA ATT GTA AAT CAT ATT C-3')

primers were used to amplify 150 nucleotide long conserved target gene flanking a highly variable type specific sequence in the L1 ORF.

The final PCR reaction mix was 25µL with 20 µL master mix and 5 µL DNA templates. The master mix was prepared from 25mMKCl, 0.4g/L Nonidet p40, 5mM Tris HCl (pH 8.8) (10xPCR buffer; MBI Fermentas GmbH, St. Leon Roth Germany), 100µM of deoxyribonucleoside triphosphate, 1.75mM $MgCl_2$ (Biozyme Scientific GmbH, Germany), 0.5U of DNA AmpliTaq polymerase (Roche Applied Biossystems, Germany) and 250nM each of the GP5+ and 5'-biotinylated GP6+ primers. The ß-globin-GP5+/6+ PCR, 50nM each of the ß-globin primers MS_3 and 5' biotinylated MS_{10} were added. Then it was stored at -20^{o}C for further use.

Then, the PCR mix was composed of 20µL PCR master mix and 5µL DNA sample and a negative control (NK) was loaded and carefully mixed. Then in the thermo cycler room a positive control (PK) was added to each plate and the PCR tubes were transferred into PCR rack. PCR conditions were as described elsewhere (Schmitt *et al.*, 2008). Briefly, the cycle time was 4-min denaturing step at 94^{o}C followed by 40cycles. Each cycle was composed of 94^{o}C denaturation for 20sec, annealing at 38^{o}C for 30sec and elongation at 71^{o}C for 80sec. The final elongation time was 4 minutes. Finally the PCR products were stored (at -20^{o}C) until further analysis.

5.6.2. Hybridization and HPV genotyping

To detect HPV genotypes, the PCR products were denatured, and hybridized with bead-coupled probes in 96 well plate. Briefly in a hybridization plate (Millipore) 10 µL PCR product and 34.4µL bead mix were mixed. Then, it was tightly closed using sticky foil (HJ-Bioanalytic GmbH, Germany) and incubated at 95^{o}C for 10minutes to denature the PCR products and hybridized with the bead-coupled mix. The plate was placed in a cooling rack (-20^{o}C) (Eppendorf) for a minute, incubated at 41^{o}C, 650RPM for 35 minutes.

In the mean time, a filter plate filled with 100µL/well PBS was incubated for 15 minutes on vacuum pump (Millipore). Besides, stain buffer was prepared from 8µL stain buffer and 5 µL Streptavidin-PE (Invitrogen) (1:1600) in a 15mL tube and covered with Aluminum foil. The filter plate was sucked using vacuum pump to remove all the liquid, PBS. Then the sticky foil was removed carefully from the hybridization plate and the hybridized DNA-bead mix was transferred into the filter plate carefully. Then, the filter plate was pumped to remove the liquid, washed once using 100µL PBS/well and tapped on green papers to remove all the liquid.

Then, 75µL/well stain buffer (streptavidin-R-phycoerythrin conjugate) was added, covered with aluminum foil and incubated at 650RPM shaker for 30minutes. Then, the plate was pumped to remove the liquid and washed three times using 100µL/well PBS each time. Finally, the beads were re-suspended in 100µL/well PBS for luminex (Luminex corp.) read-out.

The Luminex xMAP200 (Bio-Rad) instrument was used to read the signals using soft ware, Bio-plex manager 6.1 (Bio-Rad). The result were expressed as the median fluorescence intensity (MFI) of at least 50 beads per set. Results were displayed in an Excel spread sheet with the HPV types.

5.6.3. Sexually transmitted infection profiling (STIP)

STIP is a multiplex PCR amplification of target DNA from samples and bead-based hybridization method that enables the researcher to identify multiple types of infectious agents from the same sample simultaneously as described by Schmitt *et al* (2014). Briefly, the extracted DNA was amplified using agent specific primers to produce 88 to 197bp amplicons. Then, the PCR products were hybridized with luminex bead-based suspension array as described elsewhere (Schmitt *et al.*, 2014).

Bacterial vaginosis (BV) scoring

The BV status of the study participants was calculated using the relative concentration of *G. vaginalis* and *A. vaginae* to *Lactobacilli*. Therefore, *G. vaginalis*, *A. vaginae* loads were divided by the highest concentration of *Lactobacillus Spp* to determine their relative ratios to determine their BV status.

To determine the BV status of the study participants was calculated using the relative concentration of *G. vaginalis* and *A. vaginae* to *Lactobacilli*. To evaluate the microbiological disturbance or BV status, *G. vaginalis*, *A. vaginae* loads were divided by the highest concentration of *Lactobacillus* to determine their relative ratios (Table 2.3). For each probe, MFI values in reaction with no PCR product added to the hybridization mixture were considered background values. For all probes reactions, MFI value above than three times the median background result was considered to be positive.

Table 2. 3: BV scoring and description as indicated in Schmitt *et al* (2014)

BV		***A. vaginae*/ Lactobacilli**	***G. vaginalis/Lactobacilli***	***M. hominis***
score	Description			
0	Negative	Low	Low	Absent
1	Weak	Low	Low	Positive
2	Some	High or no	No or high	Absent
3-4	Strong	High	High	Absent
5	Very strong	High	High	Positive

5.7. Data Management and Statistical analysis

Socio-demographic and behavioral data was checked, validated and entered into IBM SPSS statistics v21 database system. Then independent colleagues checked data entry and validated the SPSS data. Then, before analysis, back up data was stored at a separate computer to save the original data. After validation, the data was cleaned and recoded. IBM SPSS v.21 was used for univariate, bivariate and multivariate analysis to draw

inferences. Statistical analysis of odds ratio, and significance at 95% confidence interval were used for hypothesis testing. Statistical significance was considered at $P < 0.05$.

5.8. Ethical Considerations

Ethical clearance was obtained from the Department of Microbiology, Immunology and Parasitology ethical review committee, Institutional Review Board of College of Health Sciences, AAU (March11/ 20115), and National Ethics Review Committee of Ethiopian Ministry of Science and Technology (September 7/2015) (Annexed). Moreover, the Amhara and Tigray regional health bureaus, Mekelle University and University of Gondar reviewed and approved our protocols.

The study participants were provided with information sheet if they can read or the midwives explained to them about the plan and objective of the project, its methodology, benefits and possible discomforts (harms) associated with sample collection. Their free choice (right) to participate or decline to participant in the study was explained to them. Study participant's signature or index print was obtained as indication of voluntary informed consent prior to enrollment in this study (a copy was annexed). No identification or clue of study participants was included in the questionnaires, smear, swab collection tubes or Pap or VIA reporting forms. All information collected from participants were coded (specific four digit project code) and kept confidential. Specific password was used to protect the data.

Finally, our study participants were privately informed about their HPV and STI status via the collaborating midwives. Those who were Pap/ or VIA negative during sample collection and found to be high-risk HPV positive were called and retested. Those who became Pap smear or VIA positive were treated.

Operational definitions

Pap smear results were considered to be positive if Pap smear showed CIN I, or sever

VIA was considered positive if a clear acetowhite blotch(s) was clearly identified from surrounding normal cervical epithelium

High-risk HPV refers to those samples that were identified to harbor DNA of high-risk HPV genotypes in single or multiple HPV infection with other high-risk HPV types

Multiple HPV infection refers to isolation of more than one HPV types from a single sample

Mixed HPV infection if an infection was found to contain DNA of high-risk, low risk and/ or putative high-risk (as indicated on table 6.4) HPV types.

≥1 High-risk HPV refers to the total number of high-risk HPV types in the study, i.e. the sum of single high-risk and multiple high-risk HPV types (as indicated on table 6.4).

Low risk or putative high-risk HPV infection refers to the isolation of one of or multiple low risk or putative high-risk HPV types.

Self employed refers to those women who reported that they run private business like shop, grocery, restaurant or related businesses.

6. RESULTS

6.1. Socio-demographic characteristics

Table 6.1 displays the socio-demographic characteristics of study participants. The study participants were recruited at gynecology units while seeking different services including family planning. The mean age of our study participants was 37.7(±10.3) years (range: 18-72). The highest proportion (70.3%) of participants was from the age groups of 25-34 (34.3%) and 35-44 (36.0%) years (Fig. 16). The study participants from Bahr Dar were relatively young where more than 98% of them were less than 45 years old.

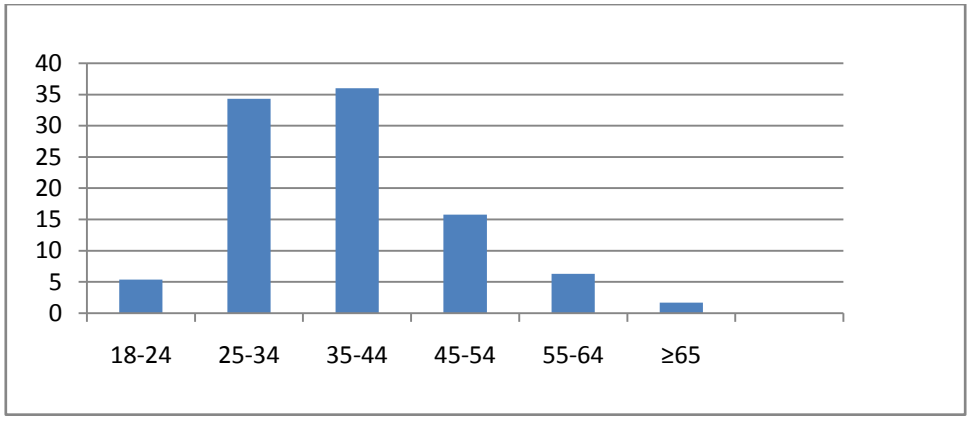

Figure 16: Proportion of study participants in the age groups

Educationally, study participants without formal education (27.4%) constituted the highest proportion followed by the most educated, college or above (26.1%) and those who attended elementary school (26.0%).

Marital status of our study participants indicated that most of them were married, 65.8%, followed by divorced 16.9% (155/915) and widow 9.3% (85/915). Among study sites, Bahr Dar (81.9%) and Mekelle (67.4%) had the highest number of married study participants while the least was reported at Gondar (59.2%). Divorced study participants were more at Gondar (26.3%) followed by Mekelle (22.5%). Single study participants

were higher at Gondar (14.5%) and Addis Ababa (11.0%) while the least was recorded at Bahr Dar (1.9%) (Table 6.1).

Table 6. 1: Demographic characteristics of study participant

Variables	**Addis Ababa n=301**	**Mekelle n=236 (%)**	**Bahr Dar n-106(%)**	**Gondar, 152(%)**	**Dessie** n=120 (%)	**Total** N=915(%)
Age group						
18-24	10 (3.3)	14(5.9)	5(4.7)	14(9.2)	6(5.0)	49(5.4)
25-34	61(20.3)	87(36.9)	59(55.7)	58(38.2)	47(38.2)	314(34.3)
35-44	93(30.9)	103(43.6)	40(37.7)	49(32.2)	44(35.8)	329(36.0)
45-54	82(27.2)	27(11.4)	2(1.9)	16(10.5)	18(15.0)	145(15.8)
55-64	40(13.3)	4(1.7)	0	10(6.6)	4(3.3)	58(6.3)
≥65	15(5.0)	0	0	1(0.7)	0	16(1.7)
Residence						
Urban	281(93.4)	221(67.0)	71(67.0)	121(79.6)	92(76.7)	786(85.9)
Rural	20(6.6)	15(6.4)	35(37.0)	31(20.4)	28(23.3)	129(14.1)
Education						
Uneducated	56(18.6)	36(15.3)	44(41.5)	65(42.8)	50(41.7)	251(27.4)
Primary	81(26.9)	78(33.1)	22(20.8)	21(13.8)	36(30.0)	238(26.0)
Secondary	91(30.2)	22(9.3)	15(14.2)	41(27.0)	18(15.0)	187(20.4)
College+	73(24.3)	100(42.4)	25(23.6)	25(16.4)	16(13.3)	239(26.1)
Marital status						
Married	188(62.5)	159(67.4)	86(81.9)	90(59.2)	79(65.8)	602(65.8)
Divorced	38(12.6)	53(22.5)	18(17.0)	40(26.3)	22(18.3)	155(16.9)
Widow	42(14.0)	18(7.6)	0		9(7.5)	85(9.3)
Single	33(11.0)	5(2.1)	2(1.9)	22 (14.5%)	10(8.3)	73(8.0)
Occupation						
Unemployed	121(40.2)	75(31.8)	14(13.2)	63(41.4)	38(31.7)	311(34.0)
Employed	77(25.6)	75(31.8)	21(19.8)	34(22.4)	27(22.5)	234(25.6)
Self Employed	93(30.9)	72(30.5)	51(48.1)	35(22.3)	24(20.0)	275(30.1)
Farmer	10(3.3)	14(5.9)	20(18.9)	20(13.2)	31(25.8)	95(10.4)
Self income						
No	83(27.6)	40(16.9)	2(1.9)	6(3.9)	6(5.0)	137(15.0)
<1500	76(25.2)	56(23.7)	29(27.4)	25(27.4)	35(29.2)	221(24.2)
1,500 - 2,999	55(18.3)	36(15.3)	30(28.3)	24(15.8)	11(9.2)	156(17.0)
3000 - 4999	44(14.6)	33(14.0)	22(20.8)	4(2.6)	5(4.2)	108(11.8)
5000+	17(5.6)	18(7.6)	1(0.9)	3(2.0)	0	39(4.3)

6.2. HPV genotyping

In this study, genital HPV types were categorized as described elsewhere (Schmitt *et al.*, 2008, 2013); 14 high-risk HPV types: HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66 and HPV 68 (with subtypes 68a and 68b). The putative high-risk HPV types include HPV 26, 53, 70, 73, and HPV 82. The low risk HPV types were HPV 6, 11, 42, 43, 54, 72, and 90. Consequently, our HPV genotyping analysis confirmed the presence of all of the HPV types included in the typing system (Table 6.3).

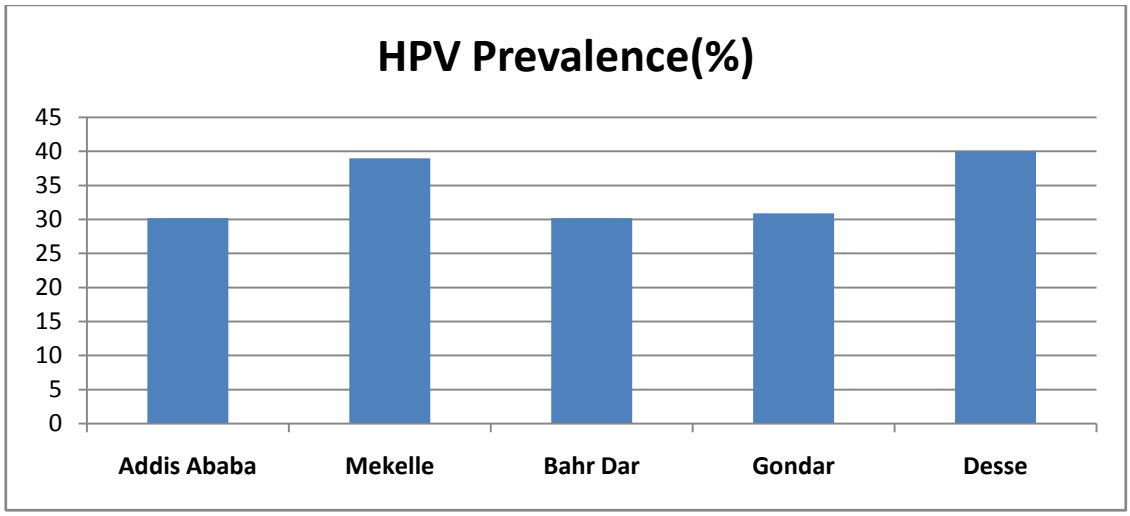

Figure 17: Site based proportion of any HPV DNA positive samples

Overall, any HPV genotype prevalence was 33.9% (310/915) (Table 6.2). The mean age of HPV positive study participants was 43.1(±11.3) years. The highest proportion of HPV infection was recorded at Dessie (40.0%) and Mekelle (39.0%) (Fig.17). Except at Bahr Dar, all the study sites had higher prevalence of HPV infection than Addis Ababa (Table 6.2). Particularly, the study participants at Dessie and Mekelle were found to be 1.54 (p=0.055) and 1.47 (p=0.034) times more likely to be HPV positive than those study participants who were at Addis Ababa.

Prevalence of HPV genotypes

Analysis of the HPV types revealed that high-risk HPV types were more prevalent than putative and low risk HPV types (Table 6. 4). Of all, HPV genotypes identified in this study, the notorious high-risk HPV 16 was the most prevalent HPV type identified. It alone covered 10.3% and 30.3% of all typed and HPV positive samples, respectively. The second most prevalent HPV type was HPV 52 with proportions of 5.6% (51/915) and 16.5% (51/310) of all and HPV positive samples, respectively. The third was HPV 56 found in 4.2% (38/915) and 12.3% (38/310) among all and HPV positive samples, respectively. The 4th most prevalent HPV types was HPV 59 constituting overall prevalence of 4.1% (37/915) and 11.9% (37/310) among all and HPV positive samples, respectively. At last, the fifth most prevalent was HPV 35 comprising 3.5% (32/915) and 10.3% (32/310) among all and HPV positive samples, respectively (fig 18).

Table 6. 2: Overall HPV prevalence among study sites

Study site	N	HPV infection n (%)		OR(95%CI)	*P*
		Negative	Positive		
Addis Ababa	301	210(69.8)	91(30.2)	1	
Mekelle	236	144(61.0)	92(39.0)	1.47(1.03, 2.11)	0.034
Bahr Dar	106	74(69.8)	32(30.2)	1.0(0.62,1.62)	0.99
Gondar	152	105(69.1)	47(30.9)	1.03(0.68,1.58)	0.881
Dessie	120	72(60.0)	48(40.0)	1.54(0.99,2.39)	0.055
Total	915	605(66.1)	310(33.9)		

On the other hand, the prevalence of the prominent high-risk HPV 18 was ranked 8th with 1.7% (16/915) and 5.2% (16/310) among all and HPV positive samples, respectively. Moreover, HPV 45 and HPV 68a were equally the least prevalent HPV types identified among the high-risk HPV types, 0.9% (8/915) and 2.6% (8/310) among all and HPV positive samples, respectively (fig. 18).

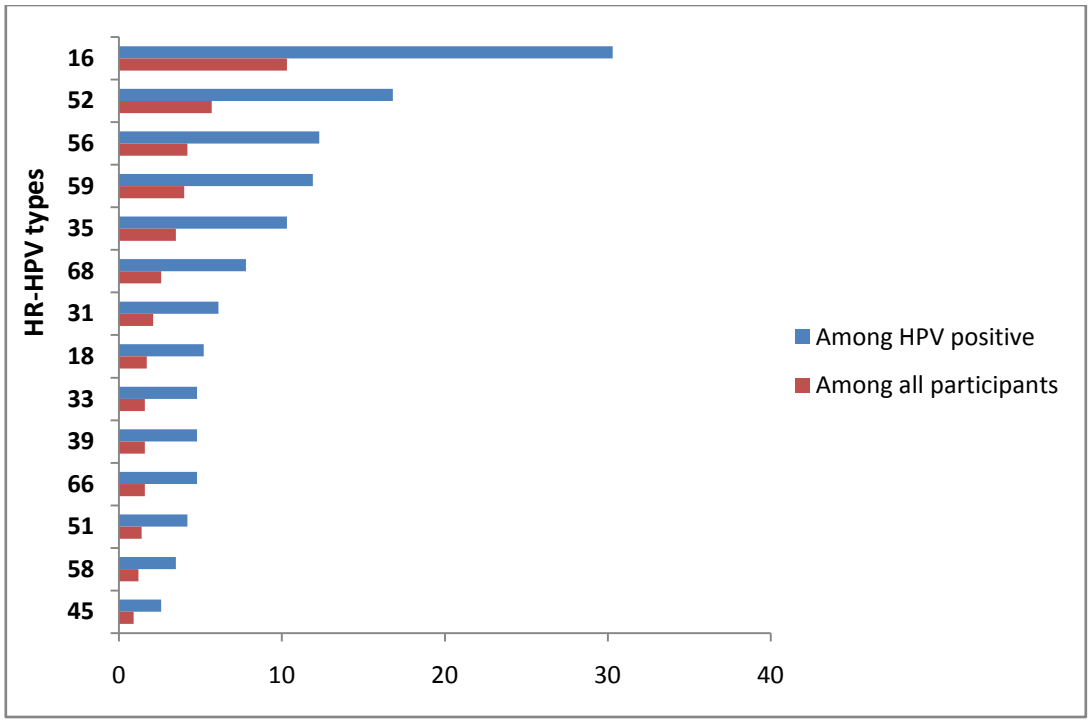

Figure 18: Proportion of hr HPV types among total and HPV positive samples

Among the putative high-risk HPV types, HPV 53 was the most prevalent with a proportion of 2.8% (26/915) and 8.4% (26/310) of all and HPV positive samples, respectively. Moreover, among the low risk HPV types, HPV 42 was the most prevalent HPV type identified with a proportion of 2.0% (18/915) and 5.8% (18/310) of all and HPV positive samples, respectively.

The overall prevalence and distribution of HPV genotypes were not significantly different among the study sites. However, the distribution of some HPV types differs between study sites. For instance, HPV 51, HPV 58 and HPV68b were not identified at Dessie; while HPV 51 and HPV 58 were moderately prevalent at Bahr Dar. Moreover, HPV 45, 66, 68a and HPV68b were not identified at Bahr Dar (Table 6.3). Therefore, the results clearly showed that the overall prevalence of HPV and HPV genotypes was different within and between study sites.

Table 6. 3: HPV types identified with their frequency

HPV Types	Addis Ababa (n=301)		Mekelle (n=236)		Bahr Dar (n=106)		Gondar (n=152)		Dessie (n=120)		Total (915)		
Hr-HPV	S	M	S	M	S	M	S	M	S	M	**S**	**M**	**Sum**
16	13	18	12	17	2	2	6	9	11	4	**44**	**50**	**94**
18	1	4	1	3	0	4	0	1	0	2	**2**	**14**	**16**
31	2	2	1	5	1	2	2	2	2	0	**8**	**11**	**19**
33	1	5	1	3	0	1	0	2	0	2	**2**	**13**	**15**
35	4	8	2	8	0	3	0	4	2	1	**8**	**24**	**32**
39	1	3	1	5	0	1	0	2	0	2	**2**	**13**	**15**
45	0	1	0	4	0	0	0	1	2	0	**2**	**6**	**8**
51	0	4	0	5	0	4	0	2	0	0	**0**	**15**	**15**
52	5	6	3	15	0	4	4	10	3	1	**15**	**36**	**51**
56	6	5	2	5	4	5	1	5	2	3	**15**	**23**	**38**
58	0	2	0	3	1	3	0	2	0	0	**1**	**10**	**11**
59	3	7	1	9	0	7	0	5	2	3	**6**	**31**	**37**
66	0	1	3	3	0	0	1	5	0	2	**4**	**11**	**15**
68	6	0	3	5	0	0	1	8	1	0	**11**	**13**	**24**
68b	4	0	3	2	0	0	1	6	0	0	**8**	**8**	**16**
68a	2	0	0	3	0	0	0	2	1	0	**3**	**5**	**8**
Putative Hr HPV													
26	0	2	0	1	0	1	0	1	0	0	**0**	**5**	**5**
53	2	5	7	7	2	3	1	4	2	3	**14**	**22**	**36**
70	4	4	2	5	1	3	1	2	3	1	**11**	**15**	**26**
73	1	1	1	3	0	1	0	4	0	0	**2**	**9**	**11**
82	1	5	3	6	5	6	0	1	0	4	**9**	**22**	**31**
Low risk HPV													
6	1	2	1	1	0	0	0	3	1	1	**3**	**7**	**10**
11	0	0	0	1	0	0	0	0	0	0	**0**	**1**	**1**
42	2	1	3	4	0	1	0	2	1	4	**6**	**12**	**18**
43	0	1	0	0	0	0	0	0	2	0	**2**	**1**	**3**
54	1	1	2	1	0	0	2	1	0	1	**5**	**4**	**9**
72	0	1	0	1	0	1	0	0	0	0	**0**	**3**	**3**
90	2	4	0	2	0	1	3	0	0	1	**5**	**8**	**13**

Note: S-single, M; multiple for HPV infections; Hr; high-risk in the samples investigated

Prevalence of multiplicity and risk category of identified HPV types

Overall, 14.5% (95%CI: 12.3, 16.7) and 19.3% (95%CI: 16.8, 21.9) of our samples harbored multiple and single HPV type(s), respectively. Among the HPV positive samples, 42.9% (133/310) found to harbor multiple HPV infection. The prevalence of multiple HPV infection did not show significant difference among the study sites. However, among HPV positive samples, the highest proportion of multiple HPV infection was identified at Gondar 51.1% (24/47) followed by Bahr Dar 50% (16/32) among HPV positive women while at Dessie, multiple HPV infection was lower 29.2% (14/48) than single HPV infections 70.9% (34/48).

The overall prevalence of high-risk, putative high-risk and low risk HPV infection was 18.7% (95%CI: 16.2, 21.2), 4.3% (95%CI: 3.0, 5.6) and 2.3% (95%CI: 1.3, 3.3), respectively. Thus, high-risk HPV types were the most prevalent and significantly different than putative and low risk HPV types. On the other hand, at least one high-risk HPV infection was 27.1% and 80.3% of overall and among HPV positive samples, respectively. At least one high-risk HPV infection was highest at Dessie (32.5%) and the least was at Bahr Dar (22.6%). On the other hand, the 95% CI analysis showed that at least one high-risk HPV infection was significantly higher (27.1%; 95%CI: 24.2,30.0) than other categories (Tables 6.4).

Therefore, there is difference among study sites in the prevalence of multiple and single HPV infection. The proportion of which HPV category, high-risk, putative high-risk or low risk HPV, was more prevalent mainly determines the outcome of HPV infection in a geographic region. In this study, infection with the high-risk HPV types was the most prevalent than the sum of putative and low risk HPV types.

Table 6. 4: Multiplicity and risk category of HPV types among study sites

	Addis Ababa(n=301)		**Mekelle(n=236)**		**Bahr Dar(n=106)**		**Gondar (n=152)**		**Dessie (120)**		**Total (915)**	
HPV infection	N	%(95%CI)	N	%(95%CI)	N	%(95%CI)	N	%(95%CI)	N	%(95%CI)	N	%(95%CI)
HPV positive	91	30.2(25.0,35.4)	92	39.0(32.7,45.2)	32	30.2(21.4,38.9)	47	30.9(23.6,38.3)	48	40.0(31.2,48.8)	**310**	**33.9 (30.8,36.9)**
Multiple	35	11.6(8.0,15.2)	43	18.2(13.3,23.1)	16	15.1(8.3,21.9)	25	16.4(10.6,22.3)	14	12.5(6.6,18.4)	**133**	**14.5 (12.3,16.7)**
Single	56	18.6(14.2,23.0)	49	20.8(15.6,25.9)	16	15.1(8.3,21.9)	22	14.5(8.9,20.1)	34	28.3(20.3,36.4)	**177**	**19.3 (16.8,21.9)**
High-risk HPV	55	18.3(13.9,22.6)	48	20.0(14.8,25.0)	13	12.3(6.0,18.5)	27	17.8(11.7,23.8)	29	24.2(16.5,31.8)	**172**	**18.8 (16.2,21.2)**
Putative Hr	8	2.7(0.8,4.5)	16	6.8(3.6,10.0)	8	7.5(2.5,12.6)	2	1.3(-0.5,3.1)	5	4.2(0.6,7.7)	**39**	**4.3 (3.0,5.6)**
Low risk HPV	6	2(0.4,3.6)	6	2.5(0.5,4.6)	0		5	3.3(0.5,6.1)	4	3.3(0.1,6.5)	**21**	**2.3 (1.3,3.3)**
Mixed HPV	22	7.3(4.4,10.2)	22	9.3(5.6,13.0)	11	10.4(4.6,16.2)	13	8.6(4.1,13.0)	10	8.3(3.4,13.3)	**78**	**8.5(6.7,10.3)**
≥1 Hr HPV	75	24.9(20.0,29.8)	70	29.7(23.8,35.5)	24	22.6(14.7,30.6)	40	26.3(19.3,33.3)	39	32.5(24.1,40.9)	**249**	**27.1(24.2,30.0)**

Demographic characteristics of study participants and HPV infections

The mean age of our study participants was 37.7(±10.3) years, range 18-72 years old. The highest HPV prevalence was among the 25-34 followed by 18-24 age groups with prevalence of 39.2% (123/314) and 34.7% (17/49), respectively. Then, the prevalence drops to 31.0% among 45-54 and keeps plateau in the remaining age groups (fig 19). Consequently, study participants who were 25-34 years old were 1.21(p=0.55) times more likely to be HPV positive than their 18-24 years old counterparts (Table 6.5).

Figure 19 shows that the highest high-risk HPV infection was recorded among 45-54 years old (21.4%) study participants while the least was among 18-24 (12.2%) years old. Thus, the youngest study participants (18-24) were found to harbor the lowest proportion of high-risk HPV (12.2%) infection and the highest proportion of low (4.1%) and putative high-risk (8.2%) HPV types. Finally, the highest proportion of mixed HPV infection was among 25-34 (13.1%) followed by 18-24 (10.2%) and ≥65 (10%) years old study participants. However, putative high-risk and low risk HPV types were absent after the age of 64.

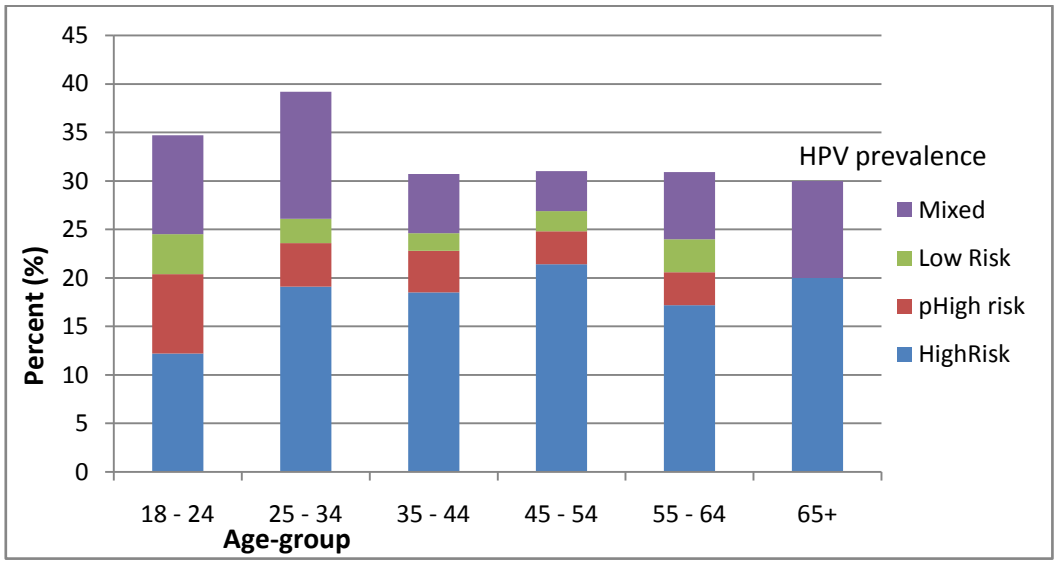

Figure 19: Prevalence of HPV along the age groups

Table 6. 5: Socio-demographic characteristics and HPV infection

Variables		**HPV**		**OR (95%CI)**	***p***
	N (%)	Negative	Positive		
Age group					
18-24	49(5.4)	32(65.3)	17(34.7)	1	
25-34	314(34.3)	191(60.8)	123(39.2)	1.212(0.65,2.277)	0.550
35-44	329(36.0)	228(69.3)	101(30.7)	0.834(0.44,1.571)	0.574
45-54	145(15.8)	100(69.0)	45(31.0)	0.847(0.427,1.681)	0.635
55-64	58(6.3)	40(69.0)	18(31.0)	0.847(0.377,1.903)	0.688
≥65	20(2.18)	14(70.0)	6(30.0)	0.807(0.262,2.479)	0.707
Residence					
Urban	786(85.9)	509(64.8)	277(35.2)	1.583(1.038,2.414)	0.033
Rural	129(14.1)	96(74.4)	33(25.6)	1	
Education					
Uneducated	251(27.4)	166(66.1)	85(33.9)	1	
Primary	238(26.0)	151(63.4)	87(36.6)	1.125(0.776,1.631)	0.534
Secondary	187(20.4)	127(67.9)	60(32.1)	0.923(0.616,1.381)	0.696
College+	239(26.1)	161(67.4)	78(32.6)	0.946(0.650,1.378)	0.773
Marital status					
Married	602(65.8)	416(69.1)	186(30.9)	1	
Divorced	155(16.9)	87(56.1)	68(43.9)	1.748(1.218,2.509)	0.002
Widow	85(9.3)	55(64.7)	30(35.3)	1.220(0.757,1.966)	0.414
Single	73(8.0)	47(64.4)	26(35.6)	1.237(0.743,2.059)	0.413
Occupation					
Jobless	311(34.0)	204(65.6))	107(34.4)	1	
Employed	234(25.6)	156(66.7)	78(33.3)	0.953(0.666,1.364)	0.794
Self employ	370(40.4)	245(66.2)	125(33.2)	0.973(0.708,1.337)	0.973
Self income					
No	137(15.0)	90(65.7)	47(34.3)	1	
<1500	221(24.2)	126(57.0)	95(43.0)	1.444(0.928,2.246)	0.103
1,500- 2,999	156(17.0)	115(73.7)	41(26.3)	0.683(0.413,1.127)	0.136
3000- 4999	108(11.8)	75(69.4)	33(30.6)	0.843(0.491,1.446)	0.534
5000+	39(4.3)	24(61.5)	15(38.5)	1.197(0.574,2.497)	0.632
Unknown	254(27.8)	175(68.9)	79(31.1)	0.518(0.864,0.556)	0.518

In this study, 85.9% (786/915) of our study participants were urban dwellers. HPV prevalence was high among urban dwellers (35.2%) than rural dwellers (25.6%). Thus, the

odds of HPV infection was 1.58 (p=0.033) times more among urban dwellers than their rural counterparts (Table 6.5).

The highest proportion of HPV infection tended to be among those study participants with the lowest level of education, elementary (36.6%) and without formal education (33.9%). However, the least was among those with secondary school education (32.1%). Thus, the odds of HPV infection was 1.13 (p=0.534) times more on those study participants who were with elementary education than their uneducated counterparts.

Table 6.5 shows that the highest proportion of HPV infection was among divorced (43.9%) while the least (30.9%) was among married study participants. As a result, divorced study participants were found to be 1.75(p=0.002) times more likely to be HPV positive than those married study participants. However, being widow or single was not statistically associated with HPV infection (p>0.05).

Behavioral characteristics of study participants and HPV infections

Table 6.6 shows the binary logistic regression of behavioral characteristics of study participants. In this study, the mean age at which our study participants married was 18.5 (±4.5) with 8 and 38 years old being the minimum and maximum age at marriage, respectively (Table 6.6). Moreover, 40.2% (368/915) and 49.7% (455/915) of our study participants were married at the age of ≤ 17 and ≥18 years old, respectively. The highest number of study participants who were married before the age of 18 were from Bahr Dar (55.7%) followed by Dessie (51.7%). On the other hand, the highest HPV infection was recorded among study participants who were married after the age of 17(36.5%). However, HPV prevalence was more or less similar among study participants who were married before the age of 18 and singles, 30.7% and 30.4%, respectively. Thus, study participants who were married ≥18 years old were 1.3 (p=0.082) times more likely to be HPV positive than those who were married ≤17 years old (Table 6.6).

Age at sexual debut is one of the main risk factors for HPV acquisition. The mean age at which the participants started sexual contact was 18.1 (±4.0) years old. While, 50.1% (458/915) of study participants reported that they initiated sexual activity at ≥18 years old, 43.1% (394/915) of them started at the age of ≤17 years old. However, the youngest and the oldest at which sexual activity started were five (1/915) and 40 (1/915) years old, respectively. The highest HPV infection was among study participants who started sexual activity at the age ≥18 (38.2%). Thus, the odds of HPV infection was 1.48 (p=0.007) times more likely among those study participants who started sexual activity at the age of ≥18 as compared to those who started before the age of 18.

The age at sexual debut showed some variation among our study participants who were living at different study sites. Majority of study participants from Bahr Dar (56.6%) and Dessie (52.5%) reported that they started sexual activity before the age of 18. However, the least proportion of study participants who started sexual activity before the age of 18 was reported from Addis Ababa (34.2%).

The number of life time sex partners a woman had is one of the independent risk factors for HPV transmission and its consequences. Of all, 49.0% (448/915) and 30.4% (278/915) study participants reported that they had only one and two lifetime sex partners, respectively. Besides, 19.0% (174/915) of them reported that they had ≥3 lifetime sexual partners. In comparison of study participants at different study sites, those who were living at Dessie (25.8%) and Bahr Dar (21.7%) were with the highest proportion of ≥3 life time sexual partners. The highest proportion of HPV infection was among women with two lifetime sex partners (41.7%) and the least among those with only one lifetime partner (27.9%). Thus, study participants with two lifetime sexual partners were 1.85(p<0.05) times more likely to be HPV positive than those women who had only one lifetime sexual partner.

The mean number of pregnancy among our study participants was 3.7 (±2.4), the highest was 15 times (1/915) a woman from Gondar. Most of our study participants, 91.6% (838/915) had been pregnant at least once in their lifetime. Among whom 15.7% (144/915) and 16.3% (149/915) were pregnant for one and two times, respectively. Besides, large proportion of our study participants, 59.6% (545/915) had been pregnant for at least 3 times in their lifetime.

The highest proportion of HPV infection was among women with only one time pregnancy (49.3%) and the least was among women with no experience of pregnancy (28.6%). Thus, as compared to study participants who were not pregnant, those with experience of only one pregnancy were 2.43(*p*=0.003) times more likely to have HPV infection. However, having two or ≥3 times pregnancy was not significantly associated (OR>1; p>0.05) with HPV infection as compared to those who never had an experience of pregnancy.

Most of our study participants were parous, 84.8%, and the mean number of children a study participant had was 3.05 (±1.9), the highest number of children was 11 (2/915) from a woman at Gondar and another at Dessie. Consequently, 19.1% (175/915) and 21.3% (195/915) of them had one and two children, respectively. Most, 44.4% (406/915), of our participants had ≥3 children per woman (Table 6.6). Besides, HPV prevalence was highest among women with one child (43.4%) followed by nulliparous study participants (40.3%). Thus, study participants with one child were 1.14 (*p*=0.58) times more likely to have HPV infection than those study participants who were nulliparous. However, having ≥3 children was found to be protective in this study (OR: 0.58; *p*=0.008) as compared to nulliparous women.

Table 6. 6: Behavioral characteristics and association to HPV infection

Variable	Addis Ababa	Mekelle	Bahr Dar	Gondar	Dessie	Total	HPV test (%)		OR(95%CI)	*P*
						n=915(%)	Negative	Positive		
Age at marriage										
≤17	81(26.9)	106(44.9)	59(55.7%)	60(39.5)	62(51.7)	368(40.2)	255(69.3)	113(30.7)	1	
≥18	166(55.1)	130(55.1)	45(42.5%)	73(48.0)	41(34.2)	455(49.7)	289(63.5)	166(36.5)	1.296(0.968,1.74)	0.082
Single	29(9.6)	0	2(1.9%)	10(6.6)	5(4.2)	46(5.0)	32(69.6)	14(30.4)	0.987(0.507,1.92)	0.970
Sex debut										
≤17	103(34.2)	109(46.2)	60(56.6%)	59 (38.8)	63(52.5)	394(43.1)	278(70.6)	116(29.4)	1	
>17	159(52.8)	127(53.8)	46(43.4%)	80(52.6)	46(38.3)	458(50.1)	283(61.8)	175(38.2)	1.482(1.112,1.98)	0.007
Life time partner										
1	158(52.2)	128(54.2)	42(39.6%)	88(57.9)	32(26.7)	448(49.0)	323(72.1)	125(27.9)	1	
2	86(28.6)	74(31.4)	41(38.7)	51(33.6)	57(47.5)	278(30.4)	162(58.3)	116(41.7)	1.850(1.350,2.54)	0.000
≥3	57(18.9)	27(11.4)	23(21.7)	13(13.6)	31(25.8)	174(19.0)	112(64.4)	62(35.6)	1.430(0.985,2.08)	0.060
History of Pregnancy										
Never	31(10.3)	11(4.7)	7(6.6)	21(13.8)	7(5.8)	77(8.4)	55(71.4)	22(28.6)	1	
1	34(11.3)	44(18.6)	19(19)	25(16.4)	22(18.3)	144(15.7)	73(50.7)	71(49.3)	2.432(1.344,4.40)	0.003
2	40(13.3)	44(18.6)	25(23.6)	20(13.2)	20(16.7)	149(16.3)	98(65.8)	51(34.2)	1.301(0.715,2.37)	0.389
≥3	196(65.1)	137(58.1)	55(51.9)	86(56.6)	71(59.2)	545(59.6)	379(69.5)	166(30.5)	1.095(0.646,1.855)	0.736
Parity										
Nulliparous	52(17.3)	26(11.0)	9(8.5)	37(24.3)	15(12.5)	139(15.2)	83(59.7)	56(40.3)	1	
1	44(14.6)	49(20.8)	25(23.6)	26(17.1)	31(25.8)	175(19.1)	99(56.6)	76(43.4)	1.138(0.724,1.788)	0.576
2	62(20.6)	63(26.7)	25(23.6)	23(15.1)	22(18.3)	195(21.3)	131(67.2)	64(32.8)	0.724(0.461,1.138)	0.161
≥3	143(47.5)	98(41.8)	47(44.3)	66(43.4)	50(41.7)	406(44.4)	292(71.9)	114(28.1)	0.579(0.387,0.865)	0.008
Abortion										
Never	141(46.8)	149(63.1)	80(75.5)	94(61.8)	68(56.7)	551(60.2)	365(66.2)	186(33.8)	1	
Yes	160(53.2)	86(36.4)	26(24.5)	58(38.2)	48(40.0)	359(39.2)	238(66.3)	121(33.7)	0.998(0.753,1.322)	0.987
STI History										
No	250(83.1)	188(79.7)	72(67.9%)	124(81.6)	85(70.8)	719(78.6)	489(68.0)	230(32.0)	1	
Yes	51(16.9)	48(20.3)	34(32.1%)	28(18.4)	35(29.2)	196(21.4)	116(59.2)	80(40.8)	1.466(1.059,2.030)	0.021

Having history of infection with STIs increases the risk of HPV infection. In that case, 21.4% (196/915) of our study participants reported that they had history of STI infection. Moreover, HPV infection was higher among women with STI (40.8%) than those without experience of STI infection (32.0%). Therefore, the odds of HPV infection was 1.47 (p=0.021) times more likely among women who had experience of STI infection as compared to those women who did not.

6.3. Prevalence of vaccine preventable HPV types

Table 6. 7: The proportion of HPV types in vaccines formulated to date

	Addis Ababa		**Mekele**		**Bahr Dar**		**Gondar**		**Dessie**		**Total**	**%(n=225)(95%CI)**
HPV	S	M	S	M	S	M	S	M	S	M		
6	1	2	1	1	0	0	0	3	1	1	10	4.44(1.75,7.13)
11	0	0	0	1	0	0	0	0	0	0	1	0.44(-0.42,1.31)
16	13	18	12	17	2	2	6	9	11	4	94	41.78(35.33,48.22)
18	1	4	1	3	0	4	0	1	0	2	16	7.11(3.75,10.47)
31	2	2	1	5	1	2	2	2	2	0	19	8.44(4.81,12.08)
33	1	5	1	3	0	1	0	2	0	2	15	6.67(3.41,9.93)
45	0	1	0	4	0	0	0	1	2	0	8	3.56(1.14,5.98)
52	5	6	3	15	0	4	4	10	3	1	51	22.67(17.20,28.14)
58	0	2	0	3	1	3	0	2	0	0	11	4.89(2.10,7.71)
Total	23	40	19	52	4	16	12	30	19	10	225	

The most important high-risk HPV types were included in the vaccines developed to fight HPV infection. The recent nine valent vaccine includes all the HPV types included in Cervarix and Gardasil, HPV6, 11, 16 and 18, and additional five most prevalent types HPV 31, 33, 45, 52, and HPV 58 (Serrano *et al.*, 2014). In this study, HPV 16 covers 85.5%, 77.7% and 41.9% of all the HPV types included in the Cervarix, Gardasil and nine valent vaccines, respectively (Table6.7). Moreover, HPV 52 (22.7%) and HPV 31(8.4%) were second and third most prevalent among vaccine types included in the 9-valent vaccine. In

comparison, HPV16 and HPV 52 showed significantly (95%CI) different proportion than other HPV types included in the different vaccines (Table 6.7).

Considering the vaccine types included in the different vaccines, there was no significant difference between the prevalence of HPV types included in Cervarix™ and Gardasil®. However, the proportion of HPV types in the nine valent vaccine were significantly higher (72.6%; 95%CI: 64.6, 80.6) than those included in the previous vaccines (Table 6.8).

Table 6. 8: Prevalence of vaccine incorporated HPV types

Vaccine	Prevalence (n=310)	95%CI
Cervarix ™	35.5% (110/310)	26.9,44.0
Gardasil ®	39.0% (121/310)	30.3,47.8
9 valent	72.6% (225/310)	64.6,80.6

6.4. Sexually transmitted infections (STI)

Overall, 17.8% (151/840) of samples harbor STIs where the top three most prevalent were *T. vaginalis,* HSV-2 and *N. gonorrhea* with proportions of 4.5% (38/840), 3.8% (32/840) and 2.3% (19/840), respectively (Fig. 20). Moreover, other most important genital pathogens identified were *M. genitalium,* 1.9% (16/840), *C. trachomatis,* 0.5% (4/840) and *T. pallidum,* 0.12%(1/840).

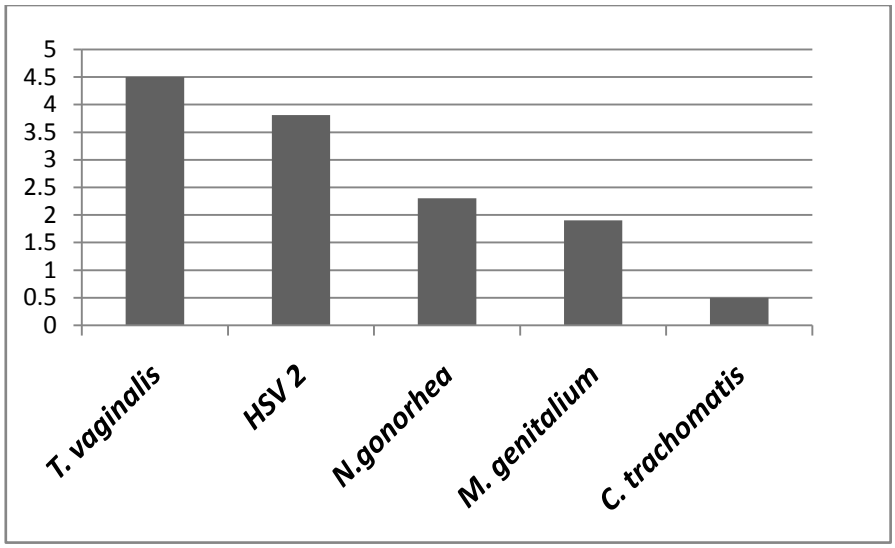

Figure 20: Prevalence of selected STIs identified

In this study, higher proportion of HPV was recorded among STI positive (45.0%) than STI negative (31.8%) study participants. Thus, STI positive study participants were 1.76 (p=0.002) times more likely to harbor HPV infection than those who were STI negative. Moreover, HSV-2 positive study participants were 3.14 (p=0.002) times more likely to harbor HPV infection than those who were STI negative (Table 6.9).

Table 6. 9: Association of selected STI to HPV infection

STI	**N**	**HPV**		**OR(95%CI)**	***P value***
		Negative (%)	Positive (%)		
Negative	693	474(68.4)	219(31.6)	1	
All STI	151	83(55.0)	68(45.0)	1.76(1.24,2.54)	0.002
HSV 2	32	13(40.6)	19(59.4)	3.14(1.52,6.47)	0.002
M. genitalium	16	9(56.3)	7(43.7)	1.67(0.614,4.54)	0.316
T. vaginalis	38	22(57.9)	16(42.1)	1.56(0.80,3.03)	0.190
N. gonorrhea	19	12(63.2)	7(36.8)	1.25(0.486,3.22)	0.640
C. trachomatis	4	1(25)	3(75)		
T. pallidum	1	1(100)	0		

6.5. Bacterial vaginosis

Among many vaginal microbiota, colonization of the vaginal epithelium with *Gardnerella vaginalis (G. vaginalis), Atopobium vaginae (A. vaginae), Mycoplasma hominis (M. hominis)* etc and loss of *Lactobacillus* is associated with bacterial vaginosis. As a result, *G. vaginalis* was the most prevalent 36.7% (310/844) followed by *A. vaginae*, 32.5% (274/844) and *M. hominis*, 14.1% (119/844) (Fig. 21).

In this study, the prevalence of single infection of *G. vaginalis, A. vaginae* and *M. hominis* were 10.9% (91/844), 7.2% (62/844) and 2.3% (19/844), respectively. Besides, more women harbored coinfection of *A. vaginae* and *G. vaginalis*, 24.9% (210/844), or all the three, 10.5% (89/844). Consequently, HPV infection was significantly higher among women co-infected with *G. vaginalis* and *A. vaginae* than those free of BV assocaited infections (OR=1.64; *p*=0.021). Moreover, study participants infected with all the three bacteria were 3.46 (p<0.0001) times more likely to have HPV infection than those study participants who were free of BV associated infections (Table 6.10).

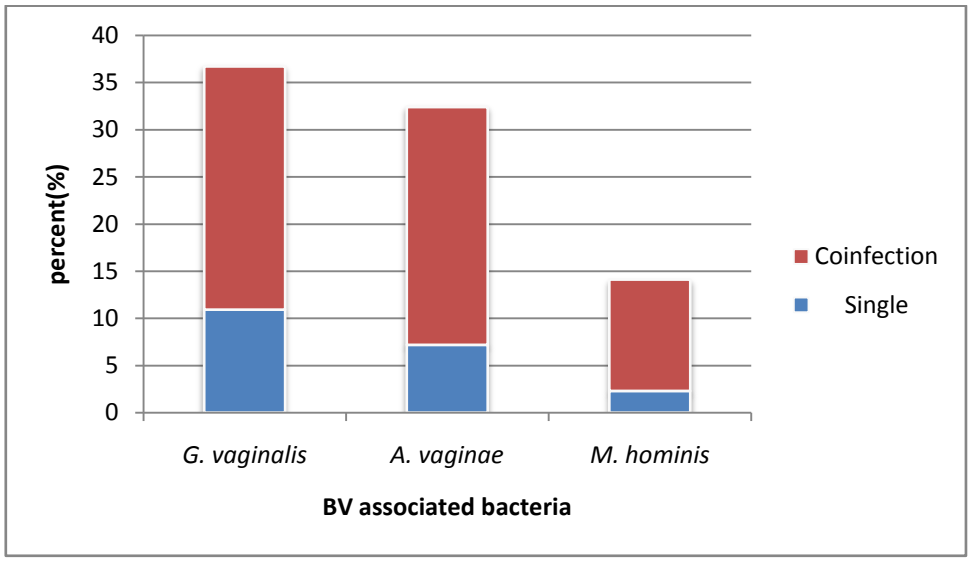

Figure 21: Prevalence of BV associated bacterial infection

Association of HPV to BV associated infections

In this study, 15.4% (130/844) of our study participants were found to have bacterial vaginosis with 4.5% (38/840) some, 7.9% (67/840) strong and 3.0% (25/840) very strong bacterial vaginosis scores. Overall, BV positive study participants harbored more HPV infection (43.8%) than their BV negative (30.9%) counterparts. Hence, BV positive study participants were 1.643 ($p<0.011$) times more likely to be HPV positive than BV negative study participants (Table 6.11).

Table 6. 10: Bacterial vaginosis associated infection and association to HPV infection

		HPV			
BV associated infections	N	Negative	Positive	OR	*p* value
Negative	451	325(72.1)	126(27.9)	1	
Positive	393	232(59.03)	161(40.97)	1.79(1.343,2.387)	0.0001
G. vaginalis	92	64(69.6)	28(30.4)	1.13(0.692,1.84)	0.628
A. vaginalis	61	39(63.9)	22(36.1)	1.46(0.830, 2.55)	0.191
M. hominis	19	12(63.2)	7(36.8)	1.51(0.579,3.91)	0.402
GV & A. vaginalis	121	74(61.2)	47(38.8)	1.64(1.08,2.49)	0.021
GV & M. hominis	8	3(37.5)	5(62.5)		
A. vaginalis & M. hominis	3	2(66.7)	1(33.3)		
AV or GV with M. hominis	11	5(45.5)	6(54.5)	3.095(0.928,10.323)	0.066
Gv, Av & M. hominis	89	38(42.7)	51(57.3)	3.46(2.17,5.53)	0.000

Gv: *Gardnerela vaginalis; AV: Atopobium vaginae*

The highest HPV infection was among those study participants who showed very strong bacterial vaginosis (56.0%) and strong bacterial vaginosis (43.3%). Thus, study participants with very strong bacterial vaginosis were found to harbor HPV infection significantly (OR=2.687; p=0.016) than their BV negative counterparts (Table 6.11).

In a separate analysis, very strong BV was not found colonized with single infections of the BV associated bacteria. While some (47.4%) and strong BV(14.9%) were dominated by *G. vaginalis* followed by *A. vaginae*. Moreover, none of the BV associated bacteria were found in very strong BV as single infection while *G. vaginalis* and *A. vaginae* coninfection constitute 40% of it. Among BV positive women (15.4%), 43.8% (57/130) were HPV positive. Overall, as BV score increases, the proportion of HPV infection increased, BV2 (36.8%), BV3/4 (44.3%) and BV 5 (56.0%) (Table 6.11). Overall, HPV infection was strongly associated (OR: 2.69; *p*=0.016) with very strong BV score (Table 6.11).

Among the HPV and BV positive study participants most of them harbor single HR (33.3%), multiple HR (24.6%) and mixed (21.1%) HPV infections. In all BV stages, high-risk HPV types were the most prevalent (Table 6.12).

Table 6. 11: Bacterial vaginosis prevalence and association to HPV infection

		HPV positivity			
BV Score	N	Negative	Positive	OR	*p value*
Negative	714	484(67.9)	230(32.1)	1	
BV positive	130	73(56.2)	57(43.8)	1.643(1.14,2.13)	0.011
Some BV	38	24(63.2)	14(36.8)	1.231(0.625,2.426)	0.547
Strong BV	67	38(56.7)	29(43.3)	1.61(0.969,2.679)	0.066
Very strong BV	25	11(44.0)	14(56.0)	2.687(1.201,6.013)	0.016

Among the BV positive samples, HPV 16 (17.5%), 52 (17.5%) and 59(15.8%) were the three most prevalent HPV types identified. However, HPV 52, 56 and 31 were the three most prevalent among women with very strong BV score (Table 6.12).

Table 6. 12: Association of HPV infection to bacterial vaginosis

HPV	BV status		BV score		
	Negative	Positive	Some BV	Strong BV	Very strong BV
Negative	484(86.9)	73(13.1))	24(32.9)	38(52.1)	11(15.1)
Positive	230(80.1)	57(19.9)	14(24.6)	29(50.9)	14(24.6)
Single HR	91(82.7)	19(17.3)	7(36.8)	10(52.6)	2(10.5)
Putative HR	27(75.0)	9(25.0)	1(11.1)	6(66.7)	2(22.2)
Low Risk	16 (84.2)	3(15.8)	2(66.7)	1(33.3)	0
Multiple HR	36(75)	12(25.0)	2(16.7)	7(58.3)	3(25.0)
Mixed HPV	60(81.1)	14(18.9)	2(14.3)	5(35.7)	7(50.0)
HPV 16	77(88.5)	10(11.8)	4(40.0)	4(40.0)	2(20.0)
52	39(79.6)	10(20.4)	5(50.0)	1(10.0)	4(40.0)
59	24(72.7)	9(17.3)	1(11.1)	7(77.8)	1(11.1)
31	11(61.1)	7(38.9)	2(28.6)	2(28.6)	3(42.9)
56	29(80.6)	7(19.4)	1(14.3)	2(28.6)	4(57.1)
35	23(79.3)	6(20.7)	1(16.7)	3(50.0)	2(33.3)
pHR					
HPV 53	25(75.8)	8(24.2)	0	5(62.5)	3(37.5)
82	25(80.6)	6(19.4)	2(33.3)	2(33.3)	2(33.3)

NB: BV= bacterial vaginosis; BV1= weak not considered as BV

6.6. Direct evaluation of the uterine cervix

In this section, two types of non molecular methods were used to diagnose HPV induced pathology indirectly, Pap smear (at Addis Ababa) and VIA (other sites). These methods detect HPV infection induced dysplasia but not HPV itself.

Pap smear test (Addis Ababa)

Among the 301 study participants, 19.6% (59/301) were diagnosed with CIN I. Consequently, CIN I positive study participants were insignificantly associated (OR=1.02; *p*=0.959) to HPV infection (Table 6.13). Moreover, one-third (30.5%) of these CIN I positive samples were HPV positive of which 77.8% (14/18) and 11.1% (2/18) were infected with high-risk and mixed HPV types. On the other hand, 19.8% (18/91) of HPV positive study participants were Pap smear positive. It was found that, 25.5% of high-risk,

12.5% of putative high-risk and 16.7% of low risk HPV infections were associated with abnormal Pap smear test (Table 6.13).

Among CIN I positive study participants, 22.0% (13/59) harbored single HPV infection. Of which, 11 harbored high-risk HPV while the remaining 2 study participants were infected with HPV 53 and HPV 42 (Table 6.13). Of all HPV genotypes, HPV 16 constituted the highest proportion, 44.4%, among CIN I positive samples. Thus, the overall HPV 16 infection was significantly associated with abnormal Pap smear test (OR: 3.3; p=0.018). Moreover, the odds of abnormal Pap smear was 4.12 (p=0.012) times more likely among those study participants who were infected with high-risk HPV types (other than HPV 16 or 18) than HPV negative study participants (Table 6.13).

Table 6. 13: Association of Pap smear test results to HPV infection (n=301)

HPV test		Pap test			*P* value
	N	Negative	CIN I	OR(95%CI)	
Negative	210	169 (80.5)	41(19.5)	1	Reference
Positive	91	73(80.2)	18(19.8)	1.02(0.548, 1.886)	0.959
High-risk	55	41(74.5)	14(25.5)	1.41(0.702, 2.823)	0.336
Putative HR	8	7 (87.5)	1(12.5)		
Low Risk	6	5(83.3)	1(16.7)		
Mixed HPV	22	20(90.9)	2(9.1)		
Total	301	242 (80.4)	59(19.6)		
16	13	8(61.5)	5(38.5)	2.5762(0.801,8.287)	0.112
16,35	3	1(33.3)	2(66.7)	3.298(1.225,8.878) all HPV 16	0.018
16,56	2	1(50.0)	1(50.0)		
18,39,59,82	1	0	1(100.0)	3.710(1.42,9.72) HPV 16 &18	0.008
35	4	2(50.0)	2(50.0)		
39	1	0	1(100.0)		
52	5	3(60.00	2(40.0)		
56,90	1	0	1(100.0)		
59	**3**	**2(66.7)**	**1(33.3)**	**4.122(1.37,12.41)**	**0.012**
42	2	1(50.0)	1(50.5)		
53	2	1(50.0)	1(50.0)		

NB: the HPV types indicated were those HPV types identified among Pap positive samples

Socio-demographic characteristics associated with abnormal Pap smear test

The mean age at which study participants diagnosed with abnormal Pap smear was 45.1(±11.0). All the age groups showed more or less similar prevalence except in the age group 45-54 that constitutes the highest proportion (25.6%). On the other hand, the demographic characteristics of study participants were insignificantly (p>0.05) associated with abnormal Pap smear. As compared to study participants in the age group 18- 34, those in the age group 45-54 were 1.69 (p=0.194) times more likely to be diagnosed with abnormal Pap smear (Table 6.14).

Moreover, marital status of study participants was not significantly associated with abnormal Pap smear (p>0.05). However, widow study participants were 1.113(p=0.798) times more likely to be diagnosed with abnormal Pap smear than those study participants who were married. Besides, as compared to jobless, employed study participants were 1.12(p=0.76) times more likely to have abnormal Pap smear test.

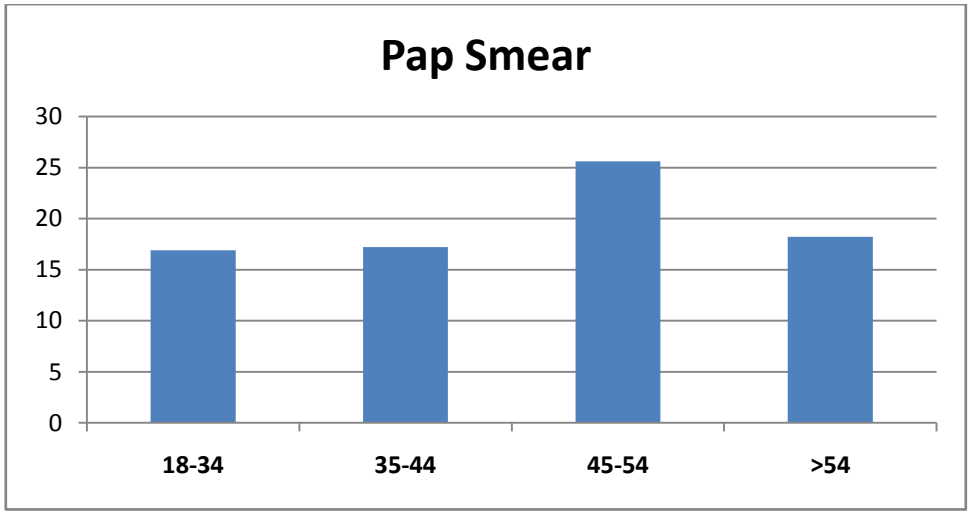

Figure 22: Prevalence of abnormal Pap smear among different age groups

Table 6. 14: The role of demographic characteristics to Abnormal Pap smear test

		Pap smear, n (%)		OR(95%CI)	P value
Age group	N	Negative	CIN I		
18 - 34	71	59(83.1)	12(16.9)	1	
35 - 44	93	77(82.2)	16(17.2)	1.022(0.45, 2.32)	0.959
45 - 54	82	61(74.4)	21(25.6)	1.693(0.77, 3.75)	0.194
≥55	55	45(81.8)	10(18.2)	1.093(0.43, 2.75)	0.851
Residence					
Urban	281	226(80.4)	55(19.6)	1	
Rural	20	16(80.0)	4(20.0)	1.027(0.33,3.20)	0.963
Education					
Uneducated	56	48(85.7)	8(14.3)	1	
Elementary	81	65(80.2)	16(19.8)	1.477(0.58, 3.73)	0.410
Secondary	91	73(80.2)	18(19.8)	1.480(0.596, 3.67)	0.398
college or above	73	56(76.7)	17(23.3)	1.821(0.72, 4.59)	0.204
Marital status					
Married	188	151(80.3)	37(19.7)	1	
Divorced	38	31(81.6)	7(18.4)	0.922(0.38,2.26)	0.858
Widow	42	33(78.6)	9(21.4)	1.113(0.49, 2.53)	0.798
Single	33	27(81.8)	6(18.2)	0.907(0.35, 2.36)	0.841
Occupation					
Jobless	121	95(78.5)	26(21.5)	1	
Employed	77	59(76.6)	18(23.4)	1.115(0.56, 2.21)	0.755
Self employed	90	76(84.4)	14(15.6)	0.673(0.33, 1.38)	0.279
Farmer	10	10(100.0)	0		
Local drink seller	3	2(66.7	1(33.3)		
Self income					
No income	83	64(77.1)	19(22.9)	1	
<1,500	76	65(85.5)	11(14.5)	0.570(0.25, 1.29)	0.179
1500 - 2999	55	49(89.1)	6(10.9)	0.413(0.15,1.11)	0.080
3000 - 4,999	44	30(68.2)	14(31.8)	1.572(0.696, 3.55)	0.277
5000+	17	10(58.8)	7(41.2)	2.358(0.79,7.04)	0.124

Role of socio-behavioral characteristics to abnormal Pap smear

Analysis of the behavioral characteristic of the study participants revealed that none of them were significantly associated (p>0.05) with abnormal Pap smear (Table 6.15). Age at

marital and sexual debut are among the most important risk factors that usually associated to abnormal cytology. However, in this study early marriage and sexual debut were not associated with abnormal Pap smear. Thus, study participants who were married after the age of 17 were 1.04 (p=0.9) times more likely to be diagnosed with abnormal Pap smear than those study participants who were married younger than 18 years old. Moreover, study participants who started sexual activity after the age of 18 were 1.38 (p=0.314) times more likely to be diagnosed with abnormal Pap smear than those study participants who started sexual intercourse before the age of 18.

Study participants who had two lifetime sexual partners constituted the highest proportion of abnormal Pap smear, 15.6% (22/86). Thus, study participants with two lifetime sexual partner were 1.47(p=0.23) times more likely to be diagnosed with abnormal Pap smear as compared to monogamous study participants.

The number of pregnancies our study participants had was differently associated with HPV infection. Study participants who had only one pregnancy were with the highest prevalence of abnormal Pap smear, 29.4 %(10/34), followed by those who had two pregnancies, 20%(8/40). Thus, only one time pregnant study participants were 1.74 (p=0.35) times more likely to be diagnosed with abnormal Pap smear than those study participants with no history of pregnancy.

Table 6. 15: Role of socio-behavioral characteristics to abnormal pap smear test

Variables	N	Pap smear n(%)		OR(95%CI)	*P* value
		Negative	Positive		
Age at marriage					
≤17	81	64(79.0)	17(21.0)	1	
≥18	166	130(78.3)	36(21.7)	1.043(0.544, 1.997)	0.900
Single	29	24(82.8)	5(17.2)	0.784(0.261, 2.361)	0.665
Sexual debut					
≤17	103	85(82.5)	18(17.5)	1	
≥18	159	123(77.4)	36(22.6)	1.38(0.736, 2.594)	0.314
Decline/Do not know	39	34(87.2)	5(12.8)	0.69(0.239, 2.020)	0.503
Life time partners					
One	158	128(81.0)	30(19.0)	1	
Two	86	64(74.4)	22(15.6)	1.47(0.784, 2.745)	0.231
≥3	57	50(87.7)	7(12.3)	0.597(0.247, 1.448)	0.254
Pregnancy					
Never	31	25(80.6)	6(19.4)	1	
one	34	24(70.6)	10(29.4)	1.736(0.546, 5.520)	0.350
Two	40	32(80.0)	8(20.0)	1.042(0.320,3.393)	0.946
≥3	196	161(82.1)	35(17.9)	0.906(0.346, 2.373)	0.840
Parity					
Nulliparous	52	40(76.9)	12(23.1)	1	
one	44	35(79.5)	9(20.5)	0.857(0.323, 2.275)	0.757
Two	62	53(85.5)	9(14.5)	0.566(0.217, 1.474)	0.244
≥3	143	114(79.7)	29(20.3)	0.848(0.395, 1.819)	0.672
STI					
No	250	201(80.4)	49(19.6)	1	
Yes	51	41(80.4)	10(19.6)	1.001(0.469, 2.136)	0.999
Abortion history					
No	160	122(76.3)	38(23.8)	1	
Yes	141	120(85.1)	21(14.9)	0.562(0.312, 1.013)	0.055
Abortion					
Never	225	175(78.8)	50(22.2)	1	
One	49	44(89.8)	5(10.2)	0.398(0.150, 1.057)	0.064
≥2	27	23(85.2)	4(14.8)	0.609(0.201, 1.842)	0.380

Visual inspection using acetic acid (VIA)

Visual inspection using acetic acid (VIA) is the means of national cervical cancer screening strategy all over Ethiopia. Thus, VIA related data was collected from all study sites out of Addis Ababa (n=614). The mean age at which VIA positive study participants detected was 36.5 (±9.5). The overall proportion of VIA positive study participants was moderately high (18.9%). Among the study participants at different study sites, overall VIA positivity was highest at Bahr Dar (28.3%) and the least was at Gondar (9.9%) (Fig.23). Consequently, the study participants at Bahr Dar were 1.55 (p=0.106) times more likely to be VIA positive as compared to those who were at Mekelle (Table 6.16).

In this study, being a farmer was associated with cervical abnormality. Hence, farmers were 2.88 (p=0.001) times more likely to be VIA positive than their unemployed counterparts. This was related to our finding where rural inhabitants were1.96 (p=0.006) more likely to be VIA positive than urban counterparts.

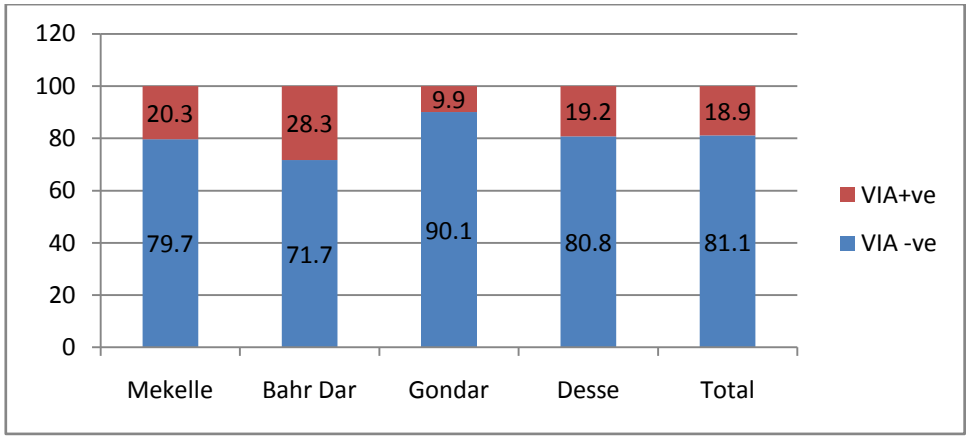

Figure 23: Proportion of VIA positive participants among the 4 study sites

Therefore, the prevalence of abnormal VIA was different among study participants living at different study sites. The odds of abnormal VIA was 1.18 (p=0.44) times more likely among those study participants who were HPV positive than those who were HPV negative.

Moreover, study participants who were infected with high-risk HPV types were found to be 1.44(p=0.065) times more likely to be VIA positive than those study participants who were HPV negative. In addition, study participants who were HPV 16 positive were 3.002(p=0.0001) times more likely to be VIA positive than those study participants who were HPV negative. Among the putative high-risk HPV types, HPV 82 was 2.15 (p=0.088) times more likely to be associated with abnormal VIA than HPV negative study participants (Table 6.17).

Table 6. 16: Overall comparison of VIA positive test among study sites

Study Sites	N (614)	**VIA**		**OR(95%CI)**	***p* value**
		Negative	Positive		
Mekelle	236	188(79.7)	48(20.3)	1	0.003
Bahr Dar	106	76(71.7)	30(28.3)	1.546 (0.912,2.622)	0.106
Gondar	152	137(90.1)	15(9.9)	0.429(0.231, 0.797)	0.007
Dessie	120	97(80.8)	23(19.2)	0.929 (0.534, 1.616)	0.794
Total	614	498(81.1)	116(18.9)	0.912(0.627, 1.329)	0.632

Proportion of HPV infection among women with abnormal VIA

In this study, HPV infection was responsible for 38.8% (45/116) of the VIA positive study participants. Overall, high-risk HPV types were responsible for most of the VIA positive samples, 88.9% (40/45), while putative and low risk HPV infection were responsible for 6.7% and 5.4% of VIA positive samples, respectively.

Consequently, high-risk HPV infected study participants were 1.3 (p=0.304) times more likely to be VIA positive than those who were HPV negative. Moreover, putative high-risk HPV infected study participants were 1.52 (p=0.211) times more likely to be VIA positive than those HPV negative study participants (Table 6.17).

Among high-risk HPV types, HPV 16 was the most prevalent among HPV positive samples and it was responsible for 33.3% (15/45) VIA positive samples. Thus, HPV 16 infected study participants were 3.002(p=0.0001) times more likely to be VIA positive than those who were HPV negative. Moreover, the second most prevalent HPV type was HPV 52 identified in 22.2% (10/45) of VIA positive samples. Among the putative high-risk HPV types, HPV 82 was the most prevalent type identified in the VIA positive samples. Thus, study participants who were HPV 82 DNA positive were 2.15 (p=0.088) times more likely to be VIA positive than those who were HPV DNA negative.

Socio-demographic characteristics of study participants and VIA test

Overall and specific age groups were not associated with abnormal VIA diagnosis. However, as compared to 18-24 years old study participants, ≥55 years old study participants were 1.9 (p=0.28) times more likely to be VIA positive (Table 6.18).

Residence of study participants was associated with abnormal VIA test. Thus, the odds of abnormal VIA was 1.96 (p=0.006) times more likely among those study participants who were rural inhabitants than those urban inhabitants. Conversely, marital status of our study participants was insignificantly (p>0.05) associated with abnormal VIA. Moreover, divorced and widow study participants were found to be 1.088 (p=0.75) and 1.53 (p=0.254) times more likely to be VIA positive than those study participants who were married.

Table 6. 17: HPV types identified in association with abnormal VIA test

		VIA test			
HPV	N(614)	Negative	Positive	OR (95%CI)	*P* value
Negative	395	324(82.0)	71(18.0)	1	
Positive	219	174(79.5)	45(20.5)	1.180(0.778,1.790)	0.436
High-risk	117	91(77.8)	26(22.2)	1.304(0.786,2.162)	0.304
pHr & Lr HPV	46	41(89.1)	5(10.9)	0.557(0.212,1.46)	0.233
Mixed	56	42(75.0)	14(25.0)	1.521(0.788,2.935)	0.211
HPV 16	63	38(60.3)	25 (39.7)	3.002(1.704,5.289)	0.0001
18	11	8(72.7)	3(27.3)		
31	15	11(73.3)	4(26.7)		
33	9	8(88.9)	1(11.1)		
35	19	18(94.7)	1(5.3)		
39	10	9(90)	1(10.0)		
45	6	3(50.0)	3(50.0)		
51	8	6(75.0)	2(25.0)		
52	39	29(74.6)	10(25.4)	1.574(0.734,3.376)	0.244
56	25	21(84.0)	4(16.0)		
58	8	6(75.0)	2(25.0)		
59	19	17(89.5)	2(10.5)		
66	10	8(80.0)	2(20.0)		
68b	11	10(90.9)	1(9.1)		
68a	6	5(83.3)	1(16.7)		
pHR HPV					
26	3	2(66.7)	1(33.3)		
53	29	26(89.7)	3(10.3)		
70	15	14(93.3)	1(6.7)		
73	9	7(77.8)	2(22.2)		
82	25	17(68.0)	8(32.0)	2.148(0.892,5.171)	0.088
Low risk HPV					
6	7	6(85.7)	1(14.3)		
11	1	0	1(100.0)		
42	15	12(80.0)	3(20.0)		
43	6	4(66.7)	2(33.3)		
54	7	6(85.7)	1(14.3)		
72	2	1(50.0)	1(50.0)		
90	7	5(71.4)	2(28.6)		
Sum LrHPV	**44**	**34(77.3)**	**10(22.7)**	**1.342(0.634, 2.843)**	**0.442**

Table 6. 18: Association of Socio-demographic characteristics and VIA

Variable	N	VIA test (%)		OR(95%CI)	*p* value
		Negative	Positive		
Age group					
18 - 24	39	30(76.9)	9(23.1)	1	
25 - 34	253	206(81.4)	47(18.6)	0.761(0.338,1.709)	0.507
35 - 44	236	196(83.1)	40(16.9)	0.680(0.300,1.543)	0.356
45 - 54	63	52(82.5)	11(17.5)	0.705(0.262,1.896)	0.489
≥55	19	12(63.2)	7(36.8)	1.944(0.590,6.412)	0.275
Residence					
Urban	505	420(83.2)	85(16.8)	1	
Rural	109	78(71.6)	31(28.4)	1.964(1.219,3.164)	0.006
Education					
Uneducated	195	153(78.5)	42(21.5)	1	
Elementary	157	128(81.5)	29(18.5)	0.825(0.487,1.400)	0.476
Secondary	96	81(84.4)	15(15.6)	0.675(0.353,1.290)	0.234
≥ College	166	136(81.9)	30(18.1)	0.804(0.477,1.355)	0.412
Marital status					
Married	414	338(81.6)	76(18.4)	1	
Divorced	117	94(80.3)	23(19.7)	1.088(0.647,1.829)	0.750
Widow	43	32(74.4)	11(25.6)	1.529(0.739,3.169)	0.254
Single	40	34(85.0)	6(15.0)	0.785(0.318,1.936)	0.599
Employment					
Jobless	190	161(84.7)	29(15.3)	1	
Employed	157	132(84.1)	25(15.9)	1.051(0.587,1.882)	0.866
Drink seller & Self	182	149(81.9)	33(18.1)	1.230(0.712,2.123)	0.458
Farmer	85	56(65.9)	29(34.1)	2.875(1.581,5.227)	0.001
Self income					
No	54	44(81.5)	10(18.5)	1	
<1,500	145	122(84.1)	23(15.9)	0.830(0.366,1.881)	0.654
1500 - 2999	101	80(79.2)	21(20.8)	1.155(0.500,2.670)	0.736
3000 - 4999	64	57(89.1)	7(10.9)	0.540(0.190,1.533)	0.247
5000+	22	17(77.3)	5(22.7)	1.294(0.386,4.343)	0.676

Conversely, being a farmer is strongly associated with increased rate of abnormal VIA. The odds of positive VIA test was 2.88(p=0.001) times more likely among those study participants who were farmers than those who were jobless (Table 6.18).

Table 6. 19: Association of social and behavioral characteristics and VIA

Variable	N	VIA test		OR(95%CI)	*P* value
		Negative	Positive		
Age at marriage					
≤17	287	225(78.4)	62(21.6)	1	
>17	289	239(82.7)	50(17.3)	0.759(0.502,1.149)	0.193
Single	17	14(82.4)	3(17.6)	0.778(0.217,2.792)	0.700
Age at sexual debut					
≤17	291	224(77.0)	67(23.0)	1	
>17	299	253(84.6)	46(15.4)	0.608(0.401,0.922)	0.019
Number of partners					
1	290	235(81.0)	55(19.0)	1	
2	192	157(81.8)	35(18.2)	0.953(0.596,1.523)	0.839
≥3	117	96(82.1)	21(17.9)	0.935(0.536,1.630)	0.812
Do not know /Decline	15	10(66.7)	5(33.3)	2.136(0.702,6.502)	0.181
Parity					
Nulliparous	87	72(82.8)	15(17.2)	1	
1	131	109(83.2)	22(16.8)	0.969(0.471,1.992)	0.931
2	133	113(85.0)	20(15.0)	0.850(0.409,1.766)	0.662
≥3	263	204(77.6)	59(22.4)	1.388(0.741,2.599)	0.305
History of pregnancy					
No	46	36(78.3)	10(21.7)	1	
Yes	568	462(81.3)	106(18.7)	0.826(0.397,1.717)	0.609
Number of pregnancies					
Never	46	36(78.3)	10(21.7)	1	
1	110	95(86.4)	15(13.6)	0.568(0.234,1.381)	0.212
2	109	93(85.3)	16(14.7)	0.619(0.257,1.491)	0.285
≥3	349	274(78.5)	75(21.5)	0.985(0.467,2.077)	0.969
Abortion					
No	391	317(81.1)	74(18.9)	1	
Yes	218	179(82.1)	39(17.9)	0.933(0.608,1.433)	0.753
Spontaneous abortion					
Never	433	353(81.5)	80(18.5)	1	
1	122	97(79.5)	25(20.5)	1.137(0.688,1.879)	0.616
2	37	26(70.3)	11(29.7)	1.867(0.886, 3.935)	0.101
STI history					
No	469	382(81.40	87(18.6)	1	
Yes	145	116(80.0)	29(20.0)	1.098(0.687,1.754)	0.697

Considering the significance level, all except sexual debut the behavioral characteristics were not significantly associated with abnormal VIA (Table 6.19). Study participants who reported that they started sexual activity after the age of 17 were found to be associated with reduced abnormal VIA (OR:0.608; *p*=0.019). Moreover, study participants married at ≥ 18 showed reduced abnormal VIA test than their counterparts married at ≤17 years old (OR<1; p>0.05) (Table 6.19).

Majority of our study participants were parous, 85.8% (527/614). The highest proportion (22.4%) of abnormal VIA was identified among those who had ≥3 children. Thus, as compared to study participants without children, study participants who had ≥3 children were 1.39 (*p*=0.305) times more likely to be VIA positive.

Among the study participants, 23.6% (145/614) reported that they had STI infection in their lifetime. Abnormal VIA test was 1.1 (p=0.7) times more likely among those with history of STI than those without history of STI infection.

6.7. Knowledge and awareness of study participants

Awareness of cervical cancer

Awareness level of cervical cancer is critical in the prevention of cervical cancer morbidity and mortality. Consequently, 33.3% (305/915) of our study participants were unaware of cervical cancer. In comparison, the least informed study participants were reported from Gondar (77.0%) while the most informed were from Bahr Dar (84%) (Fig.24). Thus, study participants at all sites, except at Gondar, were significantly (p<0.05) aware of cervical cancer than study participants at Dessie. For instance study participants at Bahr Dar were 3.14 (p<0.05) times more aware of cervical cancer than those study participants at Dessie (Table 6.20).

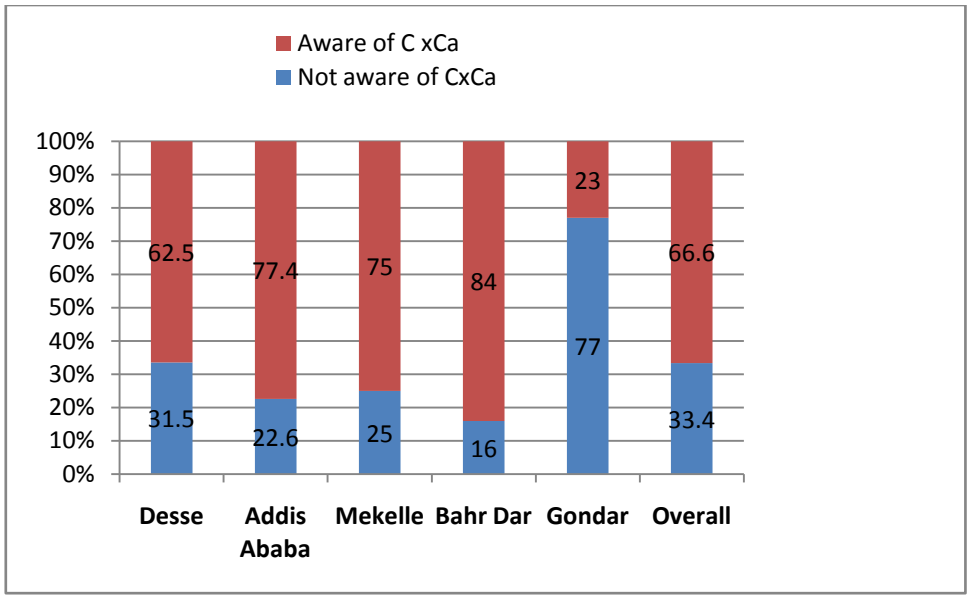

Figure 24: Awareness level of study participants at the five study sites

Among the informed study participants, the most effective sources of information were health professionals (26.4%) followed by mass media (television and radio) (18.5%). Moreover, family members were responsible for creating awareness for 6.9% of study participants (Table 6.21).

Table 6. 20: Comparison of cervical cancer awareness among study participants

Study sites	**N**	**CxCa awareness level**			
		No (%)	Yes (%)	OR(95%CI)	*P* value
Dessie	120	45(31.5)	75(62.5)	1	
Addis Ababa	301	68(22.6)	233(77.4)	2.06(1.30,3.25)	0.002
Mekelle	236	59(25.0)	177(75.0)	1.80(1.12,2.89)	0.015
Bahr Dar	106	17(16.0)	89(84.0)	3.14(1.66,5.94)	0.000
Gondar	152	117(77.0)	35(23.0)	0.18(0.11,0.30)	0.000

On the other hand, 81.2% of our study participants were unaware of the causative agent of cervical cancer. Again, 98% of study participants at Gondar (2.0%) were unaware of the

cause of cervical cancer while those at Mekelle (40.7%) were with the highest proportion of study participants aware of the cause of cervical cancer (Table 6.21).

Table 6. 21: Awareness level of study participants to cervical cancer and related issues

Variable	Addis Ababa (301)	Mekelle (n=236)	Bahr Dar (n=106)	Gondar (n=152)	Dessie (n=120)	Total (n=915)
Cxca information						
No information	68(22.7)	59(25.0)	17(16.0)	116(76.3)	45(37.5)	305(33.3)
Health staff	94(31.3)	84(35.6)	29(27.4)	16(10.6)	19(16.1)	242(26.4)
Family	32(10.7)	8(3.4)	1(0.9)	3(2.0)	19(16.1)	63(6.9)
TV or Radio	89(29.7)	32(13.6)	10(9.4)	16(10.6)	22(18.6)	169(18.5)
Mixed sources	17(5.7)	53(22.5)	49(46.2)	0	13(11.0)	132(14.4)
Know cause of Cxca						
No	265(89.2)	140(59.3)	84(79.2)	149(98.0)	104(86.7)	742(81.4)
Yes	32(10.8)	96(40.7)	22(20.8)	3(2.0)	16(13.3)	169(18.6)
What cause of Cxca						
Heredity	30(10.0)	0	0	0	0	30(3.3)
Heredity & other	0	0	3(2.8)	0	0	3(0.3)
HPV infection	9 (3.0)	50(21.2)	1(0.9)	2(1.3)	3(2.5)	65(7.1)
HPV & smoking	0	22(9.3)	0	0	0	22(2.4)
Smoking	0	10(4.2)	2(1.9)	0	0	12(1.3)
Sin	0	3(1.3)	0	0	1(0.8)	4(0.4)
Others	21(7.0)	3(1.3)	0	0	12(10.0)	36(3.9)

NB: CxCa=cervical cancer; TV=television

Knowledge about the cause of cervical cancer was minimal among our study participants. Only, 18.6% study participants responded that they knew the cause of cervical cancer. However, only 7.1% women correctly identified HPV as the main causative agent of cervical cancer. Moreover, our study participants identified heredity (3.3%), smoking (1.3%) etc causes of cervical cancer (Table 6.21). On the other hand, study participants' at different sites differ in their knowledge of the causative agent of cervical cancer. Consequently, 21.2% (50/236) of study participants at Mekelle identified HPV infection as the main causative agent of cervical cancer. The least number of study participants that

identified HPV as the main causative agent of cervical cancer were from Bahr Dar, 0.9% (1/106) (Table 6.21).

Awareness on Risk factors for HPV infection

Knowledge about the risk factors for HPV acquisition was not adequate among our study participants where 81.3% of them were unaware of it. As shown in Table 6.22, our study participants identified multiple sexual partner (9.9%), smoking (2.5%) and early marriage (1.3%) as the main risk factors for HPV infection (Table 6.22).

Awareness on intervention strategies

Adequate information on HPV and cervical cancer increases acceptance and practice of cervical cancer intervention strategies so that they could benefit from early diagnosis and management of cervical abnormalities. In this study, majority of the study participants (67.8%) reported that they were informed about cervical cancer screening. As a result, 28.7% of study participants had experience of screening at least once before recruitment in this study. Thus, large proportion of study participants did not have experience of screening. They pointed out that lack of awareness (45.5%), unaware of screening site (7.9%) and fear of cervical cancer (4.9%) were the main reasons for lack of screening. In comparison of the study sites, most of the study participants at Bahr Dar were the most informed, 86.8% (92/106) about cervical cancer screening (Table 6.23).

Table 6. 22: HPV infection associated factors (Risk factors)

Variable	Addis Ababa(301)	Mekelle (n=236)	BahrDar (n=106)	Gondar, (n= 152)	Dessie (n= 120)	Total (n=915)
Risk for HPV infection						
Multiple partners	17(6.3)	53(22.3)	7(6.6)	3(2.0)	9(7.5)	91(9.9)
Multiple partner, early marriage	0	8(3.4)	0	0	0	8(0.9)
Multiple partner, early marriage & smoking	0	4(1.7)	0	0	0	4(0.4)
multiple partner & smoking	0	11(4.7)	0	0	0	11(1.2)
Multiple partner, smoke, Heredity	0	1(0.4)	0	0	0	1(0.1)
Multiple partner & Heredity	0	1(0.4)	0	0	0	1(0.1)
Early marriage	2(0.7)	8(3.4)	0	0	2(1.7)	12(1.3)
Early marriage & Smoking	0	2(0.8)	0	0	0	2(0.2)
Smoking	0	22(9.3)	1(0.9)	0	0	23(2.5)
Heredity	0	1(0.4)	0	0	0	1(0.1)
Others	3(1.0)	4(1.7)	1(0.9)	0	1(0.8)	9(1.0)
Do not know	270 (89.7)	121(51.3)	97(91.5)	149(98)	108(90)	744(81.3)
Smoking						
Yes	5(1.7)	7(3.0)	1(0.9)	1(0.7)	3(2.5)	17(1.9)
No	293(97.3)	229(97.0)	105(99.1)	151(99.3)	117(97.5)	895(97.8)
Cooking fuel						
Animal, plant, products	52(17.3)	93(39.4)	83(78.3)	128(84.2)	89(74.2)	445(48.6)
Petrol	11(3.7)	2(0.8)	0	0	1(0.8)	14(1.5)
Electricity	117(38.9)	129(54.7)	5(4.7)	20(13.2)	13(10.8)	284(31.0)
Electricity & others	93(30.9)	5(2.1)	15(14.2)	4(2.6)	13(10.8)	130(14.2)
Mixed source	28(9.3)	7(3.0)	3(2.8)	0	4(3.3)	42(4.6)
Oral contraceptive						
No	266(88.4)	194(82.2)	103(97.2)	136(89.5)	105(87.5)	804(87.9)
< 1 years	19(6.3)	41(17.4)	3(2.8)	16(10.5)	11(9.2)	90(9.8)
1 to 5 years	12(4.0)	1(0.4)	0	0	4(3.3)	17(1.9)
≥5 years	4(1.4)	0	0	0	0	4(0.4)

Table 6. 23: Knowledge of HPV and cervical cancer prevention and control

Variable	Addis Ababa n=301(%)	Mekelle n=236(%)	Bahr Dar n=106 (%)	Gondar, n 152(%)	Dessie n= 120 (%)	Total N=915(%)
Did you know about Cxca screening						
No	52(17.3)	59(25.0)	14(13.2)	116(76.3)	53(44.2)	294(32.1)
Yes	248(82.4)	177(75.0)	92(86.8)	36(23.7)	67(55.8)	620(67.8)
Had Cxca screening						
No	173(57.5)	151(64.0)	84(79.2)	131(86.2)	112(93.3)	651(71.1)
Yes	127(42.2)	85(36.0)	22(20.8)	21(13.8)	8(6.7)	263(28.7)
Why you didn't screen						
No awareness	39.9 (120/301)	24.6 (58/236)	61.3(65/106)	79.6(121/152)	43.3(52/120)	45.5(416/915)
Not know site	2.0(6/301)	20.8(49/236)	6.6(7/106)	3.9(6/152)	3.3(4/120)	7.9(72/915)
Fear of Cxca	1.3(4/301)	11.9(28/236)	9.4(10/106)	0.7(1/152)	1.7(2/120)	4.9(45/915)
No money	0.3(1/301)	1.7(4/236)	2.8(3/106)	2.0(3/152)	2.5(3/120)	1.5(14/915)
Is Cxca preventable						
No	118(39.2)	70(29.7)	55(51.9)	91(59.9)	65(54.2)	399(43.6)
Yes	138(45.8)	142(60.2)	51(48.1)	10(6.6)	50(41.7)	391(42.7)
Do not know	45(15.0)	24(10.2)	0	51(33.6)	5(4.2)	125(13.7)
Methods of prevention						
Vaccine	7(2.3)	3(1.3)	4(3.8)	2(1.3)	1(0.8)	17(1.9)
Screening	102(33.9)	65(27.5)	25(23.6)	4(2.6)	21(17.5)	217(23.7)
No early marriage	1(0.3)	9(3.8)	0	0	2(1.7)	12(1.3)
Monogamy	15(5.0)	7(3.0)	9(8.5)	3(2.0)	7(5.8)	41(4.5)
No early marriage	0	13(5.5)	0	0	0	13(1.4)
Is Cxca treatable						
Not	47(15.6)	23(9.7)	3(2.8)	3(2.0)	30(25.0)	106(11.6)
Yes	167(55.5)	139(58.9)	70(66.0)	23(15.1)	49(40.8)	448(48.0)
Do not know	87(28.9)	74(31.4)	33(31.1)	126(82.9)	41(34.2)	361(39.5)
Therapy methods						
Not treatable	46(15.3)	21(8.9)	0	3(2.0)	30(25.0)	100(10.9)
Chemotherapy	50(16.6)	12(5.1)	6(5.7)	5(3.3)	13(10.8)	86(9.4)
Radiotherapy	25(8.3)	16(6.8)	13(12.3)	4(2.6)	5(4.2)	63(6.9)
Cryotherapy	0	53(22.5)	1(0.9)	4(2.6)	2(1.7)	60(6.6)
Surgery	19(6.3)	9(3.8)	17(16.0)	3(2.0)	9(7.5)	57(6.2)
Combined	20(6.6)	50(21.2)	28(26.4)	2(1.3)	5(4.2)	105(11.5)
Do not know	134(44.5)	75(31.8)	41(38.7)	131(86.2)	55(45.8)	436(47.7)

Our study participants lack adequate knowledge of HPV and cervical cancer prevention strategies. Thus, only 42.7% study participants were aware of the virus and its cancer as preventable maladies. Among the alternative methods of prevention, most women pointed

out regular screening as the best method of prevention (23.7%). Moreover, only 1.9% of study participants identified HPV vaccine as a first choice of prevention. Similar to other results, the least proportion of study participants, 6.6% (10/152), at Gondar were aware of cervical cancer as preventable disease.

The knowledge of our study participants about the treatment methods of cervical cancer was moderate. Slightly, less than half of the study participants (49.0%) responded that cervical cancer is treatable disease, 11.6% of them reported that they used to know that cervical cancer is not treatable disease (Table 6.23).

Cervical cancer awareness level and HPV infection

In this study, informed study participants (33.3%) showed reduced HPV infection than their uninformed counterparts (35.0%). Thus, study participants who were aware of cervical cancer were 0.93(p=0.62) times less likely to be HPV positive than those study participants who were unaware of HPV infection.

On the other hand, smoking was significantly associated (OR: 3.65; p=0.011) with HPV infection. Thus, smoker study participants were 3.65(95%CI: 1.34, 9.98) times more likely to be HPV positive than those study participants who had never been smoker (Table 6.24). Moreover, study participants with experience of cervical cancer screening were insignificantly (OR=0.95; p=0.99) had reduced proportion of HPV infection. Other factors experience of screening, oral contraceptive and knowledge of treatments methods were not associated with reduced proportion of HPV infection.

Table 6. 24: HPV infection and behavioral/awareness level of study participants

Variable	N=915(%)	HPV(%)		OR(95%CI)	*P* value
		Negative	Positive		
Cxca information					
No	306	199(65.0)	107(35.0)	1	
Yes	609	406(66.7)	203(33.3)	0.930(0.696,1.242)	0.622
Know cause of Cxca					
No	746	490(65.7)	256(34.3)	1	
Yes	169	115(68.0)	54(32.0)	0.899(0.629,1.284)	0.558
Knowledge of risk factors					
No	745	494(66.3)	251(33.7)	1	
Yes	170	(65.3)	59(34.7)	1.046(0.737,1.485)	0.801
Smoking					
No	895	596(66.6)	299(33.4)	1	
Yes	17	6(35.3)	11(64.7)	3.654(1.339,9.977)	0.011
Cooking fuel					
Electricity	284	192(67.6)	92(32.4)	1	0.200
Animal & plant	445	292(65.6)	153(34.4)	1.094(0.797,1.500)	0.580
Electricity & others	130	91(70.0)	39(30.0)	0.894(0.570,1.403)	0.627
Mixed, no electricity	42	22(52.4)	20(47.6)	1.897(0.986,3.651)	0.055
Contraceptive use					
Abstain	201	144(71.6)	57(28.4)	1	0.115
Oral contraceptive	86	58(67.4)	28(32.6)	1.220(0.707,2.104)	0.476
Oral cont with others	46	34(73.9)	12(26.1)	0.892(0.431,1.843)	0.757
Other methods	582	369(63.4)	213(36.6)	1.458(1.028,2.069)	0.035
Any contraceptive	714	461(64.6)	253(35.4)	1.386(0.984,1.954)	0.062
Know cxca screening					
No	295	195(66.1)	100(33.9)	1	
Yes	620	410(66.1)	210(33.9)	0.999(0.745,1.339)	0.993
Experience of screening					
No	652	429(65.8)	223(34.2)	1	
Yes	263	176(66.9)	87(33.1)	0.951(0.702,1.288)	0.745
Is cxca treatable?					
Not	106	76(71.7)	30(28.3)	1	0.394
Yes	448	296(66.1)	152(33.9)	1.301(0.817,2.072)	0.268
Do not know	361	233(64.5)	128(35.5)	1.392(0.866,2.237)	0.172

7. DISCUSSION

To the best of our knowledge, this is the first study conducted in Ethiopia to describe the prevalence and distribution of HPV genotypes from facilities in different parts of the country. Previous works mainly focused on biopsy samples collected from cervical cancer patients. Moreover, they were limited to four geographic locations; Jimma (Bekele *et al.*, 2010), Gondar (Abate *et al.*, 2013) and Addis Ababa (Abate, 2015) and cervical swab at Butajira (Ruland *et al.*, 2006; R. Leyh-Bannurah, Prugger *et al.*, 2014). The studies conducted at Jimma and Gondar were on cervical cancer paraffin embedded biopsy blocks.

Genotyping (HPV) of our samples revealed that the prevalence of HPV infection among the study participants was 33.9% (310/915). This finding clearly demonstrated that one in three of our study participants were positive for any type of HPV genotypes identified in the study. This prevalence rate is extremely higher, with statistically significant difference than previous studies conducted in Ethiopia.

Previously, two teams of researchers conducted cross-sectional studies at Atat hospital located at a village 110 miles South-West of Addis Ababa. Both teams recruited outpatient clients of the hospital seeking health care for different types of diseases including genital, respiratory, intestinal etc. The first (Ruland *et al.*, 2006) and the second (R. Leyh-Bannurah, Prugger *et al.*, 2014) teams reported an overall similar HPV prevalence of 15.9% (30/189) and 17.5%(94/537), respectively, among their study participants. However, the HPV prevalence of the current study is significantly different, 33.9% (95%CI: 30.81, 36.95) about two fold of the previous reports. This huge and statistically different prevalence may be explained in many ways. The major reasons may include the participants' characteristic, residence, and mainly the HPV genotyping method used and specific HPV types detected by the method.

First, the study participants in the previous studies from Atat (Ruland *et al.*, 2006; R. Leyh-Bannurah, Prugger *et al.*, 2014) were women with any type of disease who were out patient clients of the hospital. However, our study participants were women attending gynecology clinics for gynecological services including cervical screening. Therefore, high rate of HPV infection in this group of study participants may be related to the nature of complaints of the study participants.

The second reason is residence of study participants. Almost all of the study participants at Atat were rural residents, while 85.9% (786/915) of our study participants were urban inhabitants. Generally, HPV infection is more in urban inhabitants than rural counterparts. In this study, 89.4% (277/310) of HPV prevalence was from women living in urban areas. More specifically, 35.2% and 25.6% of urban and rural inhabitants were HPV positive, respectively. The finding was consistent with the perceived norm that Ethiopian rural residents are culturally and sexually conservative, low promiscuity. Hence, HPV transmission is normally low among such communities.

The third reason is that our HPV identification method was able to identify 26 HPV types, while the HC2 HPV DNA test, they used, identifies some HPV types, HPV6, 26, 42, 53, 54, 66, 68b, 70, 72 and HPV 90, HPV 26, 53, 54, 66,68b,70,72,72,73,90, less than the bead-based Luminex system we employed. Moreover, their test could not discriminate between HPV 68 and HPV 73, read both as one HPV type, while both HPV types hold different positions in the genotyping system used in this study. Indeed the bead-based Luminex XMAP2000 genotyping system (46.2%) has superiority over HCll (29.7%) in identifying HPV infection among women with ASCUS (Comar *et al.*, 2012). Thus, some of the HPV types may be missed in the previous studies at Butajira (Atat hospital). Moreover, it was confirmed that HPV prevalence rate vary geographically.

Conversely, HPV type prevalence rate reported from Eastern, Western and Southern African countries indicated that HPV prevalence among our study participants was relatively low. Besides, variable reports of overall prevalence of HPV infection and genotype distribution are common in different geographic locations. The prevalence of HPV infection in countries South of Sahara Desert was estimated to be between 12% (South Africa) and 46% (Gabon), and varies geographically (Smith *et al.*, 2008). Therefore, our result is consistent with the estimation for Sub-Sahara countries and similar to the rate of HPV prevalence reported from Rwanda, 34.0% (Ngabo *et al.*, 2016).

On the other hand, the prevalence of HPV infection in this study is significantly higher than related studies from Sudan, South Africa and Qatar. Among women attending screening facility in Sudan, the prevalence of HPV was 8.9% (12/135) (Salih *et al.*, 2010) which is the lowest HPV prevalence reported from Sub-Saharan countries. Moreover, in South Africa (Allan *et al.*, 2008) reported that overall HPV prevalence was 22.9% (221/963). Moreover, HPV prevalence reported from Middle East (Qatar) was 6.2% which is among the lowest HPV prevalence ever reported (Bansal *et al.*, 2014). Therefore, in comparison to these reports, the prevalence of HPV infection in this study was relatively higher.

In comparison, our finding is consistent with previous studies reported elsewhere. Hospital based studies reported from Korea (So, Hong and Lee, 2016) and Italy (Ripabelli *et al.*, 2010) found that HPV prevalence was 33.7% and 30.1% among their participants, respectively. Moreover, related report from India (Aggarwal *et al.*, 2006) found HPV prevalence of 36.8% among their study participants with similar characteristics to ours.

In general, the overall prevalence of HPV in this study is one of the highest HPV prevalence identified. However, it is lower than other reports from sub-Saharan countries. Two studies conducted in Kenya, Nairobi and Mombasa, on women attending family

planning center for cervical screening, found that HPV prevalence was 44.3% and 44.7%, respectively (De Vuyst *et al.*, 2010). Moreover, recently the prevalence of HPV infection among women who were recruited at cervical cancer screening centers was reported 48.1% in North-Eastern Nigeria (Manga *et al.*, 2015). Although, these reports indicated higher rate of HPV prevalence, our finding of HPV prevalence is one of the highest HPV prevalence reports to date in Ethiopia.

Table 7. 1 Comparison of HPV prevalence among African countries

Country	**N**	**HPV**			
		Negative	Positive	%(95%CI)	References
Ethiopia	915	605	310	33.9(30.81,36.95)	This study
	189	159	30	15.9(10.66,21.08)	Ruland *et al.*, 2006
	537	451	86	16.0(12.91,19.12)	Leyh-Bannurah *et al*., 2014
Kenya	429	239	190	44.3(39.59,48.99)	De Vuyst,2010
Sudan	135	123	12	8.90(4.09,13.70)	Salih *et al*., 2012
Nigeria	209	109	100	47.8(41.07, 54.62)	Manga *et al.*, 2015
South Africa	963	742	221	22.9(20.3, 25.61)	Allan *et al*., 2008

Similarly, hospital based cross-sectional studies with similar methods of HPV detection reported dissimilar findings to our study (Table7.1). The 95% CI analysis showed that the highest HPV prevalence was from Nigeria followed by Kenya with 47.8% (95%CI: 41.07, 54.62) and 44.3% (95%CI: 39.59, 48.99), respectively. Although, their HPV prevalence was not significantly different in between, it is statistically different from the studies conducted in Ethiopia. Ethiopia followed by South Africa were with medium prevalence rate of 33.9% (95%CI: 30.81, 36.95) and 22.9% (95%CI: 20.3, 25.61), respectively. Hence, the HPV prevalence of our study was statistically different from the studies conducted in South Africa and Sudan.

Our study sites are on average 400 to 1000Kms far apart from each other. Thus, we assumed that circulating HPV genotypes and their prevalence could be significantly

different among populations living in these sites. To test our assumption, we compared the prevalence of HPV infection among study sites. Thus, in comparison to study participants at Addis Ababa, HPV prevalence was higher among study participants at Mekelle (39.0%) and Dessie (40.0%). This significantly higher proportion may be due to the difference in number of lifetime sexual partners a woman had between these two sites and Addis Ababa. At Dessie and Mekelle the proportion of women with two sexual partners was 47.5% and 31.4%, respectively, while it was 28.6% at Addis Ababa. Moreover, women with ≥3 lifetime partners were 25.8% at Dessie while it was 18.9% at Addis Ababa. Therefore, the highest proportion of HPV infection at Dessie and Mekelle may be related to the higher proportion of study participants with ≥2 lifetime sexual partner at these sites than in other study sites. This is generally true that the risk of HPV infection is associated with increasing number of sexual partners.

Moreover, these two sites, Mekelle and Desse, are serving as the main military conduits to the Ethio-Eritrean war since 2000GC. As a result, prostitution has been wide spread activity in these areas. Thus, HPV infection could spill over to the public via sexual active youngsters.

On the other hand, all over the world, prevalence of HPV infection and type distribution shows regional differences. For example an IARC study reported that HPV prevalence varied nearly 20 times between populations, from 1.4% (95%CI: 0.5 to 2.2) to 25.6% (95%CI: 22.4, 28.8) from Spain to Nigeria, respectively (Clifford *et al.*, 2005). Moreover, two researches from different parts of Nigeria reported different HPV prevalence rates. The prevalence of HPV reported at Abuja (Akarolo-Anthony *et al.*, 2014) and at North-Eastern Nigeria (Manga *et al.*, 2015) were 37.0% and 48.1%, respectively. Therefore, variation in HPV prevalence with in a country and among countries is common.

High-risk HPV infection of the uterine cervix establishes the precursor and the prime cause of cervical cancer. Thus, cervical cancer is solely, ~ 99%, caused due to infection of these specific oncogenic HPV types (Bosch *et al.*, 2002). However, the distribution of these oncogenic HPV types is different in different geographic regions (Bruni *et al.*, 2010). After thorough analysis of publications (1995 to 2009) Bruni *et al* (2014) reported that HPV types 16, 18, 31, 52, and, 58 were the most prevalent HPV types isolated globally. However, regional and sub regional difference are there (Smith *et al.*, 2007). Therefore, determining circulating HPV types in a community has many implications in planning and efficiently executing overall HPV and cervical cancer related intervention strategies.

In this study, all the high-risk, putative high-risk and low risk HPV genotypes included in the typing system were identified. The five most prevalent high-risk HPV type was HPV 16 followed by HPV 52, 56, 59 and HPV 35 in their decreasing order of proportion 10.3% (94/915), 5.6% (51/915), 4.2%(38/915), 4.0(37/915), and 3.5% (32/915), respectively. The potent oncogenic high-risk HPV 16 was the most prominent and significantly higher HPV type identified in this study. On the other hand, HPV 18, the second most prevalent in different parts of the world, is the seventh most prevalent, 1.7% (16/915), of all HPV types identified in this study.

Table 7. 2 Comparison of top five prevalent high-risk HPV types in African countries

Study country	**Most prevalent HRHPV**	**References**
Ethiopia: this study	HPV 16, 52, 56, 35, 59	
Ethiopia, earlier, Atat	HPV 16, 52, 56, 31, 51	(R. Leyh-Bannurah, Prugger *et al.*, 2014)
Kenya	HPV 52, 16, 35, 66	(Vuyst, 2003)
Uganda	HPV 52, 58, 16	(Blossom *et al.*, 2007)
Nigeria	HPV 18, 16, 33, 31, 35	(Manga *et al*., 2015)

Our results were in agreement with previous reports by Leyh-Bannurah *et al* (2014) on the three most prevalent HPV types being HPV 16, 52 and HPV 56 (Table7.1). However, at Atat, HPV 31 was the fourth while it was the sixth (2.1%) most prevalent in this study.

With regard to the most prevalent high-risk HPV types, our results were different from Kenya and Nigeria reports. In Kenya (Nairobi) it was reported that HPV 52 (17.9%), HPV 16 (14.7%), HPV 35 (11.9%), and HPV 66(9.0%) were the first, second, third and fourth most prevalent HPV types identified (Vuyst, 2003). In Nigeria, it was reported that HPV 18 (44.7%), 16 (13.2%), 33(7.9%), 31(5.3%) and 35(5.3%) were the most prevalent HPV types identified with decreasing order of prevalence. In Kenya and Uganda, HPV 52 was the first most prevalent HPV type identified while it was the second prevalent type identified in Ethiopia and not in the top five in the Nigeria study (Table 7.2). This confirms regional difference of HPV genotype distribution is familiar.

In line with the above confirmed trends of geographic variation in HPV genotype prevalence, the prevalence of HPV genotypes within and among study sites was different in this study. Similarly, HPV 16 was the most prevalent HPV type in most study sites except at Bahr Dar. At Bahr Dar, HPV 56 and 59 were the first and second most prevalent HPV types identified. Moreover, the type specific prevalence of HPV types in our samples was different from other sub-Sahara countries.

The prevalence of HPV genotype in this study was different from other studies with similar study participants from central Nigeria (Abuja), Eastern Nigeria (Ibadan), and Kenya (Nairobi and Mombasa). In the Abuja (Akarolo-Anthony *et al.,* 2014) study, the five most prevalent HPV types were HPV35, 56, 58, 45, and HPV 59. While in the study at North-Eastern Nigeria, the five most prevalent HPV types were HPV 18, 16, 33, 31, and HPV 35, respectively. In their study, HPV 18 was the most prevalent with a significantly different in prevalence 44.7% than other HPV genotypes including HPV 16 (13.2%).

Moreover, the Kenya (Vuyst, 2003) study reported that HPV 52 (17.9%) was the most prevalent followed by HPV 16 (14.7%), 35 (11.6%) and 66 (9.0%). Likewise, Smith *et al* (2008) reported that the most prevalent HPV types in East Africa were HPV52, 16, 18, 53 and HPV 66. Therefore, our results were different from previously reported top five prevalent HPV types in East Africa and elsewhere.

In a large sample size study at IARC, Clifford *et al* (2005) concluded that HPV 35 (12.3%) was as high as HPV 16 (12.3%) among HPV positive Nigerian women. However, in this study, HPV 35 (10.3%) covers one-third of the prevalence of HPV 16 (30.3%) of HPV positive samples. The prevalence of HPV 16 (30.3%) is comparable to report from Scotland (31.5%), Denmark (30.5%) and England (35%) reviewed by Clifford *et al* (2005). Therefore, our study participants were more likely to have HPV 16 infection than HPV 35 like South America and European women. This difference in high-risk and low risk HPV genotypic prevalence may have implication in the vaccine type preference and instituting HPV tests or any intervention strategy in different countries (Clifford *et al.*, 2005).

The implications of elevated prevalence of HPV 16 among our study participants partly could explain the high prevalence of this genotype among cervical cancer samples analyzed in Ethiopia. In a study from Jimma, South-West of Ethiopia, composed of 122 biopsy samples of which 82 (67.2%) samples were HPV positive and 82.9% (68/82) of them were found to harbor HPV 16 (Bekele *et al.*, 2010). Similarly, a study from Gondar (North-Western Ethiopia) reported that 85%(136/160) of paraffin embedded biopsy blocks analyzed were found to have HPV 16 (Abate *et al.*, 2013). Thus, HPV 16 may be the most prevalent HPV type circulating in Ethiopia.

Among the study sites investigated, HPV 16 was significantly prevalent at Mekelle (12.3%) and Addis Ababa (10.3%). However, it was the third most prevalent HPV type (3.8%) identified at Bahr Dar preceded by HPV 56 (8.5%) and HPV 59 (6.6%). Moreover,

it was not significantly prevalent at Gondar (9.9%) than HPV 52 (9.2%). Similar reports confirming HPV type distribution difference among different study sites from the same country were reported (Vet *et al.*, 2008).

Finally, we may conclude that the most prevalent HPV types in different parts of Ethiopia differ. Our finding from the investigated study sites showed differences in genotype distribution and their prevalence. Similar trend was observed between the previous research, Leyh-Bannurah *et al* (2014) at Atat, and this study. Therefore, to avoid such confusion and have clear national data of HPV genotypes for decision making, nationwide HPV genotype investigation may be crucial before commencing HPV vaccination program.

The higher proportion of multiple HPV infection among the women participated in this study is worrying. In line with this, some researchers indicated that infection of a woman with multiple HPV types is associated with increased risk (OR:2.2; 95%CI: 1.1, 4.6) of initiating and progression to cervical cancer (Chaturvedi *et al.*, 2011). These researchers argue that presence of different high-risk HPV types in a woman may have synergistic effect to push the infection towards development of cervical dysplasia (Trottier, 2006) rapidly. Conversely, other researchers did not found any association between multiple HPV infection and development kinetics or risk of cervical cancer. Even other researchers proposed a possible antagonistic effect or competition among co-infecting HPV types (Salazar *et al.*, 2015). However, both sides might agree on the notion that multiple HPV infection is a result of risky sexual behavior. A research indicated that women with more number of sexual partners are prone to acquire multiple HPV infections (Vaccarella *et al.*, 2006). Therefore, it is advisable to evaluate the prevalence of multiple HPV infection in a population.

Our study indicated that multiple HPV infections were relatively high. Overall, the prevalence of single and multiple HPV infection was 19.3% (95%CI: 16.8, 21.9) and 14.5% (95%CI: 12.5, 16.8), respectively. However, among the HPV positive study participants, the proportion of multiple HPV infection is worrying, 42.9% (95%CI: 37.4, 48.4). This high rate of multiple HPV infection was significantly higher than other reports. Samples collected between 2007 and 2011 were analyzed and revealed that the prevalence of multiple HPV infection, of 24 most prevalent HPV types, was 4.6% (Dickson *et al.*, 2013). In line with Vaccarella *et al* (2006), 56.8 % (75/132) multiple HPV infected study participants were those who had ≥2 lifetime sexual partners (data not shown). Therefore, public awareness on risk factors and consequences of polygamy may help to reduce the prevalence of HPV infection in general and the risk of multiple HPV infection specifically.

HPV genotype distribution as multiple and single infections found to show variation between our study sites. The highest multiple HPV infection among HPV positive study participants was at Gondar. At this site, single and multiple HPV infections were 46.8% (95%CI: 32.54, 61.07) and 53.2% (95%CI: 38.93, 68.46), respectively. The second was Bahr Dar with equal proportion of single and multiple infections, 50.0%. The exact reason for the highest prevalence of multiple HPV infection at Gondar and Bahr Dar is not clear. However, these two sites are number one tourist destination sites for national and international travelers in Ethiopia. Therefore, this high flux of people from different areas to these sites may increase the HPV types circulating among sexually active individuals.

Epidemiological studies identified that HPV induced cervical cancer is exclusively associated with persistent infection of certain oncogenic HPV genotypes while other HPV types transiently colonize and induce benign lesions(Clifford, Howell-Jones and Franceschi, 2011; Senapati, Senapati and Dwibedi, 2016). So, infection with the high-risk HPV types is confirmed as the sole risk factor for cervical cancer initiation and progression (Schiffman *et al.*, 2011). Among the high-risk HPV types, over two-third (70%) of all global burden of cervical cancer is due to HPV types 16 and 18 alone (Alba *et al.*, 2009).

HPV 16 is prevalent not only among cervical cancer patients but also among women with normal cytology. In an IARC study encompassing 13 countries all over the world, showed that HPV 16 was the most prevalent with proportion of 19.7% followed by HPV 58 (7.6%), HPV31(7.5%) and HPV 18 (7.2%) (Clifford *et al.*, 2005).

Similarly, the distribution of these oncogenic HPV types varies in different parts of the world (Pande *et al.*, 2008). For instance, it was reported that HPV 16 and 18 are responsible for 74 to 77% in Europe and North America while 65 to 70% in Africa and other parts of the world (Smith *et al.*, 2007). Therefore, higher proportion of infection with high-risk HPV types in certain geographic area may be associated with increased probability of initiation and development of cervical cancer among such populations.

Correspondingly, in this study, the proportion of high-risk HPV infection was comparatively high. Of all, investigated samples, 27.2% (249/915) harbor at least one high-risk HPV type. Moreover, high-risk HPV types constitute 80.3% (78.5,87.0) among HPV positive samples. Such elevated prevalence of high-risk HPV types is distressing as the proportion of women with high-risk HPV infection have greater propensity to develop cervical dysplasia. Moreover, the elevated prevalence of high-risk HPV infection among our study participants may imply that these HPV genotypes are the dominant HPV types circulating in the population.

Proportionally, the prevalence of high-risk HPV infection among HPV positive study participants, in this study, was insignificantly lower than previous reports from Ethiopia. Previously, the prevalence of high-risk HPV infection among HPV positive study participants was 90.4% (85/94) (95%CI; 80.8, 125.1) (R. Leyh-Bannurah, Prugger *et al.*, 2014) and 83.2% (25/30) (95%CI: 53.9 to 123.0) (Ruland *et al.*, 2006). However, the 95%CI analysis showed that the prevalence of high-risk HPV infection among these studies was not significant. Therefore, these findings imply that high-risk HPV types are

the most prevalent HPV types circulating in Ethiopia. This, in turn may have implications on the high prevalence of cervical cancer and related death reported in the country.

Age of a woman is always associated with HPV infection. Researchers identified that HPV infection is highly associated with the age of a women, the young being the most infected. They indicated that although there are differences among regions and type of population investigated, the shape of the age–HPV infection curve is either declining in older ages, flat across age or U shaped (Smith *et al.*, 2008). Moreover, it was concluded that HPV prevalence in Africa and Central America show first peak at ≤25 years old, then declines and finally showed second peak ≥45 years old (Smith *et al.*, 2008). However, among our study participants the proportion of HPV infection was highest at the age group of 25-34 (39.2%) followed by young age group 18 - 24 (34.7%). Then sharply drops to 30.7% in the age group 35-44; then keeps flat at about 31.0% in the remaining age groups. This form of HPV prevalence was different from other studies. In China, it was reported that HPV infection follows a U shape curve with two peaks early 16-19 (30.55%) and later at the age group 50 to 60 years old (Wang *et al.*, 2015). On the other hand, in Denmark and Spain cervical HPV DNA prevalence review along the age groups showed sharp decline from the young to the old (Bosch, Qiao and Castellsague, 2006).

Bivariate logistic regression analysis revealed that as compared to the <25 age group, the only age group insignificantly (OR=1.212; p>0.05) associated with HPV infection was the 25-34 age group. Similar phenomenon was reported from Indian by Sarma *et al* (2013) where age was not significantly associated with HPV infection as well as young age was not associated with increased prevalence of HPV infection.

The prevalence of high-risk HPV infection was lower in age group <25 than older ages. Their proportion alternates with age groups with the second high pick at ≥65 (20.0%) years old. This elevated proportion of high-risk HPV infection in the late age group (above 50

years old) may show persistence of HPV infection or reduced immunological clearance of high-risk HPV types. On another analysis, we found that 50% (10/20) of ≥65 years old study participants had ≥2 lifetime sexual partners. Moreover, higher proportion of study participants with more than one sexual partner corresponds to the age groups with the highest proportion of high-risk HPV infection (data not shown). Moreover, 66.7% (4/6) of these study participants were infected with the potent oncogenic HPV 16 with the highest capacity to establish persistent HPV infection. Therefore, all these evidences could contribute to high level of high-risk HPV infection above ≥ 65 years old. Similar study was reported from China (Kang *et al.*, 2014) in a follow up study. Kang *et al* (2014) reported that the proportion of high-risk HPV infection was higher (19.7%) among women with ≥55 years old than the average (13.1%) rate. Conversely, other researchers indicated that multiple high-risk HPV infection are commonly high among women younger than 25 years old (Cuschieri *et al.*, 2004) and lowest (2%) above 55 study participants(Chan *et al.*, 2010).

On the other hand, low risk and putative high-risk HPV infection were not found among oldest (65+) study participants. The absence of these non-high-risk HPV types could be due to accumulation of immunity against them and older women have less probability of having new sexual partner a known risk factor for HPV acquisition.

Residence of our study participants, rural or urban, was significantly associated with HPV infection. The bivariate logistic regression analysis clearly showed that urban inhabitants were more likely to have HPV infection than their rural counterparts (OR=1.583; $p<0.05$). Similarly, it was reported that the prevalence HPV infection was much higher among urban (57.4%) than rural (25.8%) inhabitants (Baloch *et al.*, 2016). Conversely, other researchers reported that HPV infection was higher among rural inhabitants (Ali-Risasi *et al.*, 2014). However, our findings are in agreement with the perceived social norms where rural inhabitants are sexually more conservative than urban counterparts in Ethiopia.

Many large scale studies reported that HPV infection is strongly associated to women's educational and marital status. It is believed that education fosters many important factors that could reduce HPV infection and its consequences. It promotes better life style, delayed age sexual intercourse, limited number of children, reduced exposure to indoor carcinogens and finally improved socioeconomic status is a determinant factor for better health seeking behavior. In line with this notion, Ferrera *et al* (2000) HPV infection rate significantly ($p<0.00001$) reduced as the educational level of study participant's increase. Study participants who were with primary and secondary or above educational status were 0.41 and 0.08 times less likely to have HPV infection than their uneducated counterparts (Ferrera *et al.,* 2000). Consistent to these results educational status was associated with reduced ($OR<1$) proportion of HPV infection among our study participants. Although not significant ($p>0.05$), as educational level of our study participants increase HPV prevalence decreases.

Marital status is always associated with HPV acquisition. In this study, being divorced is associated strongly (OR=1.75; p=0.002) with increased risk of HPV acquisition. Moreover, being single or widow are insignificantly ($p>0.05$) associated with increased rate of HPV infection as compared to married study participants. Our results are consistent with the large cohort CLEOPATRE study (Roura *et al.,* 2012). The CLEOPATRE cohort study identified that unmarried status, more than one sexual partner, low educational status were strong independent risk factors for HPV acquisition. Similarly, a report from Mali indicated that divorced and widow women were 3.5 times more likely to have high-risk HPV infection than those married counterparts (Tracy *et al.,* 2011). Moreover, in the Danish cohort(Nielsen *et al.,* 2010), HPV acquisition was strongly associated with the number of sexual partners. They reported that study participants with ≥4 sexual partners were (OR: 15.39; 95%CI: 9.26, 25.58) times more likely to have HPV infection as compared to women with ≤1 recent partner (OR: 4.15; 95%CI: 2.45, 7.02). The strong and significant association of HPV infection with divorced women may be associated with the sexual behavior of the women after divorce.

Early age at sexual debut (<18) is usually considered as the main factors associated to HPV infection. In line with this, it was reported that early age at sexual debut was strongly associated (p=0.009) with increased rate of HPV infection (Manga *et al.*, 2015). A report identified that study participants who started sexual intercourse in the age groups of 18-19 and 20+ were 0.87 and 0.54 times less likely to have HPV infection as compared to their study participants who started sexual intercourse in the age group 12-15 years old (Ferrera *et al.*, 2000). Sarma *et al* (2013) reported similar findings where they found strong association of HPV infection with early marriage, high parity and pregnancy. However, in this study, HPV infection was significantly higher (OR=1.5; p=0.007) among those women who started sexual activity after the age of 17.

Number of partners is one of the strongest independent risk factors for HPV infection globally. Findings from the UK case control study (Green *et al.*, 2003) indicated that having ≥3 life time sexual partners was strongly and significantly associated with HPV infection and squamous cell carcinoma; (OR=3.4; 95%CI: 2.38, 4.86). In our study, HPV infection strongly associated factors were number of lifetime sexual partners and history of STI infections (p<0.05). Our results were consistent with many cohort studies (Kru *et al.*, 2001; Nielsen *et al.*, 2010; Roura *et al.*, 2012) for HPV infection.

Moreover, many epidemiologists pointed out that higher number of children and pregnancies are associated with increased risk of HPV infection. In this study, having one child or one pregnancy was associated with HPV infection as compared to women without child or pregnancy. Specifically, being pregnant for one time was significantly associated with HPV infection (OR=2.43; p=0.003). In line with this, many researchers found that high parity and pregnancy were associated with increased risk of HPV infection (Bosch and Sanjos, 2007; Gómez and Santos, 2007; Tao *et al.*, 2014). In search for HPV infection and invasive cervical cancer associated co-factors Ferrera *et al* (2000) reported that HPV infection was associated with increasing number of children or pregnancy. They reported

that those women who had 5-6, 7-10 and 11+ children were 2.56, 2.86, and 3.54 times more likely to have HPV infection.

On the other hand, the emergence of HPV vaccine and Ethiopia being in the GAVI list is an opportunity to initiate vaccination program to reduce the impact of HPV infection and cervical cancer in Ethiopia (CDC, 2013). Many researchers reported that the HPV vaccines are efficacious in preventing up to 90% the prevalence of vaccine type HPV infection (Cutts *et al.*, 2007; Castellsagué *et al.*, 2011; Guo, Hirth and Berenson, 2015). Similarly, a study from Switzerland (Jacot-guillarmod *et al.*, 2017) found that the prevalence of vaccine types was reduced from 25.4% (before vaccination) to 4.0% after 77.5% five year vaccine coverage. In this study, the prevalence of vaccine types was relatively high; Cervarix ™ and Gardasil® constituted 35.5% (110/310) and 39.0% (121/310) of the total HPV DNA identified, respectively. Moreover, those HPV types included in the new 9-valent HPV vaccine constituted 72.6 % (225/310) of HPV infections identified. As per our results, there is no significant difference between the expected impact of Cervarix and Gardasil. However, the expected impact of the 9-valent vaccine is significantly different than both Cervarix and Gardasil. Thus, it can be inferred that Ethiopia could benefit from vaccination and the GAVI initiative.

The other group of HPV genotypes worth mentioning here are the two low risk HPV types, HPV 6 and HPV 11, the most dominant (90%) causative agents of genital warts (Hartwig *et al.*, 2017). These HPV types are parts of the Gardasil and 9valent vaccines (Serrano *et al.*, 2014). However, HPV 6 with the proportion of 4.4% (10/225) was the third least prevalent HPV vaccine types identified while HPV11 with proportion of 0.4%(1/225), was only identified in a single sample with multiple HPV infection. On the other hand, as per this study, the sum of the prevalence (5.6%) of HPV types 31, 33, 45 and 58 included in the 9 valent vaccine was equal to the prevalence of HPV 56 alone (5.6%). Consequently, addition of HPV 56 and 59 alone could increase the possible coverage to 83.9%. Therefore, although the vaccines could reduce morbidity and mortality in Ethiopia,

thinking wisely to choose one of the vaccines is mandatory to maximize the benefit of protection will be vital before instituting at random.

In many epidemiological studies, HPV infection was found to associate with infection of certain STIs. Some of these sexually transmitted infectious agents include HSV-2, *Mycoplasma, T. vaginalsi, C. trachomatis, N. gonorrhea* etc. These agents are repeatedly implicated to have association with increased HPV infection and cervical cancer.

In this study, 3.81% (32/840) of study participants were infected with HSV-2. The prevalence was relatively lower than studies reported from Brazil, 15.6% (Caldeira *et al.*, 2013). Infection of HSV-2 creates favorable condition for HPV infection and cooperatively induces HPV persistence via multiple mechanisms. HSV-2 has inherent capacity to induce intense inflammation, epithelial layer disruption and host DNA destabilization (Jalouli *et al.*, 2012; Raju, 2015) which is a prerequisite for HPV infection and DNA integration. Therefore, HPV infection usually associates with HSV-2. Similarly, we identified that HSV-2 infected study participants were 3.14 ($p=0.002$) times more likely to have HPV infection than their STI negative counterparts.

Mycoplasma genitalium is associated with cervicitis, endometritis etc. In this study, the prevalence of *M. genitalium* was 1.9%. Similar results were reported from UK where the prevalence of *M. genitalium* was between 0.9 and 1.9% (Sonnenberg *et al.*, 2015) but lower than a report from Sweden, 4% (Anagrius, *et al.*, 2005) and USA 16.1% (Getman, Jiang and Donnell, 2016). On the other hand, *M. genitalium* was insignificantly ($p>0.05$) associated with HPV infection in this study.

Trichomonas vaginalis, a protozoa, is the most prevalent sexually transmitted infectious agent globally. There were 276.4 million *T. vaginalis* infection in 2008 that implies it is the

most prevalent of all STIs (Kissinger, 2015). In Africa, *T. vaginalis* prevalence was estimated to be between 6% and 42% (Ramjee, Abbai and Naidoo, 2015). However, the prevalence of *T. vaginal* in this study was 4.5%, which is lower than the estimated rate for Africa. Our results were remarkably lower than similar report from Zimbabwe (31%) and Mexico (14.2%) (Men *et al.*, 2010; Casillas-vega *et al.*, 2016).

On the other hand, the association of *T. vaginalis* to HPV infection was notable (OR: 1.56; p>0.05). As opposed to our results, *T. vaginalis* was significantly associated with low risk (OR: 2.7; 95%CI: 1.5, 5.0) and high-risk HPV (OR: 1.7; 95%CI: 1.2, 2.3) infections (Donders *et al.*, 2013). Moreover, in a similar study in Mexico, *T. vaginalis* was significantly associated to HPV infection, p<0.01 (Casillas-vega *et al.*, 2016).

Previously conducted studies in Ethiopia reported that the prevalence of *C. trachomatis* was 2% (Amsalu *et al.*, 2017). However, in this study, low level prevalence was recorded where 0.5% (4/856) of our study participants were found to harbor *C. trachomatis.* The prevalence rate is very low as compared to studies conducted elsewhere. In Nigeria (Mawak *et al.*, 2011), it was reported that the prevalence of *C. trachomatis* was 56.1% (92/164) which is very high. However, two researches from Italy reported lower prevalence of *C. trachomatis* with equivalent proportions of 5.8% and 5.2% (Marcone *et al.*, 2012; Panatto *et al.*, 2015). Comparatively, our study showed lower proportion (0.5%) of *C. trachomatis* infection among our study participants.

Chlamydia trachomatis is an intracellular bacteria transmitted via sexual contact. It is indicated that *C. trachomatis* infection is associated with intense inflammatory reaction and consequent cell lysis and disruption of mucosal integrity (Zhu *et al.*, 2016). Cervical epithelial trauma and inflammation create conducive environment for HPV infection. Moreover, it induces production of reactive oxidative metabolites that could increase host DNA damage that again could favor HPV DNA integration into the host genome (Zhu *et*

al., 2016). Thus, it is associated with HPV infection and creating conducive environment for HPV mediated cervical carcinogenesis.

Co-infection with HPV was relatively high among our study participants, which was 75% (3/4) of them were associated with multiple high-risk HPV types. Our results were comparable to finding reported from Colombia that identified 77.2% of their *C. trachomatis* positive samples were associated with multiple high-risk HPV (Quinónez-calvache *et al.*, 2016). Similarly, strong association (OR: 2.8; 95%CI: 1.6, 4.9) of *C. trachomatis* with multiple high-risk HPV infection was reported from USA cohort study (Samoff *et al.*, 2005).

Neisseria gonorrhea is a strict human pathogenic bacterium. It is one of the most prevalent sexually transmitted infectious agents with an annual incidence of more than 106.1 million individuals (Sangita *et al.*, 2016). Moreover, Some of its isolates are becoming superbugs, resisting most of the available drugs (Unemo and Shafer, 2014).

In this study, the prevalence of *N. gonorrhea* was 2.3% and insignificantly associated to HPV infection (OR: 1.25; $p>0.05$). Previously using culture method, the prevalence of *N. gonorrhea* was 5.1% at Hawassa (Hailemariam *et al.*, 2013) and Gambela 5%(Ali *et al.*, 2016). The prevalence of *N. gonorrhea* is relatively lower among our study participants than reported elsewhere. Researchers from Kenya (Nzioka, 2016) and Ghana (Duplessis *et al.*, 2015) reported that the prevalence of *N. gonorrhea* was 8% and 18%, respectively. Moreover, in USA the prevalence of *N. gonorrhea* was relatively high (6.7%) (Diag- and Jose, 2012) than among our study participants. Therefore, our finding shows that *N. gonorrhea* had relatively low prevalence among our study participants. Moreover, *N. gonorrhea* was not associated with abnormal Pap smear or VIA, in this study.

To sum up, our results indicated that the major STI agents, HSV-2, *M. genitalium, T. vaginalis, C. trachomatis* and *N. gonorhea* showed association to HPV infection. However, only HSV-2 showed significant association with abnormal VIA results.

Bacterial vaginosis is a common sexually associated disease characterized with gray-white vaginal discharge that release fishy amine odor on application of 10% KOH, vaginal pH >4.5, replacement of normal hydrogen peroxide producing *Lactobacilli* dominated vaginal microflora with over growth of *G. vaginalis* dominated anaerobic bacteria including *A. vaginae, M. hominis* etc (Livengood, 2009). In this study, the overall prevalence of BV was 15.4% (130/844). As compared to other reports, the prevalence of BV among our study participants was very low. However, there were no reports that employed similar methodology to ours to compare the prevalence rate.

On the other hand, scientific reports pointed out that BV infection is a significantly associated factor for HPV infection. In a meta analysis (Gillet *et al.*, 2011), it was reported that there is strong association (OR=1.43; 95%CI: 1.11, 1.84) between BV and cervical HPV infection that signifies BV is a risk factor for HPV infection. Moreover, employing large number of study participants, researchers found that HPV infection was strongly associated ($p<0.001$) among those women who were BV positive than their BV negative counterparts (Lu *et al.*, 2015). Similarly, our results showed that 43.8% ($p=0.011$) of BV positive study participants were significantly associated to HPV infection. Correspondingly, employing prospective cohort study, it was reported that BV positive women were found to be more susceptible (OR=1.41;95%CI: 1.25,1.59) to HPV infection than their BV negative counterparts (Watts *et al.*, 2005). Thus, it can be inferred that BV could create suitable cervical milieu to HPV infection.

In line with these findings, researchers identified that there are some insights into the mechanism by which BV infection could assist HPV infection. Hardy *et al* (2017)

identified that *G. vaginalis* establishes a biofilm that creates a conducive environment for *A. vaginae* and other polymicrobial growth. This BV associated polymicrobial growth orchestrated by *G. vaginalis* releases different enzymes that disrupts the normal vaginal epithelium enhancing its susceptibility to sexually transmitted infections. One of these BV associated enzymes is sialidase (Hardy *et al.*, 2015, 2016, 2017) that degrades the mucus layer and create microabrasions on the cervical epithelium that could enhance HPV adsorption and access to its target, basal cells. Therefore, these evidences imply that BV associated infections are important risk factors for establishing HPV infection of the uterine cervix.

It was confirmed that the vaginal microbiota is diverse and complex among BV positive than BV negative women (Gao *et al.*, 2013; Onderdonk, Delaney and Fichorova, 2016). The situation obscures to define specific or groups of etiologic agent for BV. However, infections with *G. vaginalis* and *A. vaginae* (Hardy *et al.*, 2016) as well as *M. hominis* (Schmitt *et al.*, 2014) are being reported as both specific (99%) and sensitive (95%) to define BV (Onderdonk, Delaney and Fichorova, 2016). Therefore, identifying and characterizing these two bacteria and or in association with *M. hominis* could be used to determine the presence and level of BV as described by Schmitt *et al* (2014).

In this study, HPV infection was associated with BV associated bacterial infections mainly *G. vaginalis, A. vaginae* and *M. hominis* that constitute 46.6% (393/844). Moreover, 161 (40.7%) of study participants positive for these bacteria were HPV positive. Thus, binary logistic regression analysis showed that HPV infection was significantly associated (OR=1.79; $p<0.0001$) with BV associated infections. Similar association of HPV to BV was reported elsewhere. In a meta analysis, it was indicated that BV associated infections were significantly (OR=1.43; 95%CI>1) associated with HPV infection (Gillet *et al.*, 2011). In a cross-sectional study, it was identified that *G. vaginalis* showed strong association (p=0.031) to HPV infection (Gao *et al.*, 2013).

Many researchers reported that *A. vaginae* is a good indicator of bacterial vaginosis arguing that its relatively low in women without BV (range 0 to 20%) and its higher prevalence in BV associated conditions (Ferris *et al.*, 2004; Ling *et al.*, 2010). Moreover, some researchers argue that *A. vaginae* is highly specific (96%) than *G. vaginalis* (77%) to be used as better indicator of BV (Bradshaw *et al.*, 2006). As opposed to these, our findings showed that single infections of *G. vaginalis* and *A. vaginae* were found in 47.4% Vs 13.2% and 14.9% Vs 10.4% in some and strong BV scores, respectively. Nevertheless, dual infection of *G. vaginalis* and *A. vaginae* showed strong association ($p<0.0001$) to BV than their single infections. This result entails that coexistence of *G. vaginalis and A. vaginae* has synergistic effect in the pathogenesis of BV.

The synergistic effect of these BV associated infections was confirmed in many reports. It was confirmed that more than 90% of vaginal biofilms among BV positive women harbored coinfection of *G. vaginalis* and *A. vaginae;* with *A. vaginae* constituting up to 40% of the vaginal biofilm mass (Onderdonk, Delaney and Fichorova, 2016). Moreover, many researchers indicated that *G. vaginalis* and *A. vaginae* do have symbiotic relationship where *G. vaginalis* creates anaerobic environment via its biofilm that establishes *A. vaginae* (Hardy *et al.*, 2015). In line with this, it was reported that co-occurrence of these bacteria is common among BV positive women signaling their cooperative pathogenesis (Verhelst *et al.*, 2004). Therefore, it seems that both cooperatively create the necessary cervical environment for BV initiation and progression.

A comprehensive cohort study among commercial sex workers in Rwanda, polymicrobial growth in the vagina causes changes in the mucus layer, increasing epithelia cell death, expression of proinflammatory cytokines, proteolytic activity and decreasing mucosal immunoglobulin, IgG1/2 (Borgdorff *et al.*, 2015). These findings support the idea that HPV could benefit from the immunologically suppressed and degraded vaginal epithelium to access its target cells establish infection and persistency.

At Addis Ababa, cervical abnormality was evaluated via Pap smear and results showed that CIN I was detected among 19.6% of study participants but no cancer or high-grade dysplasia case was detected. Our results were about 4 times higher than a report from Nigeria that found abnormal cytology among their study participants was 4.93% (Kolawole *et al.*, 2015). Similarly, So *et al* (2016) reported that only 11.6% of their study participants were with abnormal Pap smear of CIN I with high-risk HPV infection.

On the other hand, 30.5% (18/59) of women with abnormal cytology were HPV positive. Among these study participants, 23.7% (14/59) harbored high-risk HPV types. Although not significant (P>0.05), any HPV and high-risk HPV infections were strongly associated (OR>1) with abnormal Pap smear. In this study, the prevalence of HPV among women with abnormal cytology, CIN I, is lower than reported elsewhere. Higher prevalence of HPV infection was reported from Nigeria (Kolawole *et al* 2015) and Bangladesh (Khandker, Khan and Chowdhury, 2016) where HPV infection was 100% and 57%, respectively, among their Pap smear positive samples. In line with this, many studies conducted globally confirmed that HPV infection associated with cervical cancer initiation (zur Hausen, 2009; Ogembo *et al.*, 2015; Mendes, José and Levi, 2016; Kemunto *et al.*, 2017).

The high-risk HPV 16 is the most prevalent type identified worldwide. In 2010, it was estimated that HPV 16 contributed to 22.5% (95%CI: 21.9,23.2%) of the global HPV burden (Bruni *et al.*, 2010). In this study, among HPV positive samples with abnormal Pap smear, HPV 16 was with the highest proportion, 44.4% (8/18). Thus, HPV 16 positive study participants were 3.298($p<0.05$) times more likely to be Pap smear positive than those HPV negative study participants. Our findings were consistent with So *et al* (2016) that reported HPV 16 was the most prevalent HPV type identified among their study participants. Similarly, it is consistent with many finding all over the world that established HPV 16 as the most prevalent and effective oncogenic HPV type in inducing cervical lesions (Castle *et al.*, 2002; Vaccarella *et al.*, 2006; Winer *et al.*, 2008; Castellsagué *et al.*,

2014; Egawa *et al.*, 2015; Niyazi *et al.*, 2015; Ve and Dağilimi, 2017). However, Kolawole *et al* (2015) from Nigeria reported that HPV 16 was the third most prevalent next to HPV 35 (40%), HPV 31 (30%) among their participants with abnormal Pap smear.

The second most common HPV type among the Pap smear positive women was HPV 35. It was identified among 22.2% (4/18) of HPV positive samples. Then, HPV 39, HPV 52 and HPV 56 equally identified in 11.1% (2/18) of HPV and Pap smear positive samples. Our results were consistent with previous reports (Ogembo *et al.*, 2015) for the most prevalent HPV types among women with CIN I in East Africa, identified as HPV 16, HPV 35, and HPV 52. Overall, at least one high-risk HPV infection constituted 88.9% (16/18) of HPV positive CIN I samples. It is strongly associated to CIN I (OR, 3.471; 95%CI, 1.644, 7.330). Comparable results were reported from Beijing (China) where the prevalence of high-risk HPV infection among CIN-I study participants was 90.7% (Tao *et al*., 2014).

The overall HPV prevalence among our study participants with normal cytology was 30.2% (73/242). It is lower than the estimate of HPV prevalence among women with normal cytology for East Africa (42.2%) but higher than Western Africa (27.8%) and North Africa (12%) (Ogembo *et al.*, 2015). Similarly, Clifford *et al* (2005) reported that the rate of HPV infection among women with normal cytology in Nigeria (25.6%) was lower than our results. On the other hand, the prevalence of high risk HPV infection among our study participants with normal cytology was 16.9% (41/242). Conversely, So *et al* (2016) reported that the prevalence of high-risk HPV infection among their study participants with normal cytology was 44.4%, which is much higher than our findings.

This high proportion of high-risk HPV infection among the Pap smear negative women demands critical monitoring of possible outcome in the future. Consistent with our finding, different researchers confirmed that women who were infected with high-risk HPV

infection had high probability of developing cervical lesion. In USA researchers found that 15% of those women who were Pap smear negative with coexisting oncogenic HPV infection would have later abnormal Pap test within five years (Castle *et al.,* 2002). Moreover, in the PATRICIA study (Castellsagué *et al.*, 2014), it was reported that women infected with high-risk HPV types showed higher propensity to develop cervical CIN 1lesion with proportions 3% and 10% at 24 and 48 months follow up. Moreover, in this study, the prevalence of putative high-risk, low risk and mixed HPV infections were 2.9% (7/242), 2.1% (5/242), 8.3% (20/242), respectively.

Taking all these evidences, the elevated proportion of high-risk HPV infected women among our study participants, may increase their probability of developing cervical dysplasia in the future. Therefore, it would be imperative if cervical cancer screening includes high-risk HPV genotyping to predict and plan to manage possible consequences of high-risk HPV infection among VIA or Pap smear negative women in Ethiopia.

Abnormal Pap smear is associated with many risk factors on top of persistent HPV infection. These factors include age, young age at sexual debut, low socioeconomic status, smoking, high parity etc. In this study, it was indicated that as the age of a woman increases, the risk of abnormal Pap smear was also found to increase (OR>1; P>0.05). Our results were comparable to the reports from China that reported an increase of abnormal Pap smear with age (Tao *et al.*, 2014).

Educational level of a woman is associated with improved sanitary and living conditions that may affect the outcome of infection. Unlike other reports, our results showed that as educational level increases prevalence of abnormal Pap smear increases. Thus, women with elementary, secondary, and college or above educational level were 1.477, 1.480, and 1.82 times more likely to have abnormal Pap smear than uneducated women (p>0.05). On the contrary, in Honduras, as educational level increases, detection of abnormal cytology

decrease; i.e. women with primary and second or above educational level were 0.87 and 0.53 times less likely to have abnormal Pap smear than their uneducated counterparts, p<0.00001 (Ferrera *et al.*, 2000). Moreover, in China Tao *et al* (2014) found that education was associated with reduced level of abnormal Pap smear.

Smoking is one of the main risk factors for cervical cancer. Many large cohort studies including the EPIC cohort study confirmed that smoking is one of the major risk factors for cervical cancer (Pawlita *et al.*, 2014). However, smoking is very low among Ethiopian women and only five smokers (5/301) reported in this study site. All of them were Pap smear negative and only two were HPV 70 positive. Thus, among our study participants at Addis Ababa, smoking was not associated with abnormal Pap smear.

In low resource setting WHO recommends visual inspection using ascetic acid (VIA) as screening method. This screening method, VIA, is advantageous over other methods; cheap, none invasive and enables to combine diagnosis and treatment in the same day (WHO, 2013). Therefore, such screening methods are instrumental to reduce the consequences of cervical cancer (Peirson *et al.*, 2013) including death from cervical cancer (Shastri *et al.*, 2014). Developing countries like Ethiopia could benefit from its low cost, accessibility and simplicity of the test to curb the high rate of cervical cancer morbidity and mortality rates. The method is under use in Ethiopia as screening method. In this study, the overall prevalence of VIA positive test was 18.9% which is relatively higher than a study from India (9.9%)(Sankaranarayanan *et al.*, 2007) and 2.1% in Senegal(Irwin *et al.*, 2017). This difference mainly comes from difference in nature of the studies that ours included women attending gynecology clinics while their studies were population based. Like other studies (Gravitt *et al.*, 2010), different sites showed different prevalence of VIA positive results. Moreover, such difference among study sites could arise from the nature of the VIA tests which is subjective and has low reproducibility (Vedantham *et al.*, 2011).

It was found that 38.8% of VIA positive women harbor HPV infection while only 20.5% of HPV positive women were VIA positive. Such huge difference between VIA and HPV positive results was reported in many studies. Gravitt *et al* (2010) reported that only 16.3% of HPV positive women were VIA positive. These researchers found that 15.1% VIA positive women were HPV negative but had inflammation. Besides, not all HPV infections create cervical lesions or cancer precursors. Therefore, cervical inflammation of unknown source could increase false positive VIA results. These findings are critical in instituting screening methods, as false-positive results are associated with psychological crisis, gynecological complications, over treatment and financial burden of the client.

On the other hand, HPV 16 (33.3%) and HPV 52 (22.4%) were the most prevalent HPV types identified among VIA positive women. However, only HPV 16 was the single most important HPV type strongly associated with positive VIA results (OR: 3.002; p=0.0001).

Among the socio-demographic characteristics, age (≥55 years old) was insignificantly associated (OR: 1.944; p=0.28) with VIA positive test. In this study, employment was the main associated risk factor where being a farmer is a risk to develop cervical abnormality (OR: 2.88; p=0.001). This was related to our finding where rural inhabitants were1.96 (p=0.006) times more likely to be VIA positive than urban dwellers. This may be due to the existing condition of low sexual sanitary and high burden of inflammation among rural and farmer community than urban inhabitants that may increase positive VIA results while maintaining low proportion of HPV infection among those rural women.

Public awareness and information about cervical cancer is critical to control the disease. This information could be used for setting efficient public health policy and priorities for the best intervention strategy. However, women living in developing countries have low awareness level about cervical cancer and its intervention strategies. In line with this, it was reported that women living in Sub-Saharan countries did have different level of

awareness towards cervical cancer, from nil to high level of knowledge (Perlman *et al.*, 2014). Moreover, cervical cancer screening and vaccination programs are scarce or never practiced among such countries. Consequently, the highest burden of cervical cancer mortality and morbidity is occurring among these women in developing countries.

Similar to other women living in sub-Sahara countries, information about cervical cancer was not evenly distributed among our study participants. Thus, considerable number of our study participants was unaware of what cervical cancer mean, 33.3% (305/915). Although, this proportion of uninformed women is extremely low as compared to previous KAP studies in Ethiopia, still more work is needed to create critical mass of informed women population in Ethiopia to institute effective intervention strategies. Previously, it was reported that 81.2% of their participants living in Addis Ababa were unaware of cervical cancer and its diagnostic methods (Terefe and Gaym, 2008). However, currently, we identified only 22.7% (68/301) of study participants from Addis Ababa were uninformed about cervical cancer. The study participants from Addis Ababa reported a decade ago were 8.6 (95%CI: 14.2, 23.5) times more likely to be unaware of cervical cancer than the current study participants.

The current awareness level of our study participants is lower than studies from Niger Delta, Nigeria (Owoeye and Ibrahim, 2013) and Kinshsa, Congo (Ali-risasi *et al.*, 2014) and Qatari (Aseel, 2008) (Table 7.3). Thus, Qatari women were 0.6(95%CI: 72.7, 80.1) times less likely to be unaware of cervical cancer as compared to our study participants. Finally, study participants at the Niger Delta, Nigeria were 0.8 (95%CI: 67.5, 76.5) times less likely to be unaware of cervical cancer than our study participants.

Conversely, some studies from Africa countries reported lower level of awareness about cervical cancer. A study from Ghana showed that their study participants were 4.3(95%CI: 27.0, 36.2) times unaware of cervical cancer as compared to our study participants.

Moreover, a study from United Arab Emirates (UAE) reported that their study participants were fivefold less informed about cervical cancer 5.0(95%CI: 22.7, 34.9) than our study participants. Moreover, women from Far-East were poorly aware of cervical cancer than our study participants. Reports from China, 27.0% (Abudukadeer 2015) and Laos, 52.2% (Sichanh 2014) were less aware of cervical cancer than our study participants. However, further awareness creation programs will be crucial by emphasizing the risk factors and available intervention strategies to control HPV infection and its consequences in Ethiopia.

Table 7. 3 Compares awareness level of study participants in different countries

Variable	N	No (%)	Yes (%)	OR(95%CI)	Author
Did you know about CxCa?					
Ethiopia	915	305(33.3)	610(66.7)	1	Current study
Ethiopia	276	224(81.2)	52(18.8)	8.6(14.2,23.5)	Yifru T and Gaym A (2008)
Nigeria	386	108(28.0)	278(72.0)	0.8(67.5,76.5)	Owoeye *et al.*, 2013
Kinshsa,Congo	524	95(18.1)	429(81.9)	0.4(78.6,85.2)	(Ali-risasi *et al.*, 2014)
Ghana	392	268(68.4)	124(31.2)	4.3(27.0,36.2)	(Ebu and Mupepi, 2015)
Qatar	500	118(23.6)	382(76.4)	0.6(72.7,80.1)	(Aseel, 2008)
China	5000	3650(73)	1350(27)	5.4(25.8,28.2)	(Abudukadeer , 2015)
Laos	320	143(47.8)	177(52.2)	1.6(49.9,60.8)	(Sichanh 2014)
UAE	212	151(71.2)	61(28.8)	5.0(22.7,34.9)	(Metwali 2015)

The improvement in the awareness level of cervical cancer at Addis Ababa and all other parts of Ethiopia is largely due to the role of health professionals who played that make the difference. Moreover, mobilization or awareness programs conducted via television and radio programs and the establishment of VIA based screening method all over the country played comparable roles. Currently, cancer board is established in Ethiopia where the committee developed guidelines and the Ministry of Health declared free screening and treatment services in Ethiopia. Hence, the awareness level is increasing from time to time. However, strong effort is necessary to reach more women at the periphery who do not have access to electronic and other mass media outlets.

The study participants who had the information about cervical cancer, the most effective sources of information were health professionals, media (television and radio), and mixed sources informed 26.4% (242/915), 18.5% (169/915) and 10.7% (98/915), respectively. Moreover, family members were responsible for creating awareness to 6.9% (63/915) of study participants. Similarly, Yifru Terefe and Ashebir Gaym (Terefe and Gaym, 2008) found that health professionals were the most important source of information to their study participants. Conversely, relatives and friends were the most effective source of information about cervical cancer among Qatari women (Aseel, 2008) that created awareness for 21.6%. Moreover, a study from China (Abudukadeer , 2015) reported that friends created awareness for 35.2% which was the highest of all other means like Television, 33.9%. However, relatives were the least to deliver information to our study participants. Therefore, to maximize the awareness level in Ethiopia, engaging health professionals especially the extension health workers may be the most important means to disseminate information at the periphery where electronic media is unavailable.

Moreover, knowledge about cervical cancer was not evenly distributed among our study participants living in different study sites. Consequently, study participants from Bahr Dar were the most informed, 84% of them were aware of cervical cancer. While in a nearby site, Gondar, 76.3% of study participants were unaware of cervical cancer. Therefore, study sites noticeably showed such huge differences on the awareness level of cervical cancer. Perlman *et al* (Perlman , 2014) in a systemic review found similar results with high level of knowledge difference among different study participants at different regions of the same country. Such large information gap among women living at different sites could be minimized via programmed public sensitization programs and empowering health extension workers who were the champions in curbing maternal and child mortality rate.

Less than a fifth, 18.5% (169/915), of ours study participants responded that they knew the main causative agent of cervical cancer. However, only 7.1 % (65/915) of our study participants correctly knew that HPV infection as the main causative agent of cervical

cancer. Among other responses, smoking, and sin constitute proportions of 1.3% (12/915) and 0.4% (4/915), respectively. On the other hand, majority of our study participants did not know the risk factors for cervical cancer. Some study participants identified multiple sex partner 9.9% (91/915), smoking and early marriage as the main risk factors for cervical cancer. Moreover, 2.5% (23/915), and 1.3% (12/915) of women identified having multiple sexual partners, smoking and early marriage as the main risk factors for HPV acquisition and development of cervical cancer. Similar low level of understanding of the HPV as causative agent of cervical cancer was reported from china. In China, (Abudukadeer , 2015) reported that only 13.0% of their study participants knew about HPV.

Limitation of the study

First the study was conducted on women from gynecology clinics of the participating Hospitals that may have selection bias. Thus, our study participants may not represent the general population. Similarly, the HPV prevalence may not show the exact prevalence of HPV in the general population at the study sites. Hence, results should be interpreted with vigilance. Moreover, for explorative analysis various factors were tested for association with outcomes such as HPV positivity, positive PAP smear etc. There was no adjustment for multiple testing, therefore significant findings could also be coincidental.

8. CONCLUSIONS

Our results clearly identified that HPV infection was prevalent among our study participants. We found that almost a third of our study participants were positive for any HPV type and 27.2% were positive for high-risk HPV types.

Among the identified HPV types, HPV 16 was the most prevalent that constitutes 10.3% of investigated women and almost a third of the HPV positive women (30.3%). Moreover, it is strongly associated with cervical abnormalities. The five most prevalent HPV types were HPV 16, 52, 56, 59, and HPV 35.

Our findings showed that HPV infection and genotype prevalence was significantly different between study sites investigated with Mekelle and Dessie being with the highest HPV infection.

The overall prevalence of HPV included in the vaccine type is high, hence vaccination of women in Ethiopia would be effective in reducing the elevated morbidity and mortality.

According to this research, the factors commonly identified as associated with HPV infection includes: early marriage, early sexual debut, parity and history of pregnancy were not significantly associated with increased HPV infection or cervical abnormality.

Overall, Pap smear and VIA positivity were not significantly associated with HPV infection. However, comparison of individual HPV types, cervical abnormality was associated with HPV 16 or high-risk HPV types.

Awareness level of the investigated women on cervical cancer was good. However, their knowledge towards cervical cancer intervention was low. Health professional were the most effective source information for the investigated women. Radio and television were also important sources but limited to urban inhabitants.

9. RECOMMENDATIONS

Taking all these finding we draw the following recommendations:

- Nationwide population and facility based HPV genotype prevalence study is fundamental prior to implementing HPV vaccination program.

- Moreover, molecular epidemiology of HPV types among cervical patients and stratification of the HPV types according to pathological finding is crucial.

- Establishment of national HPV molecular diagnostic laboratory to monitor pre and post vaccination dynamics of HPV genotypes and devise recommend appropriate intervention strategy.

- Commencement of HPV vaccination and screening programs before the GAVI initiative expires.

- Cervical cancer screening program with HPV testing needs to be initiated as per the WHO recommendation.

- Awareness strategies must be inclusive. Extensive training of extension health workers about cervical cancer and the intervention strategies so that they can create critical mass of awareness in the community.

10. REFERENCE

Abate, E, Aseffa, A, El-tayeb, M, El-hassan, I, Yamuah, L, Mihret, W, et al. (2013) 'Genotyping of Human Papillomavirus in Paraffin Embedded Cervical Tissue Samples From Women in Ethiopia and the Sudan', 287(September 2012), pp. 282–287. doi: 10.1002/jmv.

Abate, SM (2015) 'Cervical Cancer : Open Access Trends of Cervical Cancer in Ethiopia', 1(1), pp. 1–4. doi: 10.4172/ccoa.1000103.

Abnormal, M, Cancer, C, Tests, S and Precursors, C (2014) 'The society for lower genital tract disorders since 1964.', (August).

Abreu, ALP, Souza, RP, Gimenes, F and Consolaro, MEL (2012a) 'A review of methods for detect human Papillomavirus infection', pp. 1–9.

Abreu, ALP, Souza, RP, Gimenes, F and Consolaro, MEL (2012b) 'A review of methods for detect human Papillomavirus infection.', Virology journal, 9, p. 262. doi: 10.1186/1743-422X-9-262.

Abudukadeer, A, Azam, S, Mutailipu, AZ, Qun, L, Guilin, G and Mijiti, S (2015) 'Knowledge and attitude of Uyghur women in Xinjiang province of China related to the prevention and early detection of cervical cancer', pp. 1–7. doi: 10.1186/s12957-015-0531-8.

Adler, DH, Wallace, M, Bennie, T, Abar, B, Meiring, TL, Williamson, A-L, et al. (2016) 'Cumulative Impact of HIV and Multiple Concurrent Human Papillomavirus Infections on the Risk of Cervical Dysplasia', Advances in Virology, 2016(October 2013). doi: 10.1155/2016/7310894.

Aggarwal, R, Gupta, S, Nijhawan, R, Suri, V, Kaur, A, Bhasin, V, et al. (2006) 'Prevalence of high--risk human papillomavirus infections in women with benign cervical cytology: a hospital based study from North India.', Indian journal of cancer, 43(3), pp. 110–6. Available at: http://www.ncbi.nlm.nih.gov/pubmed/17065768.

Akarolo-Anthony, SN, Famooto, AO, Dareng, EO, Olaniyan, OB, Offiong, R, Wheeler,

CM, et al. (2014) ‘Age-specific prevalence of human papilloma virus infection among Nigerian women’, BMC Public Health, 14(1). doi: 10.1186/1471-2458-14-656.

Alba, a., Cararach, M, Rodríguez-Cerdeira, C and Rodriguez-Cerdeira, C (2009) ‘The Human Papillomavirus (HPV) in Human Pathology: Description, Pathogenesis, Oncogenic Role, Epidemiology and Detection Techniques’, The Open Dermatology Journal, 3(2), pp. 90–102. doi: 10.2174/1874372200903020090.

Ali-risasi, C, Mulumba, P, Verdonck, K, Broeck, D Vanden and Praet, M (2014) ‘Knowledge , attitude and practice about cancer of the uterine cervix among women living in Kinshasa , the Democratic Republic of Congo’, BMC Women’s Health. BMC Women’s Health, 14(1), pp. 1–13. doi: 10.1186/1472-6874-14-30.

Ali-Risasi, C, Mulumba, P, Verdonck, K, Vanden Broeck, D and Praet, M (2014) ‘Knowledge, attitude and practice about cancer of the uterine cervix among women living in Kinshasa, the Democratic Republic of Congo’, BMC Women’s Health. BMC Women’s Health, 14(1), p. 30. doi: 10.1186/1472-6874-14-30.

Ali, S, Sewunet, T, Sahlemariam, Z and Kibru, G (2016) ‘Neisseria gonorrhoeae among suspects of sexually transmitted infection in Gambella hospital, Ethiopia: risk factors and drug resistance’, BMC Research Notes. BioMed Central, 9(1), p. 8. doi: 10.1186/s13104-016-2247-4.

Allan, B, Marais, DJ, Hoffman, M, Shapiro, S and Williamson, A (2008) ‘Cervical Human Papillomavirus (HPV) Infection in South African Women : Implications for HPV Screening and Vaccine Strategies □ ’, 46(2), pp. 740–742. doi: 10.1128/JCM.01981-07.

Ammermann, I, Bruckner, M, Matthes, F, Iftner, T and Stubenrauch, F (2008) ‘Inhibition of Transcription and DNA Replication by the Papillomavirus E8ˆE2C Protein Is Mediated by Interaction with Corepressor Molecules □ ’, 82(11), pp. 5127–5136. doi: 10.1128/JVI.02647-07.

Amsalu, A, Geto, Z, Asegu, D and Eshetie, S (2017) ‘Antimicrobial resistance pattern of

bacterial isolates from different clinical specimens in Southern Ethiopia : A three year retrospective study', 9(January), pp. 1–8. doi: 10.5897/JBR2016.0224.

Anagrius, C., Lore, B. and Jensen, SJ (2005) 'Mycoplasma genitalium: prevalence, clinical significance, and transmission', Sexually Transm Infect, 81(October 1997), pp. 458–462. doi: 10.1136/sti.2004.012062.

Araldi, RP, Muro, S, Assaf, R, Carvalho, RF De, Caldas, MA, Carvalho, R De, et al. (2017) 'Papillomaviruses : a systematic review'.

Arbyn, M, Castellsagué, X, de sanjosé, S, Bruni, L, Saraiya, M, Bray, F, et al. (2011) 'Worldwide burden of cervical cancer in 2008', Annals of Oncology, 22(12), pp. 2675–2686. doi: 10.1093/annonc/mdr015.

Arbyn, M, Sankaranarayanan, R, Muwonge, R, Keita, N and Dolo, A (2008) 'Pooled analysis of the accuracy of five cervical cancer screening tests assessed in eleven studies in Africa and India', 160(December 2007), pp. 153–160. doi: 10.1002/ijc.23489.

Aseel, MT (2008) 'Knowledge , attitude and practices regarding cervical cancer and screening among women visiting primary health care in Qatar'.

Baloch, Z, Yuan, T, Yindi, S, Feng, Y, Tai, W, Liu, Y, et al. (2016) 'Prevalence of genital human papillomavirus among rural and urban populations in southern Yunnan province, China', Brazilian Journal of Medical and Biological Research, 49(6), pp. 1–7. doi: 10.1590/1414-431X20165254.

Bansal, D, Elmi, AA, Skariah, S, Haddad, P, Abu-raddad, LJ, Hamadi, AH Al, et al. (2014) 'Molecular epidemiology and genotype distribution of Human Papillomavirus (HPV) among Arab women in the state of Qatar', pp. 1–9.

Bartek, J and Lukas, J (2001) 'Mammalian G1- and S-phase checkpoints in response to DNA damage', Current Opinion in Cell Biology, 13(6), pp. 738–747. doi: 10.1016/S0955-0674(00)00280-5.

Bautista, CT, Wurapa, E, Sateren, WB, Morris, S, Hollingsworth, B and Sanchez, JL

(2016) ‘Bacterial vaginosis : a synthesis of the literature on etiology , prevalence , risk factors , and relationship with chlamydia and gonorrhea infections’, Military Medical Research. Military Medical Research, pp. 1–10. doi: 10.1186/s40779-016-0074-5.

Bekele, A, Baay, M, Mekonnen, Z, Suleman, S and Chatterjee, S (2010) ‘Short Communication Human papillomavirus type distribution among women with cervical pathology – a study over 4 years at Jimma Hospital , southwest Ethiopia’, 15(8), pp. 890–893. doi: 10.1111/j.1365-3156.2010.02552.x.

Bergvall, M, Melendy, T and Archambault, J (2013) ‘The E1 proteins’, Virology, 445(1–2), pp. 35–56. doi: 10.1016/j.virol.2013.07.020.

Bernard, HU, Burk, RD, Chen, Z, van Doorslaer, K, Hausen, H zur and de Villiers, EM (2010) ‘Classification of papillomaviruses (PVs) based on 189 PV types and proposal of taxonomic amendments’, Virology, 401(1), pp. 70–79. doi: 10.1016/j.virol.2010.02.002.

Bertoli, C, Skotheim, JM and de Bruin, RAM (2013) ‘Control of cell cycle transcription during G1 and S phases’, Nature Reviews Molecular Cell Biology. Nature Publishing Group, 14(8), pp. 518–528. doi: 10.1038/nrm3629.

Bienkowska-haba, M, Williams, C, Man, S, Garcea, RL and Sapp, M (2012) ‘following Virus Entry Cyclophilins Facilitate Dissociation of the Human Papillomavirus Type 16 Capsid Protein L1 from the L2 / DNA Complex following’. doi: 10.1128/JVI.00980-12.

Bienkowska-Haba, MS and M (2010) ‘NIH Public Access’, 276(24), pp. 7206–7216. doi: 10.1111/j.1742-4658.2009.07400.x.Viral.

Birhanu, Z, Abdissa, A, Belachew, T, Deribew, A, Segni, H, Tsu, V, et al. (2012) ‘Health seeking behavior for cervical cancer in Ethiopia: a qualitative study’, International Journal for Equity in Health, 11(1), p. 83. doi: 10.1186/1475-9276-11-83.

Biryukov, J and Meyers, C (2015) ‘Papillomavirus Infectious Pathways: A Comparison of

Systems', Viruses, 7(8), pp. 4303–4325. doi: 10.3390/v7082823.

Blossom, DB, Beigi, RH, Farrell, JJ, Mackay, W, Qadadri, B, Brown, DR, et al. (2007) 'Human papillomavirus genotypes associated with cervical cytologic abnormalities and HIV infection in Ugandan women', Journal of Medical Virology, 79(6), pp. 758–765. doi: 10.1002/jmv.20817.

Borgdorff, H, Gautam, R, Armstrong, SD, Xia, D, Ndayisaba, GF and Teijlingen, NH Van (2015) 'Cervicovaginal microbiome dysbiosis is associated with proteome changes related to alterations of the cervicovaginal mucosal barrier'. Nature Publishing Group, 9(3), pp. 621–633. doi: 10.1038/mi.2015.86.

Bosch, FX, Broker, TR and Forman, DE Al (2014) 'NIH Public Access', Bmc, 31(0 8), pp. 11–31. doi: 10.1016/j.vaccine.2013.07.026.Comprehensive.

Bosch, FX, Lorincz, a, Munoz, N, Meijer, CJLM and Shah, K V (2002) 'The causal relation between human papillomavirus and cervical cancer', Journal of Clinical Pathology, 55(4), pp. 244–265. doi: 10.1136/jcp.55.4.244.

Bosch, FX, Qiao, YL and Castellsague, X (2006) 'CHAPTER 2 The epidemiology of human papillomavirus infection and its association with cervical cancer', International Journal of Gynecology and Obstetrics, 94(SUPPL. 1), pp. S8–S21. doi: 10.1016/S0020-7292(07)60004-6.

Bosch, FX and Sanjos, S De (2007) 'The epidemiology of human papillomavirus infection and cervical cancer', 23, pp. 213–227.

Bousarghin, L, Touze, A, Sizaret, P-Y and Coursaget, P (2003) 'Human Papillomavirus Types 16, 31, and 58 Use Different Endocytosis Pathways To Enter Cells', Journal of Virology, 77(6), pp. 3846–3850. doi: 10.1128/JVI.77.6.3846-3850.2003.

Bradshaw, CS, Tabrizi, SN, Fairley, CK, Morton, a N, Rudland, E and Garland, SM (2006) 'The association of Atopobium vaginae and Gardnerella vaginalis with bacterial vaginosis and recurrence after oral metronidazole therapy.', The Journal of infectious diseases, 194(6), pp. 828–836. doi: 10.1086/506621.

Bruni, L, Diaz, M, Castellsagué, X, Ferrer, E, Bosch, FX and de Sanjosé, S (2010) 'Cervical Human Papillomavirus Prevalence in 5 Continents: Meta- Analysis of 1 Million Women with Normal Cytological Findings', The Journal of Infectious Diseases, 202(12), pp. 1789–1799. doi: 10.1086/657321.

Brunner, A, Nemes-Nikodem, E, Mihalik, N, Marschalko, M, Karpati, S and Ostorhazi, E (2014) 'Incidence and antimicrobial susceptibility of Neisseria gonorrhoeae isolates from patients attending the national Neisseria gonorrhoeae reference laboratory of Hungary', BMC Infect Dis, 14(1), p. 433. doi: 10.1186/1471-2334-14-433.

Burd, E (2003) 'Human papillomavirus and cervical cancer', Clin Microbiol Rev, 16(1), pp. 1–17. doi: 10.1128/CMR.16.1.1.

Burd, EM (2016) 'Human Papillomavirus Laboratory Testing : the Changing Paradigm', 29(2), pp. 291–319. doi: 10.1128/CMR.00013-15.Address.

Burk, RD, Chen, Z and Van Doorslaer, K (2009) 'Human papillomaviruses: Genetic basis of carcinogenicity', Public Health Genomics, pp. 281–290. doi: 10.1159/000214919.

Caldeira, TD, Goncalves, C V, Oliveira, GR, Fonseca, T V, Goncalves, R, Amaral, CT, et al. (2013) 'Prevalence of herpes simplex virus type 2 and risk factors associated with this infection in women in southern Brazil', Revista do Instituto de Medicina Tropical de Sao Paulo, 55(5), pp. 315–321.

Calleja-macias, IE, Villa, LL, Prado, JC, Allan, B, Williamson, A, Collins, RJ, et al. (2005) 'Worldwide Genomic Diversity of the High-Risk Human Papillomavirus Types 31 , Human Papillomavirus Type 16 Worldwide Genomic Diversity of the High-Risk Human Papillomavirus Types 31 , 35 , 52 , and 58 , Four Close Relatives of Human Papillomavirus Type 16', Journal of Virology, 79(21), pp. 13630–13640. doi: 10.1128/JVI.79.21.13630.

Casillas-vega, N, Morfín-otero, R, García, S, Llaca-díaz, J, Mendoza-olazarán, S, Flores-treviño, S, et al. (2016) 'Sexually transmitted pathogens , coinfections and risk factors in patients attending obstetrics and gynecology clinics in Jalisco , Mexico', 58(4), pp. 437–445.

Castellsagué, X, Muñoz, N, Pitisuttithum, P, Ferris, D, Monsonego, J, Ault, K, et al. (2011) 'End-of-study safety, immunogenicity, and efficacy of quadrivalent HPV (types 6, 11, 16, 18) recombinant vaccine in adult women 24-45 years of age.', British journal of cancer, 105(1), pp. 28–37. doi: 10.1038/bjc.2011.185.

Castellsagué, X, Paavonen, J, Jaisamrarn, U, Wheeler, CM, Skinner, SR, Lehtinen, M, et al. (2014) 'Risk of first cervical HPV infection and pre-cancerous lesions after onset of sexual activity: analysis of women in the control arm of the randomized, controlled PATRICIA trial.', BMC infectious diseases, 14, p. 551. doi: 10.1186/s12879-014-0551-y.

Castle, PE and Giuliano, AR (2003) 'Chapter 4: Genital tract infections, cervical inflammation, and antioxidant nutrients--assessing their roles as human papillomavirus cofactors.', Journal of the National Cancer Institute. Monographs, 7234(31), pp. 29–34. doi: 10.1093/oxfordjournals.jncimonographs.a003478.

Castle, PE, Wacholder, S, Sherman, ME, Lorincz, AT, Glass, AG, Scott, DR, et al. (2002) 'Absolute risk of a subsequent abnormal Pap among oncogenic human papillomavirus DNA-positive, cytologically negative women', Cancer, 95(10), pp. 2145–2151. doi: 10.1002/cncr.10927.

CDC, G (2013) 'Human Papillomavirus (HPV) Statistics', (June). Available at: https://www.cdc.gov/std/hpv/stats.htm.

Cerqueira, C, Pang, Y-YS, Day, PM, Thompson, CD, Buck, CB, Lowy, DR, et al. (2015) 'A cell-free assembly system for generating infectious Human Papillomavirus Type 16 capsids implicates a size discrimination mechanism for preferential viral genome packaging.', Journal of virology, 90(2), pp. 1096–1107. doi: 10.1128/JVI.02497-15.

Chan, PKS, Chang, AR, Yu, MY, Li, W, Chan, MYM, Yeung, ACM, et al. (2010) 'Age distribution of human papillomavirus infection and cervical neoplasia reflects caveats of cervical screening policies', Int. J. Cancer, 126, pp. 297–301. doi: 10.1002/ijc.24731.

Chaturvedi, AK, Katki, HA, Hildesheim, A, Rodríguez, AC, Quint, W, Schiffman, M, et

al. (2011) ‘Human papillomavirus infection with multiple types: Pattern of coinfection and risk of cervical disease’, Journal of Infectious Diseases, 203(7), pp. 910–920. doi: 10.1093/infdis/jiq139.

Clad, A, Reuschenbach, M, Weinschenk, J, Grote, R, Rahmsdorf, J and Freudenberg, N (2011) ‘Performance of the Aptima High-Risk Human Papillomavirus mRNA Assay in a Referral Population in Comparison with Hybrid Capture 2 and Cytology □ ’, 49(3), pp. 1071–1076. doi: 10.1128/JCM.01674-10.

Clifford, GM, Gallus, S, Herrero, R, Mu??oz, N, Snijders, PJF, Vaccarella, S, et al. (2005) ‘Worldwide distribution of human papillomavirus types in cytologically normal women in the International Agency for Research on Cancer HPV prevalence surveys: A pooled analysis’, Lancet, 366(9490), pp. 991–998. doi: 10.1016/S0140-6736(05)67069-9.

Clifford, GM, Howell-Jones, R and Franceschi, S (2011) ‘Judging the carcinogenicity of human papillomavirus types by single/multiple infection ratio in cervical cancer’, International Journal of Cancer, 129(7), pp. 1792–1794. doi: 10.1002/ijc.25833.

Comar, M, Iannacone, MR, Casalicchio, G, Mckay-chopin, S, Tommasino, M and Gheit, T (2012) ‘Comparison of Hybrid Capture II , Linear Array , and a Bead-Based Multiplex Genotyping Assay for Detection of Human Papillomavirus in Women with Negative Pap Test Results and Atypical Squamous Cells of Undetermined Significance’, 50(12), pp. 4041–4046. doi: 10.1128/JCM.02105-12.

Conway, MJ and Meyers, C (2009) ‘Replication and Assembly of Human Papillomaviruses’, Journal of Dental Research, 88(4), pp. 307–317. doi: 10.1177/0022034509333446.

Cuschieri, KS, Cubie, H a, Whitley, MW, Seagar, a L, Arends, MJ, Moore, C, et al. (2004) ‘Multiple high risk HPV infections are common in cervical neoplasia and young women in a cervical screening population.’, Journal of clinical pathology, 57(1), pp. 68–72. doi: 10.1136/jcp.57.1.68.

Cutts, FT, Franceschi, S, Goldie, S, Castellsague, X, Sanjose, S De, Garnett, G, et al.

(2007) ‘Human papillomavirus and HPV vaccines : a review’, 38414(September), pp. 719–726. doi: 10.2471/BLT.

De, JVFJMG de ATAA and Fernandes2, M (2013) ‘Biology and natural history of human papillomavirus infection’, pp. 1–12.

Diag-, G and Jose, S (2012) ‘Prevalence of Trichomonas vaginalis and Coinfection with Chlamydia trachomatis and Neisseria gonorrhoeae in the United States as Determined by the Aptima Trichomonas vaginalis Nucleic Acid’, 50(8), pp. 2601–2608. doi: 10.1128/JCM.00748-12.

Dickson, EL, Vogel, RI, Bliss, RL and Downs, LS (2013) ‘Multiple-type human papillomavirus (HPV) infections: a cross-sectional analysis of the prevalence of specific types in 309,000 women referred for HPV testing at the time of cervical cytology.’, International journal of gynecological cancer : official journal of the International Gynecological Cancer Society, 23(7), pp. 1295–302. doi: 10.1097/IGC.0b013e31829e9fb4.

Digene corporsation (2007) ‘HC.(2 Hybrid Capture®’, (5101).

Digiuseppe, S, Bienkowska-haba, M and Sapp, M (2016) ‘Human Papillomavirus Entry : Hiding in a Bubble’, 90(18), pp. 8032–8035. doi: 10.1128/JVI.01065-16.Editor.

Dillner, J, Arbyn, M, Unger, E and Dillner, L (2010) ‘Monitoring of human papillomavirus vaccination’, pp. 17–25. doi: 10.1111/j.1365-2249.2010.04268.x.

Donders, GGG, Depuydt, CE, Bogers, J and Vereecken, AJ (2013) ‘Association of Trichomonas vaginalis and Cytological Abnormalities of the Cervix in Low Risk Women’, 8(12), pp. 1–5. doi: 10.1371/journal.pone.0086266.

Doorbar, J (2006) ‘Molecular biology of human papillomavirus infection and cervical cancer’, 541, pp. 525–541. doi: 10.1042/CS20050369.

Doorbar, J (2013) ‘The E4 protein; structure, function and patterns of expression’, Virology, 445(1–2), pp. 80–98. doi: 10.1016/j.virol.2013.07.008.

Doorbar, J, Egawa, N, Grif, H and Kranjec, C (2015) 'Human papillomavirus molecular biology and disease association', pp. 2–23. doi: 10.1002/rmv.

Duplessis, C, Puplampu, N, Nyarko, E, Carroll, J, Dela, H, Mensah, A, et al. (2015) 'Gonorrhea surveillance in Ghana, Africa.', Military medicine, 180(1), pp. 17–22. doi: 10.7205/MILMED-D-13-00418.

Ebu, NI and Mupepi, SC (2015) 'Knowledge , practice , and barriers toward cervical cancer screening in Elmina , Southern Ghana', pp. 31–39.

Egawa, N, Egawa, K, Griffin, H and Doorbar, J (2015) 'Human Papillomaviruses; Epithelial Tropisms, and the Development of Neoplasia', pp. 3863–3890. doi: 10.3390/v7072802.

Einstein, MH, Garcia, FAR, Mitchell, AL and Day, SP (2011) 'Age-Stratified Performance of the Cervista HPV 16 / 18 Genotyping Test in Women with ASC-US Cytology', (7), pp. 1185–1190. doi: 10.1158/1055-9965.EPI-11-0116.

Ferrera, A, Velema, JP, Figueroa, M, Bulnes, R, Toro, LA and Claros, JM (2000) 'Co-factors related to the causal relationship between human papillomavirus and invasive cervical cancer in Honduras', International Journal of Epidemiology, 8(1), pp. 817–825.

Ferris, MJ, Masztal, A, Aldridge, KE, Fortenberry, JD, Fidel, PL and Martin, DH (2004) 'Association of Atopobium vaginae, a recently described metronidazole resistant anaerobe, with bacterial vaginosis.', BMC infectious diseases, 4(1), p. 5. doi: 10.1186/1471-2334-4-5.

Finocchario-Kessler, S, Wexler, C, Maloba, M, Mabachi, N, Ndikum-Moffor, F and Bukusi, E (2016) 'Cervical cancer prevention and treatment research in Africa: a systematic review from a public health perspective.', BMC women's health. BMC Women's Health, 16(1), p. 29. doi: 10.1186/s12905-016-0306-6.

Gao, W, Weng, J, Gao, Y and Chen, X (2013) 'Comparison of the vaginal microbiota diversity of women with and without human papillomavirus infection : a cross-

sectional study', BMC Infectious Diseases. BMC Infectious Diseases, 13(1), p. 1. doi: 10.1186/1471-2334-13-271.

Getman, D, Jiang, A and Donnell, MO (2016) 'Antibiotic Resistance Frequency in a Multicenter Clinical Study Cohort in the United States', 54(9), pp. 2278–2283. doi: 10.1128/JCM.01053-16.Editor.

Ghittoni, R (2015) 'The role of human papillomaviruses in carcinogenesis', Ecancermedicalscience, 9, pp. 1–9. doi: 10.3332/ecancer.2015.526.

Gillet, E, Meys, JFA, Verstraelen, H, Bosire, C, Sutter, P De, Temmerman, M, et al. (2011) 'Bacterial vaginosis is associated with uterine cervical human papillomavirus infection : a meta-analysis', BMC Infectious Diseases. BioMed Central Ltd, 11(1), p. 10. doi: 10.1186/1471-2334-11-10.

Gómez, DT and Santos, JL (2007) 'HUMAN PAPILLOMAVIRUS INFECTION AND CERVICAL CANCER : PATHOGENESIS AND EPIDEMIOLOGY', pp. 680–688.

Graham, S V (2012) 'Europe PMC Funders Group Human papillomavirus : gene expression , regulation and prospects for novel diagnostic methods and antiviral therapies', 5(10), pp. 1493–1506. doi: 10.2217/fmb.10.107.Human.

Graham, S V. (2016) 'Human Papillomavirus E2 Protein: Linking Replication, Transcription, and RNA Processing', Journal of Virology, 90(19), pp. 8384–8388. doi: 10.1128/JVI.00502-16.

Gravitt, PE, Paul, P, Katki, HA, Vendantham, H and Ramakrishna, G (2010) 'Effectiveness of VIA , Pap , and HPV DNA Testing in a Cervical Cancer Screening Program in a Peri-Urban Community in Andhra Pradesh , India', 5(10). doi: 10.1371/journal.pone.0013711.

Green, J, Gonzalez, AB De, Sweetland, S, Beral, V, Chilvers, C, Crossley, B, et al. (2003) 'Risk factors for adenocarcinoma and squamous cell carcinoma of the cervix in women aged 20 – 44 years : the UK National Case – Control Study of Cervical Cancer', pp. 2078–2086. doi: 10.1038/sj.bjc.6601296.

Guo, F, Hirth, JM and Berenson, AB (2015) 'Comparison of HPV prevalence between HPV-vaccinated and non-vaccinated young adult women (20-26 years)', Human Vaccines and Immunotherapeutics, 11(10), pp. 2337–2344. doi: 10.1080/21645515.2015.1066948.

Hailemariam, M, Abebe, T, Mihret, A and Lambiyo, T (2013) 'Prevalence of Neisseria gonorrhea and their antimicrobial susceptibility patterns among symptomatic women attending gynecology outpatient department in Hawassa referral hospital, Hawassa, Ethiopia.', Ethiopian journal of health sciences, 23(1), pp. 10–18.

Hailu, A and Mariam, DH (2013) 'Patient side cost and its predictors for cervical cancer in Ethiopia: a cross sectional hospital based study', BMC Cancer. BMC Cancer, 13(1), p. 69. doi: 10.1186/1471-2407-13-69.

Hardy, L, Jespers, V, Abdellati, S, De Baetselier, I, Mwambarangwe, L, Musengamana, V, et al. (2016) 'A fruitful alliance: the synergy between Atopobium vaginae and Gardnerella vaginalis in bacterial vaginosis-associated biofilm.', Sexually transmitted infections, pp. 1–5. doi: 10.1136/sextrans-2015-052475.

Hardy, L, Jespers, V, Bulck, M Van Den, Buyze, J, Mwambarangwe, L, Musengamana, V, et al. (2017) 'The presence of the putative Gardnerella vaginalis sialidase A gene in vaginal specimens is associated with bacterial vaginosis biofilm', pp. 1–11. doi: 10.1371/journal.pone.0172522.

Hardy, L, Jespers, V, Dahchour, N, Mwambarangwe, L, Musengamana, V, Vaneechoutte, M, et al. (2015) 'Unravelling the bacterial vaginosis-associated biofilm: A multiplex Gardnerella Vaginalis and Atopobium vaginae fluorescence in situ hybridization assay using peptide nucleic acid probes', PLoS ONE, 10(8), pp. 1–16. doi: 10.1371/journal.pone.0136658.

Hartwig, S, St Guily, JL, Dominiak-Felden, G, Alemany, L and de Sanjosé, S (2017) 'Estimation of the overall burden of cancers, precancerous lesions, and genital warts attributable to 9-valent HPV vaccine types in women and men in Europe.', Infectious agents and cancer. Infectious Agents and Cancer, 12(1), p. 19. doi: 10.1186/s13027-

017-0129-6.

zur Hausen, H (2009) ‘Papillomaviruses in the causation of human cancers - a brief historical account’, Virology. Elsevier Inc., 384(2), pp. 260–265. doi: 10.1016/j.virol.2008.11.046.

Hoffmann, R, Hirt, B, Bechtold, V, Raj, K, Hoffmann, R, Hirt, B, et al. (2006) ‘Different Modes of Human Papillomavirus DNA Replication during Maintenance Different Modes of Human Papillomavirus DNA Replication during Maintenance’, 80(9). doi: 10.1128/JVI.80.9.4431.

Horvath, C a J, Boulet, G a V, Renoux, VM, Delvenne, PO and Bogers, J-PJ (2010) ‘Mechanisms of cell entry by human papillomaviruses: an overview.’, Virology journal, 7(August), p. 11. doi: 10.1186/1743-422X-7-11.

HPV/ICO (2016) ‘Human Papillomavirus and Related Diseases Report’.

Hughes, WA (2013) ‘Analysis of Human Papillomavirus Capsid Proteins: Insights into Capsid Assembly’, p. 104.

IARC group (2015) ‘Chapter 1: An introduction to the anatomy of the uterine cervix’, Colposcopy and Treatment of Cervical Intraepithelial Neoplasia: A Beginner’s Manual, pp. 1–12. Available at: http://screening.iarc.fr/colpochap.php?lang=1&chap=1.

Ibeanu, OA (2017) ‘Molecular pathogenesis of cervical cancer Molecular pathogenesis of cervical cancer a n d e s i o s c i e n c e o n o t d i s t r i b u t e’, 4047(June). doi: 10.4161/cbt.11.3.14686.

International, T, Epidemiology, C, Asthana, S and Labani, S (2015) ‘Adjunct screening of cervical or vaginal samples using careHPV testing with Pap and aided visual inspection for detecting high-grade cervical intraepithelial neoplasia’, Cancer Epidemiology. Elsevier Ltd, 39(1), pp. 104–108. doi: 10.1016/j.canep.2014.11.006.

Irwin, T, Peters, KE, Pyra, M and Traoré, F (2017) ‘Implementing visual cervical cancer screening in Senegal : a cross-sectional study of risk factors and prevalence

highlighting service utilization barriers', pp. 59–67.

Jacot-guillarmod, M, Pasquier, J, Greub, G, Bongiovanni, M, Achtari, C and Sahli, R (2017) 'Impact of HPV vaccination with Gardasil ® in Switzerland'. BMC Infectious Diseases, pp. 1–10. doi: 10.1186/s12879-017-2867-x.

Jalouli, J, Jalouli, MM, Sapkota, D, Ibrahim, SO, Larsson, P and Sand, L (2012) 'Human Papilloma Virus , Herpes Simplex Virus and Epstein Barr Virus in Oral Squamous Cell Carcinoma from Eight Different Countries', 580, pp. 571–580.

James D. Baleja, Jonathan J. Cherry, Zhiguo Liu, Hua Gao, Marc C. Nicklaus, Johannes H. Voigt, Jason J. Chen, and EJA (2009) 'Identification of Inhibitors to Papillomavirus Type 16 E6 Protein Based on Three-dimensional Structures of Interacting Proteins', Antiviral Res, 72(1), pp. 49–59. doi: 10.1016/j.antiviral.2006.03.014.Identification.

Jo, H and Kim, JW (2005) 'Implications of HPV infection in uterine cervical cancer Review Article', 3, pp. 419–434.

Johansson, C and Schwartz, S (2013) 'Regulation of human papillomavirus gene expression by splicing and polyadenylation.', Nature reviews. Microbiology, 11(4), pp. 239–51. doi: 10.1038/nrmicro2984.

Jr, MU, Castaneda, FR, Reinson, T and Männik, A (2015) 'Human Papillomavirus Type 18 cis -Elements Crucial for Segregation and Latency', pp. 1–24. doi: 10.1371/journal.pone.0135770.

Ka, N, Day, PM, Nowak, T, Selinka, H, Florin, L, Bolscher, J, et al. (2006) 'A Membrane-Destabilizing Peptide in Capsid Protein L2 Is Required for Egress of Papillomavirus Genomes from Endosomes', 80(2), pp. 759–768. doi: 10.1128/JVI.80.2.759.

Kadaja, M, Isok-paas, H, Ustav, E and Ustav, M (2009) 'Mechanism of Genomic Instability in Cells Infected with the High-Risk Human Papillomaviruses', 5(4). doi: 10.1371/journal.ppat.1000397.

Kang, L, Castle, PE, Zhao, F, Jeronimo, J, Chen, F, Bansil, P, et al. (2014) 'A prospective study of age trends of high-risk human papillomavirus infection in rural China',

BMC Infectious Diseases. BMC Infectious Diseases, 14(1), pp. 1–11. doi: 10.1186/1471-2334-14-96.

Kawana, Y, Kawana, KEI, Yoshikawa, H, Taketani, Y, Yoshiike, K and Kanda, T (2001) 'Human Papillomavirus Type 16 Minor Capsid Protein L2 N-Terminal Region Containing a Common Neutralization Epitope Binds to the Cell Surface and Enters the Cytoplasm', 75(5), pp. 2331–2336. doi: 10.1128/JVI.75.5.2331.

Kemunto, R, Gona, PN, Alaina, J, Park, HS, Bain, PA, Maranda, L, et al. (2017) 'Prevalence of Human Papillomavirus Genotypes among African Women with Normal Cervical Cytology and Neoplasia : A Systematic Review and Meta-Analysis The Harvard community has made this article openly available . Please share how this access benefits you .' doi: 10.1371/journal.pone.0122488.

Khalaf, MK, Rasheed, FA and Hussain, SA (2015) 'Association between Early Marriage and Other Sociomedical Characteristics with the Cervical Pap Smear Results in Iraqi Women', Advances in Sexual Medicine, 5(4), pp. 73–82. doi: 10.4236/asm.2015.54009.

Khandker, E, Khan, M and Chowdhury, AK (2016) 'Human papillomavirus infection among Bangladeshi women with cervical intraepithelial neoplasia and chronic cervicitis', 10(1), pp. 8–11.

Kissinger, P (2015) 'Trichomonas vaginalis : a review of epidemiologic , clinical and treatment issues', BMC Infectious Diseases. BMC Infectious Diseases, pp. 1–8. doi: 10.1186/s12879-015-1055-0.

Kolawole, O, Ogah, J, Alabi, O and Suleiman, M (2015) 'Utilization of Human Papillomavirus DNA Detection for Cervical Cancer Screening in Women Presenting With Abnormal Cytology in Lokoja , Nigeria', 8(10), pp. 8–13. doi: 10.5812/jjm.22620.

Kru, S, Chackerian, B, Brule, AJC Van Den, Svare, EI, Paull, G, Walbomers, JMM, et al. (2001) 'High-Risk Human Papillomavirus Is Sexually Transmitted : Evidence from a Follow-Up Study of Virgins Starting Sexual Activity (Intercourse) 1', 10(February),

pp. 101–106.

Lazarczyk, M, Cassonnet, P, Pons, C, Jacob, Y and Favre, M (2009) ‘The EVER proteins as a natural barrier against papillomaviruses: a new insight into the pathogenesis of human papillomavirus infections.’, Microbiology and molecular biology reviews : MMBR, 73(2), pp. 348–70. doi: 10.1128/MMBR.00033-08.

Leiman, JLS a SLM a TLSJ b CMW a RJB a G (2014) ‘Testing of Integrated Human Papillomavirus mRNA Decreases Colposcopy Referrals : Could a Change in Human Papillomavirus Detection Methodology Lead to More Cost-Effective Patient Care ?’, 5401, pp. 162–166. doi: 10.1159/000358246.

Letian, T and Tianyu, Z (2010) ‘Cellular receptor binding and entry of human papillomavirus’, pp. 1–7.

Ling, Z, Kong, J, Liu, F, Zhu, H, Chen, X, Wang, Y, et al. (2010) ‘Molecular analysis of the diversity of vaginal microbiota associated with bacterial vaginosis.’, BMC genomics, 11(1), p. 488. doi: 10.1186/1471-2164-11-488.

Lipovsky, A, Popa, A, Pimienta, G, Wyler, M, Bhan, A, Kuruvilla, L, et al. (2013) ‘Genome-wide siRNA screen identi fi es the retromer as a cellular entry factor for human papillomavirus’. doi: 10.1073/pnas.1302164110/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1302164110.

Liu, Y, Zhang, C, Gao, W, Wang, L, Pan, Y, Gao, Y, et al. (2016) ‘Genome-wide profiling of the human papillomavirus DNA integration in cervical intraepithelial neoplasia and normal cervical epithelium by HPV capture technology.’, Scientific reports. Nature Publishing Group, 6(52), p. 35427. doi: 10.1038/srep35427.

Livengood, CH (2009) ‘Bacterial Vaginosis : An Overview for 2009’, 2(1), pp. 28–37.

Longworth, MS and Laimins, LA (2004) ‘Pathogenesis of Human Papillomaviruses in Differentiating Epithelia’, 68(2), pp. 362–372. doi: 10.1128/MMBR.68.2.362.

Lowy, DR, Schiller, JT, Buck, CB, Thompson, CD, Roberts, JN and Mu, M (2006) ‘Carrageenan Is a Potent Inhibitor of Papillomavirus Infection’, 2(7). doi:

10.1371/journal.ppat.0020069.

Lu, H, Jiang, P, Zhang, X, Hou, W, Wei, Z, Lu, J, et al. (2015) ‘Characteristics of bacterial vaginosis infection in cervical lesions with high risk human papillomavirus infection’, 8(11), pp. 21080–21088.

Manga, MM, Fowotade, A, Abdullahi, YM, El-nafaty, AU, Adamu, DB, Pindiga, HU, et al. (2015) ‘Epidemiological patterns of cervical human papillomavirus infection among women presenting for cervical cancer screening in North-Eastern Nigeria’, Infectious Agents and Cancer, 10(1), p. 39. doi: 10.1186/s13027-015-0035-8.

Marcone, V, Recine, N, Gallinelli, C, Nicosia, R, Lichtner, M, Degener, AM, et al. (2012) ‘Epidemiology of Chlamydia trachomatis endocervical infection in a previously unscreened population in Rome , Italy , 2000 to 2009’, pp. 1–8.

Mawak, JD, Dashe, N, Agabi, YA and Panshak, BW (2011) ‘Prevalence of Genital Chlamydia Trachomatis Infection among Gynaecologic Clinic Attendees in Jos , Nigeria . Abstract : Introduction ’:, 12(2), pp. 100–106.

McBride, AA (2013) ‘The Papillomavirus E2 proteins’, Virology. Elsevier, 445(1–2), pp. 57–79. doi: 10.1016/j.virol.2013.06.006.

Mclaughlin-Drubin, ME and Münger, K (2010) ‘Oncogenic Activities of Human Papillomaviruses’, Virus Res., 143(2), pp. 195–208. doi: 10.1016/j.virusres.2009.06.008.Oncogenic.

Mehta, V, Vasanth, V and Balachandran, C (2009) ‘<i>Pap smear</i>’;, Indian Journal of Dermatology, Venereology and Leprology, 75(2), p. 214. doi: 10.4103/0378-6323.48686.

Men, C, Castellsagu, X, Renom, M, Sacarlal, J, Lloveras, B, Klaustermeier, J, et al. (2010) ‘Prevalence and Risk Factors of Sexually Transmitted Infections and Cervical Neoplasia in Women from a Rural Area of Southern Mozambique’, 2010. doi: 10.1155/2010/609315.

Mendes, C, José, DO and Levi, E (2016) ‘The Biological Impact of Genomic Diversity in

Cervical Cancer Development', pp. 513–517. doi: 10.1159/000449401.

Metwali, Z, Kindi, F Al, Shanbleh, S and Akshar, S Al (2015) 'Evaluating awareness and screening of cervical cancer among women in Sharjah , United Arab Emirates', 5(2), pp. 57–64.

Mitra, A, Macintyre, DA, Marchesi, JR, Lee, YS, Bennett, PR and Kyrgiou, M (2016) 'The vaginal microbiota , human papillomavirus infection and cervical intraepithelial neoplasia : what do we know and where are we going next ?', Microbiome. Microbiome, pp. 1–15. doi: 10.1186/s40168-016-0203-0.

Molijn, A, Kleter, B, Quint, W and Doorn, L Van (2005) 'Molecular diagnosis of human papillomavirus (HPV) infections', pp. 43–51. doi: 10.1016/j.jcv.2004.12.004.

Moody, CA and Laimins, LA (2010) 'Human papillomavirus oncoproteins : pathways to transformation', Nature Publishing Group. Nature Publishing Group, 10(8), pp. 550–560. doi: 10.1038/nrc2886.

Mooij, SH, Boot, HJ, Speksnijder, AGCL, Meijer, CJLM, King, AJ, Verhagen, DWM, et al. (2014) 'Six-month incidence and persistence of oral HPV infection in HIV-negative and HIV-infected men who have sex with men', PLoS ONE, 9(6), p. 668. doi: 10.1371/journal.pone.0098955.

Morris, BJ (2006) 'Cervical human papillomavirus screening by PCR : advantages of targeting the E6 / E7 region', 43(11), pp. 1171–1177. doi: 10.1515/CCLM.2005.203.

Motoyama, S, Ladines-llave, CA, Villanueva, SL and Maruo, T (2004) 'The Role of Human Papilloma Virus in the Molecular Biology of Cervical Carcinogenesis', 50(1), pp. 9–19.

Mu, V (2014) 'Why Sexually Transmitted Infections Tend to Cause Infertility : An Evolutionary Hypothesis', 10(8), pp. 1–5. doi: 10.1371/journal.ppat.1004111.

Münger, K, Baldwin, A, Edwards, KM, Nguyen, CL, Owens, M, Huh, K, et al. (2004) 'Mechanisms of Human MINIREVIEW Mechanisms of Human Papillomavirus-Induced Oncogenesis', Journal of Virology, 78(21), pp. 11451–11460. doi:

10.1128/JVI.78.21.11451.

Munoz, N, Bosch, FX, de Sanjose, S, Herrero, R, Castellsague, X, Shah, K V, et al. (2003) ‘Epidemiologic classification of human papillomavirus types associated with cervical cancer’, N Engl J Med, 348(6), pp. 518–527. doi: 10.1056/NEJMoa021641.

Muzny, CA and Schwebke, JR (2016) ‘Pathogenesis of Bacterial Vaginosis : Discussion of Current Hypotheses’, 214(Suppl 1), pp. 1–5. doi: 10.1093/infdis/jiw121.

Naghavi, M (2015) ‘Europe PMC Funders Group The Global Burden of Cancer 2013’, JAMA Oncol., 1(January 2014), pp. 505–527. doi: 10.1001/jamaoncol.2015.0735.The.

Nakahara, T, Peh, WL, Doorbar, J, Lee, D and Lambert, PF (2005) ‘Contributes to Multiple Facets of the Papillomavirus Life Cycle Human Papillomavirus Type 16 E1 ∧ E4 Contributes to Multiple Facets of the Papillomavirus Life Cycle’, 79(20), pp. 13150–13165. doi: 10.1128/JVI.79.20.13150.

Narisawa-Saito, M and Kiyono, T (2007) ‘Basic mechanisms of high-risk human papillomavirus-induced carcinogenesis: Roles of E6 and E7 proteins’, Cancer Science, pp. 1505–1511. doi: 10.1111/j.1349-7006.2007.00546.x.

Ngabo, F, Franceschi, S, Baussano, I, Umulisa, MC, Snijders, PJF, Uyterlinde, AM, et al. (2016) ‘Human papillomavirus infection in Rwanda at the moment of implementation of a national HPV vaccination programme’, BMC Infectious Diseases. BMC Infectious Diseases, pp. 1–10. doi: 10.1186/s12879-016-1539-6.

Nielsen, A, Iftner, T, Munk, C and Kjaer, SK (2010) ‘Acquisition of high-risk human papillomavirus infection in a population-based cohort of Danish women’, Sexually Transmitted Diseases, 36(10), pp. 609–615. doi: 10.1097/OLQ.0b013e3181a96d0e.Acquisition.

Nishimura, A, Ono, T, Ishimoto, A, Dowhanick, JJ, Frizzell, MA, Howley, PM, et al. (2000) ‘Mechanisms of Human Papillomavirus E2-Mediated Repression of Viral Oncogene Expression and Cervical Cancer Cell Growth Inhibition’, Journal of

Virology, 74(8), pp. 3752–3760. doi: 10.1128/JVI.74.8.3752-3760.2000.

Niyazi, M, Husaiyin, S, Han, L, Mamat, H, Husaiyin, K and Wang, L (2015) 'Prevalence of and risk factors for high-risk human papillomavirus infection: a population-based study from Hetian, Xinjiang, China', Bosnian Journal of Basic Medical Sciences, 16, pp. 46–51. doi: 10.17305/bjbms.2016.593.

Nzioka, MM (2016) 'PREVALENCE OF NEISSERIA GONORRHOEAE AND CHLAMYDIA TRACHOMATIS AND ANTIBIOTIC SUSCEPTIBILITY PATTERNS AMONG FAMILY PLANNING CLIENTS AT KENYATTA NATIONAL HOSPITAL , REPRODUCTIVE HEALTH CLINIC .'

Ogembo, RK, Gona, PN, Seymour, AJ, Soo-, H, Park, M, Bain, PA, et al. (2015) 'Prevalence of Human Papillomavirus Genotypes among African Women with Normal Cervical Cytology and Neoplasia : A Systematic Review and Meta-Analysis', (Icc), pp. 1–22. doi: 10.1371/journal.pone.0122488.

Onderdonk, AB, Delaney, ML and Fichorova, N (2016) 'The Human Microbiome during Bacterial Vaginosis', Clinical Microbiology Reviews, 29(2), pp. 223–238. doi: 10.1128/CMR.00075-15.Address.

Owoeye, IOG and Ibrahim, IA (2013) 'Knowledge and attitude towards cervical cancer screening among female students and staff in a tertiary institution in the Niger Delta', 2(1), pp. 48–56.

Ozbun, MA (2002) 'Human Papillomavirus Type 31b Infection of Human Keratinocytes and the Onset of Early Transcription', 76(22), pp. 11291–11300. doi: 10.1128/JVI.76.22.11291.

Panatto, D, Amicizia, D, Bianchi, S, Frati, ER, Zotti, CM, Lai, PL, et al. (2015) 'Chlamydia trachomatis prevalence and chlamydial / HPV co-infection among HPV-unvaccinated young Italian females with normal cytology', 11(1), pp. 270–276.

Pande, S, Jain, N, Prusty, BK, Bhambhani, S, Gupta, S, Sharma, R, et al. (2008) 'Human papillomavirus type 16 variant analysis of E6, E7, and L1 genes and long control

region in biopsy samples from cervical cancer patients in North India', Journal of Clinical Microbiology, 46(3), pp. 1060–1066. doi: 10.1128/JCM.02202-07.

Parkin, DM and Bray, F (2006) 'Chapter 2 : The burden of HPV-related cancers', 3, pp. 11–25. doi: 10.1016/j.vaccine.2006.05.111.

PATHfinder (2010) 'Combating Cervical Cancer in Ethiopia Addressing the Screening and Treatment Gap : The Single-Visit Approach', (April).

Pawlita, M, Dillner, J, Gram, IT, Tj, A, Munk, C, Bosch, FX, et al. (2014) 'Smoking as a major risk factor for cervical cancer and pre-cancer : Results from the EPIC cohort', 466, pp. 453–466. doi: 10.1002/ijc.28666.

Peirson, L, Fitzpatrick-Lewis, D, Ciliska, D and Warren, R (2013) 'Screening for cervical cancer: a systematic review and meta-analysis', Systematic Reviews. Systematic Reviews, 2(1), p. 35. doi: 10.1186/2046-4053-2-35.

PELZER, A, DUNCAN, ME, TIBAUX, G and MEHARI, L (1992) 'A Study of Cervical Cancer In Ethiopian Women', Cytopathology, 3(3), pp. 139–148. doi: 10.1111/j.1365-2303.1992.tb00039.x.

Perlman, S, Wamai, RG, Bain, PA, Welty, T, Welty, E and Ogembo, JG (2014) 'Knowledge and awareness of HPV vaccine and acceptability to vaccinate in sub-Saharan Africa: A systematic review', PLoS ONE, 9(3). doi: 10.1371/journal.pone.0090912.

Quinónez-calvache, EM, Ríos-chaparro, DI, Ramírez, JD, Sánchez, R, Patarroyo, ME and Patarroyo, MA (2016) 'Chlamydia trachomatis Frequency in a Cohort of HPV-Infected Colombian Women', 16, pp. 1–14. doi: 10.1371/journal.pone.0147504.

R. Leyh-Bannurah, Prugger, C, Koning, MNC De, Goette, H and Lellé, RJ (2014) 'Cervical human papillomavirus prevalence and genotype distribution among hybrid capture 2 positive women 15 to 64 years of age in the Gurage zone , rural Ethiopia', pp. 1–9.

Raifu, AO, El-zein, M, Sangwa-lugoma, G, Ramanakumar, A, Walter, SD, Franco, EL, et

al. (2017) ‘Determinants of Cervical Cancer Screening Accuracy for Visual Inspection with Acetic Acid (VIA) and Lugol ’ s Iodine (VILI) Performed by Nurse and Physician’, (Vili), pp. 1–13. doi: 10.1371/journal.pone.0170631.

Raju, K (2015) ‘Virus and Cervical Cancer : Role and implication : A Review’, 2(3), pp. 220–230.

Ramjee, G, Abbai, NS and Naidoo, S (2015) ‘Women and Sexually Transmitted Infections in Africa’, (July), pp. 385–399.

Rao, SR, Pindi, KG, Rani, U, Sasikala, G and Kawle, V (2016) ‘Diagnosis of Bacterial Vaginosis : Amsel ’ s Criteria vs Nugent ’ s scoring’, 4, pp. 2027–2031. doi: 10.21276/sjams.2016.4.6.32.

Richards, RM, Lowy, DR, Schiller, JT and Day, PM (2005) ‘Cleavage of the papillomavirus minor capsid protein , L2 , at a furin consensus site is necessary for infection’, (Track II).

Ripabelli, G, Grasso, GM, Del Riccio, I, Tamburro, M and Sammarco, ML (2010) ‘Prevalence and genotype identification of human papillomavirus in women undergoing voluntary cervical cancer screening in Molise, Central Italy’, Cancer Epidemiology. Elsevier Ltd, 34(2), pp. 162–167. doi: 10.1016/j.canep.2009.12.010.

Romero-pastrana, F (2012) ‘Detection and Typing of Human Papilloma Virus by Multiplex PCR with Type-Specific Primers’, 2012. doi: 10.5402/2012/186915.

Roura, E, Iftner, T, Vidart, JA, Kjaer, SK, Bosch, FX, Muñoz, N, et al. (2012) ‘Predictors of human papillomavirus infection in women undergoing routine cervical cancer screening in Spain : the CLEOPATRE study’, ??? ???, 12(1), p. 1. doi: 10.1186/1471-2334-12-145.

Ruland, AR, Prugger, C, Schiffer, R, Regidor, M, Lellé, RJ, Regidor, M, et al. (2006) ‘Prevalence of human papilloma virus infection in women in rural Ethiopia’, 21(9), pp. 727–729. doi: 10.1007/s10654-006-9055-4.

Salazar, KL, Zhou, HS, Xu, J, Peterson, LE, Schwartz, MR, Mody, DR, et al. (2015)

‘Multiple Human Papilloma Virus Infections and Their Impact on the Development of High-Risk Cervical Lesions’, Acta Cytologica, 59(5), pp. 391–398. doi: 10.1159/000442512.

Salih, MM, Safi, M El, Hart, K, Tobi, K and Adam, I (2010) ‘Genotypes of human papilloma virus in Sudanese women with cervical pathology’, Infectious Agents and Cancer. BioMed Central Ltd, 5(1), p. 26. doi: 10.1186/1750-9378-5-26.

Samoff, E, Koumans, EH, Markowitz, LE, Sternberg, M, Sawyer, MK, Swan, D, et al. (2005) ‘Association of Chlamydia trachomatis with persistence of high-risk types of human papillomavirus in a cohort of female adolescents’, American Journal of Epidemiology, 162(7), pp. 668–675. doi: 10.1093/aje/kwi262.

Sangita, R, Arpita, S, Aarohi, P and Summaiya, M (2016) ‘Surveillance of Gonococcal Infections with Assessment of Their Antibiotic Susceptibility Pattern’, (June), pp. 46–51.

Sankaranarayanan, R, Esmy, PO, Rajkumar, R, Muwonge, R, Swaminathan, R, Shanthakumari, S, et al. (2007) ‘Eff ect of visual screening on cervical cancer incidence and mortality in Tamil Nadu , India : a cluster-randomised trial’, 370.

Sapp, MJ and Weiller, F (2013) ‘HPV virions hitchhike a ride on retromer complexes’, pp. 1–2. doi: 10.1073/pnas.1305245110.

Saslow, D, Solomon, D, Lawson, HW, Killackey, M, Kulasingam, SL, Cain, J, et al. (2012) ‘American Cancer Society, American Society for Colposcopy and Cervical Pathology, and American Society for Clinical Pathology screening guidelines for the prevention and early detection of cervical cancer’, American Journal of Clinical Pathology, 137(4), pp. 516–542. doi: http://dx.doi.org/10.1309/AJCPTGD94EVRSJCG.

Schiffman, M, Wentzensen, N, Wacholder, S, Kinney, W, Gage, JC and Castle, PE (2011) ‘Human Papillomavirus Testing in the Prevention of Cervical Cancer’, 103(5). doi: 10.1093/jnci/djq562.

Schmitt, M, Bravo, IG, Snijders, PJF, Gissmann, L, Pawlita, M and Waterboer, T (2006) ‘Bead-based multiplex genotyping of human papillomaviruses’, Journal of Clinical Microbiology, 44(2), pp. 504–512. doi: 10.1128/JCM.44.2.504-512.2006.

Schmitt, M, Depuydt, C, Benoy, I, Bogers, J, Antoine, J, Arbyn, M, et al. (2013) ‘Prevalence and viral load of 51 genital human papillomavirus types and three subtypes’, International Journal of Cancer, 132(10), pp. 2395–2403. doi: 10.1002/ijc.27891.

Schmitt, M, Depuydt, C, Stalpaert, M and Pawlita, M (2014) ‘Bead-based multiplex sexually transmitted infection profiling’, Journal of Infection, 69(2), pp. 123–133. doi: 10.1016/j.jinf.2014.04.006.

Schmitt, M, Dondog, B, Waterboer, T and Pawlita, M (2008) ‘Homogeneous Amplification of Genital Human Alpha Papillomaviruses by PCR Using Novel Broad-Spectrum GP5 ∂ and GP6 ∂ Primers □’, 46(3), pp. 1050–1059. doi: 10.1128/JCM.02227-07.

Schwebke, JR, Lee, JY, Lensing, S, Philip, SS, Wiesenfeld, HC, Seña, AC, et al. (2016) ‘Home Screening for Bacterial Vaginosis to Prevent Sexually Transmitted Diseases’, 62, pp. 531–536. doi: 10.1093/cid/civ975.

Scott B. Vande Pol1, and AJK (2014) ‘NIH Public Access’, 445(0), pp. 115–137. doi: 10.1016/j.virol.2013.04.026.Papillomavirus.

Selinka, H, Florin, L, Patel, HD, Freitag, K, Schmidtke, M, Makarov, VA, et al. (2007) ‘Inhibition of Transfer to Secondary Receptors by Heparan Sulfate-Binding Drug or Antibody Induces Noninfectious Uptake of Human Papillomavirus □’, 81(20), pp. 10970–10980. doi: 10.1128/JVI.00998-07.

Selinka, H, Giroglou, T, Nowak, T, Christensen, ND and Sapp, M (2003) ‘Further Evidence that Papillomavirus Capsids Exist in Two Distinct Conformations’, 77(24), pp. 12961–12967. doi: 10.1128/JVI.77.24.12961.

Senapati, R, Senapati, NN and Dwibedi, B (2016) ‘Molecular mechanisms of HPV

mediated neoplastic progression', Infectious Agents and Cancer. Infectious Agents and Cancer, 11(1), p. 59. doi: 10.1186/s13027-016-0107-4.

Serrano, B, Alemany, L, Ruiz, PA de, Tous, S, Lima, MA, Bruni, L, et al. (2014) 'Potential impact of a 9-valent HPV vaccine in HPV-related cervical disease in 4 emerging countries (Brazil, Mexico, India and China)', Cancer Epidemiology, 38(6), pp. 748–756. doi: 10.1016/j.canep.2014.09.003.

Shafti-keramat, S, Handisurya, A, Kriehuber, E, Meneguzzi, G, Slupetzky, K and Kirnbauer, R (2003) 'Different Heparan Sulfate Proteoglycans Serve as Cellular Receptors for Human Papillomaviruses', 77(24), pp. 13125–13135. doi: 10.1128/JVI.77.24.13125.

Shastri, SS, Mittra, I, Mishra, GA, Gupta, S, Dikshit, R, Singh, S, et al. (2014) 'Effect of VIA Screening by Primary Health Workers : Randomized Controlled Study in Mumbai , India', (7). doi: 10.1093/jnci/dju009.

Sichanh, C, Quet, F, Chanthavilay, P, Diendere, J, Latthaphasavang, V, Longuet, C, et al. (2014) 'Knowledge , awareness and attitudes about cervical cancer among women attending or not an HIV treatment center in Lao PDR', BMC Cancer. BMC Cancer, 14(1), pp. 1–10. doi: 10.1186/1471-2407-14-161.

Simion, N, Căruntu, ID, Avădănei, ER, Balan, R and Amălinei, C (2014) 'Conventional cytology versus liquid based cytology in cervical pathology: Correspondences and inconsistencies in diagnosis, advantages and limits', Romanian Journal of Morphology and Embryology, 55(4), pp. 1331–1337.

Smith, JL, Campos, SK and Ozbun, M a (2007) 'Human papillomavirus type 31 uses a caveolin 1- and dynamin 2-mediated entry pathway for infection of human keratinocytes.', Journal of virology, 81(18), pp. 9922–31. doi: 10.1128/JVI.00988-07.

Smith, JS, Lindsay, L, Hoots, B, Keys, J, Franceschi, S, Winer, R, et al. (2007) 'Human papillomavirus type distribution in invasive cervical cancer and high-grade cervical lesions : A meta-analysis update', 632(7435), pp. 621–632. doi: 10.1002/ijc.22527.

Smith, JS, Melendy, A, Rana, RK and Pimenta, JM (2008) 'Age-Specific Prevalence of Infection with Human Papillomavirus in Females: A Global Review', Journal of Adolescent Health, 43(4 SUPPL.). doi: 10.1016/j.jadohealth.2008.07.009.

So, KA, Hong, JH and Lee, JK (2016) 'Human Papillomavirus Prevalence and Type Distribution Among 968 Women in South Korea', 21(2), pp. 104–109.

Sonnenberg, P, Ison, CA, Clifton, S, Field, N, Tanton, C, Soldan, K, et al. (2015) 'Epidemiology of Mycoplasma genitalium in British men and women aged 16 – 44 years : evidence from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3)', (November), pp. 1982–1994. doi: 10.1093/ije/dyv194.

Stanley, MA (2012) 'Epithelial Cell Responses to Infection with Human Papillomavirus', pp. 215–222. doi: 10.1128/CMR.05028-11.

Stanley, MA, Pett, MR and Coleman, N (2007) 'HPV: from infection to cancer.', Biochemical Society transactions, 35(Pt 6), pp. 1456–60. doi: 10.1042/BST0351456.

Stanley, M, Lowy, DR and Frazer, I (2006) 'Chapter 12 : Prophylactic HPV vaccines : Underlying mechanisms', 3, pp. 106–113. doi: 10.1016/j.vaccine.2006.05.110.

Surviladze, Z, Sterkand, RT and Ozbun, MA (2015) 'Interaction of human papillomavirus type 16 particles with heparan sulfate and syndecan-1 molecules in the keratinocyte extracellular matrix plays an active role in infection', Journal of General Virology, 96(8), pp. 2232–2241. doi: 10.1099/vir.0.000147.

Tao, L, Han, L, Li, X, Gao, Q, Pan, L, Wu, L, et al. (2014) 'Prevalence and risk factors for cervical neoplasia : a cervical cancer screening program in Beijing', pp. 1–9.

Tebeu, P, Vassilakos, P, Petignat, P and Division, G (2017) 'A review of screening strategies for cervical cancer in human immunodeficiency virus-positive women in sub-Saharan Africa', pp. 69–79.

Terefe, Y and Gaym, A (2008) 'ORIGINAL ARTICLE Knowledge, attitude and practice of screening for carcinoma of the cervix among reproductive health clients at three teaching hospitals, Addis Ababa, Ethiopia 1', 2(1), pp. 14–24.

Torre, LA, Bray, F, Siegel, RL and Ferlay, J (2015) 'Global Cancer Statistics , 2012', 65(2), pp. 87–108. doi: 10.3322/caac.21262.

Tracy, JK, Traore, CB, Bakarou, K, Dembelé, R, Coulibaly, RC and Sow, SO (2011) 'Risk factors for high-risk human papillomavirus infection in unscreened Malian women', Tropical Medicine and International Health, 16(11), pp. 1432–1438. doi: 10.1111/j.1365-3156.2011.02843.x.

Trottier, H (2006) 'Human Papillomavirus Infections with Multiple Types and Risk of Cervical Neoplasia', Cancer Epidemiology Biomarkers & Prevention, 15(7), pp. 1274–1280. doi: 10.1158/1055-9965.EPI-06-0129.

Unemo, M and Dillon, JR (2011) 'Review and International Recommendation of Methods for Typing Neisseria gonorrhoeae Isolates and Their Implications for Improved Knowledge of Gonococcal Epidemiology , Treatment , and Biology', 24(3), pp. 447–458. doi: 10.1128/CMR.00040-10.

Unemo, M and Shafer, M (2014) 'Antimicrobial Resistance in Neisseria gonorrhoeae in the 21st Century : Past , Evolution , and Future', 27(3), pp. 587–613. doi: 10.1128/CMR.00010-14.

Vaccarella, S, Franceschi, S, Herrero, R, Mun, N, Snijders, PJF, Clifford, GM, et al. (2006) 'Sexual Behavior , Condom Use , and Human Papillomavirus : Pooled Analysis of the IARC Human Papillomavirus Prevalence Surveys', 15(February). doi: 10.1158/1055-9965.EPI-05-0577.

Ve, P and Dağilimi, G (2017) 'HUMAN PAPILLOMA VIRUS (HPV) PREVALENCE AND GENOTYPE DISTRIBUTION HUMAN PAPİLLOMA VİRUS (HPV)', 8(suppl 2), pp. 109–113. doi: 10.4328/JCAM.4959.

Vedantham, H, Silver, MI, Kalpana, B, Rekha, C, Karuna, BP, Mrudula, S, et al. (2011) 'NIH Public Access', 19(5), pp. 1373–1380. doi: 10.1158/1055-9965.EPI-09-1282.Determinants.

Verhelst, R, Verstraelen, H, Claeys, G, Verschraegen, G, Delanghe, J, Van Simaey, L, et

al. (2004) ‘Cloning of 16S rRNA genes amplified from normal and disturbed vaginal microflora suggests a strong association between Atopobium vaginae, Gardnerella vaginalis and bacterial vaginosis.’, BMC microbiology, 4, p. 16. doi: 10.1186/1471-2180-4-16.

Verma, M, Erwin, S, Abedi, V, Hontecillas, R, Hoops, S, Leber, A, et al. (2017) ‘Modeling the mechanisms by which HIVAssociated immunosuppression influences HPV persistence at the Oral Mucosa’, PLoS ONE, 12(1), pp. 1–20. doi: 10.1371/journal.pone.0168133.

Verstraelen, H, Verhelst, R, Vaneechoutte, M and Temmerman, M (2010) ‘The epidemiology of bacterial vaginosis in relation to sexual behaviour’.

Verteramo, R, Pierangeli, A, Mancini, E, Calzolari, E, Bucci, M, Osborn, J, et al. (2009a) ‘Human Papillomaviruses and genital co-infections in gynaecological outpatients.’, BMC infectious diseases, 9, p. 16. doi: 10.1186/1471-2334-9-16.

Verteramo, R, Pierangeli, A, Mancini, E, Calzolari, E, Bucci, M, Osborn, J, et al. (2009b) ‘Human Papillomaviruses and genital co-infections in gynaecological outpatients.’, BMC infectious diseases, 9(1), p. 16. doi: 10.1186/1471-2334-9-16.

Vet, JNI, de Boer, M a, van den Akker, BEWM, Siregar, B, Lisnawati, Budiningsih, S, et al. (2008) ‘Prevalence of human papillomavirus in Indonesia: a population-based study in three regions.’, British journal of cancer, 99(1), pp. 214–218. doi: 10.1038/sj.bjc.6604417.

Vielfort, K, Söderholm, N, Weyler, L, Vare, D, Löfmark, S and Aro, H (2013) ‘Neisseria gonorrhoeae infection causes DNA damage and affects the expression of p21, p27 and p53 in non-tumor epithelial cells.’, Journal of cell science, 126(Pt 1), pp. 339–47. doi: 10.1242/jcs.117721.

Villa, LL (2006) ‘Biology of genital human papillomaviruses’, 94, pp. 5–9.

Villa, LL (2009) ‘Laboratory Methods for Detection of Human Papillomavirus Infection’. doi: 10.1007/978-3-540-70974-9-2.

de Villiers, EM (2013) ‘Cross-roads in the classification of papillomaviruses’, Virology, pp. 2–10. doi: 10.1016/j.virol.2013.04.023.

De Villiers, EM, Fauquet, C, Broker, TR, Bernard, HU and Zur Hausen, H (2004a) ‘Classification of papillomaviruses’, Virology, 324(1), pp. 17–27. doi: 10.1016/j.virol.2004.03.033.

De Villiers, EM, Fauquet, C, Broker, TR, Bernard, HU and Zur Hausen, H (2004b) ‘Classification of papillomaviruses’, Virology, pp. 17–27. doi: 10.1016/j.virol.2004.03.033.

Vuyst, H De (2003) The epidemiology of human papillomavirus and interaction with human immunodeficiency virus in Kenya.

De Vuyst, H, Parisi, MR, Karani, A, Mandaliya, K, Muchiri, L, Vaccarella, S, et al. (2010) ‘The prevalence of human papillomavirus infection in Mombasa, Kenya.’, Cancer causes & control : CCC, 21(12), pp. 2309–13. doi: 10.1007/s10552-010-9645-z.

Wade, R, Brimer, N and Pol, S Vande (2008) ‘Transformation by Bovine Papillomavirus Type 1 E6 Requires Paxillin □ ’, 82(12), pp. 5962–5966. doi: 10.1128/JVI.02747-07.

Walboomers, JMM, Jacobs, M V., Manos, MM, Bosch, FX, Kummer, JA, Shah, K V., et al. (1999) ‘Human papillomavirus is a necessary cause of invasive cervical cancer worldwide’, Journal of Pathology, 189(1), pp. 12–19. doi: 10.1002/(SICI)1096-9896(199909)189:1<12::AID-PATH431>3.0.CO;2-F.

Wang, R, Guo, X, Wisman, GBA, Schuuring, E, Wang, W, Zeng, Z, et al. (2015) ‘Nationwide prevalence of human papillomavirus infection and viral genotype distribution in 37 cities in China’, BMC Infectious Diseases. BMC Infectious Diseases, 15(1), p. 257. doi: 10.1186/s12879-015-0998-5.

Watts, DH, Fazarri, M, Minkoff, H, Hillier, SL, Sha, B, Glesby, M, et al. (2005) ‘Effects of Bacterial Vaginosis and Other Genital Infections on the Natural History of Human Papillomavirus Infection in HIV-1 – Infected and High-Risk HIV-1 – Uninfected Women’, 191, pp. 1129–1139.

Weyn, C, Garbar, C, Noel, J-C, Weynand, B, Verhest, A, d'Olne, D, et al. (2013) 'Inter-laboratory variability in the presence of human papillomavirus in normal and abnormal cervical cytology samples.', Cancer Epidemiol, 37(4), pp. 457–461. doi: 10.1016/j.canep.2013.03.016.

Whang, SN, Filippova, M and Duerksen-hughes, P (2015) 'Recent Progress in Therapeutic Treatments and Screening Strategies for the Prevention and Treatment of HPV-Associated Head and Neck Cancer', pp. 5040–5065. doi: 10.3390/v7092860.

White, E a, Sowa, ME, Jie, M, Tan, A, Jeudy, S, Hayes, SD, et al. (2012) 'Systematic identi fi cation of interactions between host cell proteins and E7 oncoproteins from diverse human papillomaviruses', Pnas, 109(5), pp. E260–E267. doi: 10.1073/pnas.1116776109/-/DCSupplemental.www.pnas.org/cgi/doi/10.1073/pnas.1116776109.

WHO (2011) 'WHO guidelines Use of cryotherapy for cervical intraepithelial neoplasia'.

WHO (2013) 'Guidelines for screening and treatment of precancerous lesions for cervical cancer prevention', WHO Guidelines, p. 60. Available at: http://www.who.int/reproductivehealth/publications/cancers/screening_and_treatment_of_precancerous_lesions/en/index.html.

Wiley, J (1987) 'User Information for bqpd', pp. 1–4. Available at: https://academic.oup.com/jncimono/article-lookup/doi/10.1093/oxfordjournals.jncimonographs.a003478.

Williams, VM, Filippova, M, Soto, U and Duerksen-Hughes, PJ (2011) 'HPV-DNA integration and carcinogenesis: putative roles for inflammation and oxidative stress', Future Virology, 6(1), pp. 45–57. doi: 10.2217/fvl.10.73.

Winer, RL, Feng, Q, Hughes, JP, Reilly, SO, Kiviat, NB and Koutsky, LA (2008) 'Risk of Female Human Papillomavirus Acquisition Associated with First Male Sex Partner', 197, pp. 279–282. doi: 10.1086/524875.

Wolf, M, Garcea, RL, Grigorieff, N and Harrison, SC (2010) 'Subunit interactions in

bovine papillomavirus', Proceedings of the National Academy of Sciences, 107(14), pp. 6298–6303. doi: 10.1073/pnas.0914604107.

World Health Organization (2013) 'Comprehensive cervical cancer prevention and control : a healthier future for girls and women', World Health Organisation, pp. 1–12. doi: ISBN 978 92 4 150514 7.

Yang, R, Day, PM, Iv, WHY, Lin, K, Hung, C and Roden, RBS (2003) 'Cell Surface-Binding Motifs of L2 That Facilitate Papillomavirus Infection', 77(6), pp. 3531–3541. doi: 10.1128/JVI.77.6.3531.

Zanier, K, Charbonnier, S, Sidi, AOMO, McEwen, AG, Ferrario, MG, Poussin-Courmontagne, P, et al. (2013) 'Structural Basis for Hijacking of Cellular LxxLL Motifs by Papillomavirus E6 Oncoproteins', Science, 339(6120), pp. 694–698. doi: 10.1126/science.1229934.

Zhu, H, Shen, Z, Luo, H, Zhang, W and Zhu, X (2016) 'Chlamydia Trachomatis Infection-Associated Risk of Cervical Cancer: A Meta-Analysis.', Medicine, 95(13), p. e3077. doi: 10.1097/MD.0000000000003077.

11. List of Annexes

A.1. Annexe IRB ethical clearance

	Addis Ababa University College of Health Science Institutional Review Board **'itle:** **3.2. Use of Study Assessment Form**	**SOP# AAUMF 008** **Version 2.0** **Effective date: 1 Feb. 2009** **Page 13 of 13**

ANNEX 3
Form AAUMF 03-008

IRB's Decision

Meeting No: 002/2015 Date: February, 2015
Protocol number: 056/14/DMIP Assigned No.....

Protocol Title: Molecular epidemiology of HPV and cervical cancer in North and Central Ethiopia		
Principal Investigators:	Gebremeskel G/Mariam	
Institute:	CHS-AAU	
Elements Reviewed (AAUMF 01-008)	☑ Attached	☐ Not attached
Review of Revised Application ☐ Yes ☐ No	Date of Previous review:	
Decision of the meeting:	☑ **Approved** ☐ Approved with Recommendation ☐ Resubmission ☐ Disapproved	

I.Elements approved-
1. Protocol Version No. .....
2. Protocol Version Date.....
3. Informed consent Version No. .....
4. Informed Consent Version Date .....

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

III. **TO NERC** ■

Institution Review Board (IRB) Approval: Period from **11/03/2015** to **10/03/2016**

Follow up report expected in
3 Months ___ 6 months ___ 9 months_____one year_√___

Chairperson, IRB
Dr. Yimtubezinash Woldeamanuel
Signature _____
Date: 11/03/2015

Associate Director for Research and technology transfer
Dr. Getnet Yimer
Signature _____
Date 11/03/15

A.2. Annex NRERC ethical clearance

በኢትዮጵያ ፌዴራላዊ ዴሞክራሲያዊ ሪፐብሊክ
የሳይንስና ቴክኖሎጂ ሚኒስቴር
The Federal Democratic Republic of Ethiopia
Ministry of Science and Technology

ቁጥር 3.10/049/2015
Ref. No.
ቀን Sep 7, 2015
Date

To: Addis Ababa University, College of health sciences, Ethics Review Committee

Addis Ababa

Re: Molecular epidemiology of cervical cancer and human papillomavirus in North and Central part of Ethiopia.

Dear Sir/Madam//Mr./Mrs./Dr,

The National Research Ethics Review Committee (NRERC) has reviewed the aforementioned project protocol in an expedited manner. We are writing to advise you that NRERC has granted

Full Approval

To the above named project, for a period of **one year (September 7, 2015- September 6, 2016).** All your most recently submitted documents have been approved for use in this study. The study should comply with the standard international and national scientific and ethical guidelines. Any change to the approved protocol or consent material must be reviewed and approved through the amendment process prior to its implementation. In addition, any adverse or unanticipated events should be reported within 24-48 hours to the NRERC. Please ensure that you submit biannual progress report once in six months and annual renewal application 30 days prior to the expiry date.

We, therefore, request you as PI and your esteemed organization to ensure the commencement and conduct of the study accordingly and wish for the successful completion of the project.

With regards,

Yohannes Sitotaw
Secretary of NRERC

CC. Mr. Gebremeskel Gebremariam (PI)
NRERC chairperson

ማነጋገር ቢያስፈልግዎ
You may Contact

ፖ.ሳ.ቁ. P.O.Box 2490
አዲስ አበባ ኢትዮጵያ Addis Ababa, Ethiopia E-mail most@ethionet.et
ስልክ Tel. 251-011-4-674353 Web site:-http://www.most.gov.et
ፋክስ Fax +251-011-4-66 02 41

A.3. Material Transfer Agreement

Material Transfer Agreement Form

Ministry of Science and Technology, Federal Democratic Republic of Ethiopia National Research Ethics Review Committee

Address: Tel.: +251011-4-674353 P.O. Box: 2490 Fax: +251-011-4-660241 e-mail: Addis Ababa –Ethiopia

Material Transfer Agreement

This Material Transfer Agreement (MTA) has been prepared for use by Department of Microbiology, Immunology and Parasitology (DMIP), School of Medicine, College of Health Sciences, Addis Ababa University, Ethiopia (hereinafter referred to as "Provider") and the Charité – Universitätsmedizin Berlin hereinafter referred to as "Recipient") in all transfer of research material (samples, derivatives, and specimens) related to the protocol, "Molecular Epidemiology of Human Papillomavirus and Cervical Cancer in North and Central Part of Ethiopia" .

Provider: Department of Microbiology, Immunology and Parasitology (DMIP), School of Medicine, College of Health Sciences, Addis Ababa University.

Recipient: Dr. Andreas Kaufmann Charité – Universitätsmedizin Berlin

Provider agrees to transfer to recipient's designated ("research project") the following research materials /specimen. (Cervical swab samples)

The research material will only be used for research purposes as described in the protocol by recipient's investigator in designated laboratory for the research project described below, under suitable containment conditions. This research material will not be used for commercial purposes such as screening, production or sale for which a commercialization license may be required. Recipient agrees to comply with all National and International guidelines rules and regulations applicable to the Research Project and the handling of the Research Material.

a) Are the research materials of human origin?

yes [X] No [ ]

b) If yes, are they collected according to the details in the protocol and in adherence to National Research Ethics Review Committee (NRERC) and Addis Ababa University College of Health Sciences institutional review board (IRB) Ethics Review Committee recommendations and their approval.

Yes [X] No

2. This research material and its derivatives will be used by recipient's investigator solely in connection with the following research project ("Research Project") de- 90 National Research Ethics Review Guideline Fifth Edition scribed with specificity as follows: "Molecular Epidemiology of Human Papillomavirus and Cervical Cancer in North and Central Part of Ethiopia"

3. In all presentations or written publications concerning the research project, recipient will seek agreement of provider and acknowledge provider's contribution of this research material unless requested otherwise.

4. This research material represents a significant contribution on the part of provider and is considered proprietary to provider. Recipient therefore agrees to retain control over this research Material and further agrees not to transfer the research material to other people not under her/his direct supervision without advance written approval of provider. The research material will be disposed of as agreed upon per protocol at the end of completion of the project on.

5. The provider does not take any responsibility for loss, damage, wastage or spoilage of the research material during or after shipment to the address provided by the recipient under conditions agreed to in the protocol on shipment of the samples. This research material is provided as a service to the research community. IT IS BEING SUPPLIED TO RECIPIANT WITH NO WARRANTIES, EXPRESS OR IMPLIED, INCLUDING ANY WARRANTY OF MERCHANTABILITY OR FITNESS FOR A PARTICULAR PURPOSE. Provider makes no representations that the use of the research material will not infringe any patent or proprietary right of third parties.

6. The recipient shall notify the provider in witting of any intention, improvement, modification discovery or development to the material or the information made by recipient or parties, collaborating with recipient, here in after referred to as "invention". Nothing in this agreement shall, however, be construed as conveying to the provider any rights under any patents or other intellectual property to such invention, other than as explicitly provided herein. At its option the

For Recipient:

Recipient's Investigator
Dr. Andreas Kaufmann

Signature Andreas Kaufmann

Date June 1st 2015

Duly Authorized
Dr. Anette Schröder / Dr. Frank Stief

Signature and Stamp

CHARITÉ
UNIVERSITÄTSMEDIZIN BERLIN
Technologiestiftung
Charitéplatz 1|D-10117 Berlin

Date May 26th 2015

Mailing Address for Material:
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12200 Berlin, Germany

Tele: +49 30 8445 2756
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E mail: andreas.kaufmann@charite.de

Mailing Address for Notices:

Tele:

Fax:

For Provider

Provider's Investigator

Gebremeskel Gebremariam Hidat

Signature/Stamp

Date June 2/2015

Duly Authorized

Dr. Tamrat Abebe

Head, DMIP

Signature/Stamp

Date June 2/2015

ADDIS ABABA UNIVERSITY-FACULTY OF MEDICINE
DEPARTMENT OF MICROBIOLOGY IMMUNOLOGY & PARASITOLOGY (DMIP)

Mailing Address:

Department of Microbiology, Immunology and Parasitology, School of medicine, College of Health Science, Addis Ababa University, Addis Ababa, Ethiopia

P.o.Box: 9086

Fax: 251-115513099

Mailing Address for notices:

Department of Microbiology, Immunology and Parasitology, School of medicine, College of Health Science, Addis Ababa University, Addis Ababa, Ethiopia

P.o.Box: 9086

Fax: 251-115513099

Tele: 251-5528726 Ext.439

Code

A.4. Questionnaire in English, Amharic and Tigrigna

A.4.1. English Version

Molecular epidemiology and genotyping of genital Human papillomavirus and cervical cancer in North and Central part of Ethiopia

Demographic data

Your address ❒Urban ❒Rural ☐Declined to answer Specify:Region_____Zone/town_____Wereda_____ Kebele_____
Age?_____ ❒Do not know ☐ Declined to answer
Ethnic group_____
What is the highest level of education you completed? ☐ No formal education/none ☐ Vocational training ☐ Primary school (1~8 years) ❒ College or University ☐ High Secondary School (9-10) ☐ Other specify _____ ☐ Preparatory school (10^{+1},10^{+2})
What is your occupation/source of income? ☐ House wife ☐ Student ☐ Farmer ☐ Government employee ☐ Self employed ☐ Merchant ☐ NGO employee ☐ Unemployed ☐ House servand/maid ❒Local food & drink seller (specify)_____ ❒Other specify _
Marital status: ☐ Single ☐ Married ☐ Divorced ☐ Widow ☐ other, specify_____ ☐Declined to answer
What is your average monthly income?_____/Birr ❒Do not know ☐ Declined to answer
What is your family average monthly income? _____/Birr ❒Do not know ☐ Declined to answer

Section B: Reproductive history

Have you ever been pregnant? ☐ Yes ☐ No ,if the answer is no please go to Q"**14"**
How many times have you been pregnant? _____ ☐ Declined to answer
How many children do you have? _____ ☐ Declined to answer
Have you ever experienced abortion? ❒ Yes ❒ No ☐ Declined to answer
If your answer to Q12 is "**Yes**": The number of spontaneous_____while induced abortions are_____ ☐ Decline to answer
Have you ever used a family planning method? ☐ Yes ☐ No ☐ 3.Decline to answer

Which of the following is your family planning method (you can mark more than one)?
☐ Pills, duration_____ ☐ Tubal ligation, duration_____ ☐Condom, frequency_____
☐ Abstinence ☐ Withdrawal ☐ Implant, duration____
☐ IUCD, duration_____ ☐ Injection, duration____ ☐ Lactation Amenorrhea method
☐ Sexual partner had Vasectomy ☐ Declined to answer ❒others specify _____

Section c: Knowledge/practice assessment

Have you heard about cervical cancer? ☐ Yes ☐ No ☐ Decline to answer

Who informed you about cervical cancer?
☐ Health professional ☐ Family /friends ☐ News paper ☐ TV/Radio
☐ Internet ☐ Declined to answer ☐ Other (specify) _____

Have you heard about cervical cancer screening method?
☐ Yes ☐ No ,if the answer is no please go to C 25

Have you ever had a cervical cancer screening? ☐ Yes ☐ No ☐ Decline to answer

If you did not have cervical cancer screening yet, why?
❒Fear of cervical cancer ❒Lack of money ❒Lack of awareness
❒Lack of information where screening is taking place ❒Fear of diagnostic tools insertion ❒Fear of stigma ❒ Shyness to discuss with male clinicians
❒others, specify_____

Do you know what causes cervical cancer? ❒Yes ❒ No

If your answer to Q"21" is "**Yes**" what is the cause of cervical cancer?
❒ Heredity ❒ infection with HPV ❒ smoking ❒ Sin ❒Don't know
❒others_____

Do you know the risk factors for HPV infection?
❒ Sex with multiple partners ❒Smoking ❒others_____
❒Early marriage before 18 years of age ❒Hereditary ❒Don't know

Do you think cervical cancer is preventable? ☐Yes ☐ No ☐ Do not know

If your answer to Q24 is "**Yes**" which methods can be used to prevent cervical cancer?
❒Vaccination against HPV ❒having only one sexual partner
❒ programmed screening for cervical Cancer ❒Stop smoking
❒avoid using contraceptive pills for long time ❒Avoid early marriage
❒using condom regularly ❒Do not know ❒others_____

Do you think cervical cancer is treatable? ❒ Yes ❒ No ❒Don't know

If your answer to Q26 is "Yes", what are the methods of treatment for cervical cancer?
❒Chemotherapy ❒Radiological treatment ❒Cryotherapy
❒Surgical treatment ❒combination of Chemo & Radiotherapy
❒ Chemo, Radiological & Surgical therapy ❒Don't know

Have you ever suffered from sexually transmitted diseases? ❒Yes ❒No ❒ declined to answer

Section D: Personal and behavioral issues

At what age did you get married? _____ ❒ Decline to answer ☐Do not know
At what age did you have your first sexual intercourse?_☐Decline to answer ❒Do not know
How many sexual partners have you had? _____ ☐Decline to answer
Does your sexual partner (husband) have sexual partner beside you? ❒1. Yes, if yes how many_____❒ No ❒ Do not know ❒ Declined to answer
Have you been or are you currently smoking? ❒Yes, for how long_____(years) ❒No ❒ Declined to answer
What fuel do you use to cook your food? ❒wood ❒domestic animal excreta ❒Kerosene ❒Electricity ❒Others_____

Data collector:_____sign_____date _____

A.4.2. Amharic Version

ክፍል ሀ. መሰረታዊ መረጃ

Code

የመኖሪያ ቦታ ❒ከተማ _____ ❒ገጠር_____ ☐ለመመለስ አልፈቀዱም ክልል ____ዞን _____ ወረዳ_____
ዕድሜ_____ ❒አላውቀውም ☐ ለመመለስ አልፈቀዱም
ብሄረሰብዎ_____
የትም/ት ደረጃ ☐ ያልተማረች (መደበኛ ትም/ት ያልተማረች) ☐ አንደኛ ደረጃ (1~8) ☐ ሁለተኛ ደረጃ (9-10) ❒ መስናዶ (10^{+1},10^{+2}) (12ኛ ክፍል) ☐ የሙያ ትም/ት ሰርተፊኬት ☐ ኮሌጅ ወይም /ዩኒቨርሲቲ
የሚተዳደሩበት ስራ/ የገቢ ምንጭ ☐ የቤት እመቤት ☐ ነጋዴ ☐ ተማሪ ☐ የግል ስራ ☐ የመንግስት ሰራተኛ ☐ ገበሬ ☐ ስራ አጥ ❒የግለሰብ ተቀጣሪ ☐ መያድ ሰራተኛ ☐ ምግብ ወይም መጠጥ ሻጭ (ይግለፁት)____❒ ሌላ ካለ ይግለፁ _____
የጋብቻ ሁኔታ ☐ ያላገባች ☐ ያገባች ☐ የተፋታች ☐ባልዋ የሞተባት ☐ ለመመለስ አልፈቀዱም
ወርሃዊ ገቢዎ ምን ያህል ነው?_____ /ብር ☐ አላውቅም ☐ ለመመለስ አልፈቀዱም
ጠቅላላ የቤተሰብዎ ወርሃዊ ገቢ ምን ያህል ነው?____ /ብር ☐ አላውቅም ☐ ለመመለስ አልፈቀዱም

ክፍል ለ: ስነ ተዋልዶ

አርግዘው ያውቃሉ? ☐ አዎ ☐ አላውቅም, መልስዎ **<u>አላውቅም</u>** ከሆነ ወደ ጥያቄ ለ13 ይለፉ
ለምን ያህል ጊዜ አርግዘው ያውቃሉ? _____ ☐ ለመመለስ አልፈቀዱም
ስንት ልጆች አሉዎት? _____ ☐ ለመመለስ አልፈቀዱም
ፅንስ ተጨናግፎብዎ ያውቃል? ❒ አዎ ❒ አላውቅም ☐ ለመመለስ አልፈቀዱም

ለጥያቄ 12 መልስዎ "**አዎ**" ከሆነ፡ ለምን ያህል ጊዜ በድንገት(_____)እና በራስዎ ፍላጎት(_____) ፅንስ ተጨናገፎብዎ ያውቃል.
የቤተሰብ ምጣኔ ይጠቀማሉ? ☐ 1.አዎ ☐ 2.አልጠቀምም ☐ 3. ለመመለስ አልፈቀዱም
ለጥያቄ ቁጥር 14 መልስዎ አዎ ከሆነ፤ የትኛውን ዘዴ የጠቀማሉ (ከአንድ በላይ መጥቀስ ይቻላል) ☐ ክኒን፣ ቆይታ (በዓመት)_____☐ ክንድ ውስጥ የሚቀበር ቆይታ (በዓመት)_____☐ በመታቀብ ❒አይ ዩ ሲ ዲ (IUCD)(በዓመት)____❒ መርፌ ቆይታ (በዓመት)___☐ በማጥባት ☐ የፍቅር ጓደኛ የዘር ቧንቧመቆረጥ ❒የዘር ቧንቧመቋጠር (በዓመት)_____ ☐ ለመመለስ አልፈቀዱም ❒ ኮንደም (በዓመት)___ ☐የወንድ ዘር ፍሬ ወደ ውጭ በማፍሰስ ❒ሌላ ካለ_____

ክፍል ሐ: ዕውቀት/ልምድ ጉዳዮች

ስለ የማህፀን ጫፍ ካንሰር ሰምቷልን? ☐ አዎ ☐ አልሰማሁም ❒ ለመመለስ አልፈቀዱም
ስለ የማህፀን ጫፍ ካንሰር ከማን ሰሙ? ❒ ከጤና ባለሙያ ☐ ከቤተሰብ/ ጓደኛ ☐. ከጋዜጣ ☐ ከራዲዮና ቴሌቪዥን ☐ ኢንተርኔት ☐ ለመመለስ አልፈቀዱም ❒ሌላ ካለ ይግለፁ _____
ስለ የማህፀን ጫፍ ካንሰር ቅድመ ምርመራ ሰምቷል? ☐ አዎ ☐ 2. አልሰማሁም (ወደ ቁጥር 25 ይለፉ)
የማህፀን ጫፍ ካንሰር ቅድመ ምርመራ አድርገው ያውቃሉ? ☐አዎ ☐ አላውቅም ☐ ለመመለስ አልፈቀዱም
የማህፀን ጫፍ ካንሰር ቅድመ ምርመራ አድርገው የማያውቁ ከሆነ፣ ለምን? ❒የማህፀን ጫፍ ካንሰር ፍራቻ ❒ለምርመራ የሚሆን ገንዘብ ማጣት ❒ምርመራው የት እንደሚካሄድ አለማወቅ ❒የግንዛቤ እጥረት ❒መገልልን በመፍራት ❒የመመርመሪያ መሳሪያው ፍራቻ ❒ከወንድ ሃኪም ጋር በዚህ ዙሪያ መወያየት ስለሚያሳፍረኝ ❒ሌላካለ ይግለፁ_____
የማህፀን ጫፍ ካንሰር በምን እንደሚከሰት ያውቃሉ? ❒ አዎ ❒ አላውቅም
ለጥያቄ ቁጥር 21 መልስዎ "**አዎ**" ከሆነ በምን ይከሰታል? ❒ በዘር ❒ በHPV መያዝ ❒ በማጨስ ❒ሃጥያት በመስራት ❒ ሌላ ካለ ይግለፁ_____
ለHPV ብክለት አጋላጭ ሁኔታዎች ይጥቀሱልኝ? ❒ከብዙ ወንድ ጋር ግብረ ስጋ ግኙኝነት መፈፀም ❒ማጨስ ❒ከዕድሜ በታች ጋብቻ ❒አላውቅም
የማህፀን ጫፍ ካንሰር መከላከል የሚቻል ይመስልዎታል? ☐ አዎ ☐ አላውቅም ☐ ለመመለስ አልፈቀዱም
ለጥያቄ ቁጥር 24 መልስዎ "አዎ" ከሆነ፤ የማህፀን ጫፍ ካንሰር መከላከያ መንገዶች ምን እና ምን ናቸው? ❒ የHPV ክትባት ❒ ባለማጨስ ❒ከዕድሜ በታች አለማግባት ❒አንድ ለአንድ በመወሰን ❒ወቅቱን ጠብቆ ቅድመ ካንሰር ምርመራ በማድረግ ❒ ኮንደም በአግባቡና ሁል ጊዜ መጠቀም ❒የቤተሰብ ምጣኔ ፒሎች ለረጅም ጊዜ አለመጠቀም ❒አላውቅም ❒ሌላ ካለ_____
የማህፀን ጫፍ ካንሰር በህክምና የሚድን ይመስልዎታል? ❒አዎ ❒አይድንም ❒ አላውቅም
ለጥያቄ ቁጥር 26 መልስዎ "አዎ" ከሆነ የማህፀን ጫፍ ካንሰር የሚታከመዉ በምንድ ነው? ❒በክኒን ❒በጨረራ ❒በቀዝቃዛ ናይትሮጅን ህክምና ❒በቀዶ ጥገና ❒ በቀዶ ጥገና እና በጨረር ❒ውሁድ ህክምና በክኒን እና በጨረር ❒አላውቅም
በግብረ ስጋ ግኙኝነት በሚከሰት በሽታ ታመው ያውቃሉ? ❒ አዎ ❒አላውቅም ❒ ለመመለስ አልፈቀዱም

ክፍል መ: ግላዊ እና ፀባይ ነክ ጉዳዮች

በስንት ዕድሜዎ ባል አገቡ? _____ ❒ አላውቀም ☐ ለመመለስ አልፈቀዱም	
ለመጀመሪያ ጊዜ የግብረ ስጋ ግኙኝነት ሲፈፅሙ ዕድሜዎ ስንት ነበር?___ ❒አላውቅም ☐ ለመመለስ አልፈቀዱም	
እስከ አሁን ድረስ ከምን ያህል የፍቅር ጓደኛ ጋር ወሲብ ፈፅመዋል? _____ ☐ ለመመለስ አልፈቀዱም	

የፍቅር ጓደኛዎ (ባለቤትዎ)ከእርስዎ ሌላ ሚስት (ውሽማ) አለችው? ☐ አዎ፣ ስንት_____ ☐ አላውቅም ☐ ለመመለስ አልፈቀዱም	
ከዚህ በፊት ወይም አሁን ያጨሳሉ? ❒አዎ ፤ ለምን ያህል ጊዜ_____(በዓመት) ❒ በፍፁም አላጨስም፣ ባለቤትዎስ ❒ ለመመለስ አልፈቀዱም	
ምግብ ለማብሰል የሚጠቀሙት ምንድን ነው? ❒እንጨት ❒ኩበት እና ፍግ ❒ ነዳጅ ❒ ኤክትሪክ ❒ሌላ ካለ _____	

መረጃ ሰብሳቢ፡ ስም ቀን ፊርማ

A.4.3. Tigrigna Version

Code

ክፍሊ ሀ. መሰረታዊ ሓበሬታ

መንበሪ ቦታ ❒ከተማ _____❒ገጠር_____ ☐ንምምላስ አይፈቐዳን ክልል _ዞን/ከተማ _____ ወረዳ_____
ዕድመ_____ ❒አይፈልጦን ☐ ንምምላስ አይፈቐዳን
ብሄረሰብን(ኪ)_____
ደረጃ ትም/ቲ ☐ ዘይተምሃረት ስሩዕ ትም/ቲ ዘይተምሃረት) ☐ ቀዳማይ ብርኪ (1~8) ☐ ካልኣይ ብርኪ (9-10) ☐ ናይ ሙያ ሰርተፊኬት ☐ መስናዶ (10^{+1},10^{+2}) ☐ ኮሌጅ ወይ ዩኒቨርሲቲ
የሚተዳደሩበት ስራ/ የገቢ ምንጭ ☐ የቤት እመቤት ☐ ስራሕ ዘይብላ ☐ ተምሃሪት ☐ ነጋዲት ☐ ናይ ግሊ ስራሕ ☐ ሰራሕተኛ መንግስቲ ☐ ሰራተኛ መዘድ ☐ ሕርሻ ❒ ናይ ግለሰብ ቁፃር ☐ መሸጣ መብልዕ ወይ መስተ (ይግፅኡ)_____ ❒ ካልእ እንተሃልዩ ይግለፃ_____
ኩነታት ሓዳር ☐ ዘይተመርዓወት ☐ በዓልቲ ሓዳር ☐ ዝተፋተሐት ☐ሰብዓያ ዝሞታ ☐ንምምላስ አይፈቐዳን
ወርሓዊ አታዊኪ ክንደይ እዩ?_____ /ብር ☐ አይፈልጦን ☐ ንምምላስ አይፈቀዳን
ናይ ቤተሰብኪ ወርሓዊ አታዊ ክንደይ እዩ?_____ /ብር ☐ አይፈልጦን ☐ ንምምላስ አይፈቀዳን

ክፍሊ ለ: ስነ ተዋልዶ

ጠኒሰን/ኪ ይፈልጣ ዶ? ☐ እወ ☐ 2. አይፈልጥን (ናብ ሕቶ ለ ቁ13 ይሕለፉ)
ንክንደይ እዋን ጠኒሰን/ኪ ይፈልጣ? _____ ☐ ንምምላስ አይፈቀዳን
ክንደይ ቆልዑ ወሊደን? _____ ☐ ንምምላስ አይፈቀዳን
ፅንሲ ተጨናግፍወን ይፈልጥ ዶ? ❒ እወ ❒ አይፈልጠን ☐ ንምምላስ አይፈቀዳን

ንሕቶ ቑ 13 መልሰን **"እወ"** እንተኾይኑ ንክንደይ ጊዘ ብሃንደበትን(_____)ብባዕለን ፍቓድን(_____) ፅንሲ ተጨናጊፍወን ይፈልጥ
ሐዚ/ቕድሚ ሐዚ ምጣነ ስድራ ይጥቀማ ዶ? ☐ እወ ☐ ኣይጥቀምን ☐ ንምምላስ ኣይፈቐዳን
ንሕቶ ቑ 13 መልሰን "እወ" እንተኾይኑ ኣየናይ ሜላ እየን ዝጥቀማ? (ንክንደይ ዓመት ከምዝፀንሐ ይመላእ) ☐ ክኒን (ብዓመት)_____ ❒ ኣብ ኩርናዕ ዝቐበር (ብዓመት)_____ ☐ምዕቃብ ☐ ኣይ ዩ ሲ ዲ (IUCD) (ብዓመት)_____ ☐ ጡብ ምጥባዉ ❒ኮንደም ኩሉ ሻዕ ድዩ _____ ❒መርፍእ (ብዓመት)_____ ❒ናይ ዘርኢ ቡንቧ ምቐፃር፣ ዝፀንሐሉ (ብዓመት)_____ ☐ ናይ ወዲ ፍረ ንደገ ምፍሳስ ☐ ቡንቧ ዘርኢ ናይ ፈታዊ (ሰብኣይ) ምቐራፅ ☐ ንምምላስ ኣይፈቐዳን ❒ካልእ እንተሃሊዩ_____

ክፍሊ ሐ: ፍልጠትን/ልምድ

ብዛዕባ ናይ ማህፀን ካንሰር ሰሚዐን ዶ? ☐ እወ ☐ ኣይሰማዕኩን ❒ንምምላስ ኣይፈቐዳን
ንሕቶ ቑ 16 መልሰን **"እወ"** እንተኾይኑ፣ ፈለማ ካብ መን ሰሚዐን? ❒ካብ ሓኪም ☐ ካብ ቤተሰብ/ መሓዛ ☐ ካብ ጋዜጣ ☐ ካብ ራዲዮን ቴሌቪዥንን ☐ ካብ ኢንተርኔት ☐ ንምምላስ ኣይፈቐዳን ❒ካልእ እንተሃሊዩ _____
ብዛዕባ ናይ ማህፀን ጫፍ ካንሰር ቅድመ ምርመራ ሰሚዐን ዶ? ☐ እወ ☐ ኣይሰማዕኩን (ናብ ሕቶ ቑ 25 ይሕለፉ)
ናይ ማህፀን ጫፍ ካንሰር ቕድመ ምርመራ ገይረን ይፈልጣ ዶ? ☐ እወ ☐ ኣይፈልጥን ☐ ንምምላስ ኣይፈቐዳን
ናይ ማህፀን ጫፍ ካንሰር ቅድመ ምርመራ እንድሕር ዘይገይረን፤ ንምንታይ? ❒ናይ ማህፀን ጫፍ ካንሰር ብምፍራሕ ❒ሕፅረት ንመመርመሪ ዝኸውን ሰልዲ/ገንዘብ ❒እቲ ምርመራ ኣበይ ከምዝካየድ ዘይምፍላጥ ❒ ናይ ግንዛበ ሕፅረት ❒ሰብ ከየግልለኒ ብምፋራሕ ❒እቲ መመርመሪ መሳርሒ ብምፍራሕ ❒ምስ ተባዕታይ ሓኪም ኣብዚ ነገር ምምይያጥ ስለዘሕፍረኒ ❒ ካልእ እንተሃሊዩ_____
ናይ ማህፀን ጫፍ ካንሰር ብምንታይ ከምዝመፅእ ትፈልጣ ዶ? ❒ እወ ❒ ኣይፈልጥን
ንሕቶ ቑ 21 መልሰን **"እወ"** እንተኾይኑ ናይ ማህፀን ጫፍ ካንሰር ብምንታይ ይኽሰት? ❒ በዓሌት ❒ ብረኽሲ ቫይረስ HPV ❒ ብሽጋራ ❒ሓጥያት ብምስራሕ ❒ ካልእ እንተሃሊዩ____
ንHPV ረኽሲ ዘቃልዑ ኩነታት ይጥቀሳለይ? ❒ምስ ብዙሕ ተባዕታይ ርክብ ግብረ ስጋ ምፍፃም ❒ሽጋራ ❒ቅድሚ ዕድመ ምምርዓው ❒ኣይፈልጥን
ናይ ማህፀን ጫፍ ካንሰር ምክልኻል ይከኣል ዶ? ☐ እወ ☐ ኣይፈልጥን ☐ ንምምላስ ኣይፈቐዳን
ንሕቶ ቑ 24 መልሰን "እወ" እንተኾይኑ፤ ናይ ማህፀን ጫፍ ካንሰር መከላኸሊ ሜላታት ይጥቐሳለይ? ❒ኣይፈልጥን ❒ ኽትባት ❒ ሽጋራ ዘይምትኻኽ ❒ እዋኑ ሓሊኻ ቕድመ ካንሰር ምርመራ ምግባር ❒ትሕቲ ዕድመ ግብለ ስጋ ርክብ ዘይምፍፃም ❒ምጣነ ስድራ ፒል/መርፍእ ንነዊሕ እዋን ዘይምጥቃም ❒ሓንቲ ምስ ሓደ ምውሳን ❒ ኮንደም ብኣግባቡን ኩሉሻዕን ምጥቃም ❒ካልእ እንተሃልዩ_____
ናይ ማህፀን ጫፍ ካንሰር ብሕክምና ዝድሕን ይመስለክን ዶ? ❒ እወ ❒ ኣይድሕንን ❒ ኣይፈልጥን
ንሕቶ ቑ 26 መልስኽን "እወ" እንተኾይኑ ናይ ማህፀን ጫፍ ካንሰር ብምንታይ ሜላታት ይሕከም? ❒ብኽኒን ❒ብጨረራ ❒ብሕክምና ዝሑል ናይትሮጅን ❒ብመጥባሕቲ ❒ብውሁድ ሕክምና፣ ብኽኒንን ብጨረርን ❒ ብመጥባሕቲን ጨረርን ❒ኣይፈልጥን
ብመብስዎ ብዝመሓለፉ ሕማማት ሓሚመን ይፈልጣ ዶ? ❒ እወ ❒ ኣይፈልጥን ❒ ንምምላስ ኣይፈቐዳን

ክፍል መ: ግላዊን ባህሪያዊን ጉዳያት

አብ ክንደይ ዕድመአን/ኺ ተመርዕየን/ኺ ሰብአይ አእቲወን? ___ ❒ አይፈልጥን ☐ንምምላስ አይፈቐዳን
ንፈለማ ጊዘ ርክብ ግብረ ስጋ እንትፍፅማ ዕድመአን ክንደይ ነይሩ?_____ ❒ አይፈልጥን ☐ ንምምላስ አይፈቐዳን
እስካብ ሐዚ ምስ ክንደይ ዝአክሉ ሰባት ግብረ ስጋ ፈፂመን? _____ ☐ ንምምላስ አይፈቐዳን
ናይ ፍቐሪ መሓዘአን (በዓል ገዘአን) ካበአን ወፃእ ሰበይቲ (ውሽማ) አላቶ ዶ? ☐ እወ፣ ክንደይ……..❒የብሉን ☐ አይፈልጥን ☐ ንምምላስ አይፈቐዳን
ቅድሚ ሐዚ ወይ ድማ ሐዚ ሽጋራ ይሰትያ ድየን? ❒ እወ፤ ንኽደይ እዋን___ (ብዓመት) ❒ ብፍፁም አይሰትን፣በዓል ገዘአን ከ?___❒ ንምምላስ አይፈቐዳን
ምግቢ ንምብሳል እንታይ ይጥቀማ? ❒ ዕንፀይቲ ❒ ዓኾር ወይ ድኹሚ ❒ ነዳዲ ❒ ኤክትሪክ ❒ካልእ እተሃሊዩ _____

ኣካቢ/ት ሓበሬታ ሽም	መዓልቲ ፊርማ

A.5. Standard operating procedures (SOPs)

A.5.1. PreserveCyte buffer preparation

Use reagents in PCR quality (at least analytical grade), dedicated for only PreserveCyte Preparation, use single use plastic ware, e.g. blue cap tubes

50mL (1x) PreserveCyt buffer, (50% MeOH final)

300 µl EDTA (0.5M, pH 8)

17.5 µl CH_3COOH

25 ml CH_3OH

25 mL molecular grade water

A.5.2. DNA extraction Maxwell (Promega)

Preparation of swabs

Pipet 2mL PBS /0.005% Thimerosal (With 2mL disposable filter pipets) into the original sample tubes close (storage in the 4oC fridge). Do not forget the swab control (German TK=Tupferkontrolle) which we process as an empty sample to control extraction

Vortex the sample and let it rest to 10 min. vortex during the incubation time several times to ensure that maximum amount of cells are washed off the swab/brush.

Towards the end the incubation period, vortex all the samples vigorously again

Transfer the cell suspension to 2mL safe-lock tube with filtered with filtered disposable pipet and centrifuge the sample for 5 min at 4000mL

Place some green papers in a plastic bag and pour the supernatant from the sample into it. Take care not to dispose the pellet

Transfer the pellet containing tubes to the PCR hood previously treated with UV

Use the Maxwell LEV Blood DNA kit as follows:

Mix 300µLPBS+300µL Lysis buffer+30µL Proteanase K persample (630 in total per tube)

Preheat a Thermomixer to 56^{o}C

Prepare polytrap paper

Resuspend the cell pellet in 630µL already prepared Mix buffer and mix verywell

Incubate at 56^{o}C & 600RPM for 20min

Preparation for Maxwell DNA extraction

Prepare Maxwell tube rack during the incubation period

Place LEV cartridge in the maxwell rack

Place the elution tubes in the tube rack; label and prefill them with 60µL elution buffer & close them

Remove the foil from cartridge and place the plunger in their position

After incubation for 20min, briefly centrifuge the sample tubes and transfer them to their respective well on rack

Place the fully prepared cartridge into the Maxwell instrument

Open the elution buffers very well and choose the respective program on the machin like in charite: LEV➔DNA➔Blood

When extraction is finished, open the Maxwell and directly close the elution tube, now containing the total DNA). Store your DNA into -80^{o}C until further analysis

A.5.2.1. Material requirements

Maxwell 16 LEV Blood DNA kit (cat #: AS1290)

1.5mL tubes Eppendorf

Thermomixer Biozym

Pippet boy Eppendorf

Aerosol resistance filtertips Greiner Bioone

Protrap 295PE paper Schleicher & Schuell

A.5.3. Polymerase chain reaction (PCR)

SOPS Loading of PCR

Rule: Make sure you have not entered any other laboratory room before you enter the Pre-PCR Master Mix room. Clean everything before beginning (refrigerator, cupboard doors, edges and handles)

UV eradiate equipment for 5min

Calculate the required amount of MM: 20µL/PCR tube (ready as 1mL) + 5µl template DNA = final solution 25 µL

Takeout from freezer to defreeze, quickly spin down in small table centrifuge and put inside the Pre-PCR station

Takeout PCR tubes from cabinet, close and put them in racks

Pipet 20 µLMM into each carefully

Transfer the PCR tubes into transfer racks to take them to >DNA room

Make sure the UV-Box is free of materials you used, then clean everything, put used station equipment to radiate UV for 30min

Leave them room and lock it

DNA loading

Clean everything to be used

Takeout the DNA from freezer for loading to defreeze

Put the DNA tubes in racks in logical order leaving one space between rows

Transfer the MM tubes from the transport rack to the loading rack for more stability while pipetting.

Label each MM tube with the number on respective DNA tube

Put DNA rack on left side and MM rack on right side (this depends on the peoples preferences)

For pipetting use the dedicated pipet in the paper box "for PCR loading only"

Open DNA carefully using left hand and MM right hand

Pipet 5 µL to each MM with exact the same number (mix a little by pipetting up and down once)

Always put used DNA sample one row away to monitor pipetting steps, you can use a new pipet tip box and use the tips in the same order as pipetting the samples to keep track of the order.

Write loaded DNA samples on the computer (in mean time MM+DNA tubes place in refrigerator)

Put green paper inside the rack of remains of used (loaded) DNA tubes with label "PCR loaded)

PCR room

Take lab coat

Put PCR tubes in the machine rack

Switch ON the thermocyclers, Choose profile Tina (no password)

Put the tubes in thermocycler

Take the Positive Control out of the Freezer and add to PK tube

Choose program:

Tina > Dana >TypisirungAmpliTaq> press START

The machine will ask you if you use tubes or plates, select "tubes" and press "Start" again.

Wait for machine to reach 100C and make a sound

If you wat to start a second program, press “Verlassen” and choose the same program but different cycler 2

Leave only once you heard the cover sound

Come back in 3h or next day. Store at 4C-8C in PCR room fridge (use purple racks), for long time storage transfer the tubes on to the transport racks and store the PCR product at -20°C.

A.5.4. Hybridization of PCR products for genotyping using Luminex

First each well of the hybridization plate (Millipore) was filled with5.6µL TE buffer. Then, 10 µL PCR product was added followed by 34.4µL bead mix. Then, sticky foil (HJ-Bioanalytic GmbH, Germany) was placed on the plate and tightly closed the wells. Then, the plate was incubated at 95^{o}C for 10minutes to denature the PCR products and hybridize with the bead-coupled mix. The plate was placed on -20 cooling rack (Eppendorf) to cool down (1minute). Then, the plate was incubated at 41^{o}C for 35 minute. In the mean time, a filter plate was placed on vacuum pump (Millipore) and filled with 100µL/well PBS. The filter plate was incubated for 15 minutes. Then stain buffer was prepared from 8 µL stain buffer and 5 µL Streptavidin-PE (Invitrogen) (1:1600) in a 15mL falcon tube and covered with aluminum foil to protect from light.

Then the filter plate was sucked using vacuum pump to remove all the liquid, PBS. Then after spinning for 10s, the sticky foil was removed carefully to avoid sample spillover and contamination. Then using multi channel pipette, the hybridized DNA-bead mix was transferred to the filter plate carefully. Then, the filter plate was pumped to remove the liquid, washed once using 100µL PBS/well and sucked using vacuum pump. Then the plate was tapped on green papers to remove all the liquid out of the wells. The washing step was used to remove uncoupled DNA from the mixture. Then to each well, 75 µL stain buffer (streptavidin-R-phycoerythrin conjugate) was added, covered with aluminum foil and incubated for 30minutes at 650 RPM shaker. Then the plate was pumped to remove the liquid and washed 3 times using 100 µL/well PBS each time. Then the beads were resuspended in 100 µL/well PBS and read using LuminexXMAP200.

A.6. Declaration

I hereby declare that this dissertation is my original work. I have written and submitted to AAU-CHS Department of microbiology, Immunology and Parasitology never to any other institution in any form for evaluation. All the information here is dully acknowledged and I have never used any other source except those cited ones.

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A.7. Approval letter

I have advised this PhD dissertation work and has been submitted with my approval

	Name	signature	Institution	Date
1.	_____	_____	_____	_____
2.	_____	_____	_____	_____
3.	_____	_____	_____	_____
4.	_____	_____	_____	_____
5.	_____	_____	_____	_____