CORRELATION BETWEEN AN EARLY 6 AND EARLY 7 ONCOGENES DETECTION METHOD AND CONVENTIONAL CYTOLOGY FOR CERVICAL CANCER SCREENING AT KENYATTA NATIONAL HOSPITAL, KENYA

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P150F/24677/2011

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This research thesis is my original work and has not been presented for a degree award or any other award in any other University.

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DEDICATION

This thesis is dedicated to my parents Willy and Salima Muyabwa; my siblings Philemon, Josephine, Noela, Moza, Feza and Neema. Additionally I dedicate this thesis to all my friends and classmates. Special dedication to Loise Wanjiru Muchemi for the support and encouragement.
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May God richly bless you.
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<td>A typical Glandular Cells</td>
</tr>
<tr>
<td>AMV</td>
<td>Avian Myeloblastosis Virus</td>
</tr>
<tr>
<td>ASCUSS</td>
<td>A typical Cells of Undetermined Significance</td>
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<tr>
<td>CIN</td>
<td>Cervical Intraepithelial Neoplasia</td>
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<td>DNA</td>
<td>Dioxy Ribonucleic Acid</td>
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<td>E6</td>
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<td>Invasive Cervical Cancer</td>
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<td>KNH</td>
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<td>LBC</td>
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<td>LETZ:</td>
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<td>Prefix</td>
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ABSTRACT

The oncogenic potential of human papilloma virus (HPV) early genes E6 and E7 is of interest in HPV testing for cervical carcinoma. The current study included women less than 40 years attending the Kenyatta National Hospital family planning clinic, with an objective of comparing the performance of cytological tests done in Kenya with the Pretect SEE assay developed by Norchip AS (Norway). Two hundred and four (204) samples were obtained in which the HPV E6 and E7 HPV mRNA was evaluated using the Pretect SEE assay for detection of high risk HPV types 16, 18 and 45. The HPV 35 and 52 may not be involved as the cause of invasive cervical cancer in Kenya. The real-time nucleic acid sequence-based on amplification was also included. The Pap test and cytological analysis were done locally at the Kenyatta National Hospital and the results were recorded and compared to the Pretect SEE results. Three (3) out of two hundred and four (203) women were positive with Pretect SEE (samples that expressed E6 and E7 HPV oncogenes), whereas only one sample (1) out of two hundred and three (203) samples was positive with Pap test. One sample (1) was rejected due to lack of cells. The overall diagnostic prevalence of HPV was 1.47% (3/203) after testing with pretect SEE and 0.5% (1/203) after testing the pap smears. The Pretect SEE showed a specificity of 100% and a sensitivity of 100% using Pap smear as Gold standard. The PreTect SEE showed a positive predictive value (PPV) of 33% and a 99% negative predictive value using Pap smear as the gold standard. The Pap smear showed a specificity of 100% and a sensitivity of 33% when using PreTect SEE as the gold standard. If all the PreTect SEE contains CIN2+ cases, the sensitivity, specificity, PPV and NPV would be 100%. In conclusion, the Pretect SEE™ assay was found to be more sensitive than the Pap smear. Therefore the use of PreTect SEE as a primary screening method with a high coverage rate may reduce the incidence of cervical pre-cancer to a minimum in Kenya. Using a very sensitive and specific method in a very representative female population in Kenya has strongly indicated that the prevalence of cervical cancer in Kenya may be lower due to false positive and lack of differentiation between transient and transforming infections. The estimated number of annual cervical cancer cases may be around 1000 and not 4800 as indicated by the ICO HPV Information (2012). The estimated prevalence of HPV 16 and/or HPV 18 DNA (2012) reported by ICO HPV Information Centre among cytological women in Kenya, may be less than 2.5% and not 9.1%. Around 40% of the HPV 16 and 18 HPV DNA in cytological normal cases may not express E6 and E7 mRNA. This also show that the prevalence of HIV in the whole Kenya population may not be 6.2% but rather less than 2%. The study showed that it is important to perform national screening in more than 3.2% of typical national population’s in Africa.
CHAPTER ONE: INTRODUCTION

1.1 Background Information

Cervical cancer is a malignant neoplasm arising from cells originating in the cervix uteri (WHO, 2015). One of the most common symptoms of advanced cervical cancer is abnormal vaginal bleeding, but in most cases there may be no obvious symptoms until the cancer has progressed to an advanced stage (Karlsen et al., 2013). Majority of pre-malignant lesions are asymptomatic. Cervical cancer represent 13% of female cancers and the majority (>90%) of cases are squamous cell carcinoma while the rest include adenocarcinoma (Karlsen et al., 2010). Cervical cancer is a major global health problem, with nearly 500,000 new cases occurring each year worldwide (WHO, 2014). Each year an estimated 270,000 women die from the disease with > 80% of incidence and deaths occurring in developing countries (Karlsen et al., 2015).

Almost all cervical cancers are a result of human papilloma virus (HPV) infection. There are more than 120 types of human papilloma virus (HPV) implicated in cervical cancer some of which are, 16, 18, 31, 33, 35, 39, 45, 51, 52, 56 and 58 (Karlsen et al., 2015). The diagnosis of cervical pre-cancer and invasive cervical cancer has for long relied on the cytology, histology and colposcopy for the identification of abnormal cells (FDA, 2014). Before treatment or interventions in the cervix based on cytology or colposcopy, histology has to be used as the gold standard in order to confirm the presence of histological CIN2+ or true cervical pre-cancer (Karlsen et al., 2012). True cervical pre-cancer is the only disease that may be defined as the disease that may persist into invasive cervical cancer. Therefore, an abnormal pap smear may only be an indicator that a disease process is present and further tests are required to make the complete diagnosis (Karlsen et al., 2010).
A number of publications have shown that, after the infection of the host, persistent expression of viral oncoproteins E6 and E7 occurs is established due to the integration of HPV DNA into the human genome (Molden et al., 2015). Due to the integration of HPV, loss of regulation of the E6 and E7 gene expression and the translation of proteins cause a stable high expression of the E6 and E7 proteins. These proteins bind to thousands of cell cycle regulation proteins leading to cell proliferation, cell transformation and progression to invasive cervical cancer (Xue et al., 2005).

Cytological methods especially PAP smears have been widely accepted as the primary screening methods for cervical cancer. However, the method had had challenges of false positive cases (Bauer et al., 1991). Morphological tests have many other problems such as its subjective nature, repeatability, lack of follow up, the high number of false negatives and positives. Another main problem with these morphological tests is the inability to include them in complete performance evaluation because it is not possible to define the analytical sensitivity or specificity (WHO, 2015).

Molecular diagnostic techniques for the detection of HPV are used to screen women with suspected pre-cancerous lesions combined with cytological findings (Pap smears). The common HPV molecular tests in developed countries are DNA-based showing the presence of the virus in the cervical cells of the patient but cannot differentiate between transient and transforming infection. Molecular techniques involved in the diagnosis of full-length HPV E6/E7 mRNA helps to determine which women with abnormal cervical cytology results should be referred for immediate colposcopy, biopsy and treatment (Karlsen et al., 2013).
The transient infections of HPV 35 and 52 (13%) is relatively high in Nairobi compared with HPV 16, 18 and 45 (11%) (Devuyest et al., 2002). However, the transforming infection may be transient infection of HPV 35 and 52 (10.4%) is very low compared with real transforming infection by HPV 16, 18 and 45 (78.4%) in women having invasive cervical cancer (Devuyest et al., 2007). This may indicate that it is only the transforming activity by HPV 16, 18 and 45 that is the main cause of invasive cervical cancer and that the HPV 35 and 52 and all other HPV types may only be passengers in real invasive cervical cancer cases. Even though HPV 35 and 52 may be detected in some few invasive cervical cancer cases the real cause behind these HPV 35/52 transient infection may be the transforming infection by HPV 16, 18 and 45 (Karlsen et al., 2013). The copy number of integrated HPV DNA may be unrelated to the present number of abnormal E6/E7 mRNA copies (Karlsen et al., 2011). It was very important to evaluate only transforming and not transient infection from HPV 16, 18 and 45 in Nairobi. In order to do this, the current study evaluated the utility for E6 and E7 transcript detection method for cervical cancer screening at Kenyatta National Hospital, Kenya.
1.2 Problem Statement

Cervical cancer is the second most common cancer among women in Kenya, and the second most frequent cancer among women during reproductive years (WHO, 2013). Current estimates in Kenya indicate that every year, 4802 women are diagnosed with cancer according to the estimates of 2012, 2111 women die from the disease (Ferlay et al., 2012). The most used method for cervical cancer screening in Kenya is the Pap smear; however, the main problem with Pap test is that it may miss up to 50% of the cervical cancer cases and it is both complex and expensive to maintain optimal inter and intra laboratory control in a cytology screening laboratory (Karlsen et al., 2011). Even in the countries where a more complete national screening program is done, it is missing almost 50% of the cancer positive samples. There are still a relative high number of women that become positive (4-%) without giving high enough discovery of invasive cervical cancer (ICC) in a national screening program due to these methods (Hovland et al., 2010).

The common HPV molecular tests in developed countries are DNA-based (detection of the HPV genome), showing the presence of the virus in the cervical cells of the patient but cannot separate between transient and transforming infection. Therefore, even HPV DNA tests give a high false positive in screening programs (Karlsen et al., 2011). Studies showed that the detection of oncogenic HPV E6/E7 mRNA (Pretect SEE) in cervical smears in a routine screening setting identifies prevalent CIN3 lesions with nearly 100% sensitivity and has a very high negative predictive value for disease progression during the natural course of HPV infection (Mol den et al., 2011). Thus, testing HPV E6/E7 mRNA may be used as a clinically predictive marker to enhance the net effectiveness cervical cancer screening (Karlsen et al., 2000). Therefore, this
A study was conducted to evaluate the utility for E6 and E7 transcripts detection method for cervical cancer screening at Kenyatta National Hospital, Kenya.

1.3 Justification

Cervical cancer may kill more women in Kenya than any other cancer despite the fact that treatment is effective if it is detected early. The Kenyatta National Hospital receives more than 500 referrals of cervical cancer every year. The most commonly used methods for cervical cancer screening Kenya is the Pap smear and more recently visual inspection with acetic acid/visual inspection with Lugol’s iodine (VIA/VILLI). However, the main problem with Pap test is that it may miss early lesions. The most common HPV DNA based molecular tests in developed countries cannot distinguish between transient and transforming infection resulting to high number of false positives. The early diagnosis of cervical cancer is necessary in order to treat the disease before it become invasive. m-RNA based HPV assays developed by Norchip AS (Pretect HPV proofer and PreTect SEE) are able to detect cervical pre-cancer without false positives. Therefore, the current study validated the PreTect SEE and HPV-proofer in local setting.

1.4 Research Questions

1. How are cervical lesions on conventional Pap smear in women seeking cervical cancer screening at KNH?

2. Is there expression of HPV E6/E7 mRNA in all the cervical liquid based samples?

3. What are the socio-demographic risks associated with cervical cancer in women seeking cervical cancer screening services in Kenyatta National Hospital?
1.5 Null Hypothesis

There is no difference between the conventional Pap smear and the PreTect SEE in detection of cervical pre-cancer.

1.6 Objectives

1.6.1 General Objective

To evaluate the utility of using E6/E7 transcript detection method for cervical cancer screening at Kenyatta National Hospital.

1.6.2 Specific Objectives

1. To evaluate cervical lesions on conventional pap smear screening in women seeking cervical cancer screening at Kenyatta National Hospital.

2. To determine the expression of HPV E6/E7 mRNA in all the cervical liquid based samples

3. To determine the socio-demographic risks associated with cervical cancer in women seeking cervical cancer screening services in Kenyatta National Hospital
CHAPTER TWO: LITERATURE REVIEW

2.1 Cervical cancer

Human Papilloma Viruses (HPV) are globally known as causative agents for cervical cancer. Cervical cancer is a type of cancer characterized by abnormal growth of cells within the cervix (WHO, 2015). Cancerous cells can invade or spread to other parts of the body. However, it does not often present symptoms when it is at early stage. The symptoms may include abnormal vaginal bleeding, pelvic pain, or pain during sexual intercourse. Bleeding after sex may also indicate the presence of cervical cancer (WHO, 2015). The main cause of the cancer is a virus by the name Human Papilloma Virus (HPV) (Karlsen et al., 2014). Of the more than 120 HPV types identified, about 13 have been shown to be more or less related to cervical cancer, and of these 13 types have been identified as responsible for over 90% of cervical cancer cases. Other strains of HPVs are associated with other diseases such as genital warts etc. Most if not all, HPVs are transmitted through sexual activity. However, over 70% of all men and women will once or several times in their lifetime have an HPV infection. HPV is therefore a very common infection in contrast to cervical cancer, which is a very rare disease.

Table 2.1: Epidemiologic Classification of Human Papilloma Virus Types.

<table>
<thead>
<tr>
<th>Group</th>
<th>HPV types</th>
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<tr>
<td>Established high risk</td>
<td>16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59</td>
</tr>
<tr>
<td>Probably high risk</td>
<td>26, 53, 66, 68, 73 and 82</td>
</tr>
<tr>
<td>Established low risk</td>
<td>6, 11, 13*, 40, 42, 43, 44, 54, 61, 70, 72, 81 and CP6108**</td>
</tr>
</tbody>
</table>

*Included among low-risk types by the International Agency for Research on Cancer 2007  
** Subtype of HPV 89 (Lie et al., 2008).
Once an HPV infection sets in, there is an immune response triggered. The body’s normal defenses clear many of the HPV infection. A series of host mechanisms degrade the HPV making them harmless pieces of non-infective DNA (Karlsen, et al., 2013). Those that overwhelm the natural defense mechanism reproduce and cause disease. This is the case for both cervical cancer related HPV infections and for non-cervical cancer producing HPV infections (Karlsen et al., 2008). The cancer producing HPVs invade the cells of the cervix. They incorporate themselves into the host DNA and produce a series of proteins, some that turn off the host protective mechanisms and some that promote cervical pre-cancer. The genome of the HPV has been mapped and loci identified for a number of the critical activities in the HPV life cycle.

Table 2.2: Organization of the Human Papilloma virus Genome

<table>
<thead>
<tr>
<th>Genome location</th>
<th>Genome Activity</th>
</tr>
</thead>
<tbody>
<tr>
<td>L1 and L2</td>
<td>Capsid proteins</td>
</tr>
<tr>
<td>LCR</td>
<td>Long control region</td>
</tr>
<tr>
<td>E6 and E7</td>
<td>Viral oncogenes</td>
</tr>
<tr>
<td>E1 and E8</td>
<td>Replication</td>
</tr>
<tr>
<td>E2 and E4</td>
<td>Replication and transcription</td>
</tr>
<tr>
<td>E5</td>
<td>Growth stimulation</td>
</tr>
</tbody>
</table>

Source: (Lie et al., 2008)
2.2 Human Papilloma Virus Oncogenic proteins

Based on publications of Zur et al (2008), the role of the E6/E7 onco-proteins in the carcinogenic process is well described. The activation due to loss of regulation of both myc and ras genes and the combined effects between these two genes may be compared with the loss of regulation of the E6/E7 proteins followed by loss of regulation of the E6/E7 mRNA (Karlsen et al., 2014). However, the main difference is that the E6 and E7 proteins may independently cause proliferation of cells and these two proteins can react and modify thousands of different cell cycles regulating proteins. It is very clear that continued expression of E6 and E7 is required for the maintenance of the transformed phenotype and integrated carcinogenic HPV DNA sequences that drive expression of the E6 and E7 onco-proteins are retained in almost all cervical carcinomas (Karlsen et al., 2000).

2.3 Use of the PreTect HPV-Proofer and the PreTect SEE

The use of PreTect HPV-Proofer and PreTect SEE involve two procedures; Nucleic acid amplification and Nucleic acid real-time detection with Molecular Beacons. The NASBA reaction starts with hybridization of the primer (P1) to the target RNA (red). The primer contains a 5'-terminal T7 RNA polymerase promoter sequence.

Elongation of the primer, creating a cDNA copy of the RNA template and forming a RNA/DNA hybrid.
RNase H recognizes this hybrid as substrate and hydrolyses the RNA portion of the hybrid leaving single-stranded DNA. The second primer (P2) anneals to this DNA.

Double stranded and transcriptionally active DNA promoter portion.

Recognizing the now functional promoter, T7 RNA Polymerase produces multiple copies of RNA transcripts that are anti-sense to the original target RNA sequence (Karlsen et al., 2000).

Three enzymes are involved in the amplification:

- Avian Myeloblastosis Virus Reverse Transcriptase (AMV-RT)
- Escherichia coli RNase H
- T7 RNA Polymerase

Illustration of how amplification of a single RNA target molecule is achieved by the concerted action of these three enzymes through the NASBA process (Karlsen et al., 2000).

2.4 Epidemiology of cervical cancer
In developing countries, cancer of the cervix is among the commonest cancer and therefore one of leading causes of mortality among women (Alaghebdandan et al., 2013). The World Health Organization clearly states that new cases of cervical cancer were 530,000 worldwide and the number of deaths from cervical cancer was 275,000 in 2008 (WHO, 2008). More than 90% of cases were recorded in developing countries. Fifty thousand women died of the disease and the high incidences of this type of cancer are mainly reported in Africa with exceedingly 50 per 100,000 populations and age standardize with mortality of over than 40 per 100,000 populations (WHO, 2008).

2.5 Risk factors associated with cervical cancer
Risk factors for HPV Viral persistence and development of cervical neoplasia are as follow:
Sexual factors including multiple sexual partners, sex at early age, and failure to use condoms.
The viral risk factors for HPV include viral type and variant, viral load, the presence of multiple current HPV infections and detection of HPV E6/E7 transcripts.
Non-viral factors include, long term hormonal contraceptive use, co-infection with herpes or *chlamydia*. Nutrition and smoking (Karlsen *et al.*, 2014).

### 2.5.1 Low socio demographic status

In a comprehensive analysis of association between cervical cancer and socio-economic status in England and Wales, cancer of the cervix had a highest gradient of incidence with socio-economic status than other cancers in women. This is why it is said that cervical cancer is a disease of low socio-economic status. Sexually transmitted infections are risk for women to develop cervical cancer (Batnam. *et al.*, 2010).

### 2.5.2 Age at first intercourse

First intercourse before 18 has been cited as a risk factor leading to cervical cancer (Armstrong *et al.*, 1996). It was reported in Kenya that the average age at first intercourse is 16 years among women aged 40-49 and 17 years among those aged 25-29 (UNICEF, 2007).

### 2.5.3 Smoking

Although today men smoke more than women do, it has been noticed that smoking may act either directly or via immune suppression and promotion of effect of other carcinogens, thereby increasing the risk of cervical cancer among women (Adam *et al.*, 2010).

### 2.5.4 Lack of genital hygiene

In India, a study done in Kerala has shown that the lack of genital hygiene may lead to development of infections including HPV (Varghese *et al.*, 1999).
2.5.5 Lack of screening and HIV infection

The lack of screening is one of the most important risk factors for cervical cancer (Hakama, 1991). HIV positive patients are at high-risk cervical cancer due to their compromised immunity, therefore HIV is a strong risk factor for cervical cancer development in women, and cervical cancer is an AIDS-defining illness (WHO, 2015).

2.5.6 Chlamydia

*Chlamydia* is also one of risk factors. It is a relatively common kind of bacteria that can infect the reproductive system (Adam *et al.*, 2000). It is sexually transmitted and its infection can cause pelvic inflammation, leading to infertility. Some studies have shown that there is a higher risk of cervical cancer in women whose blood test results show signs of past or current *chlamydia* infection (compared with women with normal test results (WHO, 2015).

2.6 Diagnosis for cervical cancer

2.6.1 The Pap smear

The Pap smear is the primary screening method for detection of cervical pre-cancer. This method was named after pathologist George Papanicolaou, who introduced the method in 1949 before the cause of cervical cancer was known. Since its introduction, the Pap smear has helped reduce cervical cancer incidence and mortality rates by roughly half to two-thirds. The Pap smear is a screening tool that looks for changes in cells of the transformation zone of the cervix (Karlsen *et al.*, 2013). Although the Pap smear is primary reason for the dramatic decline in death from cervical cancer, this test remains with many disadvantages (WHO, 2015). If the cell were not well collected from the cervix, or if there were any others cells obscuring visualization of the
cervical cells, a cancer or precancerous lesion may be missed (Karlsen, 2010). A typical PAP smear contains over 100,000 cells while only a few of these cells may be cancerous (Szawski et al., 2012).

Even a highly skilled cytologist can overlook a cancer cell. In fact, at least five out of every hundred abnormal pap smears may be interpreted as normal. For this reason, the PAP smear should be repeated regularly even if you have always had normal PAP smears. The American Cancer Society recommends a Pap smear every 3 years for women 21-65 years old, less frequently when combined with the Human Papilloma Virus test. Provided previous PAP smears were normal, screening can end after 65 years old. However, if past PAP smears were abnormal, your doctor may recommend continuing the Pap smear periodically (DIM, 2013).

2.6.2 Liquid Based Cytology

After a brief plateau, a further decline in cervical cancer screening was observed with the introduction of liquid-based cytology (LBC), which is the first major advance in nearly 50 years in cervical cancer screening technology (Benevelo et al., 2011). The liquid based cytology (LBC) is more generally preferred by smear takers and laboratory staff who read the smears. There is no need for smear takers to spray fix' cells on a glass slide (WHO, 2015). The Liquid Based Cytology (LBC) improves the quality of the sample and efficiency of laboratory processing by giving a clearer/cleaner preparation that helps in interpreting cell morphology. It also enables ‘automated’ slide reading (Anderson et al., 2014). Disruptive problem has been amplified with the use of liquid-based cervical cytology in conjunction with HPV testing, which represents a common approach to co-testing for cervical disease (ref). This approach of co-
testing can lead to insufficient specimen to perform multiple tests, resulting in quantity not sufficient (QNS) for one or more ancillary test (Cuschieri et al., 2014).

In the case of both unsatisfactory Pap samples and quantity not sufficient (QNS) results, Doctors will not receive the complete diagnostic information that was requested. The patient will surely be recalled to repeat the test, which is inconvenient and costly. This increases patient anxiety; and is disruptive to the clinician’s practice (Karlsen et al., 2013). Beyond the inconvenience, there is the risk that the patient might forget the repeat office visit to collect a second cytology specimen and therefore some patients develop invasive cancers without knowing. This has been shown to occur at a rate of approximately 30% for patients recalled after an unsatisfactory Pap test (Dorothy et al., 2013).

2.6.3 Testing for the Presence of HPV DNA

Testing for the presence of HPV DNA is considered a significant advance in the diagnosis of cervical cancer. However, the causative agent for cervical cancer is not the HPV DNA in itself but an integrated HPV that has lost its regulation of the E6/E7 expression (Nyard et al., 2014). Not all HPV infections, even not those related to the five most carcinogenic HPV-types, produces cancer. The reason is the body’s natural immune response can clear the HPV before it gets incorporated into the host DNA. It is only by accident when the HPV gets incorporated into the host DNA that lose the transcriptional and translational control causing stable production of abnormal full-length E6/E7 mRNA (Karlsen et al., 2013). The use of methods for the detection of the presence of HPV DNA may give a risk, but due to the very high number of transient infection, this is because a strong bias and a high number of false positives bias. The level of
underreporting (false negatives) is lower than for Pap smear, but because it is not limited to pre-cancer producing infections and because HPV-infections is very common in any population, it will cause a massive over report compared to the underlying number of histological CIN2+ cases (Hovland et al., 2013). This may be an even more problematic phenomenon in Africa due a rather low public health budget.

2.6.4 Testing for HPV mRNA

2.6.4.1 The role of E6/E7 mRNA expression in the natural lifecycle of HPV

![Diagram of Natural History of Cervical Carcinogenesis]

Figure 2.1: E6/E7 mRNA expression in the natural life cycle of HPV.

Source: Karlsen et al., (2014)

In a normal HPV infection at the lower layers of the cervical epithelium, the E6/E7 transcripts or full-length transcripts are transcribed from the p97 promoter (Karlsen et al., 2014). However, when the infected cells move up to the surface (terminal differentiation) of the cervical epithelium the p97 promoter will be more and more down regulated and finally turned off.
Therefore, the transcripts that are transcribed from this promoter in cells with normal HPV infections cannot be detected in the upper layers of the stratified squamous epithelia (Sorbye et al., 2013). In other words, this totally turns off the transcription of E6 and E7 mRNA in the upper layers of the stratified squamous epithelia. The upper part of the spinous layer, the granular layer and the conified layer (Karlsen et al., 2010).

A normal papilloma virus infection regulate down the p97 promoter in order to increase the transcription from the p670 promoter preparing the ground for viral particle production (Karlsen et al., 2004). During the terminal differentiation an increased level of E1 and E2 are produced turning down the activity of the p97 switching to the p670. The p670 promoter produces transcripts that are spliced out in order to preferential expression L1/L2 transcripts and proteins (Szarewki et al., 2012). The viral early promoter which controls E6 and E7 expression, is thought to be constitutively active during differentiation (in the lower parts of epithelia) in order to maintain the cells in a “pseudo” S phase state necessary for high-level replication of viral episome. Papilloma virus (Karlsen et al., 2014). In cervical pre-cancer cases (without normal HPV infection) this regulation and the regulation of mRNA splicing is lost and all cells coming to the cornified layer will be transcribe from the p97 promotor giving full-length E6 transcripts in addition to different variants as well as full-length E6 and E7 proteins (Holm., 2002).

Therefore, the detection of E6/E7 mRNA in the cervical mucosa indicates that something is wrong, there must be a lack of transcriptional control, which is most likely related to integration of HPV by deletion of whole parts of E1 and E2 genes (Butz et al., 2002). The splicing of HPV mRNA is probably not regulated anymore giving a possible stable production of full-length E6
mRNA as well as full-length E6 proteins (Karlsen et al., 2014). This is in particular the case detected by the HPV 16 primer-sets and probes that can only detect full-length HPV 16 E6/E7 mRNA. Stable expression of E6/E7 mRNA giving stable expression of E7 and full-length E6 proteins is by a molecular oncological definition the cause of cervical pre-cancer (Karlsen et al., 2000). Stable expression of E6/E7 mRNA giving stable expression of E7 and full-length E6 proteins is also the main cause of invasive cervical cancer and metastasis. The only possible cure of this irreversible lack of regulation and uncontrolled oncogene activity is by the remove of the pre-cancer cells by treatment or by the immune system (Karlsen et al., 2000).

E6/E7 cannot be detected in factual normal smear samples. In fact we have at least two studies showing that the E6/E7 expression is totally absent in factual CIN1 cases diagnosed defined by a panel of experienced pathologists (Karlsen et al., 2013). One option is the level of E6/E7 mRNA expression. In samples with a malignant progression the loss of regulation of E6/E7 protein or mRNA expression due to integration or instability is the real problem, proved in a high number of scientific studies (Karlsen et al., 2010). Loss of regulation is not giving different levels of mRNA expression related to different level of malignancy detected or different stages of carcinogenesis. We believe that integration or loss of regulation is resulting in any level of expression throughout the stratified squamous epithelial and is visible on the surface of the epithelium following the differentiation (Karlsen et al., 2013).

Even a very low level of mRNA expression will cause high production of full-length E6 and E7 proteins. More than 20 or 50 mRNA copies per cell in clinical samples cervical pre-cancer may be observed (Karlsen et al., 2013). The PreTect HPV-Proofer assay has documented a PPV for
CIN2+ higher than 50% in clinical studies even with strong bias from cytology or histology. This indicates that it is very likely that the PreTect HPV-Proofer assay when positive is detecting underlying pre-cancer in the majority of cases. This gives room for the possibility to give a molecular oncological definition of cervical pre-cancer: The presence of E6/E7 mRNA or proteins in the cervical mucosa is the very same as the presence of cervical pre-cancer. This also indicates that assays having more than 70-80% positivity rate (or sensitivity) towards CIN2+ cases may have an increased level of false positive or may not reflect the true oncological state of the lesion (Karlsen et al., 2014).

2.6.4.2 PreTect HPV-Proofer- and Pretect SEE more Precise Testing for Pre-Cancer

PreTect SEE is a real-time nucleic acid amplification-based (NASBA) qualitative assay for specific genotyping of E6/E7 mRNA from HPV 16, HPV 18 and HPV 45, including intrinsic sample control (Karlsen et al., 2000). The PreTect HPV-Proofer is a real-time nucleic acid amplification-based (NASBA) qualitative assay for specific genotyping of E6/E7 mRNA from HPV 16, HPV 18, HPV 31, HPV 33 and HPV 45 including intrinsic sample control. A multitude of papers recognizes that precancerous methods for detection of true cervical pre-cancer have significant advantages. The Pretect HPV proofer and Pretect SEE developed by NorChip in Norway and currently on the market in Europe have a higher sensitivity and specificity in detection of the pre-cancerous cells than cytological smear and DNA methods (Karlsen et al., 2011). The PreTect HPV-Proofer and Pretect SEE assays take advantage of several unique characteristics of the HPV and its role in cervical cancer (Karlsen et al., 2011).
2.7 Scientific evolution for HPV testing and cervical cancer screening

The diagnosis of cervical cancer has for the last 50 years relied on the Pap smear for the cytological identification of abnormal cells. This test is an aid to the clinician in diagnosing cervical cancer. An abnormal Pap smear is an indicator that a disease process may be present and further tests are required to make the complete diagnosis. While improvements to the Pap smear have been made (Liquid Based Cytology, LBC, automation), they have tended to be technical in nature and have done little to improve the clinical sensitivity for detecting histological CIN2+ (Karlsen et al., 2011). Advances in the understanding of the pathophysiology of cervical cancer, specifically the role of human papilloma virus (HPV) in the disease, has allowed for meaningful advances in the diagnosis and prevention of the disease (Molden, 2013).

As a result, a wide range of HPV-related diagnostic tools have been developed to aid in the identification of cervical cancer. These diagnostic tools targets the presence of HPV virus by detecting the presence of HPV-related DNA, RNA or mRNA or the presence of cervical cancer carcinogenesis by detecting specific abnormal mRNA activity related to the E6/E7 oncogenes that are mandatory for cervical cancer development. Current scientific literature indicate that HPV-related diagnostic tools have significantly higher sensitivity towards identification of histological CIN2+ than cytology/LBC, but, unfortunately, common for most of these HPV-related diagnostic tools are significantly lower specificity and positive predictive value (PPV) for histological CIN2+ (Karlsen et al., 2006). The lack of improvement to HPV-presentation diagnostic tools is due to the failure to utilize the knowledge that has been gained about the role that HPV has in the pathogenesis of cervical cancer (Karlsen et al., 2004).
Research based methods against Human Papilloma Virus (HPV) has been developed and evaluated in many publications since 1980's. The first papers were based on regular Southern Blot, followed by In Situ Hybridization, type-specific PCR, Consensus PCR, Northern Blot and NASBA. Later on the consensus PCR method based on MY09/11 from the L1 gene was compiled with other consensus PCR methods including Gp5/Gp6 from L1 gene and Cpl/CpIIIG from E1 (Karlsen et al., 2013).

A disadvantage with these methods was lack of HPV typing, it was not sensitive enough and was not commercialised. The research-based methods were further developed to give new modified versions of all these primer-sets (SPF and My09/11B) and the detection was based on microtiter plate, line-blot or dot-blot instead of gel-electrophoresis. Before this the first commercial kit was available based on Hybrid Capture including RNA probes and signal amplification but without target amplification (Karlsen et al., 2013).

This kit was evaluated in a number of studies with more or less success until the ALTS group and Clavet used the improved versions of the HC method, HCI and HCII in large studies. Unfortunately, the one target hybridisation probes in HCII methods was still cross reacting with a number of low-risk HPV types, it cross react with a number of other than papilloma virus targets in primary screening trials and real sensitive studies based on HCII has not been observed. Large studies on cancer using HCII have not been reported due to unknown reasons (Cuzick et al., 2011).
The main arguments of scientists are that HCII detect less than 90% of cervical carcinomas. However, the first generation Hybrid Capture Tube test detects the following high-risk types 16, 18, 31, 33, 35, 45, 51, 52 and 56. HCT was granted US FDA approval in May 1995. In March 1999 the second generation HCII (including four more types; HPV39, 58, 59 and 68 and including higher-stringency in order to increase the sensitivity) was FDA approved in 2001 but only on ASCUS and together with Thin-Prep developed by Cytyc (Cooperation). The level of detection of the second-generation HCII is rated at 5000 viral copies per sample, or one picogram of HPV DNA per samples. Finally HCII was FDA approved in Mars 2003 for primary screening together with cytology but only on women above 30 years of age. The main argument was that in several studies none of the HCII negative samples had underlying CIN II+. (Karlsen et al., 2013).

Recent reports from the ALTS study and other studies are not supporting the use of the HCII method in primary screening other than on ASCUS cases (Karlsen et al., 2013). Another commercial available method has finally evolved based on PCR consensus primer-sets in 1999 and got the name SPF10-PCR or Inno-LiPa HPV kit. Kleter and collagues discovered that 87% of the cervical carcinoma cases from Russian Cancer Center was from the Class I carcinogens (HPV 16, 18, 31, 33 and 45), while including the rest of the HPV types (HPV 35, 52, 56, 58 and 68) detected around 94% of the cervical carcinoma cases. In Severe dyskariosis (CIN III) they detected close to 90% including mainly the Class I carcinogens. No information is available of whether this commercial kit may be used in research, in routine diagnostics or in screening (Karlsen et al., 2000). Furthermore, totally different methods based on chip technology, quantitative real-time PCR, RT-PCR, sequencing and pyrosequencing were also developed. The
HPV DNAChip technology from South-Korea was commercialised giving a combination of PCR amplification and dot-blot on glass. This chip technology should detect the main so-called HR-HPV types. The real-time PCR and sequencing is not yet available as a commercial product (Maiden et al., 2013).

Finally the commercial method PreTect HPV-Proofer (NorChip, Klokkarstua, Norway) has been introduced detecting E6/E7 mRNA from the carcinogenic HPV types. By several studies the E6/E7 full-length transcripts is directly related to the presence of E6 and E7 full-length proteins. These full-length proteins are found to be the most carcinogenic cancer markers ever discovered. Eight different studies have already been done using HPV-Proofer against other relevant methods. In addition there has also been done several small-blinded country related studies. Two large studies are close to be finished including collection of biopsies either at the same time as the collection of cervical smear or after 7 month. The analytical sensitivity is down to 10 abnormal cells evaluated both by NorChip and by independent laboratory (Molden et al., 2003). The analytical specificity is 100% controlled several times both by type-specific PCR and sequencing. The clinical specificity has been evaluated in a large population study to be 97% and the clinical sensitivity is between 86 and 92% compared with CIN III and 100% compared with cervical carcinoma (Karlsen et al., 2013).

2.8 RNA verses DNA or Protein as target for routine diagnostics

DNA is the part of the inherited material that is passive target or coding sequence for RNA and Protein molecules that are going to perform the work in a biological system. RNA has the same coding sequence as DNA, but is either direct or indirectly involved in the biological machinery.
(Karlsen et al., 2010). RNA is not only the basement for translation to proteins or expressed as mRNA but it is involved in all sorts of regulation even as advanced three dimensional structures. Most of the RNA (siRNA, RNAi, tRNA, rRNA) molecules forms three dimensional structures that are directly involved in the regulation or activation of biological processes (Karlsen et al., 2012).

In contrast to DNA the RNA describe the function of both the gene and intron sequences in. The main challenge using protein as a target for routine diagnostics has been low sensitivity, reproducibility and specificity. The main challenge using DNA as target for routine diagnostics has been lack of information about biological or clinical activity. However, RNA as a target for routine diagnostics may give the information of clinical activity, regulation or processes in addition to higher or equal sensitivity, reproducibility and specificity to DNA. (Karlsen et al., 2014). Too many scientist or technicians believe that e.g. to use mRNA as a target for molecular routine diagnostics is very difficult. For more than 10 years ago new methods of isolation, purification and stabilization of mRNA was developed for routine diagnostics making the RNA very much suited as a marker for development of new diagnostics methods and even drugs.

There are at least 10 methods (many of which are commercial available) for amplification and detection of different RNA molecules: RT-PCR and other variants of PCR, LCR (Ligase Chain Reaction, Abbott), SDA (Strand Displacement Amplification, Becton-Dickinson), NASBA (Nucleic Acid Sequence Based Amplification, BioMerieux), TMA (Transcription Mediated Amplification, Gen-Probe) bDNA (Branched DNA, Chiron), CPT (DNA cleavage-based signal
amplification), HC (Hybrid Capture), Digene and Rolling circle amplification (Karlsen et al., 2012).

The main disadvantage using RT-PCR for amplification and detection of mRNA is the possible contamination of DNA and thereby the challenge of finding specific primer-sets and probes. Quantification of mRNA has been difficult due to the widely used semi-quantitative methods (mostly RT-PCR) included calibrators that are not similar to the whole coding mRNA sequence area Karlsen et al., 2011).

The method use in PreTect HPV-Proofer is the multiplex real-time NASBA method (very similar to TMA) and is a real-time and isothermal amplification and detection method generating up to $10^{14}$ copies of the target mRNA. Real-time NASBA has recently being developed for commercial absolute quantization of HIV RNA (BioMerieux). In fact multiplex routine diagnostics of RNA using multiplex real-time NASBA represent an enormous possibility for e.g. control of tumour development and progression (Karlsen et al., 2000).

2.9 Function and importance of the different E6/E7 mRNA transcripts

HPV E6 and E7 expression is primarily regulated at the transcriptional or post-transcriptional level. For example, for HPV 16, the E6 Open Reading Frame (ORF) encodes at least three distinct variants of the E6 protein, which all may have different roles in the viral life cycle (Kitcher et al., 2009). These transcripts are either unspliced (full-length E6-E7 transcript) or spliced transcripts: E6*1 is spliced from nucleotide 226 to 409 and E6*II from nucleotide 226 to 526, all being transcribed from the promoter p97 located just upstream of the second ATG of the
E6 ORF. The full-length E6 protein has been reported translated from the E6 full-length transcript. The E7 protein is likely encoded by the E6*I or E6*II and for some time it was thought that this splicing event was a means of obtaining high levels of E7 expression. Karlsen et al., 2012, states that the HPV-16 E7 protein is also translated from full-length E6-E7 mRNA structures, demonstrating that splicing is not required for E7 synthesis (Krlsen et al., 2000).

Additionally, only the full length E6 protein, not the spliced E6 variants, is found to efficiently bind to and promote the degradation of p53 (Kraus et al., 2008). It is further suggested that spliced transcripts of the HPV 18 E6 gene may encode an E6 modified protein that inhibits the full-length E6 mediated degradation of p53. Moreover, unspliced E6 mRNA is found to be more closely associated with tumorigenicity as compared to the spliced transcripts and studies including cervical cancer samples show that the full-length transcript is always present, either alone or together with the spliced transcript (Karlsen et al., 2011). E6 protein identify transcription patterns indicative of cervical disease progression and help physicians to decide clinical management (Kraus et al., 2009). Taken together, these studies point to the full-length transcript as being the transcript important for the carcinogenic process.

### 2.10 Treatment of Cervical cancer

There is no treatment for the virus itself, but there are treatments for the problems that HPV can cause (Bosch et al., 1995). Topical preparations can be used to remove visible genital warts. Large masses can also be removed surgically (Hovland et al., 2013). Premalignant lesions of the cervix may regress over time, although some progress to invasive cancer. Persistent low-grade lesions as well as high-grade lesions are usually treated to prevent cervical cancer from
developing. It is critical to follow up with confirmation after screening and treatment. Cervical is most treatable when it is diagnosed and treated early (Karlsen et al., 2010). Sometimes the treatment depends also on whether or not a woman wants to continue to have children (maintain fertility). For women who want to maintain fertility, the main treatment is radical trachelectomy with removal of pelvic lymph nodes (pelvic lymph node dissection). Another option is cone biopsy and pelvic lymph node dissection, followed by observation (WHO, 2015).

2.11 Prevention

Screening for cervical cancer is important in order to prevent development of cancer and stop it at early stage (WHO, 2015). The Papanicolaou test known again as Pap smear test has been credited with dramatically reducing the number of cervical cancer cases and mortality especially in developing countries (WHO, 2013). Screening by using this Pap test 3-5 years with appropriate follow-up can reduce cervical cancer incidence at 80% (WHO, 2015). Early molecular screening methods are encouraged in order to treat precancerous lesion.

2.12 Human Papilloma Virus vaccines

HPV prophylactic vaccines have been available since 2006 and both are registered for use in Kenya. The government plans to roll out a national immunization program for the 9 to 14 year old girls in the next two years (Becker, 2010). The national immunization program has to cover more than 70% of the target population in order to be useful. The promise of HPV vaccines does not come without challenges. Some of these reflect characteristics of the virus itself and its interaction with cervical cancer. Others reflect the challenges of stimulating an effective immune response to a mucosal infection (Kols, 2000). In animals, HPV virus does not cause any disease.
It is therefore difficult to conduct the animal research needed for development of vaccines. Some researchers instead study naturally occurring mammalian papilloma viruses, including cottontail rabbit papilloma virus, canine oral papilloma virus, and bovine papilloma virus. None of the animal models completely mimic the interaction between HPV and human host cells. It is then unclear how well the results of animal studies apply to clinical infections in humans (Karlsen et al., 2000). It is unknown precisely which elements of the human immune system are important in preventing or resolving HPV infections. Although there is evidence that immune response does play a role in controlling HPV infections, it is not known why HPV infections persist in some individuals and regress naturally in others (Karlsen et al., 2007). Vaccine developers are testing a broad range of hypotheses about what makes for an effective immune response to HPV, including testing the relative advantages of stimulating antibody- and/or cell-mediated immunity (Karlsen et al., 2014).

HPV enters the body through the mucosal membranes and does not spread systemically. Therefore, a vaccine against HPV will be most effective if it induces a strong immune response at the mucosal surface (mucosal immunity); however, some researchers argue that a systemic immune response might be sufficient (Varghese, 1999). Because HPV types differ significantly at the genetic and protein level, antibodies raised against one kind of HPV generally do not protect against other types. Preventing a majority of cervical cancer cases therefore will require a multivalent vaccine, that is, a combination vaccine effective against the common carcinogenic types of HPV (including types 16, 18, 31, and 45). Some researchers also have proposed including HPV-6 and HPV-11 in a multivalent cervical cancer vaccine, because the protection this would offer against genital warts would give men an incentive to take the vaccine. More
epidemiological research is needed to further clarify the types of HPV that are most prevalent in various regions and countries (Whitley, 2004).
CHAPTER THREE: MATERIALS AND METHODS

3.0 Study Site

This study was done at Kenyatta National Hospital (KNH), the largest Referral and Teaching Hospital in Kenya. It receives cervical cancer cases from all over the country. The hospital has a busy outpatient gynecology and colposcopy clinic that receives referrals of women with abnormal Pap smear, Visual inspection with acetic acid (VIA) or Visual Inspection with Lugol’s iodine (VILI). The hospital also runs a busy family planning clinic. (Karlsen et al., 2013).

3.1 Study Population

The target population for this study was 2040 women who were enrolled and attended the Kenyatta National Hospital family planning clinic, aged 40 years and below. Due to high sensitivity and specificity of PreTect SEE in detection of transforming infections, the best age is between 16 and 40 years. The study was done from January to May, 2015.

3.2 Study Design

This is a cross-sectional descriptive study was adopted among the women who attended KNH during the study period.

3.3 Sample Size Determination

The sample size was determined using the following formula according to Fischer et al., 1998.

Using a postulated prevalence of 12.7% (Karlsen et al., 2000).

\[
N = \frac{Z^2 P(1-P)}{D^2}
\]
Where:

\( N \) = Minimum sample size required.

\( Z \) = Normal standard deviation.

\( P \) = Postulated prevalence (12.7%)

\( D \) = 0.05 the inverse of 95% confidence limit (the allowable error)

\[ N = \frac{1.96^2 \times 12.7\% \times (1 - 12.7\%)}{0.05^2} = 170.37 \text{ Samples} / \]

The minimum sample size was to be 170 samples. However, the researcher used 10% which was 204 of the target population to cater for any data loss. According to Mugenda & Mugenda (2005) who indicated that for a large populations a sample size of 10%, 20% or 30% is appropriate data collection.

3.4 Sampling Technique

Purposeful sampling method was used, where every woman who met the criteria was enrolled in the study. All the women referred for cervical cancer screening at the family planning clinic at the Kenyatta National Hospital who were aged of 40 years.

3.4.1 Inclusion Criteria

The study had Women below 40 years seeking cervical cancers screening services at Kenyatta National Hospital family planning clinic. Women who gave consent to participate in the study and women with no history of cervical cancer.
3.4.2 Exclusion Criteria

Eligible women who failed to consent to participate in the study. Women who had history of abnormal smears or who had been treated for cervical cancer or pre-stages of cervical cancer and women above 40 years were excluded in this study.

3.5 Pap smear collection Procedure

Cells were collected by nurses who first inserted a speculum into the vagina to allow complete visualization of the cervix. Then the Cervix brush was slowly inserted into the vagina and rotated in the cervix in one direction for the recommended 3 to 5 times to obtain the Pap smear. The collected cells were immediately put on a slide and fixed with spray fixative and the collection devices were discarded. Samples were sent to the laboratory at KNH for cytological analysis.

3.6 Liquid Based Cytology (LBC) Collection Procedure

Following the conventional Pap smear technique the same brush tip was gently shaken within the fixative in the container of the PreTect. The endocervical brush is then discarded. The sample in PreTect was immediately put in an appropriate box and stored in a fridge at 2-4°C until sample collection was complete. Samples can be stored up to 4 months at this temperature without deterioration of cells to happen. The samples (204 samples) were shipped to Norway (NorChip AS, Industry 8, and 3490 Klokkarstua, Norway) for HPV RNA detection.

3.7 Storage of Samples

The PreTect TM has the ability to preserve the cells for three months at room temperature. All the samples were preserved under refrigeration at 4 to 8°C.
3.8 Laboratory Analysis

3.8.1 Pap smear test

The conventional Pap smear was carried out at KNH laboratory. Each sample on the slide was stained with routine Pap stain and viewed under a microscope. The Bethesda System of reporting Cervical-vaginal cytology for sign out of pap smears at KNH cytology laboratory was applied in this study (Karlsen et al., 2000). A slide of a sample which did not have any suspected cervical cancer was reported as negative for intraepithelial lesions. (Karlsen et al., 2000).

Table 3.1: Overview of morphological and treatment methods

<table>
<thead>
<tr>
<th>Basic Pap or cytological diagnosis at KNH</th>
<th>Abbreviation</th>
<th>Likely histological outcome</th>
<th>Other test and treatment options at KNH</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atypical squamous Cells of Undetermined Significance</td>
<td>ASCUS</td>
<td>CIN 1</td>
<td>HPV testing Repeat Smear Colposcopy Endocervical sampling</td>
</tr>
<tr>
<td>Atypical Squamous Cells – Cannot exclude High-Grade SIL</td>
<td>ASCUS-H</td>
<td>CIN2+</td>
<td>Colposcopy Endocervical sampling</td>
</tr>
<tr>
<td>Atypical glandular cells</td>
<td>AGC</td>
<td>CIN1</td>
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<tr>
<td>Low-grade squamous intraepithelial lesions</td>
<td>LSIL</td>
<td>CIN1</td>
<td>Colposcopy Endocervical sampling</td>
</tr>
<tr>
<td>High-grade squamous intraepithelial lesions</td>
<td>HSIL</td>
<td>CIN2+</td>
<td>LEEP LETZ Diagnosis Excisional Procedure</td>
</tr>
</tbody>
</table>
3.8.2 Isolation of Total HPV Ribonucleic Acid (RNA) from human cells in PreTect™

All the buffers were brought to room temperature without containing any crystals. The human cells collected in the Pretect™ were washed thereafter with the buffers provided by NorChip AS. After every wash step and centrifugation, the supernatant was discarded by aspiration using a pipette. Another buffer was added to continue the washing procedure. The supernatant was removed without disturbing the silica which was necessary for the RNA isolation. The wash procedure was completed as quickly as possible. For each wash step, all supernatant was removed by aspiration at the surface of the liquid without disturbing the silica particles. Any pause while the samples were in extraction buffer 3 was avoided, as that could result in lowering the yields of nucleic acid (Karlsen et al., 2014).

3.8.2.1 Preparation of Samples

All the samples were labeled with a new identification number after reception in the laboratory. Each sample was thoroughly mixed and 1-3 ml (Preservcyt) was aliquoted to a 10 ml sterile centrifuge tube. The selection of sample volume was considered due to amount of cells present. The cells were centrifuged for 12 minutes at 2500 rpm (1125g) and the supernatant was discarded with a Pasteur-pipette or by vacuum suction. One (1) ml of lysis buffer was added to the cells and was homogenized by vortexing. The samples were incubated at room temperature for 10 minutes or storage at 4-8 °C until isolation procedure started (Karlsen et al., 2014).

3.8.2.2 Isolation procedure

The lysed sample was transferred to 1.5 ml tube and placed directly in tube at MagRack40 and the silica suspension was prepared by vortexing until an opaque solution was formed (Karlsen et...
A hundred (100) µl of silica was added to every sample tube (1500µg silica per isolation) One rod was put in each tube and the solution was mixed using LabMix 201 (speed 3). After mixing, it was incubated for 5 minutes at room temperature and thereafter the magnetic rack in position and the particles were allowed to collect on the wall. The supernatant was removed by Pasteur-pipette and the magnet was pushed to the next row. 500µl of extraction buffer 1 was added to the tubes in the first row and mixed using LabMix201 (speed 6) for efficient washing and this step was repeated for all rows (1–5). The extraction buffer 1 was removed and then 500µl of Extraction buffer 2 was used twice in all the tubes sequentially. Five hundred (500) µl of Extraction buffer 3 was used and thereafter the contents were swirl and the rods were removed from the tubes before removal of extraction buffer 3. Fifty (50) µl Elution buffer was added and all the tubes were placed in the Thermo shaker and centrifuged at 1400rpm. The contents were incubated for 5 min at 60°C (+/-2). Samples were put back on MagRack 40 and eluates (containing isolated nucleic acids) were transferred to a 1.5 ml RNase/DNase free tube. When nucleic acids extract could not be used for amplification shortly after preparation, they were stored at 2-8°C until the next day. (Karlsen et al., 2012).

3.8.2.3 Amplification and detection of HPV Ribonucleic acid (HPV-RNA)

HPV mRNA was detected by real time Nucleic acid sequence based on amplification (NASBA) by Compton, et al. (2002). Briefly, NASBA is based on isothermal RNA amplification, accomplished by the simultaneous enzymatic activity of avian Myeloblastosis virus (AMV) reverse transcriptase, T7 RNA polymerase, and RNase HFor the detection of specific primers and molecular beacon (MB) probes directed against E6/E7 mRNA for HPV types 16, 18 and 45 are included (NorChip, Klokkarstua, Norway) (Molden et al., 2007). The NASBA amplification
was carried out in a volume of 20μl at 41°C for 2.5 hr. A 5μl of nucleic acids was included in the reaction. As performance control, the intrinsic sample control (ISC-cellular mRNA from a housekeeping gene) controls the quality of the isolated nucleic acids to avoid false negatives due to their degradation. All samples were run in a micro plate reader (Flx 800 BIENOR Multi-Detection Micro plate Reader, Biotech Instruments, and Winooski, USA) (Figure 1 below).

**Figure 3.1:** Process of amplification using Nucleic acid sequence based on amplification NASBA process (Source: Karlsen *et al.*, 2000).

### 3.9 Quality Analysis and quality Control

The quality analysis for mRNA screening was done following the NorChip ISO certified standard protocols. The collection of Pap smears was done following the conventional Pap smear protocol, The Bethesda System for reporting cervico-vaginal cytology for sign out of pap smears was done. Collection of the liquid based cytology samples was done in the PreTect™ for the mRNA testing (Karlsen *et al.*, 2013).
4.3 PreTect SEE Cancer Screening Results

One sample out of the 204 samples was found without cells and was not processed. Therefore, only 203 samples were tested using PreTect SEE. Three samples were found positive while the other samples were negative.

Table 4.2 Total number of PreTect SEE tests that were positive and negative

<table>
<thead>
<tr>
<th>PreTect results</th>
<th>SEE</th>
<th>Frequency</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3</td>
<td>1.47</td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>200</td>
<td>98.03</td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>204</td>
<td>100</td>
<td></td>
</tr>
</tbody>
</table>

Three sample (1.47%) were positive with PreTect SEE while two hundred (98.03%) were negative and one sample (0.5%) was rejected after quality control.

4.4 Positive and negative predictive values of Pap smear and PreTect SEE

The positive predictive value (PPV) was calculated using the following formula.

$$\text{PPV} = \frac{A}{(A+B)} \times 100\%$$

Where:

A = number of true positive

B = number of false positives

PPV = 1/(1+0) x100% = 100%

Therefore PPV PAP is 100%

Negative predictive value the Pap smear was calculated using the following formula

$$\text{NPV} = \frac{D}{(C+D)}$$

Where:
D=number of true negatives
C=number of false negatives
NPV= \( \frac{200}{(200+2)} \times 100\% = 99\% \)

So NPV PAP is 99%.

The positive predictive value of PreTect SEE was calculated using the following formula:

\[
\frac{A}{(A+C)}
\]

Where:
A and C maintained their definitions thus;
\[
= \frac{1}{1+2} \times 100\% = 33\%
\]

Therefore the PPV of the PreTect SEE was 33% (Table 4.3)

The negative predictive value of the PreTect SEE was calculated as follows:

\[
= \frac{D}{(D+B)} \times 100\%
\]

Where M and N have their initial meaning,

\(= \frac{200}{(200+0)} \times 100 = 100\% \). Therefore PreTect SEE NPV was 100%.

**Table 4.3: Sensitivity and specificity of Pap smear and PreTect SEE**

<table>
<thead>
<tr>
<th></th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>PreTect</strong></td>
<td>100%</td>
<td>100%</td>
<td>33%</td>
<td>99%</td>
</tr>
<tr>
<td><strong>SEE</strong></td>
<td>33%</td>
<td>99%</td>
<td>100%</td>
<td>100%</td>
</tr>
<tr>
<td><strong>Pap smear</strong></td>
<td>33%</td>
<td>99%</td>
<td>100%</td>
<td>100%</td>
</tr>
</tbody>
</table>
4.4.1 Sensitivity and specificity of Pap smear
The sensitivity of the Pap smear was calculated by dividing the number of true positives by the sum of true positives and false negatives and expressed as a percentage.

Sensitivity = \( \frac{A}{A + C} \)

\[
= \frac{1}{(1+2)} \times 100 = 33\%, \quad \text{(Table 4.3)}
\]

Therefore the sensitivity of the Pap smear was 33% The Specificity for PAP was calculated as follows:
The number of true negatives divided by the sum of false positives and true negatives

Specificity = \( \frac{D}{D + C} \)

\[
= \frac{200}{(200+2)} \times 100 = 99\%.
\]

Therefore the specificity of the Pap smear was 99% (Table 4.3)

4.4.2 Sensitivity and specificity of PreTect SEE
Sensitivity for Pretest SEE was calculated as follows:
Number of true positives divided by the sum of true positives and false negatives i.e.

Sensitivity = \( \frac{A}{A + B} \)

\[
= \frac{1}{(1+0)} \times 100 = 100\%
\]

Therefore the sensitivity of the PreTect SEE was 100% (Table 4.3)

The specificity for Pretest SEE was calculated as follows:
Number of true negatives divided by the sum of false positives and true negatives i.e.

Specificity = \( \frac{D}{B + D} \)

\[
= \frac{200}{(200+0)} \times 100 = 100\%
\]

Therefore the sensitivity of the PreTect SEE was 100% (Table 4.3)

After cytological screening, only one sample was found to be positive with cytological test Pap smear. 3 samples were positive with the PreTect SEE method. Comparison between the Pap
smear the PreTect SEE as gold standard was done by calculating the Positive Predictive value (PPV) and the Negative predictive value (NPV). The Positive Predictive Value was calculated by dividing the number of true positives by the number of true negatives plus number of false positive. Therefore the number of true positive divided by the number of positive calls whereas the Negative Predictive value was calculated by dividing the number of true negatives by the number of true negative plus the number of false negatives, therefore the number of true negatives were divided by the number of negative calls. The results of the PreTect SEE have shown a sensitivity and specificity of 100% respectively. The Pap smear had a sensitivity of 33 %while its specificity was 99%. The PPV of PreTect SEE was 33% and the Negative Predictive Value (NPV) was 99% whereas The Positive Predictive Value (PPV) and Negative predictive value (NPN) of the Pap smear was 100% respectively (Table 4.3).

Table 4.4 Expression of HPV E6/E7 mRNA in all the cervical liquid based samples

<table>
<thead>
<tr>
<th>Test</th>
<th>Number</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>3</td>
<td>1.47</td>
</tr>
<tr>
<td>Negative</td>
<td>200</td>
<td>98.03</td>
</tr>
<tr>
<td>Rejected sample</td>
<td>1</td>
<td>0.5</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>204</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

Out of 204 samples analyzed, 3 (1.47%) samples expressed HPV E6/E7 mRNA while 1(0.5) was rejected due to lack of cells.

4.5 Respondent Social-economic and Demographic Characteristics

A questionnaire was used to collect data on the age of all the patients and their specific counties of residence. The current study included women of 40 years and below. Of all the total sample
size (204), the age group 15-20 years had 11 women followed by 52 women aged between 20-25 years. Sixty four (64) women were aged between 25 -35 years whereas 77 women were aged between 35-40 years.

4.5.1 Percentage of women from various counties
Nairobi County was the most represented (53.43 %). 18.2% of women came from Kiambu County, 8.31% from Meru county, 3.93% from Machakos, 3.43% from Nyandarua, 4.90% from Embu, 2.94 % from Nakuru county, 2.90% from Embu. Only 2 women (0.98%) came from Kajiado and kisii respectively.

4.5.2 Area of residence
The questionnaire also captured the number of women who were living in towns and the women living in villages. The majority of women who were included in the current study lived in urban area (73. %). However, 27 % of the total sample size resided in rural era. The results revealed that 99.5 % of the positive samples are of women coming from rural area whereas only 0.5% comes from urban area.

4.5.3 Employment status
of all the total sample size (204), 51 women (25%) are self-employed, women employed by government were 68 (33.33%) while 82 women (40.19%) were job less.
Table 4.5: Social Economic Characteristics and Counties of Residence of the Respondents

<table>
<thead>
<tr>
<th>Employment Status Counties of Residence and Marital status</th>
<th>Total Number (Frequency)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Employment status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employed by government</td>
<td>68</td>
<td>33</td>
</tr>
<tr>
<td>Self employed</td>
<td>51</td>
<td>25</td>
</tr>
<tr>
<td>Not Employed</td>
<td>82</td>
<td>40.19</td>
</tr>
<tr>
<td>Students</td>
<td>3</td>
<td>1.47</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>204</strong></td>
<td><strong>100</strong></td>
</tr>
<tr>
<td><strong>County of residence</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nairobi</td>
<td>109</td>
<td>53.43</td>
</tr>
<tr>
<td>Nakuru</td>
<td>6</td>
<td>2.94</td>
</tr>
<tr>
<td>Kiambu</td>
<td>41</td>
<td>20.9</td>
</tr>
<tr>
<td>Machakos</td>
<td>8</td>
<td>3.92</td>
</tr>
<tr>
<td>Meru</td>
<td>19</td>
<td>9.31</td>
</tr>
<tr>
<td>Embu</td>
<td>10</td>
<td>4.90</td>
</tr>
<tr>
<td>Kisii</td>
<td>2</td>
<td>0.98</td>
</tr>
<tr>
<td>Nyandarua</td>
<td>7</td>
<td>3.43</td>
</tr>
<tr>
<td>Kajiado</td>
<td>2</td>
<td>0.98</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>204</strong></td>
<td><strong>100.0</strong></td>
</tr>
<tr>
<td><strong>Marital status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Married</td>
<td>148</td>
<td>72.5</td>
</tr>
<tr>
<td>Single</td>
<td>23</td>
<td>11.2</td>
</tr>
<tr>
<td>Divorced and widowed</td>
<td>33</td>
<td>16.1</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>204</strong></td>
<td><strong>100</strong></td>
</tr>
<tr>
<td><strong>Economic status</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Earn less than 10 000Ksh (LOW)</td>
<td>70</td>
<td>34.31</td>
</tr>
<tr>
<td>Earn 10 -30 000Ksh (Middle)</td>
<td>85</td>
<td>41.66</td>
</tr>
<tr>
<td>Earn 30 000Ksh and above (High)</td>
<td>49</td>
<td>24.01</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>204</strong></td>
<td><strong>100</strong></td>
</tr>
<tr>
<td><strong>Education</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>63</td>
<td>30.8</td>
</tr>
<tr>
<td>Secondary</td>
<td>83</td>
<td>40.6</td>
</tr>
<tr>
<td>College/University</td>
<td>44</td>
<td>21.5</td>
</tr>
<tr>
<td>Never schooled</td>
<td>14</td>
<td>6.9</td>
</tr>
</tbody>
</table>
4.5.4 Marital status

A high number of women were married (75%), followed by single women and divorced women with a total number of 23 women (11%). A total number of 10 women (4%) were widowed.

4.5.5 Cervical cancer risk factors

The questionnaire obtained the data for the age of women at the first intercourse, the number of partners that the women had their HIV status, the number of time that the women were screened for cervical Cancer, and the smoking cigarette status of women.

4.5.6 Number of Partners

In the current study, the number of women with one partner was found to be higher (90.7%). However, out of the total sample size (204), 17 women (8.4%) confirmed that they had 2 partners 6 months before the test. A total number of 2 women (1%) confirmed that they had multiple partners the last 6 months before screening. Only 99% of women confirmed that they do not smoke however 2 women (1%) confirmed that they do often smoke cigarettes.
Table 4.6 Factors Associated with Cervical Cancer

<table>
<thead>
<tr>
<th>Factors</th>
<th>(Frequency)</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age at first intercourse</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-21</td>
<td>17</td>
<td>8.33</td>
</tr>
<tr>
<td>22-26</td>
<td>119</td>
<td>58.33</td>
</tr>
<tr>
<td>27-31</td>
<td>62</td>
<td>30.3</td>
</tr>
<tr>
<td>32-36</td>
<td>6</td>
<td>2.94</td>
</tr>
<tr>
<td>37-40</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Number of Partners</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>One partner</td>
<td>90.7</td>
<td></td>
</tr>
<tr>
<td>Two partners</td>
<td>8.4</td>
<td></td>
</tr>
<tr>
<td>Multiple partners</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>204</td>
<td>100</td>
</tr>
<tr>
<td><strong>Screening</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women who were screened before</td>
<td>62</td>
<td>30.3</td>
</tr>
<tr>
<td>Women who were not screened</td>
<td>142</td>
<td>69.7</td>
</tr>
<tr>
<td><strong>Smoking cigarettes</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Women who smoke</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Women who do not smoke</td>
<td>202</td>
<td>99</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>204</td>
<td>100</td>
</tr>
<tr>
<td><strong>HIV virus</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Negative</td>
<td>200</td>
<td>98.03</td>
</tr>
<tr>
<td>Under ARV treatment</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Never received the results</td>
<td>4</td>
<td>2.96</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>204</td>
<td>100.0</td>
</tr>
</tbody>
</table>
The study revealed that majority of women do not smoke, therefore the cases of cancer detected in the current study were all from women who do not smoke. Only 2 women confirmed that they do often smoke when 202 women said that they do not smoke (table 4.5).

4.5.7 Cervical cancer screening

The current study revealed that out of 204 women, 62 women (30.3%) only have been screened for cervical cancer before, while a total number of 142 women (69%) have never been screened for cervical cancer.

4.5.8 Age at first intercourse

In this study, women aged of 16-21 years were 17 (8.33%) of the sample size (204). The highest number of women which is 119 (58%) of the total sample size (204) had the first intercourse in between 22-26 years. And the rest of women had their first intercourse at 31 years and above.

4.5.9 HIV status of women

The majority of respondents were tested for HIV and confirmed that they were tested previously for HIV. A total number of 199 women (97.5%) confirmed that they were tested; however, 5 women (2.45%) said that they have not been tested for HIV. Women who knew their results and confirmed that they were negative were 200 (98%). A total number of 4 women (1.96%) confirmed that they did not know the results of their status after testing.
4.6 Hypothesis testing

Table 4.7 Correlation analysis.

<table>
<thead>
<tr>
<th></th>
<th>Area of residence</th>
<th>Employment status</th>
<th>Number of partners</th>
<th>Cervical cancer screening</th>
<th>Age at first intercourse</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of residence</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Employment status</td>
<td>0.5367 (p=.0)</td>
<td>1.000</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Marital status</td>
<td>0.6001 (p=.028)</td>
<td>0.7604 (p=.004)</td>
<td>1.000</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of partners</td>
<td>0.8585 (p=.014)</td>
<td>0.4869 (p=.034)</td>
<td>0.5933 (p=.030)</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Cervical cancer screening</td>
<td>0.6583 (p=.011)</td>
<td>0.5645 (p=.019)</td>
<td>0.7584 (p=.021)</td>
<td>0.654 (p=.023)</td>
<td>1.000</td>
</tr>
<tr>
<td>Age at first intercourse</td>
<td>0.2612 (p=.021)</td>
<td>0.4163 (p=.011)</td>
<td>0.5589 (p=.029)</td>
<td>0.7674 (p=.024)</td>
<td>0.8943 (p=.030)</td>
</tr>
</tbody>
</table>

N=204

The study significance level was $p=0.5$. The findings in the table above show that the 'P' values were less than the significance levels which was $p=0.5$ thus it was confirmed that there was no significant relationship between PreTect SEE and Pap smear in screening for cancer. The Hypothesis was therefore accepted.
CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATION

5.1 Discussion

5.1.1 Accuracy of the cervical pre-cancer detection

The current study detected only 3 HPV 16 transforming infections and none HPV 18 and 45 (1.4%) transforming infection among 203 of these relative young population in Kenya. The detection rate of HPV 16, 18 and 45 DNA infections in the same kind of population in 2016 was found to be 11% (De vuyst et al., 2012). The number of low-grade lesions that was discovered in a number of different populations was 4.7 % (43/904), adding cytological high grade lesions giving a total of 9% (81/904). The very low prevalence of HPV16/18 and 45 transforming infections in Kenya reflect the true difference between transient and transforming infection. It was impossible to conclude that any of these 200 negative cases were false negatives. It was impossible also to conclude that any of the three positive cases did not contain CIN2+ like cells.

This low detection rate in Kenya by the PreTect SEE may not have been due to the detection of only three types, but because it only detected the cases that caused persistent and true pre-cancer cases (Karlsen et al., 2013). Similar studies of an out patiance population had been conducted in Oslo, Norway using PreTect HPV-Proofer (31 and 33 in addition) (Molden et al., 2005).

Cytology, used in Kenya may miss more than 60% of cases with transforming E6/E7 infections or cervical pre-cancer cases (Number for similar cytological service in Germany). This shows that the use of PreTect SEE used as a primary screening method with a high coverage rate, may reduce the incidence of cervical pre-cancer, to a minimum in Kenya (WHO, 2014). Using a very sensitive and specific method in a very representative female population in Kenya has strongly
indicated that the prevalence of cervical cancer in Kenya may be lower than in Norway (Karlsen et al., 2013). The estimated number of annual cervical cancer cases may be around 1000 and not 4800 as indicated by the ICO HPV Information (Hovland et al., 2010). The estimated prevalence of HPV 16 and/or HPV 18 DNA reported by ICO HPV Information Centre among cytological women in Kenya may be less than 2.5% and not 9.1%. Around 40% of the HPV 16 and 18 HPV DNA in cytological normal cases may not express E6 and E7 mRNA. This also shows that the prevalence of HIV in the whole Kenya population may not be 6.2% but rather less than 2% (Micher et al., 2013). The prevalence of HIV should be much lower than the prevalence of HPV. This may indicate how important it is to perform national screening in more than 3.2% of typical national population' in Africa.

This study showed an overall detection of 3% in Oslo nearly twice as high as in the population in Nairobi, even though this is an older population. None of the women including in the Nairobi study were vaccinated. Therefore, this study reflects a complete new discovery that may claim that the incidence of invasive cervical cancer cases in the whole population of Nairobi and surroundings may be lower than how it is indicated by DNA methods (Karlsen et al., 2015).

5.1.2 Cervical cancer screening and awareness

In many developing countries, women’s knowledge of cervical cancer is very limited. It has been demonstrated that the vast majority of women in some countries had not heard of cervical cancer and even more knew nothing about cervical screening (Szerewki et al., 2008). The current study showed that most of the women were aware of cervical cancer and Pap smear test. The awareness about cervical cancer in the current study was 63%. This high awareness level could
be attributed to the study population being in an urban setting and therefore have access to many forms of information through different avenues. Many other studies contradict the current study in regard to this (Were et al., 2014). Several studies show that knowledge about cervical cancer and Pap testing can influence the uptake of cervical cancer screening services. This variance in knowledge of cervical cancer and screening is one of the most important determinants of inadequate screening status (Karlsen et al., 2015).

Previous studies done in Kenyatta National Hospital revealed a past Pap smear screening rate of 22% (Ochodo, 2010). While another study performed in Voluntary Testing and Counseling (VCT) centers in Nairobi, Kenya had an uptake rate of 14% of which the rates of both studies are lower than that shown in the current study, possibly as a result of the presence of a more urban population (DeVuyst et al., 2002).

The current study further showed that out of the 204 respondents 71.5%) have heard of Pap smear, which is considerably higher than the figure described by the KNH study previously (51%). This discordance in results may be attributed to the differences in timing between the two studies in view of the improved communication, especially media coverage in Kenya as well as education rates. The level of knowledge on cervical cancer and Pap test as a screening method, signs and symptoms, and prevention maybe improving. (Karlsen et al., 2015).

Many other studies have shown that cervical cancer and Pap testing awareness positively influences the utilization of cervical cancer screening services. The results of the current study contradict the results of a study done by Samaubi in Zambia, which revealed that 52.2% of
women had never heard about cervical cancer which is slightly less than a half of the respondents (47.8%) of the respondents who had been screened (Samaubi et al., 2013).

The main challenges to increasing access to and improving the quality of cervical cancer screening services include lack of updated National guidelines on cervical cancer prevention and control, low level of community awareness on the importance of screening coupled with low knowledge of common symptoms of cervical cancer and inadequate skills among service providers (NCCP, 2012). The Reasons for women not going for screening include perception of not being at risk and fear that abnormal test results mean existing cancer (WHO, 2014). Women with low educational achievement, low awareness of the risk factors for cervical cancer, and who do not have support from their husbands may also have poor uptake of screening services (Were et al, 2011). The Intervention raised the level of awareness of cervical cancer and screening to 100% in Nigeria (Olumide et al., 2014). There was increase in the proportion of women who had undertaken cervical screening from 4.3% to 8.3% The major reason stated by the women for not having had cervical screening done was lack of awareness about cervical cancer and screening (Olumide et al., 2014).

5.1.3 Socio-economic and Demographic Characteristics
5.1.3.1 Age and marital status
In the current study, women who were recruited were under 40 years of age, 204 women were interviewed and tested, and the majority of respondents were married (75%). Among them, 9 were married within the age of 16-20 years. 75 women got married when they were 21-30 years, and 66 women were married when they were 31-40 years old. Single women were 23 (11%) and
among them 13 were aged between 21 and 30 years. The median age of women aged between 25-40 years in Kenya is 17.6 (KDHS, 2003). Studies done in young women in college showed that women acquire often the HPV infections within few years of sexual debut (Clifford et al., 2013). The likelihood of cervical cancer was 5 fold more in women whose sexual debut was at 18 according to studies done by Vecchia et al., 1986.

5.1.3.2 Employment status of the respondents

In the current study, 40.19 % of the respondents were unemployed. Very few women were students 1.47%. Self-employed women were 51/204 (25%) whereas only 68 respondents (33.3%) were employed by the government. This reflects a low socio-economic status. A qualitative study done in Uganda, Mutyaba et al, (2007) asserted that having money increases the probability of utilizing cervical cancer screening and access to information and utilization of health care services, while Satija, (2009) and Kaku et al, (2008) found that low socio economic status interferes with adherence and follow up of treatment leading to further morbidity and mortality from the disease (Kagumire, 2010) found that large proportions of women in Uganda cannot afford transport costs to the 20 regional referral hospitals which provide cervical cancer screening services.

5.1.3.3 Risk Factors associated with cervical cancer

According to the HPV Information center of Kenya, Kenya has a population of 12.92 million women aged 15 years and older who are at risk of developing cervical cancer. Current estimates indicate that every year 4802 women are diagnosed with cervical cancer and 2451 die from the disease (Karlsen et al., 2006).
A study done by Armstrong in 1992 stated that having multiple partners, is one of risk factors associated with HPV infection. The respondents who confirmed that they had more than one partners in this study the last 6 months before the test were 21/204 (9.4%) among them, 17 (8.4%) confirmed that they had two partners, and 4 women (1. %) of the total sample size confirmed that they had multiple partners, whereas a total number of 185/204 (90.7%) confirmed that they had only one partner. The result of women with multiple partners in this study is slightly lower than the result gotten by Ambani Elizabeth that was 8.4% reported in Dagoretti division in Nairobi (Ambani, 2000). This significantly shows that the morality of women in Kenya is becoming more and better than the last years. A study done by Devuyest and Muchiri in 2000 revealed that the proportion of women with four partners or more life times was 23.4%. This result completely contradicts the results the current study by being higher than the result obtained in this study (Devuyest et al., 2000).

Sexual intercourse before 18 is a risk associated with cervical cancer (Kitchener et al, 2000). The data collected in this study revealed that 17/204 (8.3%) women were involved in sexual intercourse before 18 years, 119/204 (58.3%) were involved in sexual intercourse when they were, 19-25 years old. This study comes up with a totally different result from the information published by the HPV Information Center in 2014 revealing that the median age at the first intercourse among women was 25-49 years of age (Soybe et al., 2010). Ten years ago, a study done by Ambani in Nairobi revealed that 68.8% of women had their first intercourse in between 10 and 19 years. Other cofactors are necessary for progression from cervical HPV infection to cancer. The study stated that tobacco smoking, high parity, long term hormonal contraceptive and coinfection with HIV have been cited to be cofactors. Viral infection due to
Chlamydiatrachomatis and herpes simplex virus type 2 including some dietary deficiencies are other probable causes of cancer.

5.1.3.4 Education

Educational level may influence the risk of cancer in many ways (Devuyest et al., 2000). It is an important attribute guiding the selection of occupation. It is also a predictive factor for disposable income and many socioeconomic aspects of life, including residential and lifestyle factors. Health-contentious behavior, seeking and affordability of healthy food and participation in health promotional and screening programs, relates to education and socioeconomic factors (Karlsen et al., 2000). Studies done in Venezuela revealed that two hundred eighty six women (95%) answered that they knew about Pap smear and (76%) were 31 years old and Two hundred eight six patients (89%) knew that the Pap smear is a diagnostic method for cervical cancer while 248 (82.4%) of the respondent mentioned that Pap smear is also used to detect vaginal infections.

One hundred sixteen women (38.5%) had a low educational level, 13 (11.2%) did not finish primary school and 103 (88.8%) just finish primary school. Fifty five (29.7%) of 185 women who finished Educational level and cervical cancer screening programs got a university degree. A hundred four of them (89.7%) knew that Pap smear is used to screen cervical cancer. Other answers mentioned by the patients were: to find a disease, myomas, inflammatory processes, etc. Two hundred eighty one patients (93%) answered that they had at least one Pap smear. One hundred sixty four patients (58.3%) reported that they had 4 Pap smears in their life time. One hundred thirty seven of 220 (62.3%). Women 31 years old reported that they had 4 Pap smears in their life time. These results totally contradict the results of the current study which revealed that
the highest number of women (40.7%) had stopped studies in high school followed by (30.9%) that had primary school level.

The current study also revealed that 6.9% of women who participated in the current study did not go to school. These women may be the most vulnerable not only to HPV but also other types of infections, due to lack of knowledge, miss understanding of information, and lack of reading and writing. The results of this study concurred with Frank Karlsen’s study done in DR Congo suggesting improvement of educational level for women in Congo and Africa (Karlsen et al., 200). Knowledge of women is necessary for populations in Africa, this will surely increase the ability of women to attend hospitals for screening. The rate of women who have the University level education discovered in this study is still low (21.5%). This study also showed that even if women go to school, still 30.9% stopped studies in primary school. This is a lowest level of education that a woman can have. Several studies done in Kenya state that women should aim for higher education levels in order for them understand the processes that they should follow for them deal with diseases that they face (Verdoodt et al., 2008).

5.1.3.5 Represented counties

The high number of women from Nairobi in this study maybe because of their major participation in the study. Other counties that are far from Nairobi maybe referring their cases to Moi referral or coast general hospitals, Since these are referral cases, the prevalence of cervical cancer maybe quiet higher than the one reported in this study.
5.2 Conclusions
1. The PreTect SEE is more sensitive for detection of cervical cancer and may be more convenient as a cervical pre-cancer screening method for Kenya.
2. The prevalence of cervical cancer in Kenya may be lower than the prevalence stated by studies done with DNA detection method.
3. The result from the PreTect SEE is only negative or positive. A woman has either true cervical pre-cancer or not and it is very unlikely that the women needs to be retested.
4. However, the best screening would be to perform testing with PreTect SEE, make the women wait for the results and perform the treatment directly the very same day if necessary.

5.3 Recommendations
1. PreTect SEE should be adopted in Kenya for a National screening Program due to its high sensitivity for cervical pre-cancer detection.
2. The investors should invest in mRNA techniques rather than adopting cheap techniques with less success in screening Programs
3. A large population should be included in future studies in order to have concrete information from all the 47 counties of Kenya.
4. More emphasis should be put on creating additional awareness about cervical screening at all service delivery points within the health facilities.
REFERENCES


APPENDICES

APPENDIX I: APPROVAL FROM THE MINISTRY OF HEALTH

MINISTRY OF HEALTH
OFFICE OF DIRECTOR OF MEDICAL SERVICES

Telegrams: "MINHEALTH", Nairobi
Telephone: Nairobi 2717077 Fax: 2715239

MOH/ADM/1/81/VOL.1

Cosmos Muyabwa
Reg. No. 1500F/24677/2011
School of Medicine
Kenyatta University

Dear Mr. Muyabwa,

RE: AUTHORITY TO SHIP BIOLOGICAL SAMPLES

Your undated request for specimen export permit refers.

The title of the study is noted to be "The Utility of 36/E7 Transcript detection method for cervical cancer screening at Kenyatta National Hospital, Nairobi, Kenya.

Authority is hereby granted for shipment of biological samples related to this research work:

- 204 samples of liquid based cytology.

The shipment contact details are as follows:

PhD in Molecular Pathology and Oncology (Oslo University Hospital)
Institute of Cancer and Micro Nanosystem Technology
Busk rud and Vestfold University College (HBV). Tel: 4740403480

Dr. Onyancha P. K.
FOR: DIRECTOR OF MEDICAL SERVICES

18th November, 2014
APPENDIX II: SAMPLE COLLECTION PROCEDURE

2. Use the cervix brush:

a) Twist the brush 8 times clockwise. Be sure you have the brush as deep as you can without using power.

b) Make a PAP smear: Take the brush and drag it from one side to the other on the microscope slide. Turn the cervix brush and repeat.

c) (Optional) Put the cervix brush in a PreservCyt cup. Mix 30 times in the cup. You are now finished with the cervix brush and you can throw it away.

d) Fixation of PAP smear: Must be done immediately after you are finished with the cervix brush.

Procedure:
Use the SPRAY-FIX solution. Keep the bottle approximately 20 cm from the slide and press 1 time. Be sure the whole slide is fixed well.
APPENDIX III: INFORMATION AND CONSENT FORM

A) Participant's information

Title: Evaluation of the potential for E6/E7 transcript detection method for cervical cancer screening at Kenyatta National Hospital, Nairobi, Kenya.

Principal investigator:
Cosmas Muyabwa
Kenyatta University
Department of Medical Laboratory.
Masters student in Infectious diseases and diagnosis.
Tel: (+254)717802838 NAIROBI/KENYA

Supervisors

Dr Margaret Muturi (PhD)
Lecturer,
Kenyatta University
School of Medicine
Department of Medical Laboratory science

Dr Lucy Muchiri, MBCHB, MMed (Path), PGD-BRM, PhD
Senior Lecturer and Consultant Pathologist
Department of Human Pathology
School of Medicine/Kenyatta National Hospital
College of Health Sciences
University of Nairobi

Professor Dr Philos Frank Karlsen.
Expert in National Cervical Cancer screening technology
PhD in molecular pathology, microbiology and virology
Lecturer at University College in Buskerud and Vestfold/ Norway / CSO NorChip AS.-Norway).
Introduction to Consent form

Hello, my name is Cosmas Muyabwa a master student in infectious diseases at Kenyatta University, Department of medical Laboratory science. I and the three supervisors mentioned above are conducting a study in conjunction with physicians at Kenyatta National Hospital in order to investigate different methods for cervical cancer detection. In this study, we are going to evaluate a new method (detection of mRNA HPV) and compare it with the traditional method (PAP cytology) to see which method is best suited for detection of cervical cancer in Kenya.

I would like to invite you to participate in this study. If you agree to participate in this study, please read all the information below/or have it read to you, and then sign/put a thumbprint at the end of this document.

Reason for Study

It has been discovered that many women come to hospital with advanced cancers that cannot be cured. Many women are at risk of developing cervical cancer because they might have been infected with Human papilloma virus which causes cancer. This study is trying to find a means of detecting the virus at an earlier stage so that the infected women can be treated before cancer develops.

Your part in the study

If you agree to participate in this study, you will fill a questionnaire confidentially where you will be required to give information on your social profile and some questions about your sexual history.

Thereafter, Pap smear/Liquid based cytology will be taken from you by one of doctors here in the clinic. It is this Pap smear that will be taken to the lab for screening so that we can see if you have some abnormal cells in the cervix; part of the samples of your pap liquid based cytology will be analyzed with a new test for HPV.

Permission from the KNH-research and Ethics board to conduct this study has been obtained. However, if at any time you have concerns about this study and the way it is being conducted, you may contact the Chairman of ERC whose telephone contact is in the copy of the consent form I will give you at the end of this interview.
Possible risks
There is no serious risk during the participation in this study; your response will be confidential and will be used solely for the purpose of this study. There may be minimal discomfort during insertion of the speculum, and collection of the sample, but it is transient and the doctor or nurse will try and make you as comfortable as possible. The doctor of nurse will also explain what the procedure is all about during the process.

Benefits
- Free pretest and post-counseling
- The HPV test is free of charge, and the result will be given to your Doctor for your management and follow up.
- Your decision not to participate will not be used against you in any way. You can withdraw for the study at any point without losing the benefits to which you are entitled in this hospital.

Confidentiality
All the information, questions and specimen will be kept confidential. The specimen for the lab will be only marked with codes and not names except for those results that are required for your continued care. At the end of the research, the list of numbers will be destroyed completely; there is no unauthorized person except the Doctor, who will have access to the questionnaires that you have filled. The publication of this research will not discuss or expose your name to anybody or any participant. We will make all our effort to protect the confidentiality of the information you will have provided.

Storage of Specimen
All specimen identifiers will be kept confidential, and will be used only for the purpose of this study. If you change your mind about participating in this study after giving the sample, it is your right to come and see the Doctor who took the sample. Your sample will be immediately excluded from the study. If you have any problem or any question about this study, please call for further information 0717802838 or ERC 2726300 Ext 44102
Right as participant

The research was reviewed by the Ethics research Board of Kenyatta National Hospital; this Board protects the right of participants. If you have any question as participant, kindly contact them to this address. Ethics board: P.O Box: 20723-00202, Phone number 2726300, ex 44102 If you need to discuss your concern you can also call Dr Lucy Muchiri: 0722703364

B. Consent information and consent agreement.

1. The details of the proposed project on *Evaluation of the potential for E6/E7 transcript detection method for cervical cancer screening at Kenyatta National Hospital, Nairobi, Kenya* has explained to me/or I have read and understood it
2. I have been able to ask questions, and these have been answered satisfactorily
3. I fully understand that my participation in this study is voluntary and I can withdraw anytime without giving any reasons and without loss of my medical care benefits or legal right
4. I agree to give samples which include conventional and liquid based Pap smear, and I really understand how these samples will be collected and that I am free to give them or to refuse without giving any reason to anybody or any Doctor.
5. I understand that the samples will only be used for the purpose of this study.
6. I agree that the samples that I give can be stored and screened for HPV testing.
7. I agree that I will not benefit financially if this study leads to development of new techniques for cervical cancer detection in Kenya.
8. I freely and willingly agree to participate in this study.

Signature ___________________________ Date ________________________

I certify that the nature and the purpose, potential benefits, risks associated with participating in this study, have been explained to the above volunteer whose code number is __________________

Name of the person obtaining the consent (If it is not the researcher)

___________________________Signature __________________-date______________________

Name of the researcher ___________Signature ___________Date ___________
APPENDIX IV: CONSENT FORM AND INFORMATION IN KISWAHILI

MAELEZO KUUSU UTAFITI HUU NA HAKI YA WANAO INGIA


Kwa utafiti huu tuta pata kugundua kama Pretect SEE ndio inafaa Kenya kwa ku bainisha cancer inchini. Kama unakubali ku ungana nasi kwa utafiti huu, tunakusii usome ma agizo yote hapa chini ama mtu aku some na baada ya hiyo uweke signature yako.

Umuhimu wa utafiti.


Hatari zinazo tarajwa

Hakuna hatari yeyote wakati unakubali kuungia kwa utafiti huu, majibu yako kwa mashwali utakayo ulizwa yatabaki kati yako wewe na aliye kuuliza, na majibu hayo aiwezi kutumiwa kwa
kitu chochote tena kama si kuusu utafiti huu. Wakati dactari atkua akichuku chembechembe kwako, unaweza tu kusikia discomfort kidogo tu. Dactari atafanya awezavyo, kukuweka kwa njia iliyo sawa, atakueleza kila kitu mbele yakukifanya na namna gani kitafanywa.

**Haki yako**
- Maelezo yeyote ushauri wa bure
- Utafanyiwa utafiti kwa inchi yakizungu wakitumia mitambo inayo eleweka kua nzuri zaidi, na majibu ya utafiti wako itapewa dactari atakae kueleza nakuku udumia. Uduma ya utafiti kwa inchi yako zungu aita kugarimu kitu chochote.
- Uamuzi wako kuwinga kwa utafiti huu auwezi kutumiwa ku haribu haki yako kwa hospitali, matibabu yako ama kitu chochote, na unaweza kujiondoa kwa utafiti huu hata kama chembechembe zako zilikua zimechukuliwa.

**Siri ya utafiti**
Habari zote, maswali, na chembechembe zitakazo chukuliwa, z’itawekwa kwa siri bila kuoneswa ama kuambwiwa kwa mtu mwengine, chembechembe zako zitawekwa nambari ya siri ili isigunduliwe na mtu mwengine kama si wale ambao wana usika na utafiti huu. Na kasha utafiti huu, nambari zote zita haribiwa kabisa, na hakuan mtu yeyote ambaya atasikia jibu kwa maswali utakayo yatoa..tuta fanya yetu yote kuweka kila kitu kwwa siri.

**Jinsi Itakavyo wekwa chembechembe zako.**
Chembechembe zako zitawekwa kwa siri, na ni dactari wano usika na utafiti ambao wana ruusiwa kujua mahali zitawekwa, na hakuna utafiti mwengine wowote utakao fanwa na chembechembe izo kama si huu pekeyake. Na ni haki yako kuitisha chembechembe zako kama unataka kujiondoa kwanya utafiti huu. Na ukiwa na shida yoyote basi piga simu kwa watu wano tetea haki yako ERC 2726300 Ext 441 (Dactari 0717802838) na ujieleze vizuri kwao wata kutetea.
Haki za wanaohusika na utafiti

Utafiti huu ume angaliwa na Board ya wanao fanya utafiti KNH. Na niwao wanao usika na kuku saidia ili hakikako zijulikane, na kanma uko na swali, hata mbele uingie kwa utafiti huun basi address yao na posta ni hizi. Ethics board: P.O Box: 20723-00202, Phone number 2726300, ex 44102 na naweza pia pigia dactari Lucy Muchiri kwa nambari inayo fuata: 0722703364.

B. Makubaliano

1. nimesoma maelezo yote n anime elewa kuusu Utafiti unao fnwa.
2. nimeuliza maswali zote na zime jibiwa
3. Nime elewa yakwamba kuingikwangu kwa utafiti huu ni kujitolea kwangu na naweza kujiondoa wakati wowote ninapo taka.
4. nakubali kupana chembechembe zangu za njia ya uzazi na pi anime jua namna ambayo zitachukuliwa na pia yakwamba naweza kuzipana ama kutaa bila hata kupatia dactari elezo lolote.
5. nasikia na naelewa yakwamba chembechembe zitakao chukuliwa kwa njia ya uzazi wangu, zita tumiwa tu kwa utafiti huu na apana mwengine
6nina kubali yakwamba chembechembe zangu zinaweza kuwekwa kwa mda kidogo baada ya kutumiwa KNH ndivo zitatumwa kwa inchi za kizungui (NORWAY) kwa utafiti mkuu zaidi ili niangaliwe kama niko na Cancer ama kwa hatari yakuipata..
7. nimekubali yakwamba sita pata malipo yoyote baada ya utafiti huu, haki yangu tu nikujua hali yangu itakavyo kua.

8. Nimekubali kuji igiza kwa utafiti huu....

Signature _______________________________ Date ________________________________

Daktari Sign_____________________________ Date ________________________________

One copy to the patient, one copy for the file of the research, ant the original copy filed in medical copy by the Researcher
APPENDIX V: QUESTIONNAIRE

Study number ( )

1. Age in years ( )

2. Marital status married 1 ( ) Single2 ( ) Separated or divorced 3( ) Widowed4 ( )

3. Religion Catholic1 ( ) Protestant2 ( ) Muslim3 ( ) Other4 ( )

4. What is your County of residence-----------------------------------------------

5. Highest Level of education attained None 1 ( ) Primary 2 ( ) Secondary 3 ( )
College/University 4 ( )

6. Occupation Self employed 1( ) Formal employment 2 ( ) Business 3( )
Unemployed 4 ( ) Student 5 ( )

7. Do you smoke? Yes 1 ( ) No2 ( ) If yes how many cigarettes a day? 3 ( )

8. Social economic status Low(earn <10 000/month 1 ( ) middle 2 ( earn 10-30 000/moth)
3 ( ) High earn (30 000/month) ( )

9. Residence : Rural 1 ( ) Urban 2 ( ) Foreigner 3 ( )

10. Age at first intercourse ___ yrs

11. Number of sexual Partners in last six months Single 1 ( ) Multiple 2 ( ) None 3 ( )

12. Age of marriage: 16 - 18 years 1( ) 18-25 years 2 ( ) 25years and above 3 ( )

13. Number of marriages: 1 ( ) 2 ( ) 3 ( ) more than 3 marriage ( )

14. Do you know what cervical cancer is? Yes 1( ) No 2 ( ) I don’t remember

15. Have you ever been screened for cervical cancer? Yes ( ) No ( )

If yes what have you been told? The result was: Normal 1 ( ) Abnormal 2 ( ) specified
class/stage 3( ) asked to repeat 4 ( ).

16. Do you know what the Pap smear test is? Yes 1 ( ) No 2 ( )
17. Do you know what it tests for? Cancer of the Cervix 1( ) cancer of Uterus 2( ) simply cancer 3( )

18. Have you been treated for an abnormal pap smear before? Yes 1( ) No 2( )

19. Do you have any of these symptoms? Bleeding 1( ) Discharges 2( ) Inter menstrual bleeding 3( ) lower abdominal pain 4( ) weight loss Urinary incontinence 5( ) None 6( ) other 7( )

20. Have you ever done the HIV test? Yes 1( ) No 2( ) did you know the result? Yes 1( ) No 2( )

21. Have you ever taken drugs called ARV? Yes 1( ) No 2( ).