Descriptive Epidemiology of Cervical Dysplasia and Inflammatory changes in Women Attending *Family Health Options, Kenya* Clinics in Nairobi

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A Thesis submitted in partial fulfillment for the degree of Master of Science in Epidemiology in the
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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university.

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DEDICATION

To my wife Wanjiku for her undying love and support, and to our children Waithera, Mburu and Wambui.

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LIST OF ABBREVIATIONS

AGC	Atypical glandular cells
AIS	Adenocarcinoma in situ
ASC-H	Atypical squamous cell – cannot exclude high grade
ASC-US	Atypical squamous cell – undetermined significance
BTL	Birth tubal ligation
CaCx	Carcinoma of the cervix
Ca in situ	Carcinoma in situ
C.I	Confidence Interval
CIN	Cervical intraepithelial neoplasia
FHC	Family Health Care
FDA	Food and Drug Administration
FHOK	Family Health Options Kenya
HPV	Human Papilloma Virus
HSIL	High grade squamous intraepithelial lesion
ITROMID	Institute of Tropical Medicine and Infectious Diseases
IUCD	Intrauterine contraceptive device
KEMRI	Kenya Medical Research Institute
LAM	Lactating amenorrhea
LSIL	Low grade squamous intraepithelial lesion
NFP	Natural family planning
OR	Odds Ratio

PV	Per vaginal
SIL	Squamous intraepithelial lesion
STI	Sexually transmitted infection
TBS	The Bethesda system
TV	*Trichomonas vaginalis*

ABSTRACT

Cancer of the cervix is the second most common cancer in women worldwide and the leading cause of cancer deaths in women in developing countries. The prevalence is highest in the developing countries, one of the reasons being lack of good screening programs. Cervical cancer can be dramatically reduced by screening of Pap or cervical smears in order to detect dysplasia or precancerous changes which can be treated to prevent development of invasive cancer. The risk factors associated with the development of cervical cancer include age at first sexual intercourse, multiple sexual partners, sexually transmitted infections (especially human Papilloma virus), smoking and not taking a Pap test, among others. The incidence of cervical cancer is also highest among poor women in developing countries. The objective of this study was to determine the prevalence of cervical dysplasia and inflammatory changes in Pap smears taken from women attending Family Health Options Kenya (FHOK) clinics in Nairobi. Family Health Options Kenya has been carrying out cervical smear screening program in various parts of the country for more than 12 years. Participants in this study were women who voluntarily went to the Nairobi clinics to have Pap smear tests. Written informed consent was obtained from each participant and a questionnaire administered by the clinician. The clinicians also routinely took the smears and filled in the Pap smear laboratory form. The Pap smears were processed and screened in the cytology laboratory by the researcher and the results given to the participants by the clinicians. The data collected was analyzed using Epi Info version 3.3 statistical software (Atlanta,

Georgia). A total of 194 Pap smears were collected and screened between the month of April and June 2008. The combined prevalence of cervical cell abnormalities and inflammatory changes was 91 (46.9%). From these cases, 27 (13.9%) were inflammatory due to various infections while 6 (3.1%) were cervical cell abnormalities and 58 (29.9%) of the rest were inflammatory changes due to other causes, mainly the use of IUCD contraception (33 cases), 1 case of atrophic cervicitis and 24 cases of non specific causes. One hundred and three (53.1%) Pap smears were negative for either inflammation or cervical cell abnormalities. The study demonstrates that cervical dysplasia and inflammatory changes are present in women attending FHOK clinics in Nairobi. However, the socio demographic characteristics of the women in these clinics indicate that the screening program was capturing women from high socio economic status. Women from poor socio economic background, low literacy and other related risk factors were not captured in the FHOK screening program. The study recommends that for a screening program to be successful, all women should have access to Pap test and that both the private and public healthcare facilities should be equipped for the same.

CHAPTER ONE

1.0 INTRODUCTION

1.1 Background

The cervix is an organ in the female reproductive system (Figure 1.1) which serves as a neck of the uterus and a passageway between the uterus and vagina. The epithelial surface lining the cervix has two types of cells. The outer part of the cervix (ectocervix) near the vagina is covered with cells called squamous epithelial cells. The cervical canal (endocervix) is lined with columnar epithelial cells that secrete mucus during ovulation. The junction between these two types of cells is called the transformation zone, which changes shape and position with age (Douglas and Rebecca, 2005).

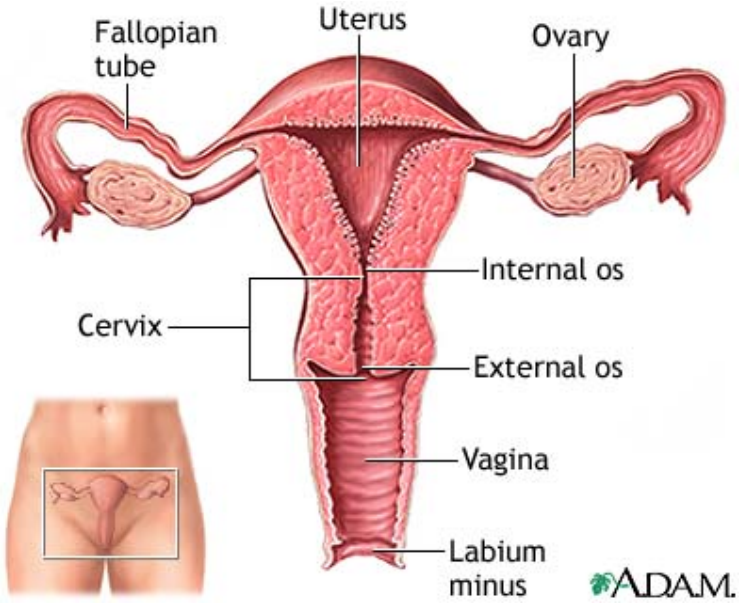


Figure 1.1: Female reproductive system showing the position of the cervix (A.D.A.M.)

Cervical dysplasia occurs when cells on the cervix have abnormalities. The condition is technically called cervical intraepithelial neoplasia (CIN) or squamous intraepithelial lesions (SIL) which refers to a preinvasive pathological intermediate of cervical cancer that is slow to progress and can be easily detected and treated. The cervix is also susceptible to other problems, including cervicitis (inflammation), sexually transmitted infections (STIs), and cancer (dysplasia is a precursor to cancer) (Douglas and Rebecca, 2005).

The most common method for diagnosing cervical dysplasia is the Pap smear or a Pap test, which was invented by the American researcher George Papanicolaou in the 1950s (Koss, 1992). Due to the effectiveness of the Pap smear in detecting cervical abnormalities, the incidence of cervical cancer has decreased by as much as 50% since 1960 while deaths from cervical cancer have decreased by as much as 70% in populations in which regular screening and treatment are the standard of care (Douglas and Rebecca 2005).

The Pap test is a simple procedure. The cervix is exposed using a metal or plastic instrument called a speculum. A small brush, swab or spatula is used to obtain a sample of cervical cells that are smeared onto a slide and subsequently examined under a microscope (Figure 1.2).

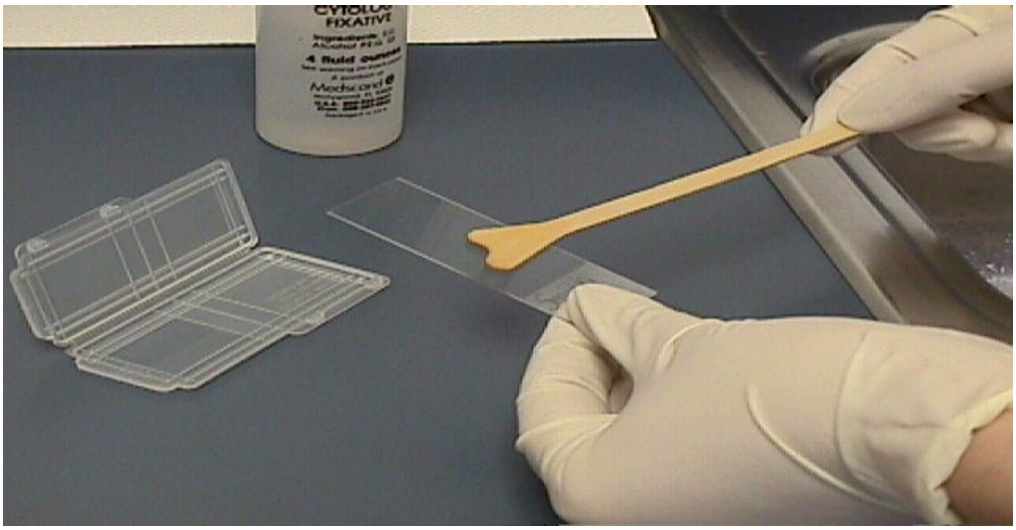
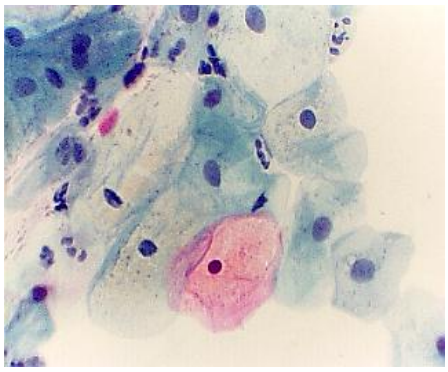
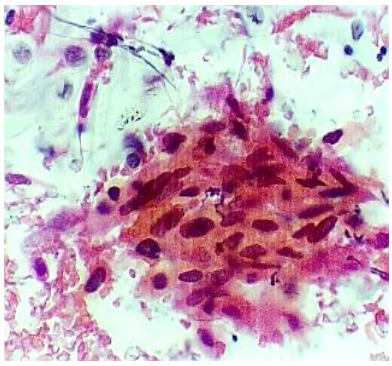


Figure 1.2: Smearing cervical cells onto a glass slide using Aylesbury spatula (NCI Bethesda System Web Atlas)

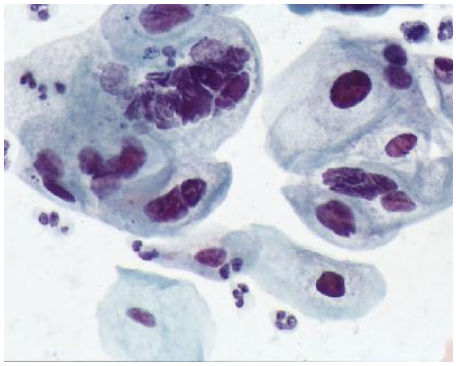
The test is a screening tool rather than a diagnostic tool and is very efficient in detecting cervical abnormalities (Lata and Margaret, 2002). The Bethesda System is commonly used to report Pap test results. The 2001 Bethesda System includes specific statements about specimen adequacy, general categorization, and interpretation of results (Barbara *et al.,* 2003). A negative test means that no abnormalities are present in the cervical tissue examined. A positive Pap test describes abnormal cervical cells as low-grade or high-grade SIL (squamous intraepithelial lesion), depending on the extent of dysplasia. Figure 1.3 below shows the cytology of cells ranging from normal (benign) to cervical cancer (carcinoma), stained with Pap stain and magnified 400 times.



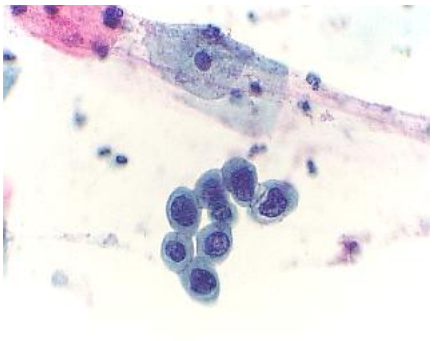
A. Benign squamous cells x 400



B.Squamous cell carcinoma x 400



C.Low grade squamous intraepithelial lesion x 400



D.High grade squamous intraepithelial lesion x 400

Figure 1.3: Cervical epithelial cells ranging from normal cells (A) to squamous cell carcinoma. B,and C show the changes in between the extremes, LSIL (C) and HSIL (D) (http://fiveprime.org/hivemind/Tags/dysplasia)

About 5-10% of Pap tests show at least mild abnormalities. However, a number of factors other than cervical cancer can cause abnormalities, including inflammation from *Candida albicans* infections, *Trichomonas vaginalis* infection and bacteria.

Candida albicans is the most common pathogenic fungi observed in the female genital tract (Koss, 1992). The fungus is made up made up of long thin filaments consisting of elongated bamboo-like spores (pseudohyphae) as shown in figure 1.4.

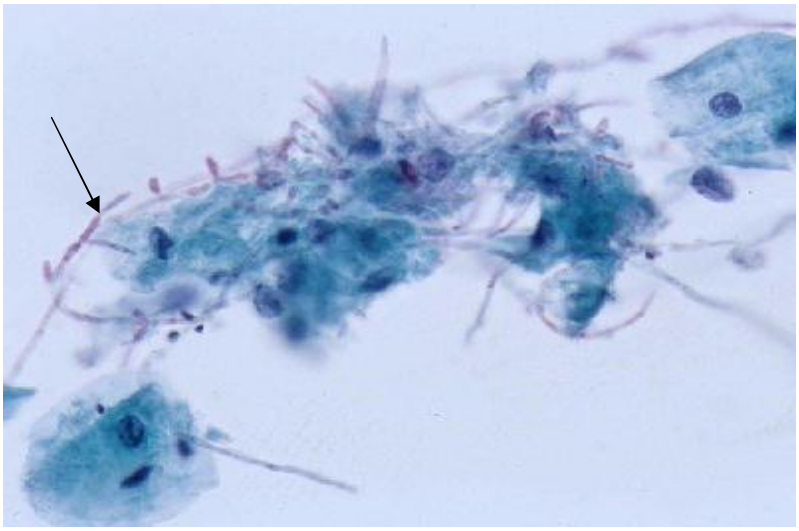


Figure 1.4: Pseudohyphae (arrowed) of *Candida albicans* within squamous epithelial cells x400 (NCI Bethesda System Web Atlas)

Trichomonas vaginalis appears as pear shaped cells with elliptical nucleus often adjacent to the cell membranes of squamous cells as seen in figure 1.5 below.

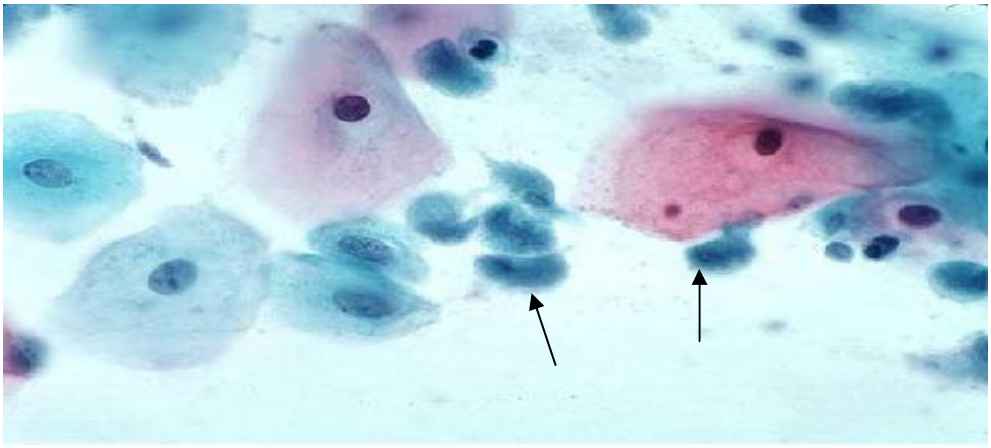


Figure 1.5: *Trichomonas vaginalis* (pointed) amongst squamous cells x 400 (NCI Bethesda System Web Atlas)

Three to six months after the infection is treated, the Pap test is repeated (Barbara *et al.*, 2003).

1.2 Problem statement

There are more than 400, 000 new cases of cervical cancer diagnosed each year. The incidence of cervical cancer is highest among poor women and among women in developing countries. The reduction in the incidence of cervical cancer is one of the major public health achievements in developed nations, largely due to the implementation of population-based screening, detection, and treatment programs for preinvasive disease. There is a need therefore to carry out a study on an ongoing screening program in Kenya and to evaluate the targeted population.

1.3 Study justification

Cancer of the cervix is among the malignancies leading in mortality of women in reproductive age in Kenya. Experience from developed countries show that mortality from cervical cancer can be markedly reduced through early diagnosis of invasive cancer and screening for pre-malignant lesions. While Pap smear screening is available in selected health facilities in the country, it is apparent that most of the women in the country have never had a chance to have the test done. There is no national Pap smear screening program by the Ministry of Health in Kenya and only scanty data are available on dysplastic cytology.

There is therefore a need to carry out a study to find out the cytology of the Pap smears and those who seek for the services from a health facility with an ongoing screening program. Carrying out an epidemiological study on FHOK clinics will aid in establishing the prevalence of cervical abnormalities and the characteristics of those who seek the test. The findings will aid in the review of the existing screening program at FHOK and to establish the socio-demographic characteristics of the women being captured in the program. These will give an insight as to whether the program is targeting the population at risk and what requires to be done to reduce the incidence of cervical cancer.

1.4 Objectives

1.4.1 General objective

To determine the prevalence of cervical dysplasia and inflammatory changes of Pap smears taken from women attending FHOK clinics in Nairobi.

1.4.2 Specific objectives

1. To determine the prevalence of cervical dysplasia and inflammatory changes.
2. To determine the relationship between cytological findings and the socio-demographic characteristics.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Development of cytology

Cytology, which is more commonly known as cell biology, is a study of cell structure, cell composition, and the interaction of cells with each other and with the larger environment in which they exist. Cytology can also refer to cytopathology, which examines cell structure to diagnose disease (Tricia, 2007). The key development of cytology as a diagnostic tool was the observation that cell samples obtained from the vagina or the uterine cervix were useful in the diagnosis of cancer of the uterus. In a book published in 1943, George Papanicolaou made key observations, supported by several illustrative case reports that the principle benefit of cytologic examination was the discovery of cancer when it was not suspected clinically or in asymptomatic patients (Koss, 1992).

2.2 Pap smear

In gynecology, the Papanicolaou test (also called Pap smear, Pap test, cervical smear, or smear test) is a medical screening method, primarily designed to detect premalignant and malignant processes in the ectocervix. It may also detect infections and abnormalities in the endocervix and endometrium (wikipedia.org/pap smear).

The endocervix may be partially sampled with whatever device used to obtain the ectocervical sample, but due to the anatomy of this area, consistent and reliable sampling cannot be guaranteed. As abnormal endocervical cells may be sampled,

those examining them are taught to recognize them. Endometrial cells may also exfoliate onto the cervix and be collected from there, so as with endocervical cells, abnormal cells can be recognized if present but the Pap test is not to be used as a screening tool for endometrial malignancy (Raffle *et al.*, 2003).

Pap smear quality is very vital for proper microscopic interpretation. Immediately after procurement, the cells are spread on a glass slide and fixed immediately in 95% alcohol to prevent air drying effect and to preserve the cellular structures (Koss, 1992). An adequate smear must also contain enough squamous and endocervical cell to ascertain that the transformation zone (where cancer is more likely to develop) has been sampled. Other features that may affect the quality of a cervical smear are blood, too many pus cells or artifactual components such as fecal material. Such artifacts may obscure the cellular structures, leading to impaired interpretation of the cytological changes.

It is generally recommended that sexually active females seek Pap smear testing annually, although guidelines may vary from country to country. The Center for Disease Control (CDC) recommends that women get a Pap test no later than 3 years after their first sexual encounter and no later than 21 years of age. Women should have a Pap test every year until age 30. After age 30, women should discuss risk factors with their health care providers to determine whether a Pap test should be done yearly. If risk factors are low and previous Pap tests have been negative, most women only need to have tests every 2-3 years until 65 years of age (CDC, 2005). If

results are abnormal, and depending on the nature of the abnormality, the test may need to be repeated in three to twelve months. If the abnormality requires closer scrutiny, the patient may be referred for detailed inspection of the cervix by colposcopy. The patient may also be referred for Human Papilloma virus DNA testing (Lata *et al.,* 2002).

2.3 Technical aspect of Pap smear collection

Samples are collected from the outer opening or os of the cervix using an Aylesbury spatula or a plastic-fronded broom by a clinician. The cells are immediately spread (smeared) on a glass slide and promptly fixed using 95% alcohol (ethanol or isopropanol) and examined microscopically in the laboratory. Since the mid-1990s, techniques based on placing the sample into a vial containing a liquid medium which preserves the cells have been increasingly used. The media are primarily ethanol based. Once placed into the vial, the sample is processed at the laboratory into a cell thin-layer, stained, and examined by light microscopy. In the last decade there have been successful attempts to develop automated, computer image analysis systems for screening. One of these has been FDA approved and functions in high volume reference laboratories, with human oversight (Biscotti *et al.,* 2005).The sample is stained using the Papanicolaou technique (Appendix 5), which results in well stained nuclear chromatin, differential cytoplasmic counterstaining, and cytoplasmic transparency (Koss, 1992). The sample is then screened by a specially trained and qualified cytotechnologist using a light microscope.

2.4 Pap smear results

About 5 to 7% of Pap smears show abnormal results, such as dysplasia, possibly indicating a pre-cancerous condition (Raffle *et al.*, 2003). A previous study carried out in a family planning clinic in Nairobi, Kenya, found a prevalence of 12% (Temmerman *et al.*, 1998). Although many low grade cervical dysplasias spontaneously regress without ever leading to cervical cancer, dysplasia can serve as an indication that increased vigilance is needed. Endocervical and endometrial abnormalities can also be detected, as can a number of infectious processes, including *Candida albicans* and *Trichomonas vaginalis* infections. A small proportion of abnormalities are reported as of "uncertain significance" (Raffle *et al.*, 2003).

2.5 Precancerous changes

Dysplasia, cervical intraepithelial neoplasia (CIN) and squamous intraepithelial lesion (SIL) are different terms or names for the same condition. Dysplasia simply means abnormal tissue development; it is still sometimes used to mean CIN, although the term is not used as frequently as in the past. Squamous intraepithelial lesion (SIL) is the more recently used term with regard to CIN, and describes the type of cervical cells that undergo changes in 80% of cervical neoplasia (Leslie, 1996). In this study, dysplasia,SIL and CIN will be used interchangeably. Squamous intraepithelial lesion is a condition characterized by new growth (neoplasia) in the

normal tissue (epithelium) of the cervix, the lowest portion of the uterus leading into the vagina. A diagnosis of SIL or CIN means that abnormal tissue has been detected in a woman's cervix.

The abnormal tissue of the cervix is collectively composed of cells that have undergone abnormal, individual changes, and which have formed lesions in the cervix. Cervical lesions can regress (grow smaller and disappear), persist or progress to early cervical cancer, more formally called cervical carcinoma in situ, and finally invasive cervical cancer. High-grade SIL (moderate or severe dysplasia, CIN II-III) is more likely to persist or progress. Low-grade SIL (mild dysplasia, CIN I) often regresses without any treatment, overcome by a successful immune system defense (Leslie, 1996). Cases of CIN are thought by some authors to progress through these stages toward cancer in a linear fashion (Rapp *et al.,* 1998; Agarastos *et al.,* 2005; Hillemans *et al.,* 2006,).

However, evidence suggests that cancer can also occur without first detectably progressing through these stages and that a high grade intraepithelial neoplasia can occur without first existing as a lower grade (Monnier-Benoit *et al.,* 2006).

Precancerous changes were initially reported using the Pap- class which gave a reporting range from I to V from a normal smear to invasive carcinoma. Table 2.1 below gives a summary of three classifications for reporting of Pap smears. The

cytological changes range from a normal smear (no abnormalities) to invasive carcinoma.

Table 2.1: Classifications used for reporting Pap smears

Cytological changes	Pap-class	CIN	Bethesda system SIL
No abnormalities	I	-	Negative for intraepithelial lesion or malignancy
Alteration of the squamous cells	II	-	- Other non-neoplastic findings (optional to report) -organisms -Reactive cellular changes
Atypical squamous metaplasia	II	-	-ASCUS
Mild dysplasia	II	I	Low-grade SIL
Moderate dysplasia	IIIA	II	High-grade SIL
Severe dysplasia	IIIB	III	
Carcinoma in situ	IV	III	
Micro invasive carcinoma	V	-	
Invasive carcinoma	V	-	

The earliest classification was the Pap class which gave the classification in severity from class I for no abnormalities to class V for invasive carcinoma. Later on, the CIN system was used to report on dysplastic cellular changes from CIN I for mild dysplasia to CIN III for severe dysplasia and carcinoma in situ. More recently, the Bethesda system has been adopted and this categorizes the dysplastic changes into low-grade SIL for mild dysplasia and high-grade SIL for the moderate and severe dysplasia and carcinoma.

2.5.1 Causes of precancerous changes

The single most frequent cause of CIN is infection with Human Papilloma virus (HPV), the virus that causes genital warts and common skin warts. The fact that sex workers have much higher rates of cervical cancer than nuns was a key early observation leading researchers to speculate about a causal link between sexually transmitted HPVs and cervical cancer (Zur and De Villiers, 1994). There are many types of HPV; some types are relatively harmless, but others can cause aggressive disease (Bosch *et al.*, 1995). It remains clear that people with greater numbers of sexual partners are at increased risk of developing genital HPV-related diseases. Co-infection with other sexually transmitted pathogens, such as HIV, may also increase the risk of developing HPV-related diseases.

Human Papilloma virus is one of the most common sexually transmitted diseases in the U.S.A. About one-third of the more than 60 identified types can be sexually transmitted. Several types cause visible genital warts, or condyloma acuminata; certain other sexually transmitted types lead to cervical, vulval and anal cancers. The types of HPV that are oncogens, or cancer-causing agents, do not cause genital warts and are usually detectable by Pap smear screening. Tests for HPV types exist but are expensive and largely unavailable (Brinton, 1992).

Women with genital warts should be treated and also examined for cervical HPV infection by Pap smear and if need be, by colposcopy (a technique that allows visual

examination of the living tissue of the vagina and cervix using a magnifying instrument).

Early detection of any HPV-induced cellular abnormalities may allow targeted surgical removal of pre-cancerous lesions prior to the development of invasive cervical cancer. In the absence of Pap testing or treatment, about 1% of women with genital HPV infections may eventually go on to develop cervical cancer (Ponten *et al.*, 1993).

Lately Merck & Co. has developed a vaccine against four strains of HPV (6, 11, 16, 18), called Gardasil™. It is now on the market after receiving Food and Drug Administration (FDA) approval (Harper *et al.*, 2006) on June 8, 2006. Gardasil is targeted at girls and women of age 9 to 26 because the vaccine only works if given before infection occurs; therefore, public health workers are targeting girls before they begin having sex. Glaxosmithkline has developed a vaccine called Cervarix™ which has been shown to be 100% effective in preventing HPV strains 16 and 18 and is 100% effective for more than four years but is yet to be approved (Diane and Scott, 2007).

2.5.2 Other risk factors

Although HPV has been discussed earlier as the most important risk factor in CaCx, there are other associated factors with the disease. These risk factors are involved with sexual encounters and the spread of HPV, predisposing factors such as smoking

and alcohol consumption. Not having taken a Pap smear is also one of the risk factors. A regular Pap smear provides an opportunity to detect pre-cancerous cells in the cervix. Three out of four women who develop cervical cancer each year have never had a Pap smear or not had one within the recommended two yearly intervals (Broder, 1992). Poor socioeconomic background makes it more difficult for women to afford this preventive care while lack of education prevents access to knowledge about the Pap test.

2.5.3 The Bethesda System

The Bethesda System (TBS) for reporting cervical or vaginal cytological diagnoses was introduced in 1988 and revised in 1991 to establish uniform terminology and standardize diagnostic reports. In addition, it introduced a standardized approach for reporting if an individual specimen is adequate for evaluation (Hudson *et al.*, 1989). The reporting system conveys laboratory findings that help physicians and their patients decide what to do about the abnormalities found on Pap tests. The System for reporting cervical cytology was then revised in 2001 (TBS-2001, Appendix 1) to reflect scientific advances achieved in the preceding decade (Mark and Abhijit, 2006).

The Bethesda System first reports on the adequacy of the sample (e.g., if endocervical cells are present) and uses descriptive terms for abnormal results. This system may describe any infection detected on Pap smear, such as fungal (e.g.,

candidiasis), bacterial, protozoal (e.g. *Trichomonas*) or viral (e.g. herpes simplex virus) infection. The results report if the Pap smear detected inflammation, squamous cell abnormalities or glandular cell abnormalities (Ronald *et al.*, 1992).

On epithelial cell abnormalities, TBS reports on the squamous cell abnormalities and glandular cell abnormalities. Reporting on squamous cell abnormalities ranges from atypical cells, Low-grade SIL, High-grade SIL to squamous cell carcinoma. Abnormalities in glandular cells range from atypical glandular cells, adenocarcinoma in situ (AIS) to adenocarcinoma. The presence of endometrial cell in women 40 years or older is also reported (Mark and Abhijit, 2006).

Cervical cancer is primarily a squamous cell cancer. A Pap smear result of atypical squamous cells of undetermined significance (ASCUS) indicates abnormalities that do not fit the criteria for SIL, but which are significant. An estimated 20% of women with ASCUS results will go on to develop SIL or invasive cancer (Agorastos *et al.*, 2005).

2.6 Epidemiology

Cervical cancer is the second most common cancer in women worldwide and the leading cause of cancer deaths in women in developing countries (Diane and Scott, 2007). Worldwide, there are more than 400, 000 new cases of cervical cancer diagnosed each year (WHO, 2006). Older women are at the highest risk for cervical cancer. Although girls under the age of 15 rarely develop this cancer, the risk factor

begins to increase in the late teens. Rates for carcinoma in situ reach the peak between the ages of 20 and 30 years. In the United States, the incidence of invasive cervical cancer increases rapidly with age for African-American women over the age of 25. The incidence rises more slowly for Caucasian women. However, women over age 65 account for more than 25% of all cases of invasive cervical cancer (Lata and Margaret, 2002).

The incidence of cervical cancer is highest among poor women and among women in developing countries. In the United States, the death rates from cervical cancer are higher among Hispanic, Native American, and African American women than among Caucasian women. These groups of women are much less likely to receive regular Pap tests. Therefore, their cervical cancers usually are diagnosed at a much later stage, after the cancer has spread to other parts of the body (Lata and Margaret, 2002).

In Kenya, as in most parts of Africa, cancer of the cervix is a very common disease accounting for 70-80% of all cancers of the genital tract (Nyong'o and Meheus, 1994). Where cancer registries exist in the country, cancer of the cervix represents up to 37% of all histologically proven cancers in women (Lowe *et al.,* 1981). Cervical cancer is second at 19.60% to breast cancer (21.40%) in frequency of all malignant tumors reported at the cancer registry in KEMRI (Nairobi Cancer registry, 2006). In Kenya the peak age of cervical cancer is 35 to 45 years, a time when the women are

of great demand to their families as well as the economy (Nyong'o and Meheus, 1994).

2.7 The value of cytological screening

The value of cytological screening for cancer of the cervix is now proven in developed countries where early detection through screening programs have reduced the incidence of invasive cancer, and thereby lowered the associated mortality (Ponten *et al.,* 1993). Cytological screening reduces mortality from cervical cancer by earlier diagnosis of invasive disease (Hans-Olov *et al.,* 1994). Since this screening tool was developed there has been a 70% decrease in cervical cancer deaths over the last 50 years in populations where the services have been utilized. Pap smear testing has proven to be one of the most successful screening tests in the history of medicine (Walboomers *et al.,* 1999). Not having taken a pap test then, can be considered as a risk factor to cervical cancer.

Alternative methods to cytology for screening involve visual inspection of the cervix with acetic acid or DNA testing for HPV. Cervical-cancer screening strategies incorporating visual inspection of the cervix with acetic acid or DNA testing for HPV in one or two clinical visits are cost-effective alternatives to conventional three-visit cytology-based screening programs in resource-poor settings (Sue *et al.,* 2005).

Today, a dichotomy exists between developing and developed nations; the incidence of cervical cancer in the latter has fallen dramatically (Agorastos *et al.,* 2005), while

the disease continues to be the second most common cancer in women worldwide (Park *et al.,* 1998). The reduction in the incidence of cervical cancer is one of the major public health achievements in developed nations, largely due to the implementation of population-based screening, detection, and treatment programs for preinvasive disease.

In Kenya, cervical cytology was introduced at the Kenyatta National Hospital in 1969 and these services have been offered to women attending gynecology and family planning clinics only. More recently other major private hospitals such as Nairobi Hospital and Aga Khan University Hospital have been offering such services. However, it is apparent that the majority of women do not live in Nairobi, and therefore to date, only a small portion of the Kenyan population has been able to benefit from the screening.

The Family Health Options Kenya, formerly The Family Planning Association of Kenya has had a cervical screening program for more than 12 years. Family Health Options Kenya has 9 clinics in 6 provinces in Kenya, 3 of which are in Nairobi. The Association is therefore suited with a broader national outlook of private non profit care networks in terms of diversity of the Pap smear cytology. By carrying out a descriptive study of the women attending the Nairobi clinics and the cytological findings, a better understanding of the cervical abnormalities and associated factors can be achieved.

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Study design

This descriptive cross sectional study design was used to establish the cytological findings of the Pap smear tests from FHOK clinics in Nairobi. The study entailed looking at the quality of smear, inflammation, dysplasia and also establish the socio-demographic characteristics of women seeking the screening services. This was carried out at three FHOK clinics in Nairobi.

3.2 Study population

All women attending the three FHOK clinics in Nairobi and who requested or accepted to have Pap smear test and who also provided a written and signed consent to participate in the study. The participants were living in Nairobi and the surrounding rural and urban areas. Some of the participants were referred for a Pap test from various parts outside Nairobi.

3.3 Study setting

Family Health Options Kenya headquarters is situated at the Lang'ata road and Mbagathi road junction in Nairobi West. The cytology laboratory is found in this facility alongside the clinical services. There were two other outreach Pap smear collection clinics in Nairobi at Ribeiro house, Luthuli Avenue and Phoenix House,

Kenyatta Avenue and the smears were processed and screened at the cytology laboratory in Nairobi West.

3.4 Inclusion criteria

All women above 18 years and seeking to have Pap test and willing to provide informed consent were included in the study.

3.5 Exclusion criteria

Women below 18 years or those who did not provide consent to participate in the study.

3.6 Sample size

The sample size was based on the formula (Fisher, 1960):

$$n = \frac{z^2 * pq}{t^2}$$

Where z = Confidence Interval (1.96 for 95% CI)

p = prevalence of inflammation and dysplasia

q = (1-p)

t = precision (5% = 0.05)

A prevalence of 12% is assumed (Temmerman *et al.*, 1998)

This gave a minimum sample size of 163. An extra 19% was added to take care of spoilt questionnaires, giving a sample size of 194 during this study.

Starting April 2008, Pap smears were consecutively collected, processed and analyzed until the sample size was achieved in June 2008.

3.7 Sampling

3.7.1 Recruitment

Study subjects were recruited from the three FHOK clinics. Those recruited were women who voluntarily reported to the clinics for Pap test or those referred for the test by clinicians. Before recruitment they were asked to give written informed consents. The attending clinician explained details about the study, including potential risks and benefits of participation. The prospective participants granted voluntary consent after all relevant information had been provided in a language and style they understood. The study clinician assigned a participant identification number (PIN) to each woman who consented to participate. This was to ensure anonymity for the study participants. The clinician administered a structured questionnaire and filled in the laboratory request form. Each questionnaire was labeled with the participant's PIN.

3.7.2 Collection of Pap smear

The clinicians from each clinic obtained the Pap smears from the consenting women. Each specimen was collected using a Pap smear kit consisting of spatula, glass slide with frosted end and a cytological fixative. Using a pencil, the clinician labeled the slide with the participant's PIN on the frosted end of the slide. The clinician then took and fixed the Pap smear on the labeled slide and sealed the kit to prevent breakage of the slide.

3.7.3 Transport of Pap smears

Each Pap smear kit was sealed accordingly and all the kits packed inside a box. The corresponding questionnaire, consent and laboratory request forms were placed in envelops and sealed. All these were carried by the researcher to the cytology laboratory for further processing.

3.7.4 Laboratory examination

In the laboratory, the specimen packages were received and recorded. A file was opened for the questionnaires and consent forms. The smears were stained using Pap staining kit (Appendix 6). The slides were then examined by the researcher under the light microscope and reported using the Bethesda System 2001 (Appendix 1) for adequacy quality, inflammatory changes and dysplastic changes. The standard operating procedure for Pap smear as set out by the Bethesda Interobserver Reproducibility Study (BIRST) (Mark and Abhijit, 2006) was used to examine the smear. All slides with cellular abnormalities were sent to a pathologist for review, while 10% of all the screened slides were also reviewed by the pathologist for quality assurance.

3.7.5 Dissemination of Pap smear results

After examining the Pap smears, the results were immediately sent to the clinics in sealed envelops. These results were given and explained to the participants by the attending clinician. Those with cervical abnormalities were treated according to

FHOK standards and given an appointment for a repeat Pap test in six months. Participants with High grade Squamous Intraepithelial Lesions (HSIL) and Cervical cancer CaCx were referred for further medical attention at the provincial and referral hospitals.

3.8 Variables

Variables were captured in the Pap test request form and the questionnaire. All information, apart from the name of the patient was transferred from the request form to the consolidated laboratory form for data processing.

The socio-demographic variables captured were age, marital status, number of children, age at first delivery and occupation. The level of education, religion, history of smoking/ alcohol consumption, previous Pap smear and residence were also captured.

The medical history was on last menstruation, state of the cervix, and contraception.

3.9 Data collection

In the laboratory the Pap smears were screened, processed, and the findings recorded in the Pap test forms (Appendix 2) and the consolidated laboratory report form (Appendix 3). The Pap test form with the results filled was taken back to the clinics for the necessary dissemination and action by the clinicians. The consolidated report and the questionnaire were used for data analysis.

3.10 Data analysis

Data was entered, cleaned and analyzed in Epi Info for windows 2005 version 3:3:2. Double entry of collected data was done during the study to minimize entry errors and missing data. Each questionnaire was given a unique identifier number for proper verification. Analysis included the different types of computations. Frequencies for categorical variables and the mean, median and standard deviation of continuous variables were used to describe the sample. Percentage prevalence was calculated as follows:

$$\text{Percentage Prevalence} = \frac{\text{Total number of cases} \times 100}{\text{Total number of Pap smears}}$$

Chi Square tests and Fisher Exact tests, Odds Ratio and Logistic regression were used to determine the significance of association between the risk factors and the cytological findings.

3.11 Ethical considerations

Approval to do the study was obtained from Jomo Kenyatta University of Agriculture and Technology, Centre for Microbiology Research Scientific Committee (SC), Kenya Medical Research Institute Scientific Steering Committee (SSC) and the National Ethical Review Committee (NERC). Consent was also obtained from the Chief Executive Officer of FHOK where the study was done. Written informed consents (Appendix 5) were obtained from the study participants. The names of the participants were not indicated on the questionnaires. The data was

backed up in a Compact disk and a Flash disk and was only accessible to the chief researcher to maintain confidentiality.

All the laboratory reports were sent to the respective clinicians where the results and the relevant follow ups were done according to the FHOK requirements.

CHAPTER FOUR

4.0 RESULTS

4.1 Socio demographic characteristics

4.1.1 Background of participants

There was a 100% of the response to the questionnaires administered, giving a sample size of 194.The average age of the women seeking Pap smear screening from the three FHOK clinics in Nairobi was 36.8 years (median 37 years), the youngest being 21 years and the oldest 57 years and a mode of 39 years. All women apart from 5 resided in Nairobi and its environs of Kiambu, Thika and Ngong. The five who came from the furthest were each from Dar-es-saalam, Kendu Bay, Siaya, Kericho and Nyandarua.

4.1.2 Awareness

Majority (146) of the women (75.1%) learnt about Pap tests from clinicians while 28 (14.9%), were informed by friends and another 15 (7.8%) got their information from the media. The other 5 (2.6%) learnt about the Pap test by other ways as seen in Figure 4.1 below.

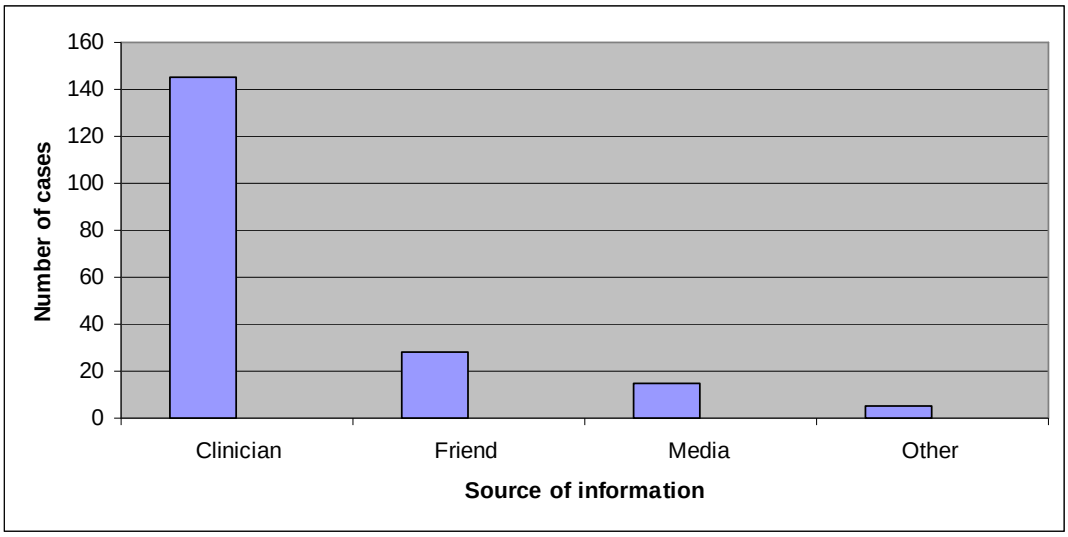


Figure 4.1: Source of information about Pap test form women attending FHOK clinics, Nairobi (April to June 2008)

All the study participants knew about the Pap test. One hundred and thirty four of those who took the Pap test were repeat tests, having previously had one or more than once tests done with a cumulative percentage (Cum Percent) of 69.1%. Only 60 (30.9%) were taking the test for the first time (Table 4.1).

Table 4.1: History of taking a Pap test in study participants at FHOK clinics, Nairobi (April to June 2008)

History of Pap test	Number	Proportion (%)
More than once	84	43.3
Once	50	25.8
Never	60	30.9
Total	194	100

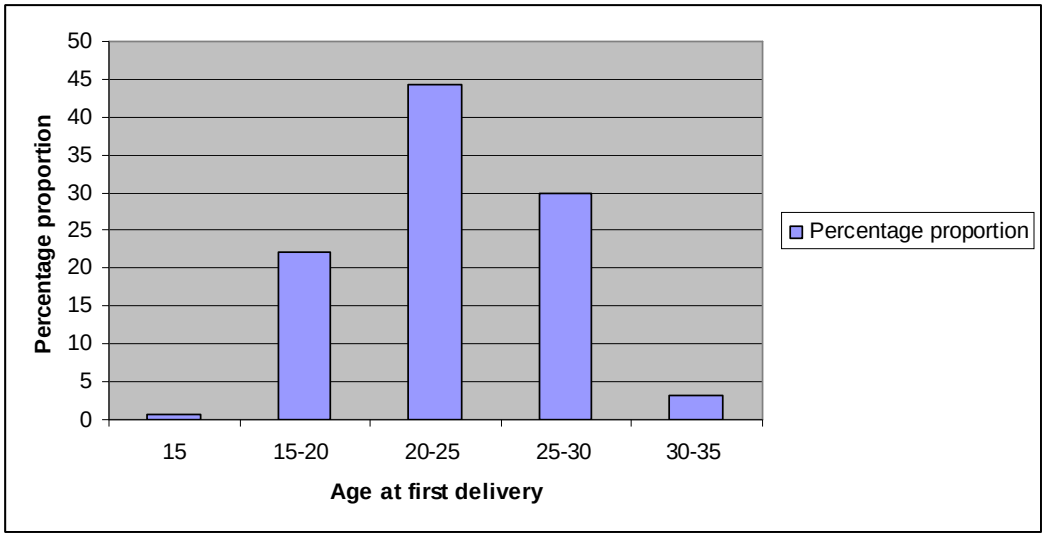
4.1.3 Childbirth

Majority of women had already given birth and only 14 (7.2%) had not experienced childbirth. Of the women who had given birth, majority (56.2%) had 2 or 3 children. Those having 4 children or less were 91.8% and only a few (8.1%) had 5 children or more as shown on table 4.2.

Table 4.2: Number of births per woman attending FHOK clinics, Nairobi (April to June 2008)

Number of births	Number	Proportion (%)
0	14	7.2
1	36	18.6
2	59	30.4
3	46	23.7
4	23	11.9
5	8	4.1
6	3	1.5
7	3	1.5
11	2	1
Total	194	1.00

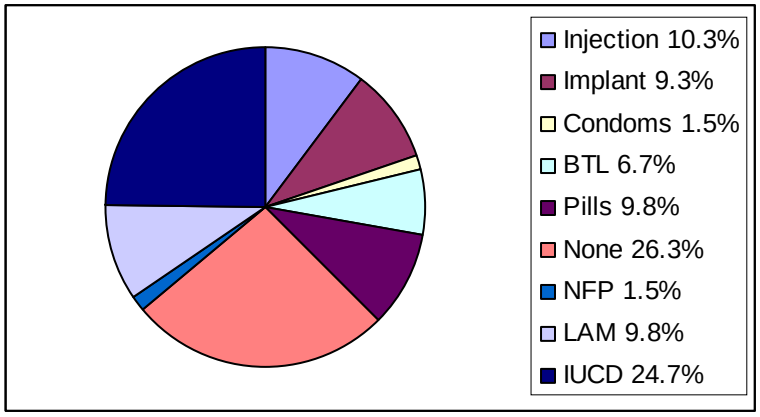
The youngest age at first delivery was 15 years in one woman and the oldest age at first delivery was 35 years. Many women gave birth for the first time between 20 and 30 years (96.1%). Figure 4.2 shows the ages of women at their first childbirth.



<15years – 1, >15-20 years – 40, >20-25 years – 80, >25-30 years – 54, >30-35 years - 6

Figure 4.2: Age at first child delivery for women attending FHOK clinics in Nairobi (April to June 2008)

Majority of women (72.2%) were using various forms of contraceptives ranging form Birth tubal ligation (BTL) to hormonal contraception. Those who never used contraception were either using natural family planning (NFP) or no method at all. Those using lactating amenorrhea (LAM) as contraceptive method were mothers attending routine post natal clinic while some of those not using any method were postmenopausal women. A summary of the birth control methods used is given in figure 4.3.



Key: BTL – Birth tubal ligation. IUCD – Intrauterine contraceptive device
LAM – Lactating amenorrhea. NFP – Natural family planning

Figure 4.3: Contraceptive methods used by women who took the Pap test at FHOK clinics, Nairobi (April to June 2008)

4.1.4 Marital status, education, religion and employment

The FHOK clinics were mostly attended by married women (86.6%) and the rest were single, separated or widowed (Table 4.3).

Table 4.3: Marital status of women attending FHOK clinics, Nairobi (April to June 2008)

Marital status	Number	Proportion (%)
Married	168	86.6
Separated	1	0.5
Single	22	11.3
Widowed	3	1.5
Total	194	1.00

The only unemployed women in the study (14.4%) were housewives. The rest of them were in gainful employment with a sizeable proportion (31.45%) doing business (Figure 4.4). However, all the housewives were married to spouses who had some formal employment or in business. Seventy two (42.9%) of the spouses of the married women were doing business too.

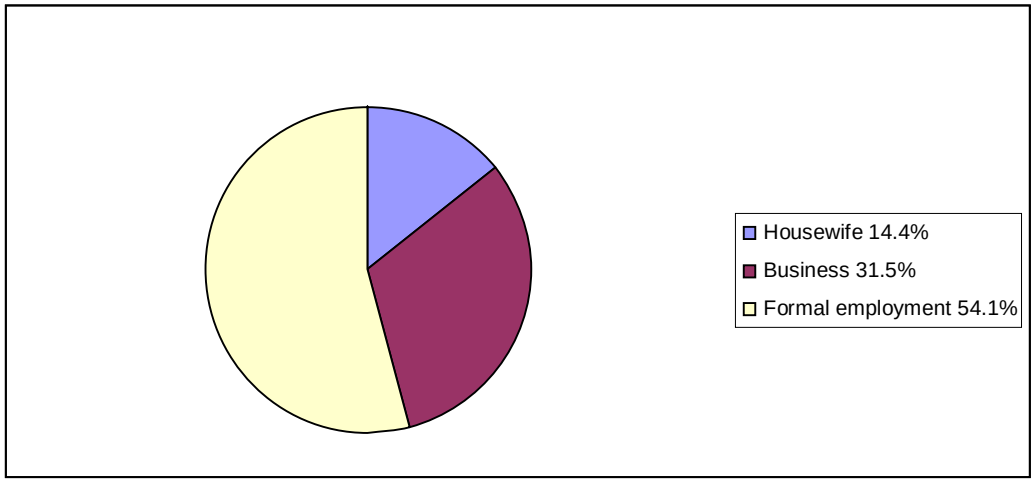


Figure 4.4: Occupation among the participants at FHOK clinics, Nairobi (April to June 2008)

The dominant religious affiliation was Christianity with 184 (94.8%) and the rest 10 (5.2%) were Muslims.

Women who sought the Pap test were all literate with175 (90.2%) having gone through secondary and post secondary education and only 19 (9.8%) had primary school level of education, Table 4.4

Table 4.4: Education level of women attending FHOK clinics, Nairobi (April to June 2008)

Education level	Number	Proportion (%)
Post secondary	84	43.3
Primary	19	9.8
Secondary	91	46.9
Total	194	100

4.1.5 Social lifestyle of women attending FHOK clinics in Nairobi

Five women (2.6%) were smokers while 6 (3.1%) had stopped smoking. This gave a cumulative percent of 5.7% for those who had some experience of tobacco smoking. The rest (94.3%) had never smoked, Table 4.5.

Table 4.5: History of tobacco smoking among the women taking the Pap test at FHOK clinics, Nairobi (April to June 2008)

History of smoking	Frequency	Proportion (%)
Smoking	5	2.6
Stopped	6	3.1
Never	183	94.3
Total	194	100

As shown in table 4.6, there were more participants who had some experience with alcohol consumption compared to smoking, with 47 women (24.2%) taking alcohol

regularly and 26 (13.4%) having stopped. This means that 37.6% of the participants had some experience with alcohol consumption.

Table 4.6: History of alcohol consumption among the study participants at FHOK clinics, Nairobi (April to June 2008)

History of alcohol consumption	Frequency	Proportion (%)
Drinking	47	24.2
Stopped	26	13.4
Never	121	62.4
Total	194	100

All the women who were smokers were also among the 47 who were taking alcohol, Table 4.7.

Table 4.7: History of smoking among the women taking alcohol attending FHOK clinics, Nairobi (April to June 2008)

History of smoking	Frequency	Proportion (%)
Never	39	83.0
Smoking	5	10.6
Stopped	3	6.4
Total	47	100

4.2 Magnitude of inflammation and cervical abnormalities

4.2.1 Inflammation and cervical abnormalities

Between the month of April and June 2008, a total of 194 Pap smears were collected from the three FHOK clinics in Nairobi. The combined prevalence of inflammation and cervical abnormalities was 91 (46.9%). A total of 27 (13.9%) was the prevalence of inflammatory cases due to various infections while 6 (3.1%) out of the total collected were cervical abnormalities. Inflammatory changes due to other causes were 58 (29.9%) and these were mainly with the use of IUCD contraception (33 cases), 1 case of atrophic cervicitis in a post menopausal participant and 24 cases of non specific causes. The rest of the 103 (53.1%) cases were negative for either inflammation or cervical cell abnormalities. The summary of the cytological findings is shown in figure 4.5 below.

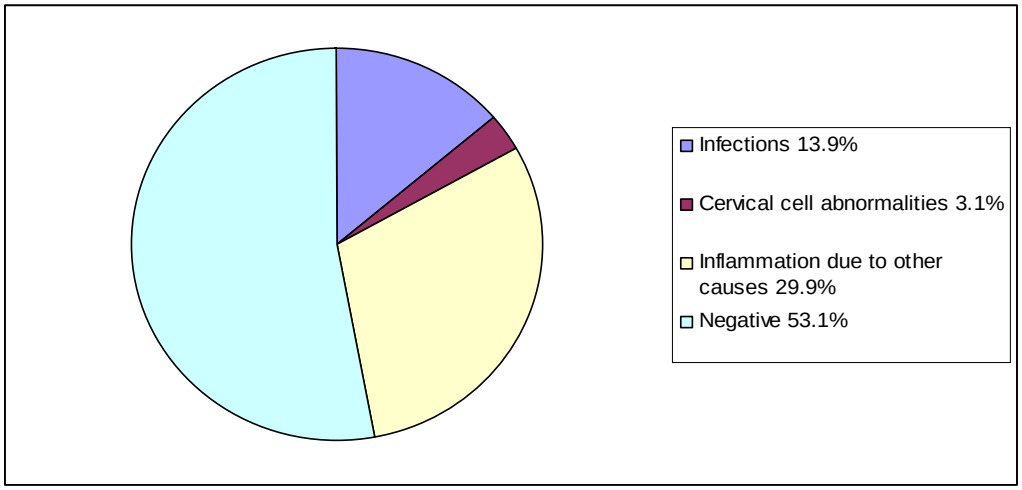


Figure 4.5: Percentage prevalence of cytological findings (N=194) among all study participants at FHOK clinics, Nairobi (April to June 2008)

Among the 91 cases that showed cytological changes of infection or cervical cell abnormalities, the leading cause of infection was due to *Candida* (15.4%). Inflammation due to other causes was found in IUCD users (36.3%). Figure 4.6 below gives a summary of the main causes of cytological changes.

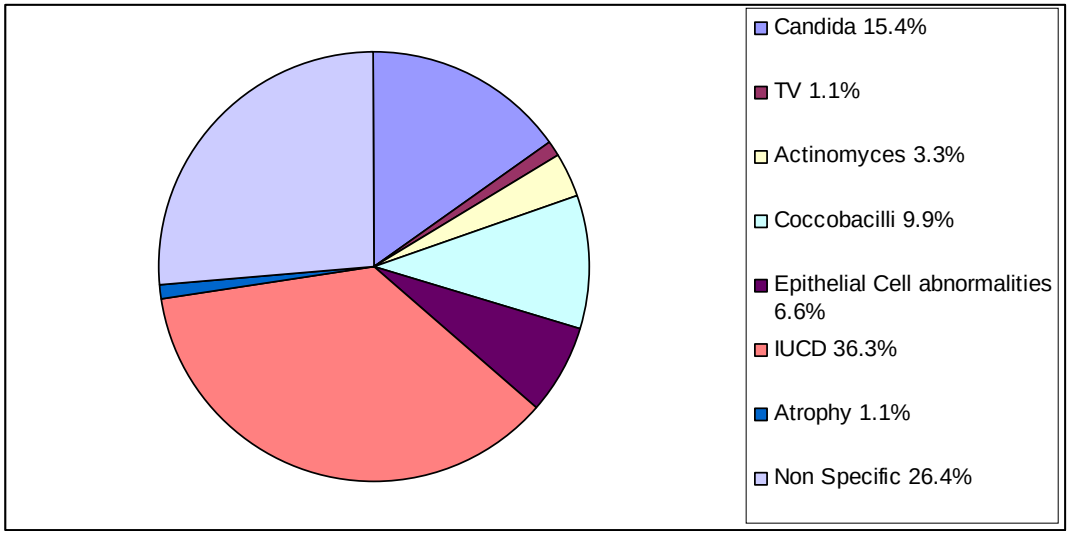


Figure 4.6: Inflammation and cervical abnormalities in all Pap smears (N = 91) among all study participants at FHOK clinics Nairobi (April to June 2008)

Candida appeared in two forms in the smears: the yeast form (conidia) and the fungus form (pseudohyphae). In some cases of *Candida* infection there were no changes in the smear pattern except the presence of the fungus while in other instances there was mild inflammation affecting squamous and endocervical cells. Trichomonad infection in the single Pap smear appeared as grey-green round or elliptical structures and was accompanied by moderate inflammation. Morphologically, *Actinomyces* appeared as a “ball” of filaments, with single, slender filaments spreading peripherally. Coccobacilli were identified as short rods stained

dark blue with the Papanicolaou stain. The organisms were found accumulated on the surfaces of squamous epithelial cells and in some cases observed in the background. Inflammation in IUCD users was characterized by the presence of polymorphonuclear leucocytes (pus cells) in the cellular background. Non specific inflammation meant that pus cells were identified without evidence of accompanying cause of inflammation and in one case of atrophic smear pattern in a post menopausal participant. Epithelial cell abnormalities were lumped together as all neoplastic cellular changes ranging from LSIL to CaCx. These were identified and categorized depending on the cells affected and the severity of the nuclear-cytoplasm changes. Koilocytocis, a cellular change caused by the presence of HPV was identified in two of the abnormal epithelial cell abnormalities.

4.2.2 Inflammatory changes due to infections

The total number of cases of inflammation due to infections was 27. Among these, the leading cause was *Candida* (51.9%) and only one case was due to *Trichomonas* (3.7%). The other two causes of inflammation were *Actinomyces* (11.1%) and Coccobacilli (33.3%). These are shown in Table 4.8.

Table 4.8: Causes of infections among the cases of inflammation (N=27) among women attending FHOK clinics Nairobi (April to June 2008)

Cause of infection	**Number of cases**	**Prevalence (%)**
Candida	14	51.9
Coccobacilli	9	33.3
Actinomyces	3	11.1
Trichomonas	1	3.7
Total	27	100

4.2.3 Inflammation due to other causes

Some Pap smears (N = 58) had evidence of inflammation due to other causes as indicated by the presence of pus cells and reported as mild (+), moderate (++) and severe (+++). Of these 24 (58.7%) were non specific causes and 33(41.3%) were in those participants using IUCD for contraception and one case of atrophic cervicitis. These are summarized in figure 4.7 below.

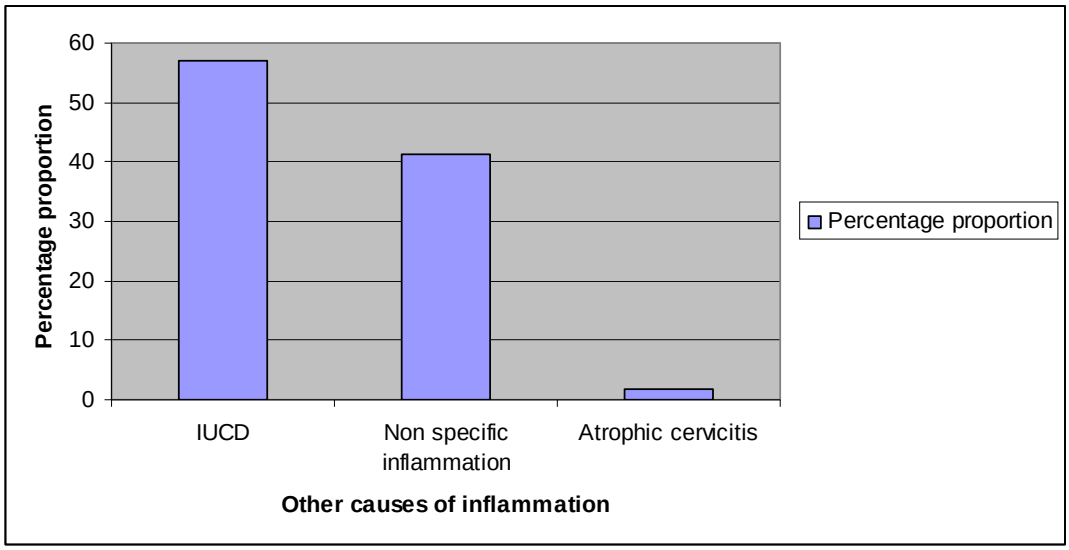


Figure 4.7: Other causes of inflammation (N = 58) among the study participants at FHOK clinics, Nairobi (April to June 2008)

4.2.4 Cervical cell abnormalities

There were 6 (3.1%) cases of cervical cell abnormalities ranging from dysplasia to squamous cell carcinoma. These were 2 LSIL, 3 HSIL and 1 CaCx. Two HPV cellular changes were seen, in one of the LSIL and the other in one of the HSIL cases as shown in Table 4.9. One of the HSIL cases had evidence of AGC – Endocervical.

Table 4.9: Cervical cell abnormalities among all cell abnormalities and inflammations in the study participants at FHOK clinics, Nairobi (April to June 2008)

Cellular abnormality	Number of cases	HPV changes	AGC – Endocervical
LSIL	2	1	-
HSIL	3	1	1
CaCx	1	-	-
Total	**6**	**2**	**1**

4.3 Socio demographic characteristics and the cytological findings

4.3.1 Characteristics of participants with epithelial cell abnormalities

All the 6 cases of epithelial cell abnormalities ranging from LSIL to CaCx were married women. The mean age of women with cervical cell abnormalities was 39.2 ± 9.9 years, the youngest being 25 years and the oldest 51 years and age did not seem to have any influence on cellular abnormalities though half of these women had some history of alcohol consumption, there was no significant association between cervical abnormalities and alcohol consumption.

Statistical significance of association between the socio demographic characteristics and epithelial cell abnormalities were computed using contingency tables, logistic regression and in a few cases by linear regression. The outcomes of relationships were considered significant with the p-value less than. 0.05. Table 4.10 below shows a summary of statistical significance of the findings.

Table 4.10: Statistical significance of relationship between socio demographic characteristics and cervical abnormalities among the study participants at FHOK clinics, Nairobi (April to June 2008)

Socio demographic characteristics among the cellular abnormalities	P-value at 95 % confidence interval	Test	Odds Ratio	Conclusion of significance in association
Age	0.458	Logistic regression	-	Association not significant
Religion	0.967	Logistic regression	-	Association not significant
History of smoking	0.299	2x2 table, Fishers Exact test	3.56	Association not significant
History of alcohol consumption	0.394	2x2 table, Fishers Exact test	1.73	Association not significant
History of Pap test	0.603	2x2 table, Fishers Exact test	0.89	Association not significant
Number of children	0.060	Logistic regression	1.36	Association not significant
Age at first birth	0.967	Logistic regression	1.005	Association not significant
Education	0.5109	Logistic regression	2.28	Association not significant
Marital status	0.417	2x2 table, Fishers Exact test	-	Association not significant

The odds of cervical abnormalities in women who were exposed to smoking was more than three and a half times that of non smokers but the association in this study

was not significant at the 95% level of confidence. The history of alcohol consumption gave higher odds (OR 1.73) with cervical abnormalities but the association again was not significant (95% C.I.) Similarly the odds of cervical cancer and education (OR 2.28) and the number of children (OR 1.36) though high, were not statistically significant (95% C.I.).

The only case of CaCx was in a postmenopausal woman aged 51 years who had given birth 11 times with the first birth having been at the age of 16 years. This woman had never had a Pap test before and had traveled from Siaya with clinical symptoms of per vaginal (PV) bleeding. She first heard of a Pap test from a clinician who advised her to take the test. Socially, the woman was currently taking alcohol but had stopped smoking and was the only one in this category with a primary school level of education.

The women with precancerous abnormalities (LSIL and HSIL) had an education level of secondary and beyond. Although the case of CaCx had primary level of education, education did not seem to have any statistical significance when compared with other cellular abnormalities. Table 4.11 gives a summary of these findings.

Table 4.11: Epithelial cell abnormalities of Pap test and socio demographic characteristics of study participants at FHOK clinics, Nairobi (April to June 2008)

Abnormality	Age	No. of births	Age at First birth	Drinking	Smoking	History of Pap	Education
LSIL	48	6	33	No	No	Once	Secondary
HSIL	40	3	21	No	No	Never	Post sec
HSIL	31	1	26	No	No	Once	Secondary
HSIL	40	2	23	Stopped	No	Once	Secondary
CaCx	51	11	16	Yes	Stopped	Never	Primary
LSIL	25	0	n/a	Yes	No	Once	Post sec

4.3.2 HPV infection

Infection with HPV was found in 2 study cases with epithelial cell abnormalities. There were no cases of HPV in the rest of the women in this study, even in the group with other infections. The HPV infection was significantly associated (p-value of <0.05 at 95% C.I.) with epithelial cell abnormalities.

4.3.3 Other infections and inflammatory changes

There were four other sources of infection identified, namely, *Candida*, *Trichomonas*, Coccobacilli and *Actinomyces*. Most of these infections were not statistically associated with the socio demographic characteristics. However, infection with coccobacilli, *Actinomyces* and TV were only found in married women but the association was not statistically significant. It was noted that 2 of the 3 cases of actinomyces were using IUCD for birth control but the statistical analysis did not show any significant association (at 95% C.I.), although the odds of the infection

was more (OR 2.23) in IUCD users than non users. Similarly, non specific inflammatory changes in the Pap smears were not significantly associated with specific socio demographic characteristics in the study. Table 4.12 below gives a summary of various statistical computations of significance of associations between socio demographic characteristics and inflammatory changes.

Table 4.12: Statistical significance of relationship between socio demographic characteristics and inflammation in Pap smear results of study participants at FHOK clinics, Nairobi (April to June 2008)

Socio demographic characteristics among the inflammations	P-value at 95 % confidence interval	Test	Odds Ratio	Conclusion of significance in association
Marital status	0.3439	2x2 table, chi square (1.36)	1.69	Association not significant
Age	0.0777	Linear regression	-	Association not significant
Smoking	0.337	2x2 table, Fishers Exact test	2.18	Association not significant
Alcohol	0.897	2x2 table, chi square (0.02)	0.96	Association not significant
Taking Pap	0.583	2x2 table, chi square (0.30)	1.19	Association not significant
Number of children	0.518	Linear regression	-	Association not significant
Age at first birth	0.418	Linear regression	-	Association not significant
Education	0.904	Chi square (2df) 0.20	-	Association not significant
Religion	0.396	2x2 table, Fishers Exact test	0.69	Association not significant

The odds of inflammation in Pap smear results in married women was more than one and a half times (OR 1.69) that of the women who were not married, but this association was not significant at 95% level of confidence. The odds of inflammation in Pap smear results for women who were smoking was more than twice (OR 2.18) compared to non smokers but again the association was not significant at 95% confidence interval. Similarly although the odds of inflammation and religious affiliation was low (OR 0.69), the association was not significant at 95% confidence interval.

CHAPTER FIVE

5.0 DISCUSSION

5.1 HPV in cervical cell abnormalities

The Pap smear results of women attending FHOK clinics in Nairobi showed a low prevalence of cervical cell abnormalities and a higher prevalence of inflammatory changes. Squamous cell carcinoma and precancerous changes accounted for 3.1% while inflammatory changes accounted for 43.8%. The cervical cell abnormalities included 2 cases of LSIL, 3 cases of HSIL and one case of squamous cell carcinoma. Human Papilloma virus, the main predisposing cause of precancerous cellular changes was found in 2 out of the 6 cases. A national community based cross-sectional study describing the characteristics of 5453 women between the age of 35 and 60 years conducted in Egypt (Howayda *et al.*, 2007) estimated prevalence of CIN and invasive lesions at 3.1% and 0.04%, while the prevalence of HPV was 2.6% and was positive in 94.3% of cervical lesions confirming that it is the main causing agent. In the Egyptian study, cervical epithelial abnormalities were mostly found in middle income, married, with three and more children, mostly uneducated and not working.

In a previous study carried out at Kenyatta National Hospital Cytology Laboratory (Nyong'o, 1991) out of 280 cases of dysplastic cervical smears, 20% showed evidence of HPV infection. Just like in the previous studies, in this study, the presence of koilocytes (Appendix 7) was used as the cytologic evidence of HPV.

Human papilloma virus infection can be reliably diagnosed cytologically if both nuclear and cytoplasmic changes are present.

In this study, there was some significant association of the HPV and dysplasia. In another study done in Nairobi in 2003 on clients attending a family planning clinic 44.3% of clients were infected with HPV (De Vuyst *et al*., 2003). The Pap smear and DNA extraction, detection and typing were used in the above study, accounting for the higher detection of the virus. Like in our study, the women scored low for the classic risk factors for cervical cell abnormalities (sexual and Pap smear history). In all the studies done, it was evident that there was high prevalence of HPV in women with a high a prevalence of precancerous conditions.

5.2 Awareness of cervical cancer screening

Although the Pap smear has its limitations, it is the best way currently available for preventing the development of cervical cancer (Kurman and Solomon, 1994). The fundamental purpose of screening for cervical cancer is to capture precancerous cytological changes before the progress to fully blown carcinoma. During the period of our study, 5 cases of precancerous cytology were found, and this was an important detection in line with the aim of the screening. These women with dysplasia were on routine screening on advice from clinicians.

Three out of four women who develop cervical cancer each year have never had a Pap smear or not had one within the recommended two yearly intervals (Burkadze and Gulisa, 2004). The case of squamous cell carcinoma in this study had never had

a Pap test before. Although the patient was advised by the clinician to take a pap test, she had already reported to the clinic with clinical signs of PV bleeding. Health professionals play an important role in encouraging women to have a Pap smear. It was evident that many of the women (75.1%) who had a Pap test at the FHOK clinics learned about it from the clinicians. Others learnt about the Pap test from the clinicians while attending the prenatal and postnatal clinics. This was evident in the numerous cases where the Pap smears from the participants were taken during routine postnatal clinics, 6 weeks after childbirth.

Even if a woman is embarrassed about having a Pap smear, research has shown that most women will accept their doctor's advice about having it.

5.3 Inflammation in cervical cytology

During the screening process, encounters with inflammation were very common. In general, the Pap smear is insensitive for the diagnosis of lower genital tract infections, but it may be reasonably specific (www.medscape.com). *Candida*, *Actinomyces*, TV and coccobacilli were the specific causes (13.9%) identified in the pap smears. Vaginal infections are common cause of inflammation of the genital tract in all women; some are associated with sexual activity while others, such as vaginal candidiasis, are not. Normally 10 – 20% cases of candidiasis may be present among women (Leslie, 1995). In this study, *Candida* was the most prevalent (51.9%) among the 27 cases of assorted infections.

Coccobacilli in this study were an overgrowth of a mixture of bacterial flora. This was the second most prevalent (33.3%) among the identified causes of inflammation. Overgrowth of mixed bacterial flora, including *Gardnerella vaginalis*, which overtake the normal *Lactobacillus* bacteria population in the vagina may sometimes be confirmed as bacterial vaginosis after proper microbiological investigation (Until recently "*Gardnerella*" was the term commonly used to designate this infection). A condition rather than a true infection, bacterial vaginosis represents an alteration of the normal environment of the vagina. It is associated with sexual activity but not considered sexually transmitted although an abnormal vaginal discharge and a strong ("fishy") odor usually occur, an estimated 50% of women have no symptoms. If present, the odor tends to be most pronounced directly after intercourse (Leslie, 1995).

Evidence of the presence of sexually transmitted organisms may be found on Pap smears. In some cases, this evidence is specific (e.g., finding a trichomonad), whereas in other cases the evidence may be nonspecific (e.g., inflammatory cells).*Trichomonas vaginalis* is a common protozoan causing sexually transmitted infection among women. In this study there was only one (3.75%) case found.

Actinomyces infection is usually detected in IUCD users (Koss, 1992). The infection was not highly prevalent in this study (11.1%) among the identified causes of infection and more so among the IUCD users. Only one case was observed among the 36 women using IUCD for contraception. This can be attributed to the possibility that many of the IUCD users had taken their Pap tests more than once and therefore

had had treatment previously. However, inflammation characterized by presence of pus cells was evident in 33 (91.6%) of the IUCD users and with a significant statistical association (OR 25.9 at 95% C.I.). This was 41.25% of all the (80) cases of inflammation.

Inflammation is a common finding in Pap smears as was evident in our study. However, the inflammation is only significant if it is obscuring or severe and if any infectious cause is associated.

5.4 Risk factors in socio demography

Socio demographic characteristics of the participants pointed to a study population that had prior knowledge of the Pap test. One of the risk factors associated with cervical cancer was having never taken a Pap test. At least 134 (69.1%) had taken the test before. Most of them (75.1%) had learnt about it from a clinician. The level of knowledge about Pap test in this study group was evidently high. In a national screening program done in Hong Kong (Topical Health Report No. 4, 2004) the better healthy educated and health- conscious women were the ones who got screened. In our study, literacy level was also notably high, with only 19 (9.8%) having primary level of education.

Another risk factor associated with cervical cancer was early age of sexual intercourse. Although the participants were not directly asked about their first sexual encounter, the indicator used was the age at first delivery. The youngest participant gave first birth at the age of 15 years, and 22 (12.2%) of the 181 women had given

birth at an age of 19 years and below. Of note was that the only case of CaCx gave first birth at an age of 17 years.

The other risk factors associated with CaCx were tobacco smoking and taking of alcohol. In this study participants who smoked were 5 (2.6%) while 6 (3.1%) had stopped smoking. Alcohol consumption was, however, more popular with at least 47 (24.2%) being consumers and 26 (13.4%) having stopped. Again there was no significant association between these two habits and cervical abnormality in the study participants (p value >0.05). Although the case of CaCx participant had had an exposure to both alcohol and tobacco smoking and should have given a classic example, the data available could not give statistical significance.

In a population based cross sectional study by Delia and others, examining low-grade cervical abnormalities and which they found compatible with those from studies of more severe cervical lesions, lending added support to the hypothesis that smoking predisposes to development of a spectrum of cervical abnormalities. Women reporting current/recent smoking had an increased likelihood of cervical abnormalities while women who had never smoked but who reported recent passive smoking exposure also had a greater likelihood of abnormal test results (OR 1.4, 95% CI 1.0–2.0) (Delia *et al.,* 1999). In another study in South Africa, alcohol use was found to be associated with an increased prevalence of HPV infection and HPV-16 IgA. Past and current cigarette smoking were associated with reduced levels of HPV-16 IgG antibodies, and current smoking was positively associated with increased levels of HPV-16 IgA antibodies (Dianne *et al.,* 2008). Similar population

studies would be recommended in Kenya especially targeting populations exposed to smoking and alcohol use.

Economically, the majority (166) of participants were in some form of employment and only 28 (14.4%) were housewives with employed spouses. This meant that the participant could afford to pay for the services which cost Ksh. 600.

What came out openly on the religious affiliation of the participants was that only 10 (5.2%) Muslims took the Pap test at FHOK during that period. None of the Muslim participants had epithelial cell abnormalities. Similarly, although all the cases of cervical cell abnormalities were married, marital status gave no significant association in this study (p- value >0.05). There was no literally evidence associating married women to having less exposure to risk factors for CaCx.

5.5 Limitations of the study

The greatest limitation of this study was in capturing some risk factors from the participants, especially, on sexual encounters. The participants who attended FHOK clinics in Nairobi were exposed to the knowledge of cervical cancer screening and could afford to take a Pap test. Sexually transmitted infections were not common although HPV was found in the cases of abnormal cytology. It appears that the women clientele at FHOK in Nairobi may not be from the high risk category.

The current results may not be applicable to the whole of Nairobi city or to other parts of the country (especially the rural areas) because this study was based in an urban setting and a special category of women. Nevertheless, the findings may serve as a model for possible scenarios in other urban centers in the country. Validity of the results rather than their ability to be generalized was a guiding principle for this study.

The fact that this study was based on women seeking private health care services which may be out of reach to many in the urban centers and rural settings made the results not replicable or generalizable. This would mean that the prevalence calculated underestimated the true magnitude of the problem.

CHAPTER SIX

6.0 CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

Cervical dysplasia and inflammatory changes were present in women attending FHOK clinics in Nairobi. The predisposing etiologic factor, HPV, was evident within the attendees of this private health care facility. Out of the 6 cases positive for cervical abnormalities, 2 had HPV.

What the study could not capture well were other predisposing factors such as sexual encounters and high risk sexual behavior. Sexual encounter was pegged on the age at first delivery but the risky sexual behaviour was not well captured. Cases of infections were 13.4% among all the participants and only one had TV, the only case that could be linked to sexual transmission. The participants in this study were a selective category that did not reflect the real situation in the urban and more so in the rural setting.

Most women (85.6%) studied were employed while the rest were married to employed spouses.

Many of the women (90.2%) were educated to secondary school level and beyond while only 9.8% were educated up to primary school level. Most of the women were aware of the Pap test. It was evident then that the captured data portrayed women who knew about Pap smears and had due advantages such as accessing and affording the test. This kind of findings may not necessarily reflect the results of other healthcare facilities in Kenya whether in the rural or urban settings.

6.2 Recommendations

- There is a need to capture all categories of women in the screening of cervical cancer.
- It is evident that precancerous changes and inflammations can be found in women without regard to the socio demographic characteristics and therefore, all women should have access to Pap smear test in order to detect cervical abnormalities in prevention of cervical cancer which is incurable.
- Since FHOK offers private healthcare at a fee, the following would be recommended:

  i) Step up screening awareness, especially, to high risk groups using clinicians and other health personnel.

  ii) Maintain the consistency in screening as evidenced by the number of participants who had previous tests.
- Other private healthcare facilities and the public health care should also be actively involved in the campaign on screening for cervical cancer, so as to capture precancerous cases and inflammations and to treat the women.
- It is only through concerted efforts of both the private sector and the government that the incidence of cervical cancer can be reduced as evidenced in the developed countries.
- The creation of awareness should go hand in hand with the provision of accessible and affordable services.

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APPENDICES

Appendix 1

The 2001 Bethesda System for Reporting Cervical Cytologic Diagnoses

Specimen adequacy
Satisfactory for evaluation
Presence or absence of endocervical or transformation zone components or other quality indicators such as partially obscuring blood or inflammation
Unsatisfactory for evaluation (specify reason)
Specimen rejected or not processed (specify reason)
Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormalities

General categorization (optional)
Negative for intraepithelial lesion or malignancy
Epithelial cell abnormality
Other

Interpretation/result
Negative for intraepithelial lesion or malignancy
Organisms
Trichomonas vaginalis
Fungal organisms morphologically consistent with *Candida* species
Shift in flora suggestive of bacterial vaginosis
Bacteria morphologically consistent with *Actinomyces* species
Cellular changes consistent with herpes simplex virus
Other non-neoplastic findings (optional to report)
Reactive cellular changes associated with:
Inflammation (includes typical repair)
Radiation
Intrauterine contraceptive device
Glandular cells status posthysterectomy
Atrophy

Epithelial cell abnormalities
Squamous cell
Atypical squamous cells (ASC)
ASC of undetermined significance (ASC-US)
ASC, cannot exclude high-grade squamous intraepithelial lesion (ASC-H)
Low-grade squamous intraepithelial lesion (LSIL)
Encompassing: human papillomavirus, mild dysplasia, and cervical intraepithelial neoplasia (CIN) 1
High-grade squamous intraepithelial lesion (HSIL)
Encompassing: moderate and severe dysplasia, carcinoma in situ, CIN 2, and CIN 3
Squamous cell carcinoma

Glandular cell
Atypical glandular cells (AGC)
Specify endocervical, endometrial, or glandular cells not otherwise specified
Atypical glandular cells, favor neoplastic
Specify endocervical or not otherwise specified
Endocervical adenocarcinoma in situ (AIS)
Adenocarcinoma
Other (list not comprehensive)
Endometrial cells in a women 40 years or older

Adapted from. The 2001 Bethesda System: terminology for reporting results of cervical cytology. JAMA 2002;287:2116

Appendix 2

Cytology Pap Smear Test Form

LAB E Cyto No . _____

FAMILY PLANNING ASSOCIATION OF KENYA
P.O. BOX 30581, TEL: 604296/7, 603923/7, FAX: 603928, NAIROBI

CLINIC NAME: _____ **Box** _____ **Tel.**_____

PAPSMEAR TEST FORM

Client Name: _____ **Receipt No .** _____

Age _____ **Client No .** _____

LMP: _____ **Date of smear** _____ **Para:** _____

On Contraceptive: _____
Clinical Summary (Please Include any relevant previous findings)

REPORT

INFECTION		NEOPLASTIC CHANGES			
NIL		CIN (class)			SIL (Class.)
POLYMORPHS		CIN - 0		NORMAL	Normal
T.V		CIN - 1		MILD DYSPLASIA	Low Grade
CANDIDA		CIN - 2		MOD. DYSPLASIA	High Grade
ACTINOMYCETES		CIN - 3		SEVERE DYSPLASIA	High Grade
VIRAL CHANGES		CIN - 4		CA. INSITU (CIS)	High Grade
OTHERS (specify)		CIN - 5		INVASIVE (CA)	Invasive

SUGGESTION

Treat, REPEAT Immediately, AFTER--------Therapy, 3,6,12 Months

COMMENT: **Observe, Refer to sessional Doctor**

Lab. Technologist: _____ **Signature:**_____ **Date:**_____

Family Health Clinic, Nairobi West, Langata/Mbagathi Road Junction

Appendix 3

Consolidated laboratory report

Accession Number: _____

Age: _____ Para: _____ LMP_____ Date of smear: _____ Contraception: _____

Clinical summary:

_____

_____

1. Specimen Adequacy

[ ] Adequate

[ ] Inadequate, Reason – [ ] Scanty

[ ] No endocervical cells

[ ] Hemorrhagic

[ ] Pus cell

[ ] Other, specify _____

2. Result

[ ] Negative for intraepithelial lesion or malignancy

Inflammation

[ ] *Candida*

[ ] T. V.

[ ] *Actinomyces*

[ ] Coccobacilli

[ ] Other, specify

Reactive Changes

[ ] Repair

[ ] IUCD

[ ] Atrophy

[ ] Radiation

[ ] Other, specify

Epithelial cell abnormality

[ ] HPV changes

[ ] ASC - US

[ ] ASC – H

Glandular cell abnormality

[ ] Endometrial cells present > 40 years

[ ] AGC – Endocervical

[ ] AGC – Endometrial

[ ] LSIL

[ ] HSIL

[ ] Squamous cell carcinoma

[ ] AIS

[ ] Adenocarcinoma

Appendix 4

Questionnaire

Questionnaire number_____

1. Residence: _____

2. Occupation: _____ **3.** Spouse's occupation: _____

4. Age at first delivery: _____ years

Please tick whichever is applicable

5. Level of education

[ ] None

[ ] Primary school

[ ] Secondary school

[ ] post secondary school

6. Religion

[ ] Christian

[ ] Muslim

[ ] Other, specify _____

7. History of tobacco smoking

[ ] Never

[ ] Stopped

[ ] Smoking

9. History of alcohol consumption

[ ] Never

[ ] Stopped

[ ] Drinking

10. History of a Pap smear test

[ ] Never

[ ] Once, specify when _____

[ ] More than once, specify when ___

11. Marital status

[ ] Married

[ ] Single

[ ] Divorced

[ ] Widowed

8. How did you learn of a Pap test? [ ] Separated

[ ] Clinician [ ] Media

[ ] Friend [ ] Other, Specify _____

Appendix 5

CONSENT FORM

Study title: DESCRIPTIVE EPIDEMIOLOGY OF CERVICAL DYSPLASIA AND INFLAMMATORY CHANGES IN WOMEN ATTENDING FAMILY HEALTH OPTIONS KENYA CLINICS IN NAIROBI.

Investigator: Raphael Kinuthia Mburu, TM 313-0111/2006
MSc Medical Epidemiology
Institute of Tropical Medicine and Infectious Diseases.
KEMRI / JKUAT
P.O. Box 54840- 00200, Nairobi. Mobile phone, 0722837449

Email, kinuthiaraf@yahoo.com

Introduction

This Consent Form contains information about the research named above. In order to be sure that you are informed about being in this research, we are asking you to read (or have read to you) this Consent Form. The purpose of this consent form is to give you the information you will need to help you decide whether or not to participate in the study. Please read the form carefully. You may ask questions about the purpose of the research, what we would ask you to do, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. This consent form may contain some words that are unfamiliar to you. Please ask us to explain anything you may not understand. When we have answered all your questions, you can decide if you want to participate in the study or not. This process is called 'informed consent. You will also be asked to sign it (or make your mark in front of a witness). We will give you a copy of this form for your records.

Purpose for the Research

We are asking you to participate in this study to help us to find out more about the women who visit the FHOK clinics for a Pap smear test and the results of the tests. The findings from this study will help to establish the women captured in the cervical screening program that has been ongoing in FHOK for many years with the aim of enhancing the program.

Your part in the Research

If you agree to be in this research study today, we will:

- Ask you questions to see if you are eligible to be in the study
- Get your permission to participate in the study by signing this form
- Ask you questions about your personal life
- Take a Pap smear.

Benefits

This study will not benefit you directly but the results that will be obtained will aid in recommendation of how to improve or offer quality screening services to prevent cervical cancer.

If You Decide Not to Be in the Research

You are free to decide if you want to be in this research. Your decision will not affect the health care/service you would normally receive.

Confidentiality

We will protect information about you and your taking part in this research to the best of our ability. We will use a code on the interview form and your name will not appear on any reports.

Leaving the Research

If you choose to be in the study, you can still decide not to complete the interview. If you leave the study, please tell the interviewer why you are leaving.

Your rights as a Participant

This research has been reviewed and approved by the KEMRI/ National Ethical Review Committee and Jomo Kenyatta University of Agriculture and Technology. These committees review research studies in order to help protect participants. If you have any questions about your rights as a research participant you may contact:

- The Secretary KEMRI Ethical Review Committee at Tel. 020-272-2541, or
- The Director ITROMID, Jomo Kenyatta University of Agriculture and Technology, P.O. 62000, Nairobi

Participant Agreement

The study described above has been explained to me. The risks, benefits and procedures involved in the study titled have been read and explained to me. I have had an opportunity to ask questions about the research and they have been answered to my satisfaction. If I have future questions about the research, I can ask the investigator. I volunteer to participate in the research.

_____

Participant's Name (printed)

_____ _____

Signature of Participant **Date**

Thumb print for volunteers who cannot sign_____

If volunteers cannot read the form themselves, a witness must sign here:

I was present throughout the entire informed consent process with the participant. All questions from the subject were answered and the participant has agreed to take part in the research.

_____

Printed Name of Witness

_____ _____

Signature of Witness **Date**

I certify that the nature and purpose, the potential benefits, and possible risks associated with participating in this research have been explained to the above individual.

_____

Printed Name of Person Who Obtained Consent

_____ _____

Signature of Person Who Obtained Consent **Date**

Appendix 6

Pap Staining set up

Pap Smear slide fixed in 95% Alcohol for at least 15 minutes

1. 10 dips	2. 10 dips	3. 4 minutes	4. 5dips
50% Alcohol	**Distilled water**	**Harris Hematoxylin**	**Acid water**

5. 10 dips	6. 1 minute	7. 10 dips	8. 10dips
Distilled water	**Bluing solution**	**50% Alcohol**	**95% Alcohol**

9. 1½ Minutes	10. 10 dips	11. 10 dips	12. 3 minutes
Orange G - 6	**95% Alcohol**	**95% Alcohol**	**Eosin Azure 36**

13. 20 dips	14. 10 dips	15. 10 dips	16. 15 minutes
Absolute Alcohol	**Alcohol/Xylene 50:50**	**Xylene**	**Xylene**

Mount the slide using DPX and a cover slip. Leave to dry.

Appendix 7

Koilocytes indicating HPV infection in LSIL 400x

Gay Family Options.org