EFFECTS OF DEPOT MEDROXY PROGESTERONE ACETATE USE ON CERVICAL CYTOLOGY OF HIV POSITIVE WOMEN AT A COMPREHENSIVE CARE CLINIC IN KISUMU, KENYA

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UNIVERSITY OF NAIROBI

A DISSERTATION SUBMITTED IN PARTIAL FULFILMENT FOR THE AWARD OF DEGREE IN MASTERS OF SCIENCE IN CLINICAL CYTOLOGY

UNIVERSITY OF NAIROBI

2011

DECLARATION:

This is my work and to the best of my knowledge has not been submitted for any other degree course in any other university.

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DEDICATION

To all those infected and affected by HIV and those dealing with terminal illnesses.

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ACKNOWLEDGEMENT

I would like to express my sincere appreciation and great gratitude to all those who have contributed directly or indirectly in enabling me to successfully complete this dissertation.

- To the almighty God for He gave me life and strength to conduct this study and my family for their moral and material support throughout this study.
- My supervisors Dr. Emily Rogena, Prof. Christine Kigondu for their keenness and guidance during the whole study process which has honed my research skills and for their support in supplying the initial materials at the beginning of the study
- FACES team in Kisumu, Dr. Elizabeth Bukusi,programme officer, coordinators, Dr Lisa Dillabaugh, Dr.Patrick Oyaro, Dr.Kate Kidiga, Alinda Ndega,Anrica Atieno-the research assistant who worked tirelessly during the whole study, The laboratory staff, Mr. Albert Okumu, Mr.Raphael Ondondo, Noel Odhiambo,Carol Dande, The information technology team,Mr. Kevin Owino,
- Dr Lucy Muchiri who did the quality assurance check and consultancy.
- Dr. Edwin Waloong who provided me with the impetus to do this study and for his material and moral support.
- Mr. Walter Akello level 5 student at UON who helped in processing of the specimens.
- The lecturers my colleagues, Mmed and Msc classes, for their positive scientific input and constructive criticism.
- Carolyn Imbayi and Sarah Ngugi who endured past open hours in the department giving me time to screen and report my slides.

LIST OF ABBREVIATIONS

AGUS	Atypical Glandular Cells of Undetermined Significance
AIDS	Acquired Immunodeficiency Syndrome
ARV	Anti Retrovirals
ASCUS	Atypical Squamous Cell of Undetermined Significance
BMD	Bone Mass Density
CCC	Comprehensive Care Centre
CD4	Cluster of Differentiation of T lymphocytes
CI	Confidence Interval
CIN	Cervical Intraepithelial Neoplasia
COC	Combined Oral Contraceptive
CPR	Contraceptive Prevalence Rate
Dd	Day
DITRAME	Diminution De La Transmission Mere-Enfant
DMPA	Depot Medroxyprogesterone Acetate
DPX	Distrene Dibutylphalate Xylene
E A	Eosin Azure
ERC	Ethics and Research Committee
FACES	Family Aids Care and Education Services
FFPRHC	Faculty of Family Planning and Reproductive Health
FHI	Family Health International
FP	Family Planning
HAART	Highly Active Antiretorviral Therapy
HEC	Human Ectocervical Cells
HIV	Human Immunodeficiency Virus
HPV	Human Papilloma Virus
HSIL	High Grade Squamous Cell Intraepithelial lesion
ICC	Invasive Cervical Cancer
IUCD	Intra-Uterine Contraceptive Device
KDHS	Kenya Demographic and Health Survey

KNH	Kenyatta National Hospital
LEEP	Loop Electrosurgical Excisional Procedure
LSIL	Low Grade Squamous Cell Intraepithelial lesion
Mm	Month
NICHD	National Institute of Child Health and Human Development
O G	Orange Green
PAP	Papanicolaou
PID	Pelvic Inflammatory Disease
RCTP	Research Care and Training Program
SCC	Squamous Cell Carcinoma
SEROCO	Serocoverter Cohort
SGN	Signature
SILS	Squamous Intraepithelial Lesions
SIV	Simian Immunodeficiency Virus
SOPs	Standard Operating Procedures
SPSS	Statistical Programme for Social Studies
STI	Sexually Transmitted Infections
TZ	Transformation Zone
UNAIDS	United Nations Progrmme on Aquired Immuno-Deficiency Syndrome
UNFPA	United Nations Populations Fund
UNICEF	United Nations Childrens Fund
UON	University of Nairobi
VIA	Visual Inspection with Acetic Acid
WHO	World Health Organization
YRS	Years

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ABSTRACT

Background: The use of the long acting hormonal contraceptive Depot Medroxy-Progesterone Acetate (DMPA) has been increasing in the last decade. The hormonal effects of this contraceptive agent on human cervical epithelium have been underreported in the literature especially for the women infected with the Human Immune-deficiency Virus (HIV). Of concern is the increased risk of developing pre-malignant lesions that lead to cervical cancer if not detected early. Contraception in HIV-infected women can decrease the number of unintended pregnancies and thus reduce maternal death and vertical transmission of HIV. The relationship between DMPA, HIV and cervical cytology is unclear.

Objective: This study set out to determine the hormonal effects of DMPA on cervical smears among HIV positive women by determining cervical changes and lesions of HIV positive women on DMPA. These were compared with cervical changes seen in HIV positive women who were not on hormonal contraception. The relative risk of abnormal cytology associated with the DMPA use for at least 6 months in HIV positive women was estimated.

Design: This was a case control study where cases were selected from among HIV positive women using DMPA while controls were selected from HIV positive women who were not using any method of contraception.

Setting: The study was carried out at the research training and care programme/Comprehensive Care Centre (CCC) clinics at Lumumba Health Centre and Kisumu District hospital sites where HIV positive women receive treatment and follow up. Cytomorphological changes and cytohormonal changes were interpreted at the University of Nairobi (UON) laboratory.

Materials and Methods: HIV positive women who sought services from CCC's clinics were recruited during the study period. A total of 126 HIV positive women were included in the final statistical analysis and were grouped into women who were using DMPA (63 cases) and those who were not on any contraceptive method (63 controls). Structured questionnaires were used to collect clinical and demographic data while CD4 counts data was obtained from participants' files. Cervical smears were then collected and processed for cytological examination and classified according to the Bethesda system 2001. Smears were further examined for squamous cell folding and clustering, intermediate cells predominance, lactobacilli amounts, atrophic pattern, navicular cells and premalignant lesions.

Results: The mean age of DMPA users group (cases) was 27.5 years while that of the DMPA non-users (control) group was 29.5 years. There was a statistically significant difference of progesterone dependent changes observed in DMPA users and non users, P=0.023 OR 2.4(1.1-5.1). Progesterone pattern was seen in 45.0% of DMPA users and 25.4% in DMPA non users. For pattern description, increased amounts of Lactobacilli (28.0.%) were observed more in DMPA users while folding of the cytoplasm was described more in non- users (31.3%.). Generally there was no statistically significant difference of abnormal cytology in DMPA users and non users P=0.080, OR 2.7 (0.9-8.2) when compared to normal cytology. The prevalence of High grade squamous intraepithelial lesion (HGSIL) was 6.3% in DMPA users and 3.2% non-users, P=0.680, Low grade intraepithelial lesion with HPV effect (LGSIL and HPV) was 6.3% in DMPA users and 11.1% in non-users, P=0.344 OR 0.5 (0.2-2.0) and Atypical squamous cells of undetermined significance (ASCUS) 6.3% in DMPA users and 1.6% in non-users, P=0.365, OR4.2 (95% CI 0.5-38.7. Atypical glandular cells of undetermined significance (AGUS) were reported in DMPA users was 3.2% only, P=0.496, OR 2.2 (CI 0.2-25.3).

Conclusion: Certain progesterone-dependent effects like squamous folding of the cytoplasm, crowding, presence of lactobacilli and intermediate predominance were identified more in DMPA users while squamous crowding and folding of the cytoplsmic edges of cells were identified more in the non users. There is no increased risk of cervical intraepithelial neoplasia in patients using DMPA.

Recommendations

1. Further evaluation of abnormal cytology in HIV positive women on DMPA using other methods of cervical cancer screening like liquid based cytology and HPV- DNA testing strategies should be explored.
2. Family Planning services and cervical smear screening should fully be integrated into Comprehensive Care Centre's.

CHAPTER ONE: INTRODUCTION AND LITERATURE REVIEW

1.0: INTRODUCTION

United Nations Programme on Human Immuno-deficiency Virus-Aquired Immuno-Deficiency Sydrome/World Health Organization (UNAIDS/WHO) estimates that 33.3 million people are living with HIV/AIDS world-wide at the end of 2009. In Sub-Saharan Africa the total number of people living with HIV in 2009 was 22.5 million; 68% of the global total. Sub-Saharan Africa has more women than men living with HIV (slightly > 50%) predominantly infected via heterosexual transmission [1]. A higher proportion of women age 15-49 (8.8 percent) than men (5.5 percent) are infected with HIV according to Kenya Aids Indicator Survey (KAIS) 2007, meaning that 3 out of 5 HIV-infected Kenyans are female [2]. There is wide variation in contraception prevalence worldwide ranging from 8% of women aged 15–49 years in Western Africa up to 78% in northern Europe [3]. As a region, sub-Saharan Africa has a contraceptive prevalence, of 22 per cent, women of reproductive age who are married or in union using contraception[3]

In respect to contraceptive practice in women, 32% use female sterilization, 22% use intrauterine devices and the use of oral contraceptive pill at 14%. These figures account for more than two thirds of all contraceptive practice worldwide. In less developed countries 70% of contraception users rely on female sterilization and intrauterine devices in part because they are advocated by healthcare services as a result of cost effectiveness in terms of pregnancy prevention and service provision [4]. In Kenya, the contraceptive prevalence rate is 39.3% with DMPA use reported at 16% with 24.5% having unmet need for family planning [4]. In studies of women with HIV infection, it is estimated that approximately 70% have been sexually active [5]. In a cohort of Irish HIV positive women

only 57% of the sexually active women used a reliable method of contraception [5]. The French Serocoverter Cohort (SEROCO) study on the impact of HIV diagnosis on sexual and contraceptive behaviour found that of the sexually active women 20% were not using any contraception, 24% became pregnant, and 63% of conceptions ended in abortion [6]. Effective contraception use is variable, and unplanned pregnancy frequently reported. In the African Diminution De La Transmission Mere-Enfant (DITRAME) project 39% of women with HIV infection used contraceptives; factors significantly related to contraceptive use were marital status and level of education [7]. The incidence of further pregnancy was 16.5 per 100 women years at risk; 50% of these pregnancies were unplanned and one third terminated by abortion, significant determinants of pregnancy were death of the previous child, cessation of breast feeding, and cessation of postpartum abstinence [7]. Whether hormonal contraception, and in particular COC and DMPA use, alters the risk of HIV acquisition is a critical unresolved public health issue. A large, multi-country study sponsored by the National Institute of Child Health and Human Development (NICHD) to specifically evaluate the relationship between hormonal contraception and HIV acquisition found no statistically significant association between the use of either COC's or DMPA and HIV acquisition [8]. Responding to family planning needs is an important component of care, which includes contraceptive options [9, 10]. DMPA is mostly used by HIV-infected women due to tolerability and limited interaction with antiretroviral therapy [9]. This makes Contraception a major component of Comprehensive Care Centers (CCC).

Studies in *Macaca mulatta* monkeys have shown that experimental Simian Immunodeficiency Virus (SIV) transmission increased almost eightfold in the

progesterone-treated animals a factor that maybe attributable to vaginal thinning. Conversely, macaques treated with oestrogen appeared to be protected against vaginal SIV transmission. This suggests that the hypoestrogenic effect associated with DMPA use may be responsible for the increase in SIV acquisition. Although the dose of progesterone in those primates was not analogous to the DMPA dose used in humans, in a later primate study with DMPA in which progesterone levels were comparable with those in humans using DMPA for contraception, significant thinning of the vaginal epithelium still occurred and was reversible after cessation of progesterone [11]. In humans, DMPA use has been associated with increased acquisition of HIV in sex workers and increased shedding of HIV among sero-positive women although DMPA has not been associated with HIV in large cross-sectional studies of family planning clinic attendees [8]. The mechanism by which DMPA users might be at risk for HIV is not known. However, hydrogen peroxide (H_2O_2)-producing *Lactobacillus* inhibits HIV in vitro and in one report, the acquisition of HIV was significantly higher in women without *Lactobacillus* or with abnormal vaginal flora compared with those with H_2O_2-positive *Lactobacillus* in the vagina [12]. DMPA has been found to induce hyperplasia of the cervical columnar cells, thus increasing the number of target cells at risk for infection [13].

Few studies have evaluated the relationship between DMPA, a progestin-only injectable contraceptive, and cervical neoplasia [14, 15, 16]. A recent meta-analysis found only a slight increase in cervical neoplasia risk associated with long-term DMPA use [14]. A WHO collaborative study to determine whether the long-acting contraceptive DMPA alters risk of cervical cancer conducted in two hospitals in Bangkok, Thailand, and in one hospital each in Chiang Mai, Thailand, Mexico City, Mexico, and Nairobi, Kenya found

no trends in risk with duration of use or times since initial or most recent exposure were observed in a normal population [17]. Women with HIV infection, like other women, may wish to plan pregnancy, limit their family, or avoid pregnancy. Health professionals should enable these reproductive choices by counseling and appropriate contraception provision at the time of HIV diagnosis and during follow up.

1.1. LITERATURE REVIEW

1.1.1 HIV versus Contraception

An estimated 14 million women in sub-Saharan Africa are HIV infected and these women deserve access to evidence-based family planning services. Increasing contraceptive use by HIV-infected women can decrease the number of unintended pregnancies and thus reduce maternal death and vertical transmission of HIV [18]. More than 20 years into the HIV/AIDS epidemic World Health Organization (WHO) has developed evidence-based guidelines on contraceptive eligibility which directs family planning providers and HIV clinicians regarding contraception choices [19]. Unprotected intercourse can result in two life threatening conditions for women in sub-Saharan Africa: pregnancy and HIV/AIDS. The probability of an uninfected woman acquiring HIV with one act of penile–vaginal intercourse ranges from 1/1000 to 1/10, depending on cofactors such as HIV disease severity, presence of concomitant sexually transmitted infections (STI's) or male circumcision status [20,21,22].

Comparably, the probability of becoming pregnant with one act of unprotected intercourse is estimated to be 1/25 to 1/3, dependent upon co-factors such as day of the menstrual cycle and past history of sexually transmitted infections which can result in tubal infertility [23, 24]. Although conception is most likely to occur in the 6 days prior to

ovulation, the timing of this fertile window is unpredictable [23, 25]. Contraceptive use to protect against unintended pregnancy must be used correctly and consistently with every act of intercourse. Fourteen million women are estimated to be infected with HIV in sub-Saharan Africa and the reproductive health statistics for these women are staggering [1]. One in 22 women has a lifetime risk of dying due to pregnancy complications. Although the unwanted pregnancy rate in sub-Saharan Africa is estimated to be 20–40%, only 21% of partnered women are using modern contraception, and an estimated 35% of women have an unmet need for contraception [26, 27]. Contraception provision is cost-effective, can improve the quality of life of HIV-infected women and their families, and can ultimately reduce the number of infants born with HIV [28,29,30,31].

1.1.2. Contraceptive Choices in HIV infection

Contraception is an integral part in HIV CCC's thus, counselors should help each HIV-infected woman assess her contraceptive needs, review all the contraceptive options available to her, and determine whether she and her partner will be able to use a particular method or combination of methods safely, correctly, and consistently [32]. The major issue with the use of COC in HIV-positive patients is interaction with medications that lower the efficacy of the pill [33, 34]. Such patients must be advised to use condoms at all times along with the pill with double the dose or tricycling. The COC interacts with liver enzyme-inducing drugs. These drugs when acted to by these enzymes become substrates of the cytochrome p450 CYP 3A4 system of enzymes present in the microsomal system of hepatocytes in the liver and enterocytes in the small intestine. Antiretroviral drugs that induce cytochromes—for example, ritonavir, nevirapine, increase the hepatic metabolism of hormonal contraception. Inhibitors of antiretroviral

drugs cause decreased clearance and increased plasma concentrations of substrate drugs. When both drugs are substrates their interaction is more uncertain and may result in increased or decreased plasma concentrations. Some drugs exhibit two or all three of these properties—for example, efavirenz [35]. Implantable contraceptives have been found to be highly acceptable in Kenya, but no data exist regarding their use in HIV-infected women. Implants could be a highly desirable method since placement requires minimal skill, and the implants are long-acting [36].

Many studies have investigated contraceptive use by HIV infected women. One of the most persistent questions about the intrauterine contraceptive devices (IUCD's) is whether it increases the risk of pelvic inflammatory disease (PID). Infectious organisms, most often those causing gonorrhea or chlamydia, are the direct cause of PID. The majority of evidence indicates that a woman who does not already have an STI cannot get PID just from having an IUD inserted [38]. Complications of PID can be severe. PID can permanently damage the lining of the fallopian tubes and may partially or totally block one or both tubes enough to cause infertility. However, well-designed studies find no significant increase in infertility associated with IUCD use [37]. WHO guidelines on medical eligibility criteria informs programs and providers that IUCD's should not be inserted in women with current purulent cervicitis, chlamydia, or gonorrhea, or in women who are at very high individual risk of chlamydia or gonorrhea, therefore performing a STI risk assessment and physical examination is essential to safe use of IUCD's [19]. Thus, IUCD's are readily acceptable and should be adopted by the majority of patients. IUCD's may be extremely beneficial than the combined oral contraceptive (COC) because of reduced pill burden and instant reversibility [38].

Depot medroxy-progesterone acetate (DMPA) is another good contraceptive method of choice due to good compliance rate. DMPA requires less user participation and has a lower rate of first-year failure associated with typical use (0.3–3.0%). However it has a setback; this product is thought to cause some reduction in bone mineral density (BMD) as does HIV and its treatment. Notably, the loss of BMD seen among current users of DMPA is reversible following cessation of use. Results of two cross-sectional studies included in the meta-analysis, one in postmenopausal women and the other in reproductive-age women showed that BMD in former users of DMPA and Mirena (a progesterone based contraceptive) was not significantly different from that of never-users [39,40,41,42]. Patients to be given this treatment should be chosen carefully and need follow-up. Where a family is complete, permanent methods (for the male, vasectomy and for the female, sterilization) are appropriate. Hormonal contraceptives are the most popular and most effective nonsurgical methods for spacing and fertility control in the world. However, there is concern about the link between hormonal contraceptives and the risk of cancer, including cervical cancer [17,43].

1.1.3. Cervical Neoplasia and HIV Infection

Cervical cancer is the second most common cause of cancer mortality in women worldwide. Cervical cancer incidence has decreased in developed countries, where cervical screening is widespread. However, it remains common in less developed countries where access to screening is limited, and is the leading cause of cancer death among women in Africa [13,44,45,46]. It is accepted that the necessary cause of cervical Squamous Intraepithelial lesions (SILs), which are precursors of invasive cervical cancer, is Human Papillomavirus (HPV) infection [47, 48]. It has also become clear that HIV-

positive women carry an increased risk of persistent genital HPV infection and therefore have an increased risk of HPV-associated lower genital tract neoplasia, particularly Cervical Intraepithelial Neoplasia (CIN). Higher incidence and prevalence of CIN have been reported in HIV-1 positive women [45, 49, 50]. Other established risk factors for CIN or cervical cancer include smoking, increasing parity, early age at first intercourse, multiple sexual partners, and infection with other sexually transmitted diseases [14, 15, 51, 52].

In recognition of the increased risk of Invasive Cervical Cancer (ICC) in HIV-positive women cervical cancer was included as an AIDS defining illness in 1993 [53]. Since then there have been a number of case reports of rapidly progressing cervical cancer in HIV-positive women [53, 54]. In the first year following the Centers for Disease Control and Prevention (CDC) expanded case definition 1.3% of the women reported with AIDS in the United States were reported as having cervical cancer [55]. Hormonal contraceptives also seem to increase the risk of cervical cancer in most populations studied [56, 57]. A recent meta-analysis by the International Collaboration of Epidemiological Studies of Cervical Cancer included 24 studies conducted worldwide. It found elevated risk of cervical carcinoma associated with both oral and injectable contraceptives, increasing with duration of use [58]. Some studies of populations in sub-Saharan Africa, however, have not shown an increased risk [16].

The role of HIV in the etiology of cervical cancer is also unclear, especially in Africa. Immuno-suppression is a risk factor for HPV infection and/or detection, and there is consistent evidence that HIV-positive women have higher prevalence of HPV infection, more persistent infection, and resulting higher rates of pre-invasive cervical lesions [50, 51, 58,59,60,61]. However, the evidence is mixed for an increased risk of invasive

cervical cancer (ICC) associated with HIV [43]. In the United States and Europe, large studies have shown 5- to 8-fold increases in the risk of ICC associated with HIV infection or AIDS [62, 63]. In contrast, in a study of South African women between 1998 and 2001, Moodley and coauthors found increased risk associated with HIV for Low-grade Squamous Intraepithelial Lesions (LSIL) and High-grade Squamous Intraepithelial lesions (HSIL), but no significant increase in the risk of ICC [odds ratio (OR), 1.17; 95% confidence interval (CI), 0.75-1.85] [64]. Several authors have suggested that in Africa, where treatment for HIV is limited, the life span of HIV-positive women may be too short for ICC to develop [44,45,60,62].

In another recent study from South Africa, HIV-1 infection was associated with increased risk of cervical cancer (OR, 1.6; 95% CI, 1.3-2.0;) [65]. In a cross-sectional study conducted in Senegal of commercial sex workers and women presenting to an outpatient infectious disease clinic between 1994 and 1998, HIV was associated with both HSIL and ICC (ORs of 3.7 and 6.7, respectively, for HIV-1 infection, and 7.1 and 16.0 for HIV-2 infection). However, that study was limited by a small number of invasive cancers and lack of histological confirmation of disease status. Other studies have also suggested higher risks associated with HIV-2 than with HIV-1 [45, 63].

The WHO Collaborative Study of Neoplasia and Steroid Contraceptives performed a case-controlled study in four countries, including Kenya. It was found that the relative risk of invasive cervical cancer in women who ever used DMPA was estimated to be 1.11 (0.96—1.29). There were no trends observed in risk with duration of use or since initial or most recent exposure in the general population [17]. Another study carried out in 325 women using progesterone only pills, DMPA or Norplant implants for

3 years or more also failed to find any significant cervical changes during use. The exception was the fact that there was a slight increase in the prevalence of intraepithelial lesions and higher incidence in HIV positive women[45, 66].

1.1.4. ARV and cervical changes

The gaps in knowledge regarding biologic interactions between contraceptives and HIV and antiretrovirals (ARVs) need to be closed and knowledge gained applied to practice. Previous reports have suggested that 20% to 35% of HIV infected women without previous evidence of cervical disease will develop SIL within 3 years [50, 66]. Highly active antiretroviral therapy (HAART) has greatly reduced morbidity and mortality in HIV patients, but its effect on the evolution of cervical cytological changes in HIV-positive women is full of controversy [67,68,69,70]. Currently, HAART is recommended when CD4 level is lower than 350 cells/mm^3 but it is not known whether HAART could have an effect on the evolution of cervical cytological changes when the immunological status is above 350 cells/mm^3 [71].

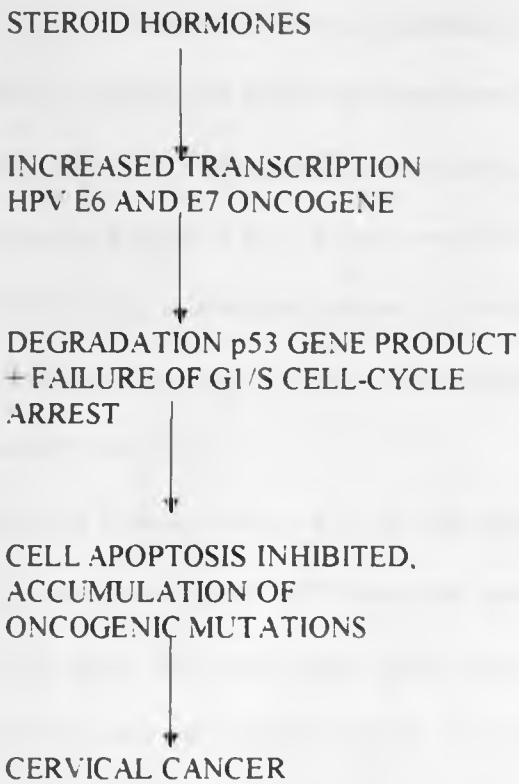
Significant progress has been made in the development of HAART. With the advent and widespread use of combination HAART, AIDS-associated mortality and morbidity have fallen dramatically in industrialized countries [72, 73, 74,75,76,77]. In developed healthcare systems where combination HAART is available, the prospect for long-term survival from HIV/AIDS has been demonstrated and consequently preventive health care strategies become relevant[78]. For HIV infected women, this means consideration of the risk to the cervix. In resource-poor healthcare settings the non availability of combination HAART means that women are at risk of dying early from AIDS and, although the risk of cervical cancer is significantly increased, death from opportunistic infections is likely

to precede cervical pathology. WHO sponsored a study on systematic review on the effect of HAART on CIN in women with HIV which revealed that CIN has not been adequately addressed by the studies in this review[79]. There is still a need to conduct large multicentre trials to address this question. The current recommendations of close cervical cancer screening surveillance in women infected with HIV should still be followed even in women on HAART until there is enough evidence to suggest otherwise[79].

1.1.5. DMPA use and Uterine Cervical Cytology

Data with respect to cytological alterations during the extended use of progesterone only contraceptives are scarce. In one study, in amenorrheic women using DMPA, it was observed that the chronic use of DMPA was associated with cervical cytology that was inconclusive or of difficult diagnosis [80]. These investigators reported an atrophic cell pattern with various grades of nuclear atypia, a finding also reported in cervical cytology examinations carried out in the puerperal period in which the action of progesterone is similar. A limitation of many previous studies is the lack of information concerning the role of HPV with steroids in cervical carcinogenesis. Moodley, et al. suggested that steroid hormones may enhance the expression of two HPV oncogenes, E6 and E7 by influencing gene function by inhibiting the p53 gene-mediated cell apoptosis[81].

Proposed interaction between steroid hormones, HPV p53 oncogene in cervical cancer [81]



Several epidemiologic studies have established a strong link between infection with high-risk types of HPV (16, 18), precursor lesions and cervical cancer [82, 83, 84]. The E6 and E7 early genes of HPV 16 encode the main transforming proteins [85]. The protein products of these early genes interfere with the normal function of the products of tumor suppressor gene p53 and retinoblastoma protein (pRB). The E6 gene product binds to p53 tumor suppressor gene product and promotes its degradation [85, 86, 87]. It has been established that certain nucleotide sequences of HPV type 16 offer responsiveness to glucocorticoids and progesterone [88]. Pater et al. reported that HPV was capable of oncogenic transformation of baby rat kidney cells in the presence of progesterone

(norgestrel) [89]. Subsequently, in a study involving Human Ectocervical cells (HEC) and HPV 16 containing three consensus glucocortoid response elements (16GREcs), it was shown that viral E6-E7 oncogene RNA was increased by hormones substantially more in HEC-16GREcs than in wild-type HPV 16-immortalized HEC [90]. Progesterone has been shown to increase HPV 16 E6/E7 oncogene transcription in HPV 16 containing cell lines, while the progesterone antagonist RU 486 has been shown to abrogate this effect [91]. The results of the WHO Study of Neoplasia showed an overall relative risk of 1.2 in women who had used the long acting DMPA. Further, a risk estimate of 9 was reported from one participating center (Chile) [92].

Another study evaluated the risk of cervical cancer in women exposed to oral contraceptives in association with HPV positivity and found that risk of invasive squamous cervical cancer for women who tested positive for HPV DNA is increased three-fold if they have used oral contraceptives for 5 years or longer [93]. Castle et al. found a small increased risk of CIN2 and CIN3 with current injectable contraceptive use [15]. A recent meta-analysis found only a slight increase in cervical neoplasia risk associated with long-term DMPA use (5years) [14]. DMPA has been found to induce hyperplasia of the cervical columnar cells, thus increasing the number of target cells at risk for infection [13]. Hormones influence cervical epithelial differentiation and maturation [80, 93, 94]. DMPA decreases cell maturation and promotes the appearance of an atrophic epithelium, which could make histologic features of CIN more difficult to detect among DMPA users [95, 96, 97]. Harris et al. found that DMPA use does result in thinning of the vaginal epithelium, perhaps reflecting the loss of glycogen [98].

One other study commented that atrophic epithelium may be more susceptible to damage, making it more vulnerable to HPV infection and could explain finding that DMPA use was positively associated with oncogenic HPV infection [97].

1.2. RATIONALE

The cytomorphology of cervical smear of HIV positive women on DMPA can be confusing; the progesterone pattern imparts an atrophic cell pattern with various grades of nuclear atypia thus masking abnormal cytology leading to possible misdiagnosis [80]. The effect of DMPA on cells of the cervix is not clear in HIV positive women. The hormonal effects of injectable contraceptive agents on cervical epithelium are underreported in the literature especially for the long acting implantable progesterone-only contraceptive or injectable DMPA [99]. Few studies have evaluated the relationship between DMPA, a progestin-only injectable contraceptive and cervical neoplasia [14, 15, 16].

Moreover studies that examine the risk of cervical neoplasia and/or carcinoma in DMPA users report contradictory results for example, one study that specifically addressed the risk of cervical cancer associated with DMPA among oncogenic HPV-positive women found no association, whereas Castle et al found a small increased risk of CIN2 and CIN3 with current injectable contraceptive use. This is in contrast to another finding of an inverse association between DMPA use and both CIN1 and CIN2-3 or carcinoma among oncogenic HPV–positive women [15, 16, 98]. A recent meta-analysis found only a slight increase in cervical neoplasia risk associated with long-term DMPA use (5years) [14]. African studies on the effect of DMPA on cervical cytology in HIV population are lacking and no local studies have been published.

Contraception plays an important role in Comprehensive HIV management since it increases identification of unrecognized HIV infected women in need of contraception. CDC guidelines recommend that routine baseline prenatal screening should include an HIV test for women residing in communities with a high incidence of HIV infection among women of child bearing age [100]. This expands the delivery and benefit of antiretroviral therapy and decreases mother-to-child transmission [98]. Health care providers thus need to be well versed in the contraceptive options available to women and the clinical issues related to their use. The great majority of HIV infected women are in their reproductive age, and no decrease in fertility has been clearly demonstrated [101]. Therefore, contraceptive methods offered should consider reproductive wishes, interaction with HIV disease, anti-retroviral therapy, and other commonly used medications.

Hormonal contraceptives are the most popular and most effective nonsurgical methods for spacing and fertility control in the world. However, there is concern about the link between hormonal contraceptives and the risk of cancer, including cervical cancer [17,32]. Studies summarized in 2006 IARC monograph show that there is sufficient evidence in humans for the carcinogenicity of COC's and sufficient evidence in experimental animals for the carcinogenicity of ethinylo estradiol plus ethynodiol diacetate and mestranol plus norethynodrel. The overall evaluation was that COC's are carcinogenic to human.[101] For the progesterone based contraceptives, IARC has documented that there is inadequate evidence in humans for the carcinogenicity of progestogen-only contraceptives but there is sufficient evidence in experimental animals for the carcinogenicity of Medroxy-progesterone acetate. The overall evaluation was that

progestogen-only contraceptives are possibly carcinogenic to humans. [101]. DMPA has been found to induce hyperplasia of the cervical columnar cells, thus increasing the number of target cells at risk for infection [13]. This issue is particularly relevant because DMPA is a common form of contraception used by HIV-infected women due to tolerability and limited interaction with antiretroviral therapy [102].

Cancer of the cervix is an AIDS defining malignancy common in Kenya particularly in HIV positive women co-infected with HPV. It has also become clear that HIV-positive women carry an increased risk of persistent genital HPV infection and therefore have an increased risk of HPV associated lower genital tract neoplasia, particularly CIN. Higher incidence and prevalence of CIN have been reported in HIV-1 positive women [45,49,50,51]. This study was undertaken to establish if there exists a relationship between DMPA use and abnormal cytology in HIV positive women. The outcome may help the health provider in better management of these women.

1.3. RESEARCH QUESTION

What are the differences in the cervical cytology of DMPA and non-DMPA use in HIV positive women in Kisumu, Kenya?

1.3.1. Objectives

Broad objective was:

To determine the effects of Depot Medroxy-Progesterone Acetate use on cervical cytology of HIV positive women

Specific Objectives were:

1. To determine cervical changes and lesions of HIV positive women on Depot Medroxy-Progesterone Acetate (DMPA).
2. To compare these cervical changes with changes seen in HIV positive women not on hormonal contraception.
3. To estimate the statistical significace of abnormal cytology associated with the use of DMPA for at least 6 months in HIV positive women.

CHAPTER TWO: METHODOLOGY

2.1. Study design

2.1.1. Study Area

This was a case control study.

The study was conducted at the Research Training and Care Program/Family Aids Care and Education Services (RTCP/FACES) comprehensive care clinic in Lumumba Health Centre -Kisumu. Kisumu is in Nyanza Province in Western Kenya, on the edge of Lake Victoria. It has a population of approximately 505,000; HIV prevalence is 15.3% [2]. Lumumba Health Centre is Kisumu's busiest municipal maternity facility. Services are provided free of charge to the general population. These include provision of comprehensive care to HIV positive individuals: prevention, diagnosis and treatment of opportunistic infections, provision of antiretroviral therapy to both adults and children, provision of family planning services in the Family Planning (FP) clinic. Laboratory investigations offered include CD4 levels, liver and renal function tests, hematocrit, urinalysis and testing for serum cryptococcal antigen, syphilis, malaria parasites and pregnancy testing. At the family planning clinic 38% of the women use modern contraceptives of which 40% use DMPA as a contraceptive of choice. On average 15 HIV positive women attend the FP clinic per day. Provider initiated cervical screening is performed at the facility using the Visual Inspection with Acetic Acid (VIA). Colposcopy and Loop Electrosurgical Excision Procedure (LEEP) are performed on women with abnormal cytology.

Kenyatta National Hospital (KNH) is the largest referral hospital serving East and Central Africa.KNH is also a teaching hospital for medical and paramedical students and serves as the teaching hospital for the University of Nairobi (UoN).

2.1.2 Study population

HIV positive women who were attending the Research Training and Care Program/Family Aids Care and Education Services (RTCP/FACES) comprehensive care clinic in Lumumba Health Centre- Kisumu. All participants between the ages of 18yrs and 49 yrs were counseled and consent form administered.

2.1.3 Study eligibility criteria

2.1.3.1. Inclusion criteria for cases were:

- HIV positive women
- Aged between 18-49 years
- Ability to consent to participate in the study
- CD4 cell counts $\geq$ 350cells/mm^3 within the previous 3 months
- Asymptomatic for AIDS
- On DMPA for at least 6 months
- No history of uterine cervical neoplasia
- No history of total hysterectomy

Inclusion criteria for controls were:

- HIV positive women
- Aged between 18–49 years
- Ability to consent to participate in the study
- CD4 cell counts ≥ 350cells/mm^3 within previous 3 months
- Asymptomatic for AIDS
- Not on any steroid contraception
- No history of uterine cervical neoplasia
- No history of total hysterectomy

2.1.3.2 Exclusion Criteria for cases and controls were:

- Women on active treatment for tuberculosis
- Have history of uterine cervical neoplasia
- Have history of total hysterectomy
- Pregnant or Lactating
- CD4 counts < 349cells/mm^3 within the previous 3 months
- Douched within 48hrs prior to sample collection
- Not consented to the study
- Aged less than 18 and more than 49 years
- Women on ARV'S

2.1.4. Sample Size determination

The prevalence rate of DMPA use is 16%.Using this prevalence rate the sample size was calculated using the formula below [103]

$$n = (\frac{r+1}{r}) \frac{(\bar{p})(1-\bar{p})(Z_{\beta} + Z_{\alpha/2})^2}{(p_1 - p_2)^2}$$

For 80% power, Z_{β}= 0.84

For 0.05 significance level, $Z_{\alpha/2}$=1.96

r=1 (equal number of cases and controls)

The proportion exposed in the control group is 16%, $\bar{p}$ = 0.218

p1 is the proportion of cases exposed is 0.67 with p1-p2= 0.2

Odds ratio of ≥ 2.0

n= 67 per group

2.2. Recruitment and Counseling

All HIV positive patients 18 yrs to 49yrs attending the FACES clinic were sampled. Cases were matched for age ± 2.5years. These were HIV positive women on DMPA for at least six months. Suitable study cases were sampled from the records in the family planning (FP) clinic by the research assistant. Data retrieved from the files included CD4 levels and duration of DMPA use. Those who were coming in for the first time to seek family planning services were enrolled as the controls. All participants were counseled by the research assistant working at the CCC and consent form administered, Appendix 1. The participants were all women who gave consent to participate in the study at the clinic. The benefits of the study were communicated to the participants. All participants were asked about the contraceptive method of choice. Demographic and medical information was obtained from the study participants using a standard

questionnaire Appendix 2. Abnormal cytology (any smear on microscopy that was reported as low grade intraepithelial lesion-LSIL or high grade intraepithelial lesion - HSIL or Squamous Cell Carcinoma (SCC) was communicated to the clinician for further management of the client.

2.3. Specimen Collection

A physical and gynecological examination was performed on each study participant by both the clinician and nurse manning CCC clinics followed by collection of the cervical smear while there ensured proper labeling sample collection and fixation. A warm sterile speculum was inserted in the cervix to visualize the transformation zone (TZ) and cervical os. Specimen for cervical smear was obtained from the TZ of the cervix using a cyto-brush. The specimen was smeared onto a clean labeled glass slide then fixed in 95% ethanol and sent to the cytology laboratory for analysis. The Bethesda system was used to classify the cervical smears [114] (Appendix 3). The smear was considered satisfactory if it was composed of ectocervical, squamous metaplastic cells and endocervical cells reflecting the TZ of the cervix. External quality control was done by having every 10^{th} cervical smear re-examined by a blinded pathologist.

2.4. Biosafety measures and smear processing procedures

During collection of samples, the clinician nurses and research assistant ensured that aseptic procedures were employed. Personal protective clothing was worn and sterile equipment used to ensure that cross contamination was avoided. Protective gear was worn during processing and storage of samples. All the cervical smears collected were processed for direct microscopy using the pap staining technique [115] (Appendix 4). All

slides were reviewed by a consultant pathologist supervising the study. The Bethesda system 2001 was used in the reporting of the cervical smears.

2.5. Quality Assurance

The study was conducted by well trained suitably qualified personnel during recruitment, specimen handling and analysis of the results. Strict adherence to protocol during sample collection preservation and processing was followed. Aseptic technique was adhered to and reagents were prepared in accordance with standard operating procedures (SOPS). All the slides were reviewed by a consultant pathologist supervising the study. The Bethesda system 2001 was used in the reporting of the cervical smears.

2.6. Ethical Consideration

Approval for the study protocol was sought from the Kenyatta National Hospital/University of Nairobi (KNH/UoN) Ethical and Research Committee (ERC) prior to commencement of the study. Informed consent was obtained from each study participant. Clients were subjected to a comprehensive questionnaire regarding the use of contraceptives. Sample collection was collected according to standard procedure. Confidentiality of the results was maintained. All cases and controls were coded. Abnormal results were communicated to the clinician/caregivers. Participants in whom disease was diagnosed were counseled, treatment provided and follow up done by the clinic doctor. There was no prejudice on recruitment and treatment of cases and controls.

2.7. Data Management and Analysis

Data was collected and stored in hard cover books, spreadsheets and in soft copies. Data entered in hard cover books was stored in a lockable cabinet while those saved in soft copies were password protected. Only authorized persons were allowed to access the information. The data was cleaned for errors and inconsistent (conflicting) answers, missing entries and duplicate entries hence ensuring high quality data. The data was captured on Statistical Package for Social Sciences (SPSS) version 17. Analysis was done using the SPSS statistical package version 17.0. Summary statistics was determined during the analysis and presented as proportions and percentages in the form of tables and graphs. T-test was be used to compare difference in mean for two groups for continuous variables. Chi-square test of independence was be used to assess the association between two nominal or categorical variables. Odds ratios were used to measure the magnitude of association while 5% level of significance with p-values of <0.05 was be considered to be significant. Fisher's exact test was used in place of chi-square test in cases where the sample size was very small (counts less than 5).

CHAPTER THREE: RESULTS

3.1: Demographic characteristics of study participants

This study enrolled a total of 140 HIV positive women. Eight were excluded from analysis either because their smears were inadequate, too thick or too thin to be reported or obscured by staining scum. Smears from six DMPA users were further excluded from analysis because they had used DMPA for less than six months. Final analysis was done for 63 cases and 63 controls reaching a sample size of 126 women. The cases in the study were current users of DMPA while the controls were non-users. The cases had a mean age of 27.5(±5.6) years while the controls had a mean age of 29.5 (±7.7) years old. The cases also had a median CD_4 level of 674cells/mm^3 (IQR 550-935 cells/mm^3) while that of the controls was 575cells/mm^3 (IQR 468-724 cells/mm^3. Both the DMPA users and DMPA non- users had a high proportion of women who were married 66.7% and 60.3% respectively (Figure 1).

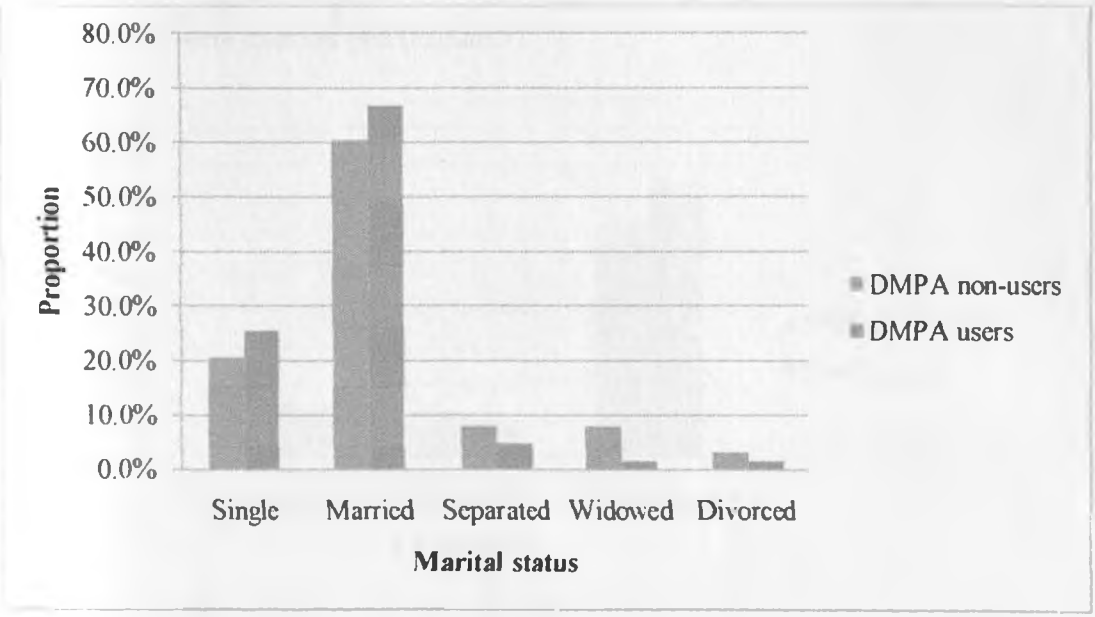


Figure 1: Marital status of DMPA users (n=63)and DMPA non- users (n=63)

Majority of the women had had a primary level of education. Of the DMPA users 52.4% had attained a primary level of education while for the DMPA non users 54.0% had attained the same level of education (Figure 2).

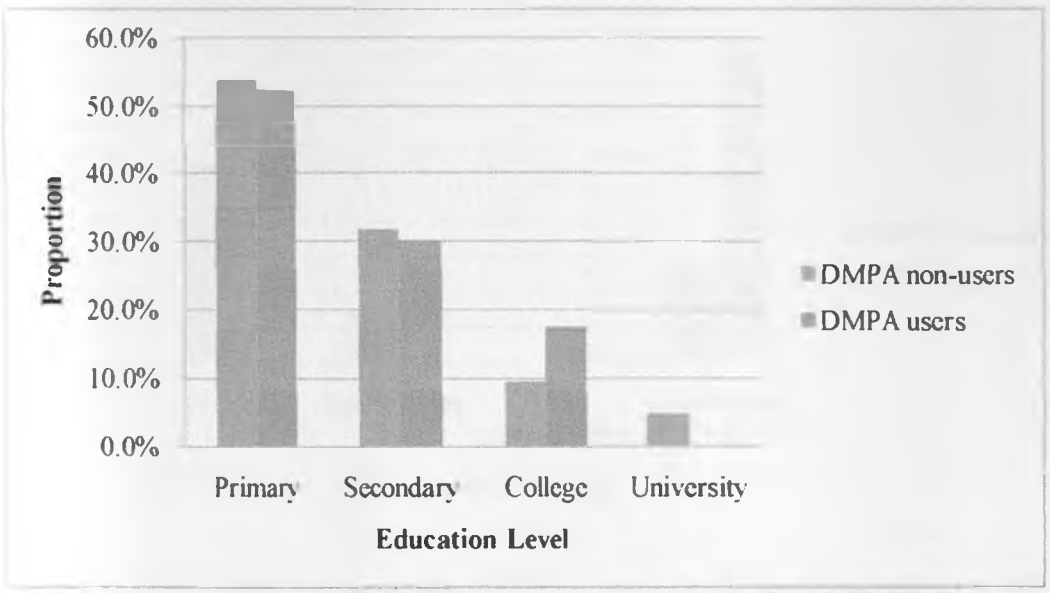


Figure 2: Level of education of DMPA users (n=63) and DMPA non users (n=63)

Likewise, high rate of unemployment was observed in all the groups with76.2% DMPA users being unemployed while 63.5% of those not using any contraception method (non-DMPA users) were unemployed (Figure 3).

Figure 3: Occupations DMPA users (n=63) and non users (n=63)

Among the DMPA users, 92.0% had previously used the injectable DMPA in the 6 months preceding the study as a method of contraception as compared to 28.6% of controls who were non-DMPA users. For the DMPA users, 1(1.6%) had used spermicides, 4.8% had used oral contraceptives and 1(1.6%) was using natural methods (Figure 4).

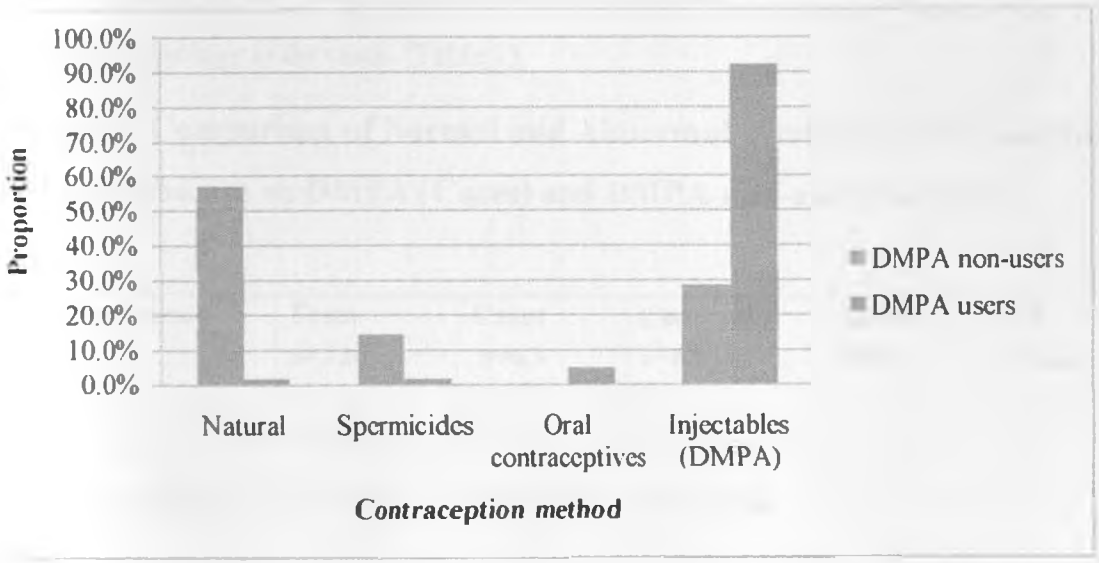


Figure 4: Previous contraception method DMPA users (n=63) and non-users (n=63)

Other findings in the cervical smears for both groups were infections that included bacterial vaginosis which was highest at 69%, candidiasis (16%), trichomoniasis (10%) and a combination of trichomoniasis and candidiasis at 5%. There was no statistically significant differences (P=0.683) between the DMPA users and no-users.

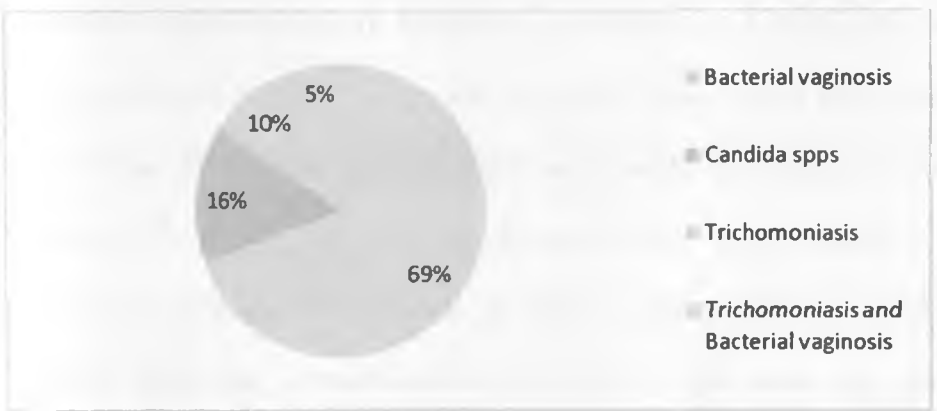


Figure 5: Infections seen in the cervical smears of DMPA users and non-users n =126

3.2: Cytomorphology of the Cervical Smears

The prevalence of abnormal cervical cytology for all HIV positive women in this study was 16.7%. There was no statistical significance in the abnormal cytology of cervical smears of DMPA users and non users, P=0.639 though there were higher numbers of abnormal cytology in the cases, (Table 1).

Table 1: Comparison of Normal and Abnormal cytology in HIV positive women who are on DMPA (Cases) and DMPA non-users (controls)

Cytology result	Total n=126 N %	Cases n=63 N %	Controls n=63 N %	OR (CI 95%)	P value
Normal cytology	104 82.5	51 (81.0%)	53 (84.1%)		
Abnormal cytology	22 17.5	12 (19.0%)	10 (15.9%)	1.2 (0.5-3.1)	0.639

More HIV positive women on DMPA reported High grade Squamous Intraepithelial Lesion (HSIL) than HIV positive women not on DMPA at 6.3%,but this was not statistically significant P=0.680. Low grade Squamous Intraepithelial lesions with HPV effect (LGSIL and HPV effect) were reported more in non-DMPA users at 11.1% than in DMPA users 6.3% but also not statistically different 0.344. Atypical squamous cells of undetermined significance (ASCUS) were identified in the cervical smears of 2(3.2%) DMPA users which was the same in the non users 2(3.2%) smear was reported as ASCUS. Generally these SIL's were not statistically significant, Table 2.

Table 2: Cytology findings in HIV positive women who are on DMPA (Cases) and DMPA non –users (controls)

Cytology result	Total n=126 N %	Cases n=63 N %	Controls n=63 N %	OR (CI 95%)	P value
Normal	103 81.7	51 80.9%	52 82.5%		
ASCUS	4 2.9	2 3.2%	2 3.2%	4.2 (0.5 -38.7)	0.365
AGUS	3 2.2	2 3.2%	0 0.0%	2.2(0.2-25.3)	0.496
HGSIL	5 3.6	4 6.3%	2 3.2%	2.1 (0.4 – 11.7)	0.680
LGSIL&HPV	11 8.0	4 6.3%	7 11.1%	0.5 (0.2 – 2.0)	0.344

3.2: Progesterone Dependant Changes on Squamous Cells of the Cervix

There was statistically significant difference in progesterone dependent changes in DMPA users P=0.023 as compared to non users. Progesterone pattern was approximately two times more likely to be demonstrated in HIV positive women on DMPA than in those HIV positive women not using any method of contraception (DMPA non users);OR 2.4 (1.1-5.1), Table 3.

Table 3: Progesterone effects on the squamous cells of the cervix of HIV positive women who were DMPA users and non users

Progesterone patterns	Case		Control		OR (95% CI)	P value
	No	%	No	%		
Progesterone pattern						
Yes	28	44.4	16	25.4	2.4 (1.1 – 5.1)	0.023
No	35	55.6	47	74.6		
Progesterone pattern description						
Atrophic	5	17.9	3	18.8		
Folding and clustering	4	14.3	4	25.0		
Folding and lactobacilli	3	7.1	0	0.0		
Folding of cytoplasm	4	14.3	4	25.0		
Intermediate+++	2	7.1	1	6.2		
Lactobacilli+++	9	32.1	4	25.0		
Navicular cells	1	3.6	0	0.0		
Total	**28**		**16**			

Smears with no progesterone pattern were identified, and then compared to those exhibiting the progesterone changes where cells lie flat on the slides (Figure $6a_1$; $6a_2$[106]). The progesterone effects that were seen on squamous cells included predominance of para-basal cells leading to an atrophic smear (Figure $6b_1$; $6b_2$[99]). Other patterns demonstrated in the smears were the predominance of intermediate cells that cluster and fold their cytoplasmic edges ($6c_1$; $6c_2$[99]); folding of the cytoplsmic edges and higher amounts of lactobacilli presence (Figure $6d_1$; $6d_2$[99]), folding of cytoplasmic edges making them not to lie flat (Figure $6e_1$;$6e_2$[107]). Other effects include, intermediate cells predominance in a smear rather than the superficial cells that indicate full maturation of the cervix due to estrogen effect, (Figure $6f_1$; $6f_2$[108]), cytolysis due to higher amounts of *Lactobacilli* leaving bare nuclei (Figure $6g_1$;$6g_2$[106]) and cells that are glycogenated giving them a boat shape because of presence of glycogen – navicular cells (Figure $6h_1$;$6h_2$[99]).

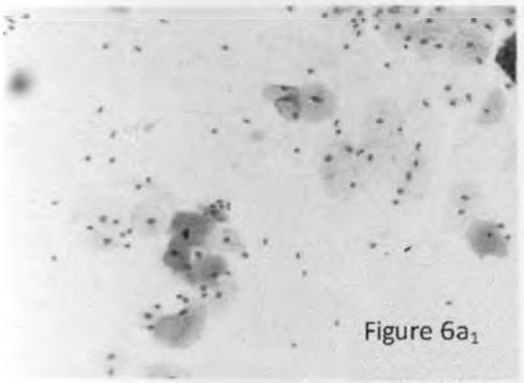


Figure $6a_1$

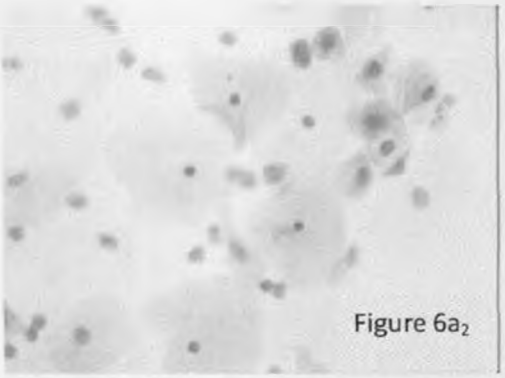


Figure $6a_2$

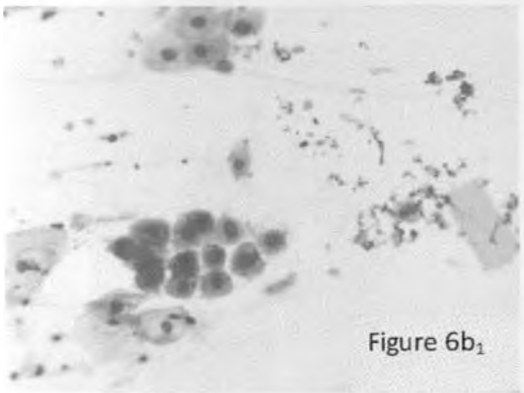


Figure $6b_1$

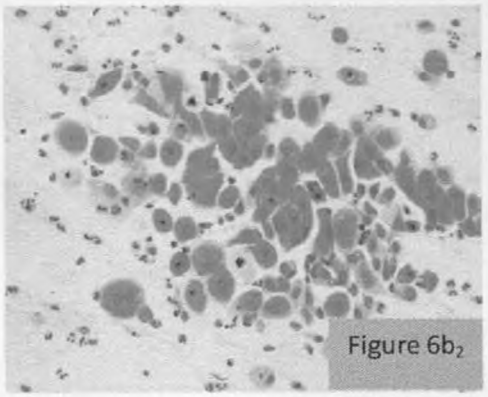


Figure $6b_2$

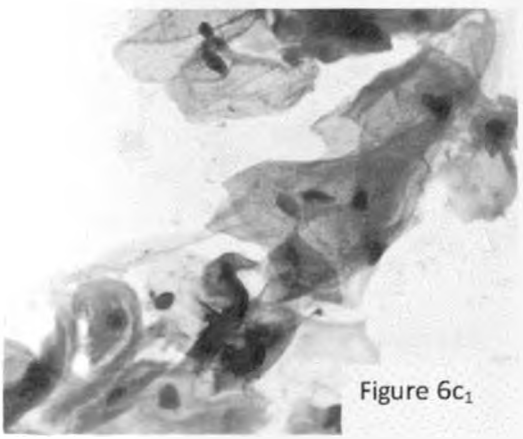


Figure $6c_1$

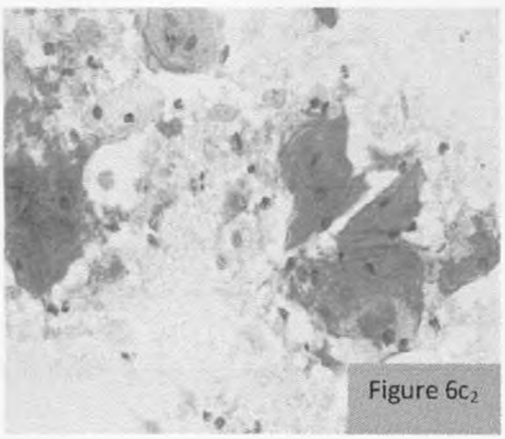


Figure $6c_2$

Figure $6a_1$: Normal cervical smear without progesterone pattern (Pap stain x 20)

Figure $6a_2$: Normal smear showing mature epithelium of the ectocervix Pap stain x 40 (Courtesy of Ducatman and Cibas[104])

Figure $6b_1$: Smear demonstrating atrophy Pap x40

Figure $6b_2$: Cervical smear from a postmenopausal patient demonstrating atrophy.Pap stain, x320. (Courtesy of Kaptain et al [98])

Figure $6c_1$: Smear demonstrating cells clustering and folding of cytoplasmic edges (Pap x63)

Figure $6c_2$: Cervical smear demonstrating squamous cell crowding, curling of cytoplasmic edges, and cytolysis;Pap stain x400. (Courtesy of Kaptain et al [98]).

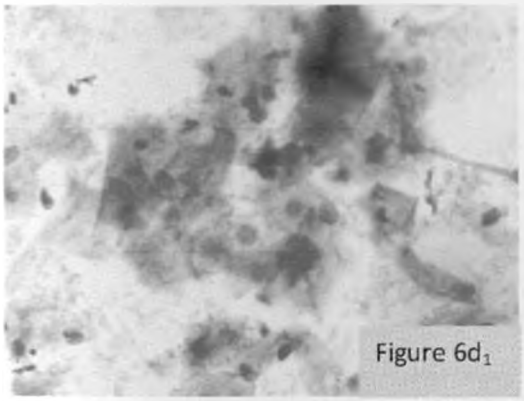


Figure $6d_1$

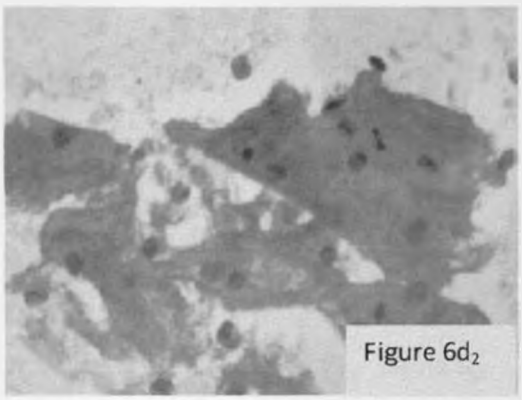


Figure $6d_2$

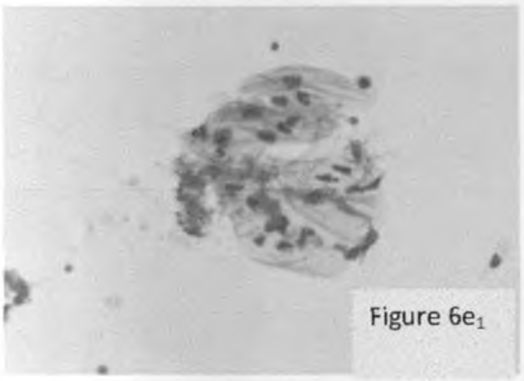


Figure $6e_1$

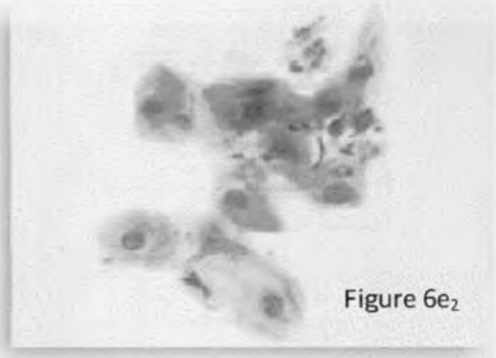


Figure $6e_2$

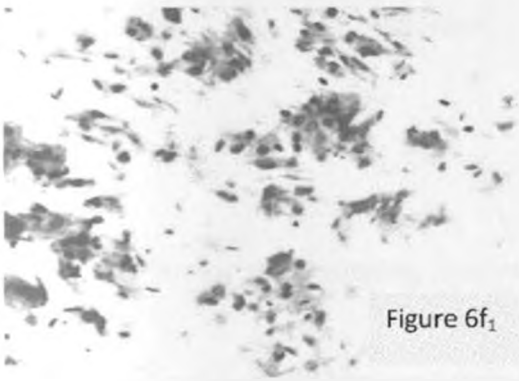


Figure $6f_1$

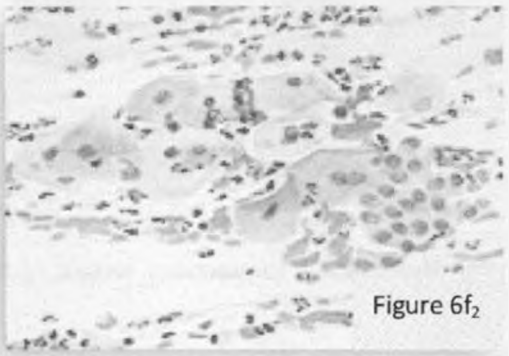
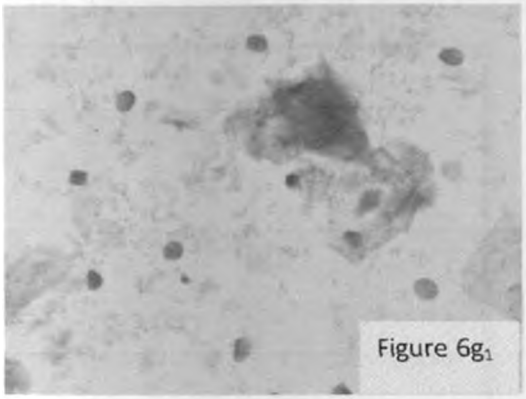
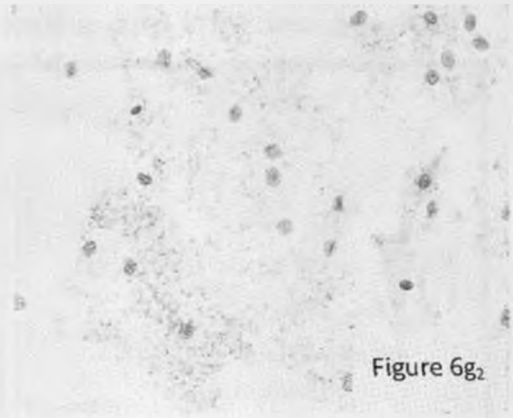
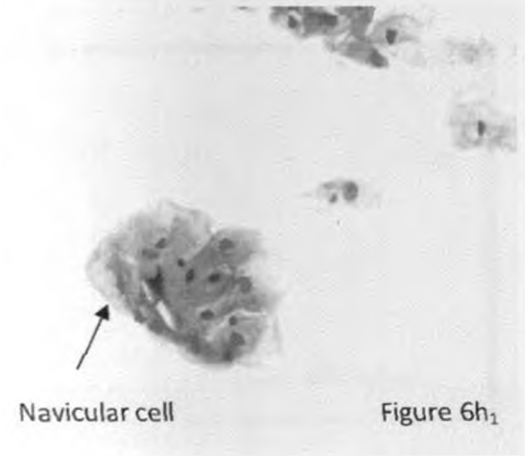


Figure $6f_2$

Figure $6d_1$: Smear demonstrating folding of cells and higher amounts of lactobacilli (x63)
Figure $6d_2$: Cervical smear demonstrating cell curling and many lactobacilli can clinging to cells Papstain x63. (Courtesy of Kaptain et al [99])Figure $6e_1$: Smear demonstrating folding of cytoplasmic edges of cells (Pap stain x63)
Figure $6e_2$: Smear demonstrating folding of cytoplasmic edges of cells Pap stain x63(Courtesy of Papilla et al [107])
Figure $6f_1$: Smear demonstrating intermediate cell predorminance (Pap stain x20)
Figure $6f_2$: Smear demonstrating intermediate cell predorminance (Courtesy of Cchieng[108])







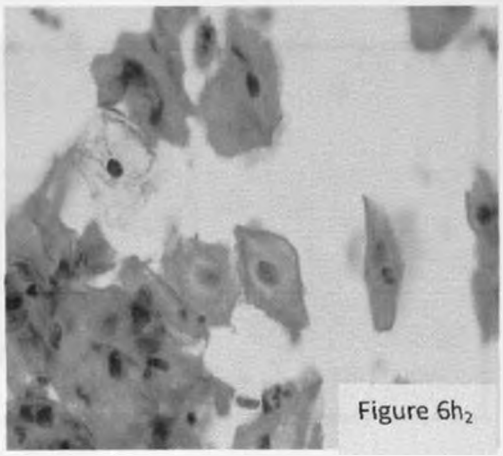


Figure $6g_1$: Smear demonstrating cytolysis due to higher amounts of Lactobacilli (Pap stain x63)

Figure $6g_2$: Lactobacilli. There are bare nuclei of the intermediate cells, due to cytolysis by these organisms (Courtesy of Ducatma and Cibas[106])

Figure $6h_1$: Smear demonstrating presence of navicular cells (Pap stain x40)

Figure $6h_2$: Cervical smear demonstrating a navicular cells containing central glycogen with rounded cytoplasmic edges. Pap stain, x630(Courtesy of Kaptain et al [99])

3.3: Cytomorphology of premalignant lesions and infectious agents

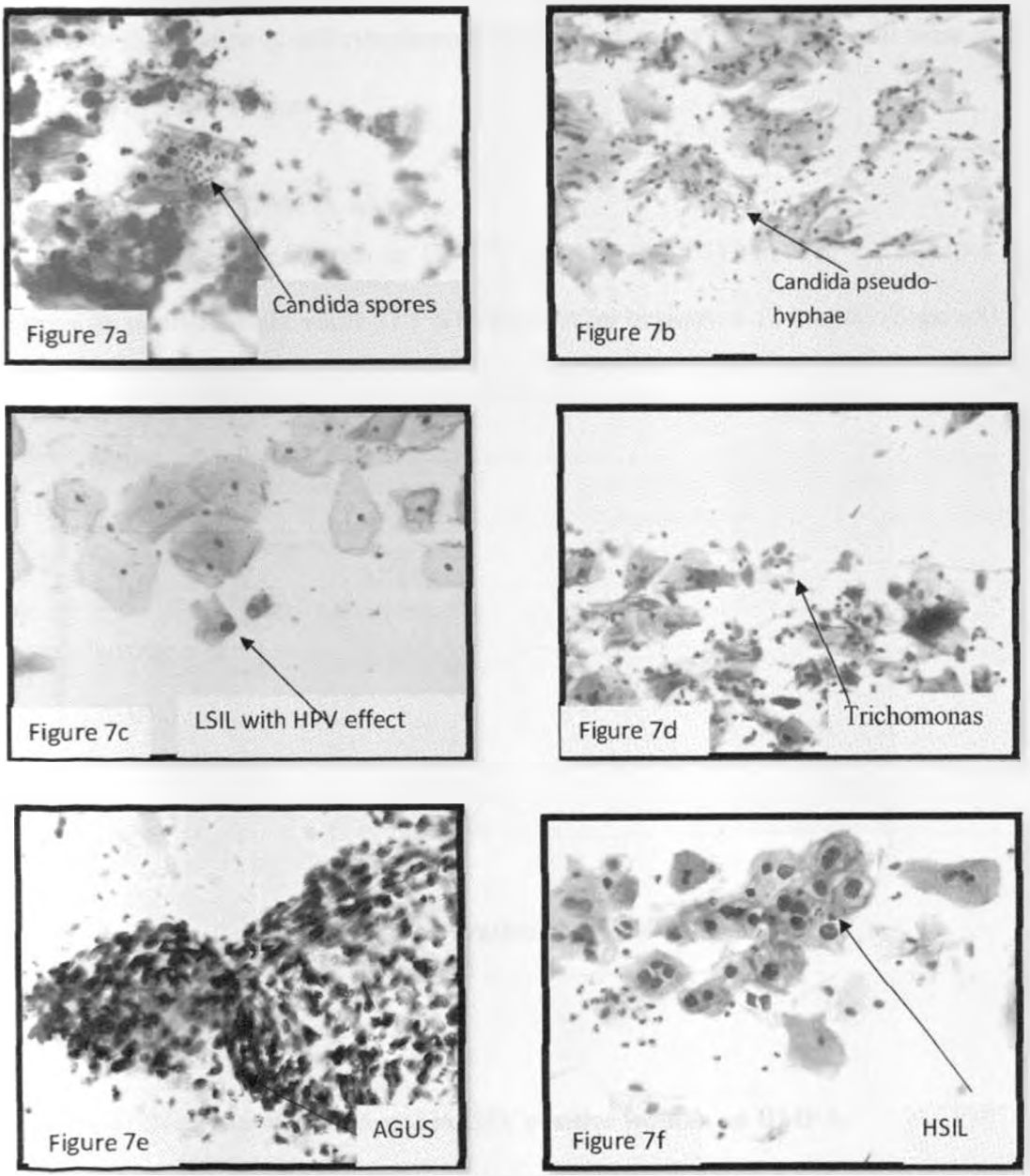


Figure 7: Cytomorphology of Pre-Malignant Lesions and infectious agents

Figure 7a: An illustration of Candida spps spores in cervical smears (Pap x63)
Figure 7b: Candida spps pseudo-hyphae in cervical smear (Pap x63)
Figure 7c: LSIL with HPV effect (Pap x40)
Figure 7d: Infestation of Trichomoniasis (Pap x63)
Figure 7e: Dyskaryotic glandular cells -AGUS (Pap x63)
Figure 7f: Severe dyskaryosis -HSIL (Pap x63)

For the controls (16)25.4% demonstrated progesterone pattern with 25.0% exhibiting folding of the squamous cell cytoplasm and increased amounts of lactobacilli were found in 4(25%) of these women.

3.4: Duration of DMPA use

For those HIV positive women on DMPA, majority (62.9%) had used the injection for more than twelve months while 37.1% had used it for between 6-12 months (Figure 8).

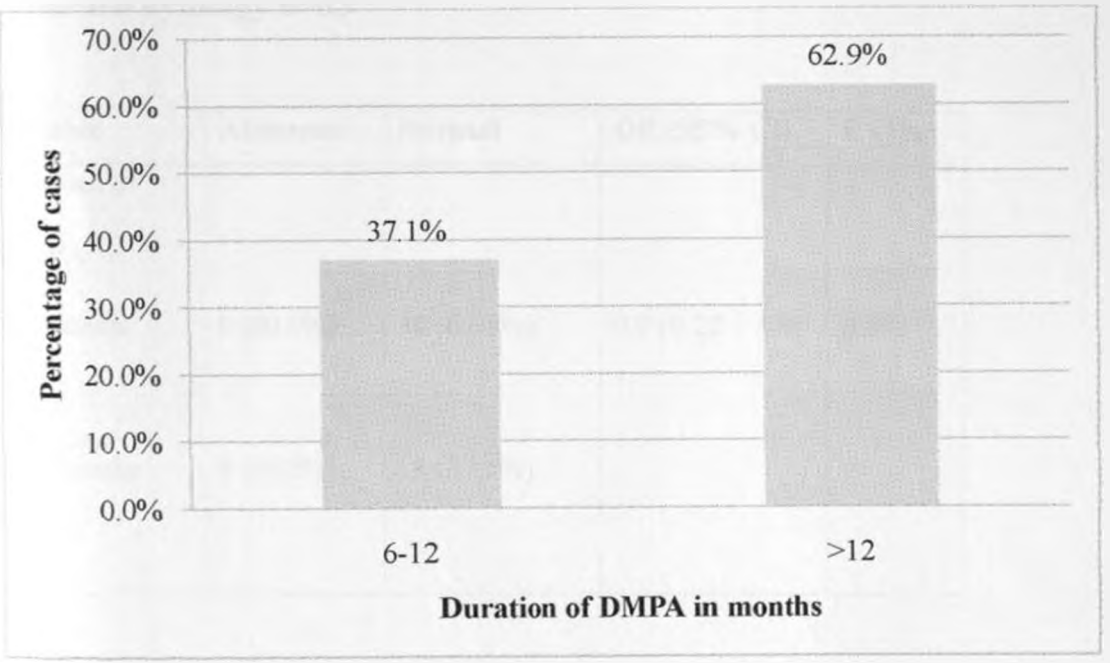


Figure 8: Duration of DMPA use in HIV positive women on DMPA

With respect to duration of DMPA use and abnormal cytology in the current DMPA users (cases), 60.0 % of the smears were reported as abnormal in cytology from women who had used the injectable for more than twelve months as compared to 40.0% smears from women who had used DMPA for six to twelve months O R 0.9 (C I 0.2-3.6) Table 4.

Table 4: Duration of DMPA use by HIV positive women in relation to abnormal cytology n=63

Variable	Abnormal	Normal	OR (95% CI)	P value
Duration of DMPA				
>12 months	6 (60.0%)	30 (62.5%)	0.9 (0.22 – 4.9)	0.882
6-12 months	4 (40.0%)	18 (37.5%)		

CHAPTER FOUR: DISCUSSION

The injectable DMPA as a method of contraception is an effective means of preventing pregnancy, having greatly improved the well-being of women and their families alike. HIV positive women are no exception to this fact despite their immune status. The impact of DMPA on the cervical epithelium in HIV positive women has become an important concern of women's healthcare with some studies suggesting that it may increase the risk of cervical cancer while others dispute this fact [97, 109]. The aim of this study was to determine cytomorphological findings of cervical smears in HIV positive women using DMPA as a method of contraception.

Women cases enrolled in this study were in their prime reproductive year's [age (mean 28.5±6.8)] with a mean CD4 count of 668.2 ± 228.2cm^3 and used DMPA as their choice method for contraception. Masaad et al [50] in their study on prevalence of cervical neoplasia in HIV positive women recruited women who were older (mean age of 35±5 years). The prevalence of an abnormal cytology was 16.7% in this study which compares well with other studies/reports. The risk of having CIN in the setting of HIV appears to be related to the degree of immuno-suppression. Wright *et al.* found that in women with CD4 counts < 200x10^6/L the prevalence of CIN was 29% *v* 16% in women with CD4 counts >500 x10^6/L [108]. A study done in the obstetrics and gynaecology department at the UON in 2009 by Waweru et al found a prevalence rate of CIN in the general population of women was 6.9% [111].

In general, infectious agents were prevalent in both the study group and control group. These agents included Bacterial vaginosis 69%, Trichomoniasis, 10% and Candidiasis 16% and co-occurrence of Trichomoniasis and Candidiasis 5%. This is in agreement with other authors who reported infection of Trichomoniasis at 28%,Bacterial

vaginosis at 51.4% and co-occurrence of Trichomoniasis and Bacterial vaginosis at 17.5% in HIV positive patients irrespective of their hormonal status [112].

With respect to the effects of DMPA use on cytomorphology of the cervical cells, HIV positive women on DMPA had more significant progesterone dependent changes that included, atrophic smears, folding of cytoplasm cell, crowding and abundant lactobacilli in most of the smears. Full maturation of the cervix is attained by adequate amounts of eostrogen hormone together with other factors, intermediate cells mature to superficial cells that protect the cervix against adverse injury. This compares well with a study by Kaptain et al on hormonal effects of Depo-Provera (a hormonal contraceptive) in cervical smears who reported squamous cell curling, crowding, atrophic smears and cytolysis in Depo-provera users [99]. Folding of cytoplsamic edges and squamous cell crowding was more pronounced in those women in the control group.

The Pap smear is an effective test for cervical cancer screening. The relationship between pre-invasive cervical cancer and the progesterone-only contraceptives is controversial because in order to arrive to conclusive findings several confounding factors have to be factored into the study. These factors include a series of sexually related factors, such as age at first sexual intercourse, number of sexual partners, and sexual conduct of the partners and the use of protective barrier methods [113]. There was no statistically significant difference in the lesions found in the DMPA users and none users (Control) P=0.639. This is consistent with natural history studies for HPV and cervical lesions that show women who become infected with HPV in their 20s generally get rid of their infection, and it is those with persistent HPV infection after the age of 30years that are at risk of progression to high grade lesions and invasive cervical cancer

[113]. In this study four women (6.3%) on DMPA and seven women (11.1%) of the controls had LSIL which is a form of pre-malignant lesion that usually revert spontaneously to normal without therapy in 85% of case [114]. In a large sample-sized study of 3374 women using different methods of contraception, spontaneous regression of dysplasia was seen in 85 (40.2%) of the 94 post treatment cases and in all 47 pretreatment dysplasia. Among 686 women using levonorgestrel contraception, complete regression of 19 cases of dysplasia was observed on follow-up [98].

This study did not show a significant link between pre-malignant lesions in relation to duration of DMPA use P=1.000, OR 0.9 (CI 0.2-3.6). Likewise, in a large sample-sized study, there was no causal association with moderately increased risk (up to a factor 1.5) between the use of DMPA and the risk of cervical neoplasia [58]. Although some studies have reported that DMPA has been shown to increase the risk of cervical carcinoma in situ the cervical lesions induced by DMPA did not progress to invasive disease [115]. Large case-control studies by WHO found that the use of DMPA does not increase the risks of breast, endometrial, ovarian or cervical cancer [17, 63]. The strongest variable linked to risk of pre-invasive cervical lesions was the duration of use and high parity the latter is a real risk factor in developing countries [17, 63]. The World Health Organization study indicated that the relative risk of in situ cancer in DMPA users was small and the risk decreases with time after use[17]. However, this finding could be related to uncontrolled variables, such as sexuality related factors [49]. In the WHO study the authors concluded that "It can be stated with a fair degree of confidence, however, that use of DMPA as a contraceptive for over four years is not associated with an increase in

risk of adenomatous cervical carcinomas, and risk is not altered even after a potential latent period of over 12 years since initial exposure" [17].

LIMITATIONS

This study had a few limitations. The study was carried out over a short period of time and on minimum number of cases hence it could not provide information on the incidence of CIN as would a prospective cohort study. Furthermore, there were no facilities to analyze for HPV infection and the specific strains which are the causative agents of cervical dysplasia which progresses to cancer. HPV testing would have identified high risk types that would have helped in close monitoring of affected clients. In addition, the young age of the study population and their fewer years of contraceptive use probably led to limited power to assess the association between long-term (>5 years) DMPA use and CIN. In this study, two women in their early 4th decade had used DMPA for $\geq$ 3 years. Some smears were excluded from data analysis because they were unsatisfactory for evaluation when classified according to Bethesda classification (Appendix 3).

CHAPTER FIVE: CONCLUSIONS AND RECOMMENDATIONS

CONCLUSIONS

There was no increased risk of pre-malignant lesions in DMPA users as compared to DMPA non-users.

Certain progesterone dependent effects like squamous folding of the cytoplasm, crowding, presence of lactobacilli and intermediate predominance were identified more in DMPA users while squamous crowding and increased numbers of lactobacilli were identified more in the control group.

RECOMMENDATION

1. Further evaluation of abnormal cytology in HIV positive women on DMPA using other methods of cervical cancer screening like liquid based cytology and HPV-DNA testing strategies should be explored.

2. Family Planning services and cervical smear screening should be integrated into Comprehensive Care Centre's and a long term surveillance studies on cervical changes in HIV/AIDS during contraceptive use monitored.

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Appendix 1: Informed Consent Document

TITLE OF STUDY: EFFECTS OF DEPOT- MEDROXY PROGESTERONE ACETATE USE ON CERVICAL CYTOLOGY OF HIV POSITIVE WOMEN

Informed consent explanation for patients aged 18 years and above

I am Violet Kisato, a master's degree student in Clinical Cytology at the University of Nairobi. I am conducting a study on the effects of Depot Medroxy-Progesterone Acetate (DMPA) use on cervical cytology of HIV positive women. The gynecologist/research assistant will collect a sample from you from the cervix that was be analyzed in Cytology laboratory at the University of Nairobi. We will be comparing the cervical epithelium of those women using DMPA as a method of contraception with those who are not on any method of contraception. We will be looking for any changes in the cervical epithelium including any cervical lesions which will help us to identify the most reliable approach to manage you as our patient and therefore help you make better choices for a healthy life. If you agree to be a participant in this study, we will take a cervical smear from you. We will use aseptic technique and we was ensure complete confidentiality of your test results. The slides will be stored at cytology laboratory up to ten years for further evaluation if deemed necessary with permission from KNH/UON/ERC. This study has been approved by KNH/UON/ERC.

Risk / benefits

During this procedure there will be no long-lasting effect. However, you may feel a brief moment of pain or fear. You may have slight pains after the test and this will go away in a few hours. You will not be given any monetary benefits; neither will you incur any costs. The study will benefit you in that this will be part of your medical checkup and will be done free. The results will be disseminated to the management of FACES at Lumumba

health centre for evaluation. These results will benefit the policy makers since they will provide guidelines on management of HIV positive women on hormonal contraceptives.

Participant's rights

Your participation in this study is voluntary and if you decline to participate, you will not be denied any services that are normally available to you.

Duration of participation

This study will only require one cervical sample. There is no follow-up or further information needed.

Assurance of confidentiality of volunteer's identity

Records relating to you will remain confidential. You will be given a signed copy of the consent form.

Contact information

If you have questions now or in the future regarding your rights or this study or research related injury, you may ask any of the field officers involved in this study or contact Violet Kisato, MSc student at the University of Nairobi on 0722931801 or Chairperson, KNH/UON/ERC P.O. BOX 20723 - 00200. Nairobi. Tel#:020-2725272.

CONSENT FORM

Consent from the patient

The above details about the study and the basis of participation have been explained to me and **I agree** to take part in the study. I understand that I am free to choose to be part of the study or not. **I give my consent** to be screened for cancer of the cervix.

Patient signature/ Thumb mark: .....

Witness' signature: .....

Gynecologist's/Research assistant signature.....

Date: .....

Appendix 2: Questionnaire

EFFECTS OF DEPOT- MEDROXY PROGESTERONE ACETATE USE ON CERVICAL CYTOLOGY OF HIV POSITIVE WOMEN

Socio-Demographic Data

Study No: ☐☐☐

Clinic No: ☐☐☐☐☐☐☐

Date: ☐☐☐

dd mm yy

DOB ☐☐☐

dd mm yy

Type of study participant: ☐ Case ☐ Control

Religion: ☐ Christian ☐ Moslem ☐ Other specify_____

Marital Status: ☐ Single ☐ Married ☐ Separated ☐ Widowed ☐ Divorced

Educational Level: ☐ None ☐ Primary ☐ Secondary ☐ College ☐ University

Occupation: ☐ Professional ☐ Housewife ☐ Unemployed

CONTRACEPTION:

Previous Family Planning Method in the past one year:

- ☐ Natural
- ☐ Spermicides
- ☐ Oral Contraceptive Pills
- ☐ Injectable (DMPA)
- ☐ Intrauterine Devices
- ☐ Others specify_____

Current contraceptive: ☐ None ☐ DMPA

Duration of depot medroxyprogesterone acetate (DMPA) use (months):_____

CD4 level counts.....cells/mm^3

CLINICAL OBSERVATION:

☐ Normal ☐ Erosion

☐ Inflammed ☐ Suspicious

☐ Others specify: _____

Provisional diagnosis: _____

DOCTOR'S NAME_____ Sgn_____

LABORATORY REPORT

BETHESDA CLASSIFICATION [100]

Suitability ☐ Yes ☐ No Specify_____

Adequacy ☐ Yes ☐ No Specify_____

Negative	☐ ASCUS	☐	HGSIL	☐
Inflammatory	☐ LGSIL	☐	HPV	☐
Reactive	☐ AGUS	☐	Glandular-Neoplasia	☐

Progesterone pattern ☐ Yes ☐ No

If yes describe: _____

Date:

Appendix 3: BETHESDA SYSTEM 2001

SPECIMEN TYPE: *Indicate conventional smear (Pap smear) vs. liquid-based vs. other*

SPECIMEN ADEQUACY

- Satisfactory for evaluation (describe presence or absence of endocervical/transformation zone component and any other quality indicators, e.g., partially obscuring blood, inflammation, etc)
- Unsatisfactory for evaluation (specify reason)
    - Specimen rejected/not processed (specify reason)
    - Specimen processed and examined, but unsatisfactory for evaluation of epithelial abnormality because of (specify reason)

GENERAL CATEGORIZATION (optional)

- Negative for Intraepithelial Lesion or Malignancy
- Epithelial Cell Abnormality: See Interpretation/Result (specify 'squamous' or 'glandular' as appropriate)
- Other: See Interpretation/Result (e.g. endometrial cells in a woman > 40 years of age)

AUTOMATED REVIEW

If case examined by automated device, specify device and result.

ANCILLARY TESTING

Provide a brief description of the test methods and report the result so that it is easily understood by the clinician.

INTERPRETATION/RESULT

NEGATIVE FOR INTRAEPITHELIAL LESION OR MALIGNANCY (when there is no cellular evidence of neoplasia, state this in the General Categorization above and/or in the Interpretation/Result section of the report, whether or not there are organisms or other non-neoplastic findings)

ORGANISMS:

- Trichomonas vaginalis
- Fungal organisms morphologically consistent with Candida spp

- Shift in flora suggestive of bacterial vaginosis
- Bacteria morphologically consistent with Actinomyces spp
- Cellular changes consistent with Herpes simplex virus

OTHER

- Endometrial cells (in a woman > 40 years of age)
  (Specify if 'negative for squamous intraepithelial lesion')

EPITHELIAL CELL ABNORMALITIES

SQUAMOUS CELL

- Atypical squamous cells
  - of undetermined significance (ASC-US)
  - cannot exclude HSIL (ASC-H)
- Low grade squamous intraepithelial lesion (LSIL)
  - encompassing: HPV/mild dysplasia/CIN 1
- High grade squamous intraepithelial lesion (HSIL)
  - encompassing: moderate and severe dysplasia, CIS/CIN 2 and CIN 3
  - with features suspicious for invasion (if invasion is suspected)
- Squamous cell carcinoma

GLANDULAR CELL

- Atypical
  - endocervical cells (NOS or specify in comments)
  - endometrial cells (NOS or specify in comments)
  - glandular cells (NOS or specify in comments)
- Atypical
  - endocervical cells, favor neoplastic
  - glandular cells, favor neoplastic
- Endocervical adenocarcinoma in situ
- Adenocarcinoma
  - endocervical
  - endometrial
  - extrauterine
  - not otherwise specified (NOS)

Appendix 4: Papanicolou Staining Technique

Principle of the stain

Haematoxylin stains the nuclei blue by dye lake formation. The eosin azure solution being acidic stains the cytoplasm which is basic so that the eosin has affinity for the mature cells while light green has affinity for the young cells. Orange G also being an acidic dye has an affinity for the cytoplasm and stains keratin.

Staining technique

1. The smear is fixed in 95% ethanol.
2. Hydrate smears by passing them through ethanol grades of 80%, 70% and then 50%.
3. Rinse smears in distilled water 10 dips.
4. Stain in Harris haematoxylin for 3 minutes.
5. Rinse in tap water.
6. Differentiate in 0.05% acid water 10 dips
7. Rinse in tap water and blue in Scott's tap water 10 dips
8. Rinse in 95% ethanol 10 dips
9. Stain in O.G 6 for 1½minutes
10. Rinse in 95% ethanol 10dips
11. Stain in E.A.50 for 3 minutes
12. Rinse in 95% ethanol 10 dips
13. Dehydrate in changes of absolute ethanol 10 dips each
14. Clear in 3 changes of xylene 10 dips each
15. Mount in D.P.X

Reagent preparation

Harris alum hematoxylin

To prepare 2000mls

1.	Hematoxylin	10gm
2.	Absolute alcohol	200ml
3.	Ammonium alum	200gm
4.	Distilled water	2000ml

5. Mercuric oxide 6gm
6. Glacial acetic acid 80ml

Method of preparation

1. Dissolve Hematoxylin in absolute alcohol (Solution 1).
2. Dissolve Ammonium alum in water (Solution 2).
3. Mix solution 1and solution 2 and heat to boil.
4. Add mercuric oxide and cool rapidly.
5. Add glacial acetic acid.
6. Solution is ready for use as soon as it cools.
7. Filter before use.

E.A.36

To prepare 4 litres

1. Light green 2gm
2. Bismark brown 2gm
3. Eosin yellow 10gm
4. Phosphotungstic acid 8gm
5. Distilled water 1200ml
6. Absolute ethanol 2800ml
7. Glacial acetic acid 40ml

Method of preparation

1. Dissolve in order of light green then bismark brown then eosin yellow into water.
2. Add phosphotungstic acid slowly in stages while agitating
3. Add alcohol slowly while agitating
4. Add glacial acetic acid then agitate

ph of E.A.36 should be 4.5-5 to achieve maximum results

O.G 6

To prepare 4 litres

1. Orange green(O.G) 9.4 gm
2. Phosphotungstic acid 0.6gm
3. Distilled water 1200ml
4. Absolute ethanol 2800ml

Method of preparation

1. Dissolve O.G in water then phosphotungstic acid
2. Add absolute ethanol

0.05%acid alcohol

To prepare I litre of 0.05%acid alcohol

1. Distilled water 999.5ml
2. Conc.HCL 0.5ml

Scotts tap water

To prepare 1000ml

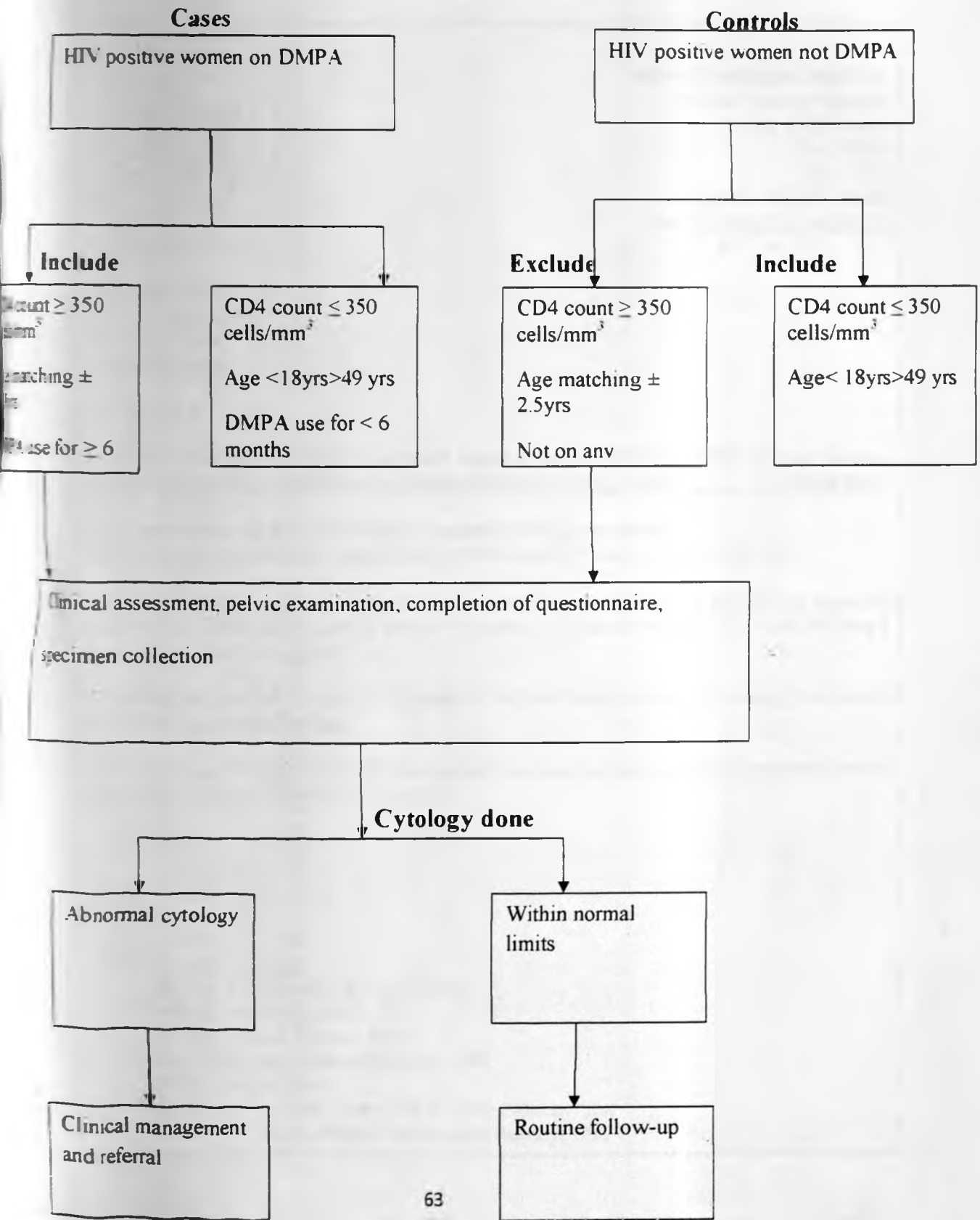
1. Sodium bicarbonate 3.5gm
2. Magnesium sulphate 20gm
3. Distilled water 1000ml
4. Thymol 1 tablet

Quality check

1. Stains were be stored in dark coloured, stoppered bottles.
2. Fresh amounts of Haematoxylin was be added to replace stain loss due to evaporation.

3. O.G and E.A was be replaced as soon as the cells appear without crisp staining colours.
4. Water rinses were be done under running tap water.
5. Alcohol was be replaced on a rotating basis
6. Xylene was be changed as soon as it becomes tinted with any of the cytoplasmic stains or becomes slightly milky due to presence of water.
7. An agitation of the slides by dipping was done to remove excess dye.
8. Dipping was be done gently to avoid cell loss and the slide carrier was not hit the bottom of the staining dish.

Appendix 5: Flow chart on recruitment and consenting



Appendix 6: Ethics Committee Approval

KENYATTA NATIONAL HOSPITAL
Hospital Rd. along, Ngong Rd
P O. Box 20723. Nairobi
Tel 726300-9
Fax: 725272
Telegrams: MEDSUP", Nairobi
Email: KNHplan@Ken.Healthnet.org

Ref KNH-ERC/ A/494

31st May 2010

Kisato Violet Afandi
Dept of Human Pathology
School of Medicine
University of Nairobi

Dear Ms. Afandi

RESEARCH PROPOSAL: "EFFECTS OF DEPOT MEDROXY PROGESTERONE ACETATE USE ON CERVICAL CYTOLOGY OF HIV POSITIVE WOMEN AT A COMPREHENSIVE CARE CLINIC-KISUMU" (P121/4/ 2010)

This is to inform you that the KNH/UON-Ethics & Research Committee has reviewed and **approved** your above revised research proposal for the period 31st May, 2010 to 30th May 2011.

You will be required to request for a renewal of the approval if you intend to continue with the study beyond the deadline given. Clearance for export of biological specimens must also be obtained from KNH/UON-Ethics & Research Committee for each batch.

On behalf of the Committee, I wish you a fruitful research and look forward to receiving a summary of the research findings upon completion of the study.

This information will form part of the data base that will be consulted in future when processing related research study so as to minimize chances of study duplication.

Yours sincerely

PROF A N GUANTAI
SECRETARY, KNH/UON-ERC

c.c. Prof. K. M. Bhatt, Chairperson, KNH/UON-ERC
The Deputy Director CS, KNH
The Dean, School of Medicine, UON
The Chairman, Dept. of Human Pathology, UON
The HOD, Records, KNH
Supervisors: Dr. Emily Rogena, Dept.of Human Pathology, UON
Prof. C. Kigondu, Dept.of Human Pathology, UON

Appendix 7: The Faces Experience

The Family aids care and educational services (FACES) is an HIV/AIDS treatment programme in Nyanza region. FACES is collaboration between KEMRI and university of Califonia, San Francisco (UCSF) funded through PEPFAR. My experience while doing my research at FACES was resourceful. FACES has got many programmes running therefore staff meetings are held every Thursday to update everyone else is updated on what is happening in other projects and progress reports presented. FACES focuses on improving the health of the whole family as a unit. The programme supports those who are infected and affected by having home visits, helping them form study groups and monitoring their progress.The staff with whom you will have the privilege of working with are experts in their own fields and are impressively resourceful, and will serve as a daily source of knowledge, encouragement, and inspiration.

This study was a sub study of Dr Waloong E., Mmed Pathology student at the UON who was studying cytokines levels in HIV positive women. Since the study population was the same; we merged our questionnaires so that it was easy to do the recruitment and enrollment hence making it comfortable for the study participants. We thought this was a good approach because they did not have to fill two questionnaires. There is a cervical cancer screening room next to the laboratory where Visual Inspection with Acetic acid is done. Despite this being a health centre, the laboratory can carry out all the tests for monitoring their HIV positive clients. We also set up a cytology bench in the laboratory, prepared standard operating procedures (SOP's) and trained the laboratory staff on Pap staining technique. By the end of our study this bench was up and running and included the Heamatoxylene and Eosin staining technique which was to process fine needle aspirates. These were reported by Dr.Waloong and the provincial pathologist.

This is a great organization to work with because of the amount of support you get– from the clinicians,nurses,laboratory staff and everyone in between. Their clients end up satisfied and have a very high positive look to life and when you meet them they tell you they are healthy and not being eaten up by the virus. FACES is a big family with each dedicated and inspiring family member welcoming you with open arms.