DETERMINATION OF PERFORMANCE CHARACTERISTICS OF ENDOMETRIAL TAO BRUSH IN THE DETECTION OF ENDOMETRIAL CANCER AND ATYPICAL HYPERPLASIA

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Determination of Performance Characteristics of Endometrial Tao Brush in the Detection of Endometrial Cancer and Atypical Hyperplasia

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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

This thesis is unequivocally dedicated to my husband and my beloved parents and who have been my source of inspiration and strength throughout this research, who continually provided their moral, spiritual and emotional support.

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ACRONYMS AND ABBREVIATIONS

ACOG	American College of Obstetricians and Gynecologists
ACS	American Cancer Society
AUB	Abnormal Uterine Bleeding
CAH	Complex Atypical Hyperplasia of the Endometrium
CB	Cell Block
CCEC	Clear Cell Endometrial Cancer
COCs	Combined Oral Contraceptives
CS	Conventional Smear
CT	Computed Tomography
D & C	Dilation and Curettage
EC	Endometrial Cancer
EEC	Endometrioid Endometrial Cancer
ES	Endometrial Sampling
FDA	Food and Drug Administration
FIGO	International Federation of Gynecology and Obstetrics
JKUAT	Jomo Kenyatta University of Agriculture and Technology
MLBC	Manual Liquid Based Cytology
NEEC	Non-Endometrioid Cancers

PET	Positron Emission Tomography
PI	Principal Investigator
PPAP	Polymerase Proofreading Associated Polyposis
PPAP	Polymerase Proofreading Associated Polyposis
SEIC	Serous Endometrial Intraepithelial Carcinoma
SOPs	Standard Operating Procedures
TCGA	The Cancer Genome Atlas
US	United States

ABSTRACT

Endometrial cancer is the most frequent gynecologic malignancy in countries that are developed with 54,870 new cases projected in the United States in 2022. In Kenya, cancer of the uterine corpus has an incidence of 1.7%. It is usually common among postmenopausal women with an average age of 60 years when they are diagnosed and early detection is crucial for favorable outcomes. In Kenya there is no screening tool for Endometrial Cancer. The brush is easy to use, can be used in an ambulatory setting without anesthesia, and is less painful than the dilatation and curettage technique which is the gold standard. The current study aimed at determining the performance characteristics of Endometrial Tao brush in the detection of Endometrial Cancer and Hyperplasia with atypia using Manual Liquid Based Cytology Samples and Conventional cytology samples compared with the biopsies from Dilatation and Curettage. Sixty women were evaluated for Hyperplasia with atypia and Endometrial Cancer using the Endometrial Tao brush and Dilatation and Curettage Method. Manual Liquid Based Cytology specimens were fixed in a liquid fixative which was formulated (containing 10% formalin, sodium chloride, isopropyl alcohol, and sodium citrate) and then vortexed. 2mls of the polymer solution was added to the deposit and a smear was made on the slide, it was then oven dried and fixed with 95% alcohol for 15 minutes. The slides were then stained with papanicolaou stain and examined. The Conventional cytology samples were fixed with 95% alcohol for 15 minutes, stained with papanicolaou stain and then examined. Histology samples were fixed with 10% formalin and then underwent dehydration, clearing, infiltration, embedding, sectioning, staining with Hematoxylin and Eosin and finally examination under the microscope. Cytological findings were categorized as Unsatisfactory for evaluation, Negative endometrium, Endometrial hyperplasia without atypia, hyperplasia with atypia and Malignant neoplasms. Histopathological results were used as the reference for determining the performance characteristics of cytological smears. The agreement level between the two methods was determined using Cohen's Kappa test. Histopathological diagnoses from the 60 patients comprised 14 (23%) Endometrial Cancers, 8 (13%) Complex hyperplasia with atypia, 16 (27%) Simple and mild hyperplasia without atypia, 3 (5%) Non-Diagnostic, and 19 (32%) patients with Negative Endometrial Histology. The Manual Liquid Based Cytology specimens diagnosed as Malignant, Atypical hyperplasia, Endometrial hyperplasia without atypia, Unsatisfactory, and Negative were 12 (20%), 9 (15%), 16 (27%), 3 (5%), and 20 (33%) specimens correspondingly. The Conventional Cytology specimens diagnosed as Malignant, Atypical hyperplasia, Endometrial hyperplasia without atypia, Unsatisfactory, and Negative were 15 (25%), 8 (13%), 13 (22%), 6 (10%), and 18 (30%) specimens accordingly. The specificity and sensitivity of cytology using Tao Brush for detecting endometrial cancer and hyperplasia with atypia were 100% and 95.45% for the Manual Liquid Based Cytology specimens and 95% and 90.91% for Conventional specimens respectively. The Cohen's kappa value was 0.912 and 0.891 for Manual Liquid Based Cytology specimens and Conventional specimens respectively. Given the auspicious specificity and sensitivity of the endometrial Tao brush in this current study, direct sampling of the endometrium with the Tao brush is a useful tool in the assessment of unusual uterine bleeding, particularly in the detection of cancer of the endometrium. This therefore will improve treatment outcomes for patients with Endometrial cancer and prolong the quality of life.

CHAPTER ONE

INTRODUCTION

1.1 Background

Endometrial Cancer is the most frequent malignancy of the Female Reproductive Tract in countries that are developed and some which are developing (Horta & Cunha, 2019a). The likely outcome, the chance of recovery or recurrence of uterine cancer is dependent on many factors such as the age of the patient, the histological stage and grade, depth of invasion into the myometrial and the cervix, and the existence of metastases to the lymph nodes(Narice *et al*., 2018). Unlike cervical carcinoma, in which screening and evaluation can be done routinely, only women with signs and symptoms of cancer of the endometrium usually look for medical assistance for them to be evaluated, diagnosed and treated (Levy-Zauberman *et al*., 2017).

There would be a social and economic value from a tool that can be used for screening due to the incidence of Uterine carcinoma which is increasing all over the continent (Horta & Cunha, 2019a). This will eventually result in earlier diagnosis and treatment of carcinoma of the endometrium. Sadly, there is no efficient and cheap program for screening and early detection of cancer of the endometrium has been adopted currently unlike for cancer of the cervix (Du *et al.,* 2016).

Dilatation and curettage is still the gold standard technique for endometrial malignancy evaluation, however, it has numerous shortcomings; apart from it being expensive, it's possibility to cause perforation, (Levy-Zauberman *et al*., 2017), D&C at times results in false-negative diagnoses since it can only assess less than 1/2 of the endometrium in roughly sixty percent of dilatation and curettage procedures (Williams *et al*., 2008).

The need for admission and the risks of hemorrhage and perforation caused by the dilatation and curettage technique limits its usage as a tool for screening endometrial malignancies (Narice *et al.*, 2018). In the recent past, various endometrial tools have been developed and proposed for use (Van Hanegem *et al*., 2016). Patients who are at a high risk of having carcinoma of the uterine cavity would benefit from a cheap and

efficient screening tool (Levy-Zauberman *et al*., 2017). Hence, there is an urgent need for embracing the use of alternative devices for the detection of endometrial malignancies in their early stages.

1.2 Statement of the Problem

Endometrial cancer is the most common gynecologic malignancy in developed and developing countries with 54,870 new cases projected in the United States in 2022. It is the 4th most frequent carcinoma in females after breast, lung, and colorectal cancers. In Kenya, cancer of the uterine corpus has an incidence of 1.7%. The low incidence is majorly contributed by lack of an effective screening test for endometrial Cancer in Kenya. Early detection is crucial for favorable outcomes since about 90% of cases screened early can be cured with treatment.

Endometrial cancer dispenses a clinical and diagnostic enigma owing to its difficulty in detection using the Papanicolaou (Pap) screening method, as it fails to detect abnormal endometrial cells in about fifty percent of cases. Consequently, direct endometrial sampling is a costly procedure since it is done by collecting biopsy or curettage under dilatation among symptomatic women.

Even though advances in endometrial carcinoma detection have accomplished a certain rate of success, it is now believed that screening of postmenopausal women using proper cytological sampling methods will help improve early detection of the disease and hence prolong life.SAP-1 devices, Endometrial Tao brush, Aspiration devices, and Pipelle are among the tools used to screen the Endometrial lining. Out of these screening tools, the Endometrial Tao Brush is the utmost auspicious uterine sampler for screening for malignancies in the endometrium. Endometrial Tao Brush is easy to use, can be used in the absence of anesthesia in an ambulatory setting and it turns out to be well tolerated by women.

Therefore, this study determined the performance characteristics of endometrial cytology for the detection of malignancy and atypical hyperplasia using conventional and manual liquid-based cytology specimens collected with the Endometrial Tao brush

sampler among women diagnosed with endometrial malignancies using imaging techniques attending Kenyatta national hospital, Kenya.

1.3 Justification

Endometrial cytology using Tao Brush for direct sampling of the uterine cavity is a relatively painless, reliable and cheap technique for detecting lesions from the endometrium and previous studies demonstrated that its specimen satisfaction was 89.9 to 100% while the pathological accuracy was 91.0 to 100%. Tao brush can sample a representative section of the endometrial cavity and can be done in an outpatient setting with the least anguish and distress to the patient.

The use of Endometrial Tao Brush for screening of endometrial cancer and its precursors will facilitate early detection of cancer and therefore improve treatment outcomes for patients with Endometrial cancer. Endometrial Tao brush rarely causes hemorrhage and perforation and has minimal risk of infection. It also has a sheath that is pulled back after the collection of endometrial cells hence there is no contamination with the endocervical cells and there is no need for hospitalization since it is done without the need for general anesthesia.

This will encourage more patients to get screened and hence facilitate early detection of cancer and therefore improve treatment outcomes for patients with Endometrial cancer and prolong the quality of life.

1.4 Research Questions

i. What will be the significant difference between Conventional and Manual liquid-based specimens in the detection of endometrial malignancies?
ii. What will be the performance characteristics of endometrial cytology using Tao brush for the detection of endometrial malignancies?
iii. What will be the discordance rate and diagnostic level of agreement between Endometrial Brush Biopsy (Tao brush) and histopathological findings (Gold Standard) in the detection of endometrial cancer and atypical hyperplasia?

1.5 Hypothesis

1.5.1 Null Hypothesis

There will be no significant performance difference between the endometrial brush Biopsy (Tao brush) and dilatation and curettage (Gold standard) in the detection of endometrial cancer and atypical hyperplasia.

1.5.2 Alternative Hypothesis

There will be a significant performance difference between the endometrial brush biopsy (Tao brush) and dilatation and curettage (Gold standard) in the detection of endometrial cancer and atypical hyperplasia**.**

1.6 Objectives

1.6.1 General Objective

To determine the performance characteristics of endometrial Tao brush in detection of endometrial cancer and atypical hyperplasia.

1.6.2 Specific Objectives

i. To compare conventional and manual liquid-based specimens collected with Tao brush in the detection of endometrial lesions.
ii. To evaluate the performance characteristics of endometrial cytology using Tao brush for the detection of endometrial malignancies.
iii. To determine the discordance and diagnostic level of agreement between endometrial brush cytological sampler (Tao brush) with histopathological findings (Gold standard).

CHAPTER TWO

LITERATURE REVIEW

2.1 Introduction

Endometrial carcinoma stands as the fifth most frequent cancer in females globally its incidence is rising in countries that are developing (Horta & Cunha, 2019a). Endometrial hyperplasia is a proliferation of the lining of the uterus and can be a prototype of some types of endometrial cancer. Endometrial Hyperplasia can be classified into two categories; atypical hyperplasia and hyperplasia without atypia (Me *et a*l., 2018).

Endometrial cancer is most frequently diagnosed in women who are postmenopausal (Van Hanegem *et al*., 2016). Nevertheless, fifteen percent to twenty-five percent of cases are found in pre-menopausal women (Williams *et al*., 2008). Four percent of cases arise in females under forty years, seven percent to ten percent under 45 years. Endometrial Adenocarcinoma of the endometrium is often diagnosed in its initial stages, it's well-differentiated and is the most common subtype (Visser *et al*., 2017b). In young females, 5 and 10year disease-free survival (DFS) after standard surgical treatment (bilateral salpingo-oophorectomy and hysterectomy) is ninety-nine percent and ninety-eight percent respectively. Young women with endometrial cancer also are more likely to have early-stage low-risk tumors (Yang *et al*., 2019).

The key risk factor for endometrial endometrioid adenocarcinoma and endometrial hyperplasia is obesity (Njoku *et al*., 2019). Obesity is linked to the marginal conversion of androgens to estrogens by adipose tissue. Other risk factors comprise hyperinsulinemia, hypercholesterolemia, a sedentary lifestyle, diabetes, nulliparity, hypertension, anovulation and early menarche but most of the factors are associated with, and not independent of obesity (Aue-Aungkul *et al*., 2018). Management for EEC and atypical hyperplasia is majorly based on progestin's, administered orally or through a levonorgestrel-releasing intrauterine system (IUS) (Horta & Cunha, 2019a).

2.2 Epidemiology

Cancer of the Endometrium stands as the most prevalent malignancy of the female reproductive tract (Visser *et al*., 2017b). Cancer of the Endometrium is the sixth cause of mortality among females in the United States and the eight cause of death associated with cancer among European women (Van Hanegem *et al*., 2016). It is also the 4th most frequent carcinoma in females after breast, lung, and colorectal cancers (Van Hanegem *et al*., 2016). In Kenya the incidence of Endometrial is 1.7%. The death rate of Uterine cancer has had an upsurge of greater than a hundred percent during the previous twenty years, rising by eight percent since 2008 (Rodriguez *et al*., 2019). The average age of patients when they are diagnosed is sixty-three years, with ninety percent of cases arising in women above 50 years of age. Only twenty percent of patients who have endometrial cancer are diagnosed before menopause (Du *et al*., 2016). In addition, more than ninety percent of ECs are sporadic while the rest are inherited. Sporadic cases are classified clinicopathologically as those dependent on estrogen Endometrioid endometrial cancers (Type I) and Non-endometrial endometrial Cancers (Type II) (Du *et al*., 2016).

2.2.1 Epidemiological Risk Factors

Greater risk for being exposed to EEC, a tumor type dependent on estrogen, is mainly related to early menarche, obesity, diabetes, unopposed estrogen therapy use, late menopause and nulliparity (Rodriguez *et al*., 2019). Old age is a risk factor for clear cell and serous Endometrial Cancer. The use of tamoxifen upsurges the risk of having Endometrial Cancer; histological subtypes found in tamoxifen users are, high-grade endometrioid ECs, serous Endometrial Cancer and Carcinosarcomas *(*Aue-Aungkul *et al*., 2018). Other risk factors may include being older than 50 years, family genetic history of EC, diabetes mellitus, hypertension, thyroid disease and obesity. Tumors of Type II origin are commonly found in black females who are older than fifty years (Doll *et al.*, 2020). Approximately seventy percent of obese women are diagnosed with EC in its early stages hence the risk of death somewhat rises as body weight increases. Shielding factors may include grand multiparity and former use of COCs for more than a year (Cakmak & Oge, 2020).

2.2.2 Genetic Risk Factors

Cowden Syndrome, Polymerase Proofreading Associated Polyposis (PPAP) and Lynch Syndrome are associated with increased genetic risk for developing Cancer of the Endometrium (Woolderink *et al*., 2020).

Lynch Syndrome is a predisposition syndrome to cancer that is vastly penetrant and is initiated by a germline mutation which is monoallelic in a mismatch repair gene, specifically *PMS2, MSH6, MSH2* or *MLH1* (Woolderink *et al*., 2020). Carriers of mutation are at greater risk of getting Endometrial Carcinomas and colorectal cancer, the two major tumors caused by this Syndrome, also stomach cancer, cancer of the kidney, small intestine, Ovarian cancer, biliary tract and the skin. Roughly two to six percent of Endometrial Cancers are attributed to Lynch Syndrome c (Woolderink *et al*., 2020).

Cowden syndrome is a disorder in which Phosphatase and tensin homolog (*PTEN*) carriers of the mutation have an augmented susceptibility for acquiring multiple hamartomas, breast cancer, endometrium, thyroid, kidney, colorectal and the skin (Vermij, 2020). Polymerase Proofreading Associated Polyposis is a dominant cancer susceptibility syndrome that is autosomal and it is accredited to mutations of the germline in the exonuclease domain of *POLD1* or *POLE*. The carriers of PPAP mutation are at increased risk of acquiring Colorectal, Endometrium, brain and breast cancer (Vermij, 2020).

2.3 Pathogenesis of Endometrial Cancer

More than ninety percent of ECs are sporadic while the rest are hereditary. Sporadic cases are classified clinic-pathologically as estrogen-dependent EECs (type I) and NEECs (type II) (Du *et al*., 2016). With developments in molecular genetics, the pathogenesis of hereditary and sporadic ECs has progressively been elucidated, and the pathogenic mechanisms have also been appraised concerning types I and II (Ohgami, 2014).

2.3.1 Genetic Mechanisms Involved in Type I Endometrial Carcinoma

2.3.1.1 The Role of Phosphate and Tensin Homolog (PTEN)

Endometrioid adenocarcinoma encompasses a range of genetic alterations. The gene encoding of PTEN, which is found on chromosome ten, is one of the most commonly altered and it encodes tyrosine kinase with an enzyme and lipid phosphatase (Koninckx *et al*., 2019). The Phosphatase activity of PTEN's is involved in cell migration and spreading, the focal adhesions inhibitions and MAPK signaling stimulated by growth factor. Hence, loss of Phosphate and Tensin Homolog expression can lead to abnormal growth of cells, migration spreading and apoptosis escape (Koninckx *et al*., 2019). Up to eighty-three percent of EAs and fifty-five percent of lesions that are precancerous show loss of Phosphate and Tensin Homolog expression, an initial occurrence in endometrial cancer carcinogenesis (Bulun *et al*., 2019).

2.3.1.2 The Role of Microsatellite Instability (MSI)

Another significant genetic variation found in twenty to forty-five percent of endometrial endometrioid adenocarcinomas is Microsatellite instability(MSI) (Koninckx *et al*., 2019). Microsatellite instability originates from damaged DNA repair mismatch, leading to the buildup of inaccurate insertions of nucleotides, misincorporations and deletions when DNA replication is done (Koninckx *et al*., 2019). Failure to activate the mismatched repair gene MutL Homolog 1 by its promoter methylation seems to be the utmost common source of MSI in endometrial cancer of sporadic origin (Vermij, 2020). In addition to MutL Homolog 1 (MLH1), the expression loss of MSH6 or MSH2 is another case of repair genes that are mismatched affected in ECs. Mechanisms of Inactivation of MutS Homolog 2 (MSH2) are not clear yet, this is caused by the fact that mutation and promoter methylation of the gene is seen rarely (Tran & Gehrig, 2017).

2.3.1.3 The Role of KRAS Proto-Oncogene

The Kirsten rat sarcoma viral oncogene homolog (KRAS) proto-oncogene translates a small cellular membrane of the inner plasma GTPase that works like a molecular

switch (on/off). Once it is activated, it activates and recruits proteins required for the proliferation of cell signaling cascades (Bulun *et al*., 2019). These signaling tracks are mainly related to the growth of tumors and differentiation. Thereby the Kirsten rat sarcoma viral oncogene homolog Proto-oncogene mutations are activated of which are present in ten to thirty percent of Endometrial endometrioid adenocarcinomas (Bulun *et al*., 2019). Kirsten rat sarcoma viral oncogene homolog mutations are there in 13% of patients with endometrial hyperplasia without atypia and 22% of those with atypia, demonstrating that KRSA mutation might be an initial occurrence in endometrial cancer carcinogenesis. KRAS mutations arise more commonly in tumors with Microsatellite instability, proposing that both genes occur concurrently before clonal expansion. contrastingly, PTEN and Kirsten rat sarcoma viral oncogene homolog (KRAS) mutations don't seem to coexist in tumors(Koninckx *et al*., 2019).

2.3.1.4 The Role of E-Cadherin and B-Catenin

Gene encoding mutations in b-catenin are identified in about twenty percent of endometrial cancers. b-catenin is a dual-purpose protein that controls gene transcription and the coordination of adhesion of cell-cell components (Bulun *et al.*, 2019). It becomes incorporated into the E-cadherin intracellular domain; whose extracellular domain is also important for the adhesion cell cells. Both b-catenin and E-cadherin are vital constituents of adherens junctions formed by a protein complex, which are essential for creating and sustaining epithelial cell barriers and layers. The role of b-catenin in endometrial carcinogenesis is not known yet. There is no relationship of gene mutations of b-catenin with PTEN mutations, KRAS or with MSI have been established, signifying that the Wnt signaling pathway plays an independent role, such as maintenance of CSC potency in carcinogenesis of endometrial lesions (Vermij, 2020).

2.3.2 Molecular Genetic Mechanisms in Type II Endometrial Carcinoma

The main gene alterations of endometrial carcinoma (type II) and their frequencies are as follows;

2.3.2.1 The Role of p53

Tumor protein p53 is the most outstanding genetic alteration, arising in about 90% o adenocarcinomas of the serous subtype, which are the most predominant type II endometrial tumor (Vermij, 2020). The p53 mutations do not seem to coexist with Phosphate and Tensin Homolog mutations are nearly always linked with aneuploidy. In contrast to endometrial endometrioid adenocarcinomas, mutations in PTEN, KRAS genes MSI mutations are very rare amongst serous adenocarcinomas. Microsatellite instability has been identified only in serous adenocarcinomas and mixed endometrioid and not in pure serous adenocarcinomas (Vermij, 2020).

2.3.2.2 The Role of p16

p16 is a protein that slows down cell division by decelerating the process of progression of the cell cycle to the S phase from G1, hence a tumor suppressor gene. It is located on chromosome 9p21 and it encodes a protein that regulates the cell cycle (Ohgami, 2014). The inactivation of the p16 gene is seen in roughly forty-five percent of adenocarcinomas of the serous subtype but it leads to uncontrolled cell proliferation in less than ten percent of endometrial endometrioid adenocarcinomas (Tran & Gehrig, 2017). Furthermore, most EA with inactivation of p16 is linked to prognosis which is poor and advanced tumor stage (Tran & Gehrig, 2017).

2.3.2.3 The Role of HER-2 Oncogene

The Human Epidermal Growth Factor Receptor 2 oncogene encodes tyrosine kinase which is a transmembrane receptor, allied in cell-signaling pathways (Tran & Gehrig, 2017). HER-2 proteins form hetero or homo-dimers when HER3, HER1 or HER4 of similar EGF receptor family as HER2 is activated, leading to transduction of the signal. Human Epidermal Growth Factor Receptor 2 oncogene activates pathways signaling that involve PI3K, STAT PKC, and MAPK molecules, which are crucial for cell proliferation promotion and apoptosis inhibition. Overexpression of HER2 and gene amplification has been detected in approximately 45 and 70% of serous adenocarcinomas (Koninckx *et al*., 2019).

2.3.2.4 The Role of E-Cadherin

E-cadherin is a trans-membranous protein with one intracellular domain and five extracellular domains (Tran & Gehrig, 2017). E-cadherin, the adhesion protein plays an essential part in the epithelial morphogenesis process. E-cadherin protein expression is downregulated during the late stages of progression of epithelial tumor and metastatic potential acquisition. A decrease in E-cadherin protein expression is linked to a decreased cohesion of cell-cell and may facilitate the progression of cancer by increasing cellular metastasis and/or invasion and proliferation. Amongst clear cell and serous adenocarcinomas, negative E-cadherin protein expression is ascertained in 62% of cases and reduced E-cadherin expression in 87% of cases (Rodriguez *et al*., 2019).

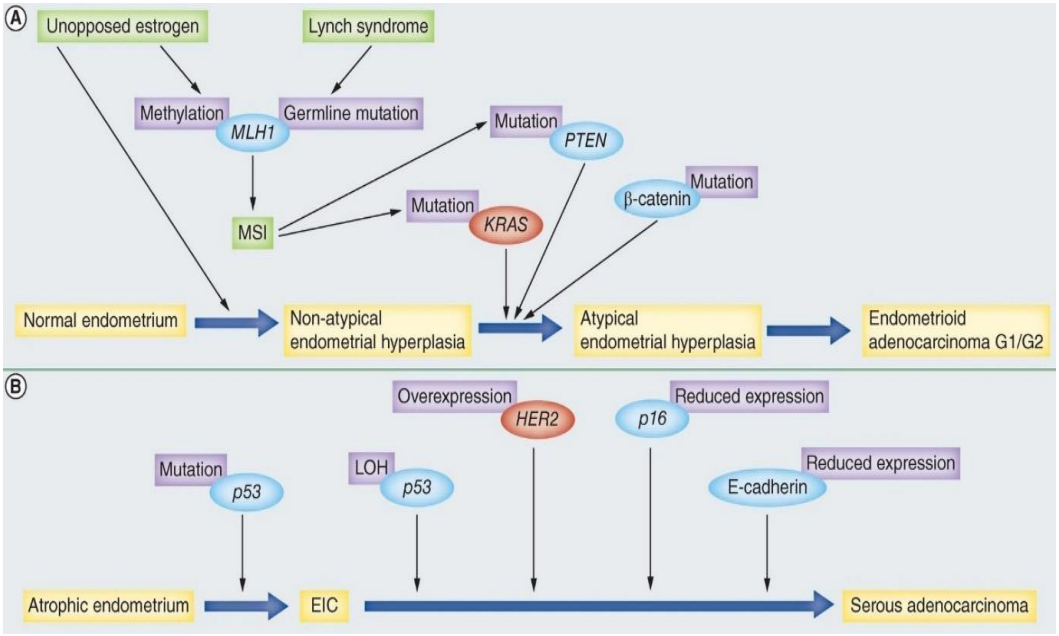

Figure 2.1: Models for Type I and Type II Endometrial Carcinogenesis

Adopted from (Rodriguez et al., 2019)

(A) Type I and (B) type II. The blue boxes and red indicate tumor suppressor genes and oncogenes respectively

2.4 Endometrial Cancer Screening and Diagnosis

2.4.1 Screening and Prevention

The American Cancer Society recommends that women 65 years and above should be well conversant with the symptoms and risks of EC and are advised to go for screening if symptoms arise (Wyckoff *et al*., 2016). Evidence supporting the screening of women who are asymptomatic is not there at the moment, except for those with or who are at a higher risk of acquiring Lynch syndrome. It is recommended that an endometrial screening and evaluation should be done yearly on women from the age of 35 due to a twenty-two percent to fifty percent risk of getting EC (Woolderink *et al*., 2020). Women diagnosed with this syndrome ought to be counseled to have a menstruation chart and any abnormal bleeding should be reported, also those who are 40 years and above and are not planning to get pregnant later in life can opt for a prophylactic hysterectomy (Braun *et al*., 2016).

Recommendations for endometrial cancer screening for patients under tamoxifen therapy are not there yet; nevertheless, for those who present with abnormal uterine bleeding, a diagnostic checkup should be considered. Endometrial cancer can be prevented by managing risk factors such as diabetes, obesity and hypertension (Tran & Gehrig, 2017). Progesterone addition for women on hormonal therapy has displayed a decrease in the risk of contracting endometrial cancer (Woolderink *et al*., 2020).

2.4.2 History, Physical Examination, Laboratory Evaluation and Diagnostic Studies

2.4.2.1 History

The most common clinical presentation of EC in women who are postmenopausal is vaginal bleeding. Most postmenopausal women with EC are diagnosed in the initial stages hence increasing the possibility of successful treatment. Nevertheless, only ten-twenty percent of postmenopausal females who are evaluated for AUB are diagnosed with EC since atrophy of the endometrium is the commonest cause of bleeding in postmenopausal women (Narice *et al*., 2018). All postmenopausal bleeding should be

examined, particularly in the presence of endometrial cancer and atypical hyperplasia risk factors. The ACOG recommends that women who are 45 years and above with abnormal bleeding of the uterus be assessed for EC or those younger than 45 years with a history of unopposed estrogen exposure. Tissue sampling of the endometrium or ultrasonography can be used for evaluation (Van Hanegem *et al*., 2016).

2.4.2.2 Physical Examination

Physical tests outcomes in EC patients are only a few. A checkup of the pelvic should be done to assess other causes of abnormal bleeding, such as the cervix or the vagina. Palpation of the adnexa and the uterus should be done for assessment of unusual masses. Physical examination outcomes which are abnormal may be indicative of more advanced disease (Colombo *et al*., 2016).

2.4.2.3 Laboratory Evaluation

Endometrial cancer evaluation has no particular laboratory tests. Laboratory tests ought to consist of pregnancy tests in women of reproductive age. A partial thromboplastin time, full haemogram test and prothrombin time could also be done for women with abnormally heavy bleeding. Occasionally, the presence of cells from the endometrium cells in a Papanicolaou smear report can indicate the presence of cancer of the endometrial although Pap smears tests aren't considered as part of the assessment and evaluation of EC (Braun *et al*., 2016)

2.4.2.4 Diagnostic Studies

Endometrial biopsy or transvaginal ultrasonography are recommended by most guidelines during the initial stages of endometrial cancer evaluation. The ACR Appropriateness Criteria has tables that clearly outline the imaging studies preferred for evaluation of AUB both Pre and Post-menopausal, pre-treatment and follow-up of EC patients based on the options available, the degree of invasiveness and preference of the patient. (Levy-Zauberman *et al*., 2017).

2.4.3 Imaging Techniques

2.4.3.1 Ultrasound

Transvaginal ultrasonography (TVUS) is inexpensive, quick and the patient is not exposed to ionizing radiation hence it is mostly used in the initial evaluation of postmenopausal women with abnormal bleeding history. Endometrial Cancer normally presents as endometrium thickening and TVUS diagnosis of EC is based on anteroposterior dimension measurement of the endometrial thickness (Williams *et al*., 2008). The specificity and sensitivity of TVUS in detecting Endometrial Cancer are approximately 61% and 96% respectively, when the threshold of endometrial thickness is 5 mm in women who are postmenopausal (Tsai & Goldstein, 2012). For proper evaluation of deep invasion into the myometrium, a meta-analysis recommends specificity of 71-90% and sensitivity of 68-100%. Moreover, a thin endometrium has a very high negative predictive value. Also, One is more exposed to EC when the endometrium has margins that are poorly defined and echotexture is heterogeneous (Tsai & Goldstein, 2012).

2.4.3.2 Computed Tomography

On Computed Tomography, Cancer of the Endometrium is seen as a hypo enhancing and a hypoattenuating mass in the uterine canal (Aue-Aungkul *et al*., 2018). Computed Tomography has poor differentiation of soft tissue and hence it's not commonly used in the local staging of Endometrial Cancer but it is used to evaluate EC in its advanced stages by detecting distant and nodal metastases. The specificity and sensitivity of CT in myometrial invasion evaluation ranges from 42% to 75% and from 40% to 83% respectively (Audebert, 2018).

2.4.3.3 Magnetic Resonance Imaging

MRI is well-thought-out to be the utmost precise modality of imaging for the local staging pretreatment of Endometrial Cancer and it also has excellent delineation of soft tissues. Endometrial Cancer is enhanced less than the myometrium on post-

contrast dynamic images. The overall accuracy of MRI staging is stated to be 83-92% (Njoku *et al*., 2019).

2.4.3.4 Computed Tomography / Positron Emission tomography

Computed Tomography / Positron emission tomography (CT/PET) permits for metabolic and anatomic information acquisition simultaneously and has turned out to be an important diagnostic tool for inpatient surveillance with different cancer types and oncologic staging. Fluorodeoxyglucose (FDG) normally amasses in lesions that are malignant secondary to their glucose metabolism which is high (Levy-Zauberman *et al*., 2017). Though Endometrial Cancer shows strong FDG uptake, restriction of the value addition of Computed Tomography/ Positron emission tomography in early staging of Endometrial Cancer is caused by inadequate physiologic uptake and spatial resolution in women who are pre-menopausal. Nevertheless, Computed Tomography/ Positron emission tomography is extremely specific and sensitive for detecting para-aortic or pelvic lymphadenopathy which is positive and distant metastases in high-risk patients with Endometrial Cancer (Audebert, 2018).

2.4.4 Endometrial Biopsy

2.4.4.1 Classification by Histopathology

Histopathologically, Ec's are classified into two; the commonest which accounts for about 90% of the neoplasms is the EA (type I) and they are linked to Obesity, hypertension and estrogen excess (Yang *et al*., 2019). These types of growth often arise in patients with a history of endometrial hyperplasia, it has a good prognosis, occurs in the early stages post menopause and generally falls under low histological grade (Visser *et al*., 2017a).

Endometrial Cancer, Type II include the serous and clear-cell Carcinosarcomas (Tran & Gehrig, 2017). Endometrial cancers of Type II origin usually occur in females who are older, spreads like ovarian cancer and carry a worse prognosis. High-grade tumors have a poor prognosis and they include grade 3 EA tumors and all cancers of type II origin (Tran & Gehrig, 2017).

Endometrioid adenocarcinoma is the commonest histological subtype of EC which roughly accounts for 70–80% of Endometrial Cancer Cases (Horta & Cunha, 2019b). NEEC comprises of clear-cell and serous carcinomas and approximately account for 1–5% and 5–10% of EC respectively. The other ECs types which are aggressive are differentiated and undifferentiated Endometrial Cancer and uterine Carcinosarcomas (Bianchi *et al*., 2019).

Endometrial cancer rates and related mortality are rising amongst women of all backgrounds, but in the past decade, the rates amongst Black women have risen most. This is supported by the fact that the rates of hysterectomy among black women are is higher than in white women, hysterectomy-adjusted rates of endometrial cancer show a disproportionate rise in incidence (Doll *et al*., 2020). There is also an increase in the incidence of tumors with aggressive, non endometrioid histologic features amongst Black women and the cause of such is unclear, although early access to appropriate care may contribute to these variances. Henceforth, more studies need to be done to find out why black women are more exposed to non-endometrioid tumors more than women of other races (Doll *et al*., 2020).

2.4.4.2 Grading

Tumors are graded according to the FIGO system, Grade 1 are tumors without solid areas, Grade 2 are neoplasms with less than fifty percent solid area and Grade 3 are tumors in which the solid area occupies greater than fifty percent (Mittal *et al*., 1988).

2.4.4.3 Stage

Staging of Endometrial Cancer is done based on the surgicopathologic FIGO 2009 system and tumor–node–metastasis (TNM) system (Horta & Cunha, 2019a). Total abdominal hysterectomy, para-aortic and pelvic lymphadenectomy, peritoneal lavage, bilateral salpingo-oophorectomy and exploratory laparotomy are among the findings used for the surgicopathologic staging system. Surgical staging is not done in patients who have severe morbidity secondary to comorbidities and those who are at greater risk for mortality. Patients with high-grade tumors or deep myometrial invasion always receive Para-aortic lymphadenectomy (Horta & Cunha, 2019b).

Staging is done in the FIGO system from Stage I to IV and from TX to T4 in TNM System as shown below, (Doll *et al*., 2020).

Table 2.1: FIGO Staging of Endometrial Cancer

Adopted from (Doll *et al*., 2020)

TNM system, T category	FIGO system	T criteria
TX		Primary tumor cannot be assessed
T0		No evidence of primary tumor
T1	I	Tumor confined to corpus uteri, including endocervical; glandular involvement
T1a	IA	Tumor limited to the endometrium or invading less than half of the myometrium
T1b	IB	Tumor invading one half or more of the myometrium
T2	II	Tumor invading the stromal connective tissue of the cervix but not extending beyond the uterus. Does not include endocervical glandular involvement
T3	III	Tumor involving serosa, adnexa, vagina or parametrium
T3a	IIIA	Tumor involving serosa, adnexa, or both (direct extension or metastasis)
T3b	IIIB	Vaginal involvement (direct extension or metastasis) or parametrial involvement
T4	IVA	Tumor invading the bladder mucosa, bowel mucosa or both. Bullous edema is not sufficient to classify a tumor as T4

2.4.4.4 Types of Endometrial Malignancies

2.4.4.4 .1 Serous Endometrial Cancers

SECs are normally estrogen-independent and arise in an atrophic endometrium. These are high-grade tumors, preceded by SEIC, often diagnosed in the late stages with an increased risk of recurrence. The p53 stabilization or *TP53* mutations occurrence in serous endometrial intraepithelial carcinoma supports this anomaly being an initial occurrence in the pathogenesis of Serous endometrial cancers (Koninckx et al., 2019).

2.4.4.4.2 Uterine Carcinosarcomas

Uterine Carcinosarcomas are alleged to originate from Endometrioid Endometrial Carcinomas of high histologic grades, monoclonal, SEC or other subtypes which are aggressive that have undergone metastasis from biphasic tumors to form components that are sarcomatous (Horta & Cunha, 2019a). Since they originate from ECs which are aggressive, the genes which commonly mutate of somatic origin in Uterine Carcinosarcomas are also frequently mutated in other Endometrial Cancers, SECs tumor types most closely resemble Uterine Carcinosarcomas, thus upholding the likelihood that epithelial-mesenchymal transition backs to the prognosis which is poor for females with Uterine Carcinosarcomas (Horta & Cunha, 2019b).

2.4.4.4.3 Clear Cell Endometrial Cancers

Multiple studies have presented that Clear cell endometrial cancers that are clinically diagnosed share features of mutational with both Serous endometrial cancers and EECs but a clear molecular etiology of Clear cell endometrial cancers remains unclear (Wyckoff *et al*., 2016). However, a clinical diagnosis of Clear cell endometrial cancers embodies a challenge since the classification of this tumor Histopathologically is puzzling. Clear cell endometrial cancers have been the focus of exome sequencing studies and targeted gene sequencing but have not been analyzed using integrated analyses similar to techniques used by TCGA (Wyckoff *et al*., 2016).

2.4.4.4.4 Endometrial Hyperplasia

EH is a pathology of the uterine lining demonstrating a range of endometrial changes morphologically. An increase in the glandular to the stromal ratio of the endometrium is the main characteristic when compared to normal endometrium which is proliferative (Aue-Aungkul *et al*., 2018). The importance of Endometrial Hyperplasia clinically is in its related risk to progress to EEC and other precursor lesions of EC. Standardized management of patients with Endometrial Hyperplasia can be perplexing since EH systems of histopathological classification display varying and wide ranges of diagnostic reproducibility (Taraboanta *et al*., 2020).

World Health Organization classification of EH in 2014 has two categories: atypical hyperplasia and hyperplasia without atypia. This classification is based on new findings on molecular genetic changes. Hyperplasia without atypia usually regresses and genetic changes are not significant. Atypical EH shares many mutations (paired box gene2 inactivation, microsatellite instability, KRAS, phosphatase and tensin homolog and β- catenin mutation) with EEC and has a high co-incidence or conversion to endometrial cancer (Lv *et al*., 2020).

Hyperplasia without or with atypia has different degrees of progression to cancer. Atypical endometrial hyperplasia progresses to cancer at a higher rate as compared to Hyperplasia without atypia since thirty-six percent to fifty-nine percent of women with atypical EH undergo hysterectomy (Cakmak & Oge, 2020).

2.5 Cytology

2.5.1 Exfoliated Endometrial Cells

Exfoliated Cells from the Endometrium are small and they are normally arranged in tight ball-like clusters seen. The nuclei are a bit smaller as compared to those of an intermediate cell. The pattern of the chromatin poses a difficulty to determine due to dark nuclei and cells which overlap, nucleoli are not easily seen, Mitoses is not evident, Cytoplasm is very scanty, Karyorrhexis is often present and its occasionally vacuolated

(Horta & Cunha, 2019b). Ill-defined Cell borders are present and the clusters of endometrial cells which are Double-contoured may be seen (Horta & Cunha, 2019b).

According to the 2014 Bethesda System, it is stipulated that cells from the endometrium which are exfoliated should only be reported in females forty-five years and above. There is no need of reporting the presence of cells from the Endometrial which are Benign-appearing in females under forty-five years, even if they are noted out of the menstrual phase since they portray little or no significant indication of Cancer of the Endometrium (Horta & Cunha, 2019b).

2.5.2 Endometrial Sampling

Endometrial sampling is a technique used to obtain samples from the uterine lining endometrial to screen and evaluate women with AUB histologically. ES is normally done to evaluate the pathologies of the endometrium that are significant such as EC and EH in women who on imaging such as Ultrasonography have a thick wall of the endometrium of 5mm and above, postmenopausal bleeding and glandular cells which are atypical on a cervical smear (Bianchi *et al*., 2019).

Former studies have shown that the endometrial sampling performance is as great when compared to D&C or aspiration techniques such as Vabra aspirator. Endometrial sampling has been shown to have a specificity of 99%–100% and a sensitivity of 90% in cases of bleeding post-menopause in diagnosing EC and EH with dilatation and curettage or hysterectomy with biopsy as a gold standard of diagnosis (Van Hanegem *et al*., 2016).

The insufficient volume of endometrial tissue obtained is the major shortcoming of endometrial sampling. Some types of Endometrial sampling have been known to yield insufficient samples for certain diagnoses pathologically in six to thirty-three percent of cases. About six percent of women who are postmenopausal with specimens that are non-diagnostic after ES for AUB on further assessment and evaluation are found to have significant endometrial malignancies (Van Hanegem *et al*., 2016).

2.5.3 Endometrial Sampling Devices

2.5.3.1 Tao Brush

The Endometrial Tao Brush was introduced and approved by the FDA in 1993 for general medical use. To commence the collection of cells from the endometrium, the sheath is pulled back, and then the Tao brush is inserted into the uterine cavity at the level of the fundus through the cervical canal. The 3 -3.5cm brush is then rotated at 360° three to five times to collect cells from the endometrium. The outer sheath is then pushed back to the tip, and the device is removed from the uterine cavity. The endometrial brush is then cut off and immersed into liquids meant to preserve the cells and its sent for cytological assessment and diagnosis (Du *et al*., 2016). The Endometrial Tao Brush can be used in an ambulatory setting, without anesthesia (local or regional), as seems to be tolerated well by women and it is simple to use. Pathological accuracy with the Endometrial Tao Brush, according to previous articles from the year 1997 to 2016, was 91.0 to 96.0 % while the Sample satisfaction was 89.9 to 100 % (Du *et al*., 2016).

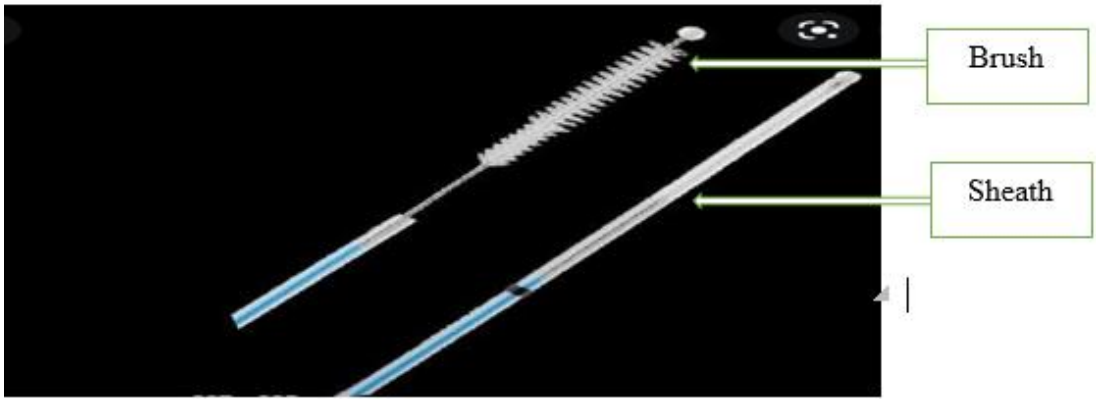

Figure 2.2: Tao Brush

Adopted from (Du *et al*., 2016)

2.5.3.2 Pipelle

Pipelle is a polypropylene flexible device that operates using a suction mechanism. It is an ideal ambulatory endometrial biopsy procedure since it can be inserted into the

cervical canal without dilatation. Pipelle is the most commonly used technique for evaluating the uterine canal (Taraboanta *et al*., 2020).

Pipelle endometrial biopsy device samples only 4% of the uterine cavity and has a sensitivity up to 97%. A positive biopsy can help the patient in avoiding the inconveniences caused by the dilatation and curettage procedure, but a nonspecific finding requires further evaluation. The core technique for sampling the uterine lining is the endometrial biopsy with a Pipelle cannula. The Pipelle endometrial biopsy is affordable, efficient, safe and an accurate technique for sampling women with EC symptoms (Du et al., 2016).

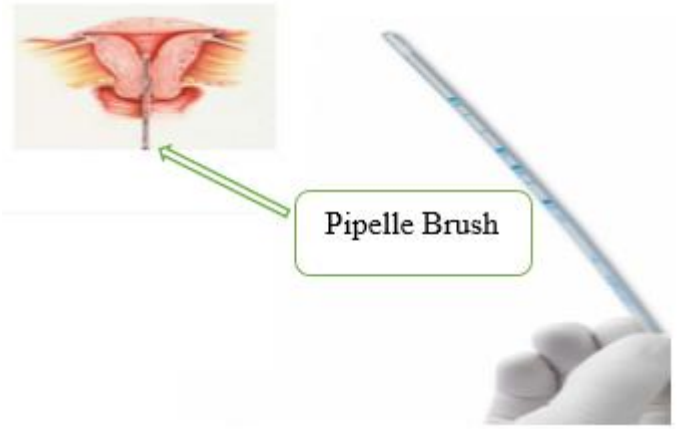

Figure 2.3: Pipelle Brush

Adopted from (Taraboanta *et al*., 2020)

2.5.3.3 Aspiration Devices

Aspiration technology was described in 1934 by Bela Lorincz, as a technique with minimal complications that could be used in an ambulatory setting. In the recent past, aspiration technology is a reliable, safe and simple method for endometrial malignancies screening and evaluation. Amongst the aspiration devices in the market, the Vabra aspirator is the one that is mostly used in clinical trials to screen and evaluate endometrial malignancies (Du *et al*., 2016).

The Vabra aspirator device is a metal cannula with an external diameter of 3 mm and a length of 24 cm. It also has an aperture of 1.5 × 16 mm at the inner side of the curved ending, the cannula is connected to a receptacle of plastic material, which contains a plastic sieve to retain the tissue fragments. After the cannula is inserted into the uterine cavity, the pump is switched on. An index finger is used to cover the two proximal openings in the cannula to create negative pressure while holding the plastic receptacle of the Vabra aspirator. As a result of this, the uterus lining is emptied by suction, and then the cannula is withdrawn from the uterus briefly. The procedure is then repeated several times to make sure that the whole interior surface of the uterus is sampled (Levy-Zauberman *et al*., 2017).

In a study done, the samples obtained using aspiration devices among 150 patients were adequate for cytology in 93 % of the cases. A sensitivity of 88.2 % and a specificity of 88.7 % were found in a symptomatic group of 100 women, and, only 2 % of the results were false negative in comparison to histological diagnosis. The use of the aspiration technique in endometrial cell sampling also seems to be promising as a screening tool in asymptomatic women (Du *et al*., 2016).

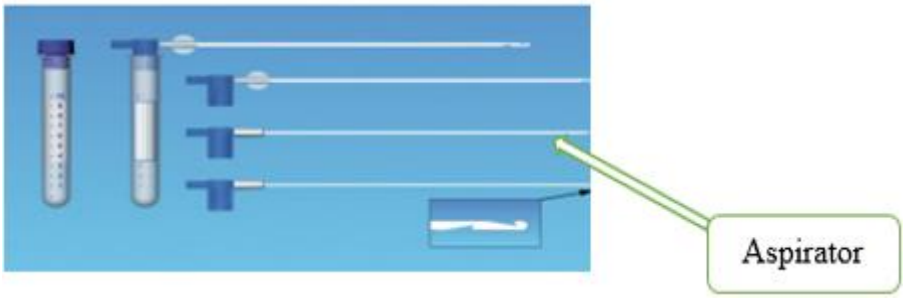

Figure 2.4: Vabra Aspirator

Adopted from (Du et al., 2016)

2.5.3.4 SAP-1 Device

The SAP-1 device was patented and received permission in 2001 to be used in China. The sampling device has a sheath that is 25 cm in length and is roughly 3 mm in diameter. The role of the shielding sheath outside the loop is to prevent contamination

with vaginal and cervical cells when removing the device from the uterine canal (Du *et al*., 2016). To collect cells from the endometrium, the device is first inserted into the uterine canal to the level of the fundus and then the outer sheath pulled back, and then the loop is rotated in a clockwise direction 15 times. After collecting enough endometrial cells, the outer sheath is pushed to the tip and the device removed from the uterine canal. The SAP-1 sampler may become a reliable technique for screening endometrial cancer and its precursors, particularly in asymptomatic and postmenopausal women (Du *et al*., 2016).

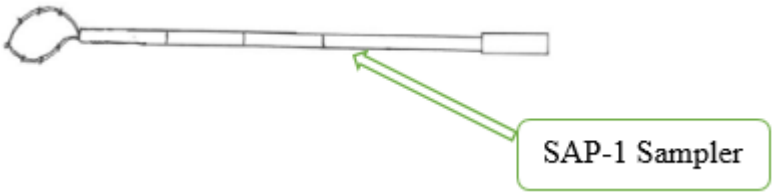

Figure 2.5: SAP-1 Sampler

Adopted from (Du *et al*., 2016)

2.5.3.5 Li Brush

The Li Brush received a patent in 2014 (ZL.201420720356.8) and it was invented to correct the limitations of the other samplers such as pipelle and SAP-1 Sampler which are not able to sample the uterine horns. The Li Brush was designed as an inverted cone, similar in shape to the uterine cavity when compared with other samplers *(Du et al*., 2016). Theoretically, this Brush can collect more cells from the endometrial lining than the other samplers, particularly cells in the uterine horns, hence allowing a more accurate diagnosis of endometrial lesions. Clinical trials of the Li Brush have been launched and they are ongoing in outpatient and inpatient clinics in the Department of Gynecology of the First Affiliated Hospital, Xi'an Jiaotong University (Lv *et al*., 2020)

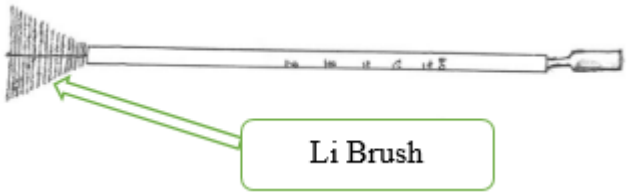

Figure 2.6: Li Brush

Adopted from (Lv *et al*., 2020)

2.5.4 Liquid Based Cytology

LBC is a new method approved by the FDA in 1996 for preparing cervical samples for cytological screening and examination. Liquid-Based samples for cytological examination are prepared by dispensing the sample after collection into a container with preservative fluids (Du *et al*., 2016). LBC can lessen the obscuring factors such as blood and mucus and offer thin-layer samples for cytology screening and examination. Cell block can be made from the remaining cytological samples (Ranjana & Sadhna, 2016). The glandular architectures and the morphology of cells from the endometrial are critical in Endometrial Carcinoma diagnosis and treatment. Liquid-Based Cytology is useful for examining the details of the cell, while assessment of the architecture of the glands depends indirectly on the cell clumps' size, shape and structure (Du *et al*., 2016). CB can maintain the tissue architecture, cell morphology and thus it is a valuable complement to LBC smears for conclusive diagnosis (Ranjana & Sadhna, 2016).

2.6 Endometrial Cancer Treatment and Management

Endometrial cancer Management is divided into non-surgical and surgical therapies (Agarwal *et al*., 2019). All patients with endometrial hyperplasia which is a precursor lesion should be tested so that concurrent adenocarcinoma can be ruled out. Hysterectomy is the ultimate treatment for complex atypical EH. Surgical options may include minimally invasive and abdominal techniques such as laparoscopy (Agarwal *et al*., 2019). Hysterectomy can be done without or with bilateral salpingo-oophorectomy. Supracervical procedures as treatment are not endorsed by ACOG since they can cause an infection and hence leave behind the residual disease (Agarwal *et al*., 2019). Surgery of the Abdomen is associated with longer hospital stays, recovery and more pain when it is compared to laparoscopy. If the carcinoma is identified, additional procedures may be necessary (Johnston *et al*., 2019). Most patients with EH may not have cancer. Patients with EH without atypia, or multiple illnesses prohibiting

surgery, and those who are yearning to have children in the future, can be treated with nonsurgical options (Taraboanta *et al*., 2020).

2.6.1 Treatment

The most commonly preferred treatment option is oral progesterone therapy and the levonorgestrel-releasing intrauterine system which is used to stabilize the disease and prevent its progression to EC (Bulun *et al*., 2019; Chandra *et al*., 2016). The common side effects of treatment, including surgical menopause, infertility, sexual dysfunction, lymphedema of the lower extremities, fatigue and distress, impact considerably on the patient's quality of life. Lifestyle changes such as eating a diet that is high in vegetables and fruits and being physically active may relieve symptoms and improve the quality and quantity of life in females who have undergone endometrial cancer treatment (Horta & Cunha, 2019b).

2.6.2 Surgical Approaches

Surgery is often the main treatment method for EC and consists of total hysterectomy along with bilateral salpingo-oophorectomy, and removal of lymph nodes *(Agarwal et al*., 2019). In some circumstances, pelvic washings are done, the omentum is removed, and/or peritoneal biopsies are done to stage the disease (Braun *et al.*, 2016). If cancer has spread throughout the pelvis and abdomen, a debulking procedure may be done. Laparotomy has been associated with more complications post-operative than laparoscopy which is widely embraced (Braun *et al*., 2016).

2.6.3 Adjuvant Radiotherapy

Patients with low-grade carcinoma overall survival are not affected by Radiation therapy. It is linked to increased morbidity and reduction in quality of life when used in patients with low-risk EC (Braun *et al*., 2016). Patients who are medically inoperable receive Radiation therapy as an option. The adjuvant chemotherapy which is often administered in combination with EBRT is mainly recommended for cancers of serous subtype and in advanced stages of Endometrial cancer. The presence of

clinicopathological risk factors is the basis for indication of adjuvant treatment (Braun *et al*., 2016).

2.6.4 Chemotherapy and Hormone Therapy

Chemotherapy uses one or more anti-cancer drugs as part of a standardized chemotherapy regimen. Chemotherapy may be given with an intent to cure or it may aim to prolong life or to reduce symptoms (Braun *et al*., 2016). The goal of chemotherapy is to inhibit the proliferation of cells and tumor multiplication, thus avoiding metastasis and deep invasion. Tumor growth Inhibition can occur at several levels within the cell and its environment (Braun *et al*., 2016). Progesterone therapy is normally given to women who wish to preserve fertility and present with EC Stage I and they are advised on immediate total hysterectomy once they complete childbearing to avoid progression to High-risk Endometrial Cancer subtypes (Braun *et al*., 2016).

2.6.5 Immunotherapy

Immunotherapy represents the most auspicious treatment approach in neoplasm affecting the Female reproductive organs (Horta & Cunha, 2019b). Ongoing and Current studies are trying to improve clinical responses through immunotherapies strategies (Horta & Cunha, 2019b). EGF, angiogenesis, Insulin-like Growth Factor and genomic instability represent the hallmarks of Endometrial cancer most investigated in the recent past which has achieved clinical results which are significant. The use of hormonal therapy, PARP-inhibitors and anti-angiogenetic agents is a strategy that is promising in the treatment of Endometrial Cancer (Du *et al*., 2016).

Adoptive T cell therapies as an immunotherapeutic approach are receiving increased attention for Endometrial cancer but for BiTE antibodies treatment, only trials which are preclinical have been done on USC stem cells and hence it has not been implemented yet. As single treatments modalities such as radiotherapies and chemotherapies in advanced recurrent and metastatic cancer may be ineffective (Tran & Gehrig, 2017).

CHAPTER THREE

METHODOLOGY

3.1 Study Site

The project was carried out at Kenyatta National Hospital, a referral and a teaching hospital for all cadres of healthcare workers. The hospital serves patients from Kenya and the greater East Africa Region. Patient recruitment was done at the Daycare Gynaecological Theatre 66 and the Histopathology aspect of the Project was carried out at the Department of human pathology, school of medicine, College of health sciences, University of Nairobi, while the Manual Liquid based cytology and Conventional cytology analysis was carried out at Jomo Kenyatta University of Agriculture and Technology (JKUAT), Department of Medical Laboratory Sciences Laboratory, Kenya.

3.2 Study Design

The study design was a Prospective Cross-sectional Study of Patients suspected to have Endometrial cancer and its precursors diagnosed through Several imaging techniques such as transvaginal ultrasonography, positron emission tomography (PET), computed tomography (CT), MRI, PET/CT and PET/MRI which are used as diagnostic tools for preoperative staging of Endometrial Cancer (Braun *et al*., 2016).

3.3 Study Population

The study population was pre and postmenopausal women diagnosed with Endometrial malignancies and its precursors through Several imaging techniques scheduled to undergo dilatation and curettage procedure.

3.3.1 Inclusion Criteria

a. Women above 40yrs old scheduled to undergo dilatation and curettage
b. Women willing to provide a written and signed informed consent

3.3.2 Exclusion Criteria

a. Women who have been diagnosed with Endometrial Cancer through biopsy and histopathology.
b. Women with other types of gynaecological malignancies and those who had undergone hysterectomy were not eligible.

3.4 Variables

3.4.1 Independent Variables

a. Demographic and Clinical Information/data (advancement in age, early menarche, late menopause, obesity, parity, family history of EC, exposure to radiation, infertility, Long-term use of estrogen therapy, tamoxifen therapy and hypertension (Rodriguez *et al*., 2019).

3.4.2 Dependent Variables

b. Histopathological and Cytopathological parameters (tumor size, type, tumor invasion and metastasis).

3.5 Sample Size Determination

Table 3.1: Sample Size Determination

	Sample size when all the pairs agree with each other (k = 0).					
		Tolerance probability β				
		80%	85%	90%	95%	99%
	0.01	161	189	230	299	459
	0.05	32	37	45	59	90
Discordance	0.10	16	19	22	29	44
Rate α	0.15	10	12	15	19	29
	0.20	8	9	11	14	21

This formula by Jason J Z Liao (2010) allows for k individual discordant pairs (K=0). It determines Sample sizes for tolerance probabilities, β, and different discordance rates, α, based on inequality (Ã, 2010). If k pairs of the samples n are allowed to be discordant so that an agreement is implied between two methods of measurement, then sample size decreases as the discordance rate increases and it increases as the tolerance probability increases(Ã, 2010).

If; Tolerance probability, β = 95%

Discordance rate, α = 0.05

Then, n = 59 Samples

Hence, 59 sample pairs were required to claim agreement at a discordance rate of 0.05 and a tolerance probability of 95% when there is no discordant pair (k = 0)

3.6 Sampling Method

The convenience sampling method was used to recruit subjects to the study until the required sample size was achieved.

3.7 Research Design and Methods

3.7.1 Recruitment and Data Collection

A questionnaire Prepared by the PI was used to interview the Subjects attending the Kenyatta National Hospital Gynaecology and Obstetrics clinic 66 to obtain demographic data. Qualified Physician interviewed the participants to obtain both demographic and clinical data which were recorded for further analysis. Patients of age 40yrs and above undergoing dilatation and curettage who were diagnosed with endometrial cancer by clinical and imaging tests were recruited into the study. The counseling session was carried out by a qualified nurse or Gynaecologist or a physician at the clinics and the patients were asked to sign an informed consent form after the benefits and the risks of the study were explained to them.

3.7.2 Laboratory Procedures

3.7.2.1 Sample Collection

The study population were the symptomatic spectrum of women undergoing D&C of age 40 years and above. The Patients were examined using the questionnaire clinically and with the relevant imaging findings. After obtaining a signed informed consent, all the women sequentially proceeded to endometrial cytology using Tao Brush and then Dilatation and curettage (biopsy) endometrial sampling. This was done by a gynecologist.

To begin the collection of cells from the walls of the endometrium, the sheath was pulled back, and then the brush was inserted into the uterine canal at the level of the fundus through the cervix with the help of the speculum and the dilator (Du *et al*., 2016). The 3- 3.5cm brush was then rotated at 360° about three to five times to collect the cells from the endometrium. The protective outer sheath was then pushed back to the tip, and the device was removed from the endometrial canal. For the Conventional smear, the material obtained from the endometrial Tao brush was spread on glass slides and fixed immediately with 95% ethanol for at least 15 minutes.

3.7.2.2 Laboratory Analysis

For Manual Liquid Based Cytology, the brush was then cut off and immediately immersed into a container with the fixative solution. The solution contained 50 ml of 10% formalin, 0.5 gm of sodium citrate, 0.5 gm of sodium chloride and 50 ml of isopropyl alcohol in 100ml (Manoli *et al*., 2017). The specimen collected with the endometrial Tao brush was mixed with equal volumes of fixative solution. Centrifugation was done at 2000 rpm for five minutes. The supernatant was then decanted and excess fixative blotted, 1-2 ml of polymer solution (Containing 10 ml of polyethylene glycol, 2 ml of Poly-L-lysine, 2 gm of agarose and 88 ml of distilled water) was then added to the deposit and it was centrifuged again for five minutes at 2000 revolutions per minute (Manoli *et al*., 2017). The deposit was then pipetted onto a glass slide in a circular motion, the slides were placed on a metal tray and dried in a hot air oven at fifty degrees Celsius for fifteen minutes and they were further fixed by immersing in 95% alcohol for fifteen minutes (Manoli *et al*., 2017).

3.7.2.3 Staining and Examination

The Conventional and Manual liquid-based smears were stained using the Papanicolaou method for cytomorphological diagnosis. Material from the endometrial canal which is Properly collected usually contains clusters of endometrial stromal cells and numerous glandular fragments (Gupta *et al*., 1995). All smears were screened and the results from Conventional and MLBS were compared. The Cyto-histologic correlation was done for each sample. Cytological diagnoses were classified as non-diagnostic (inadequate), negative, atypical, or positive for malignancy according to the International Federation of Gynecology and Obstetrics (FIGO system) (Fulciniti et al., 2018). All cytological smears which were suspicious and positive for Malignancy were blindly and independently re-evaluated by board-certified pathologists.

For the D&C procedure, specimens were collected in buffered 10% formalin and were allowed to fix for 24 hours. A detailed examination was done grossly and bits were given for further processing. The specimens then underwent the tissue processing process from fixation, dehydration, clearing, infiltration by wax, embedding/blocking, sectioning and Paraffin-embedded H and E stained sections were obtained then

screened and signed out under light microscopy examination. Histopathology and cytology reports were then given to the patients. Histopathologic findings were used as the reference gold standard for determining the performance characteristics of cytology in the detection of endometrial cancer and atypical hyperplasia (Levy-Zauberman *et al*., 2017).

3.8 Laboratory Procedures Summary

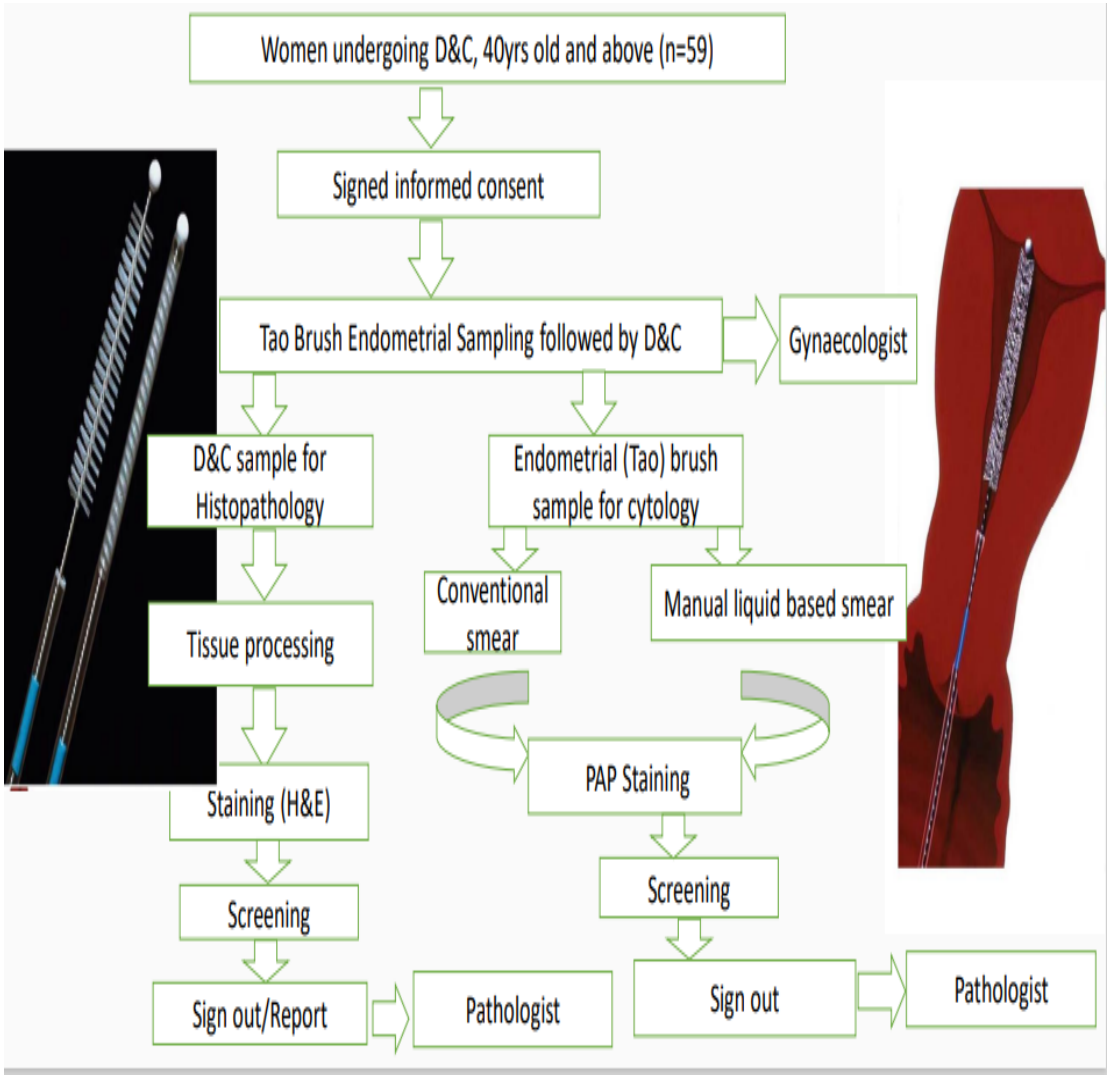

Figure 3.1: Laboratory Procedures Summary

3.9 Quality Assurance

The study was conducted by the PI and qualified personnel at Kenyatta National Hospital during sample collection, processing and Result analysis. Standard operating procedures (SOPs) were used during Dilatation and Curettage procedure, endometrial Tao brush sampling, preparation of the specimen and reporting.

3.9.1 Pre-analytic standardization

Tao brush sample was fixed immediately in 95% alcohol for the conventional smear, for MLBC the head of the Tao brush was cut immediately and placed into a container with a special fixative solution and for D&C specimen, the specimen was immersed in 10% Neutral buffered formalin (NBF) and allowed to fix for 24-48 hours.

3.9.2 Analytical standardization

Staining with Papanicolaou stain and hematoxylin and eosin was done by the PI under the supervision of a qualified laboratory technologist while following SOPs.

3.9.3 Post-Analytical standardization

The conventional and Manual Liquid-Based smears were screened by the Principal Investigator, reported and signed out by the supervisors who are certified by the board as consultant anatomic pathologists. The turn-around time for reporting and signing out was two weeks and the results were submitted to the clinician.

3.10 Ethical Approval

The Ethical approval was obtained from the Jomo Kenyatta University of Agriculture and Technology Institutional Ethics Review Committee (Protocol Number: JKU/ISERC/02316/0650) and also from the Kenyatta National Hospital-University of Nairobi (KNH-UoN) Ethics and Research Committee (Protocol Number: P22/01/2022). A research permit was also obtained from National Commission for Science, Technology and Innovation (License Number: NACOSTI/P/22/18677).

Permission was also sought from KNH administration before the commencement of the study.

3.11 Data Analysis and Presentation

Data was collected using questionnaires, dummy tables and laboratory analysis then stored in file maker software. The results were expressed as a mean standard deviation (parametric) and as median and range (nonparametric). Cohen's kappa test was performed with a 95% confidence interval to determine the agreement between CS and MLBC. Sensitivity and Specificity was also used to determine the performance characteristics of the Endometrial Tao Brush. All data analysis was performed using the IBM SPPS 21 Statistical analyzer. Data was presented as means and standard error of means in tables or graphs.

3.12 Research Finding Dissemination

Publishing findings in an international peer-reviewed journal and presenting data in an international scientific forum.

CHAPTER FOUR

RESULTS

4.1 Demographic Features of the Study Population

Most patients recruited into the study were between 52-61 years old. The following number of patients in the different age brackets were diagnosed with endometrial cancer 2 (42-51 yrs.), 6(52-61 yrs.), 2(62-71 yrs.), and 2(72-82 yrs.) respectively. The following were also diagnosed with atypical hyperplasia respectively using Manual liquid-based cytology 1(32-41 yrs.), 3(42-51 yrs.), and 5(52-61 yrs.) as shown by Table 4.1 below.

Table 4.1: Age Distribution

Age Range (Years)	Negative	Endometrial hyperplasia without atypia	Atypical Endometrial hyperplasia	Malignant	Non-Diagnostic	TOTAL
32-41	2 (6%)	0 (0%)	1 (7%)	0 (0%)	0 (0%)	3
42-51	5 (15%)	2 (7.5%)	3 (20%)	2 (10%)	0 (0%)	12
52-61	10 (30%)	12 (45%)	5 (33%)	6 (30%)	2 (40%)	35
62-71	1 (3%)	2 (7.5%)	0 (0%)	2 (10%)	1 (20%)	6
72-82	2 (6%)	0 (0%)	0 (0%)	2 (10%)	0 (0%)	4
TOTAL	20	16	9	12	3	60

For the patients who were diagnosed with cancer; the average age at the time of diagnosis was 60 years, average parity of 4 children, average weight of 69kgs, onset of menopause at 50yrs, first full term pregnancy at 20yrs and onset of menarche at 15yrs. For those diagnosed with atypical hyperplasia; the average age at the time of diagnosis was 51yrs, average parity of 3 children, average weight of 75kgs, onset of menopause at 47yrs, first full term pregnancy at 23yrs and onset of menarche at 15yrs as shown by figure 4.1 below.

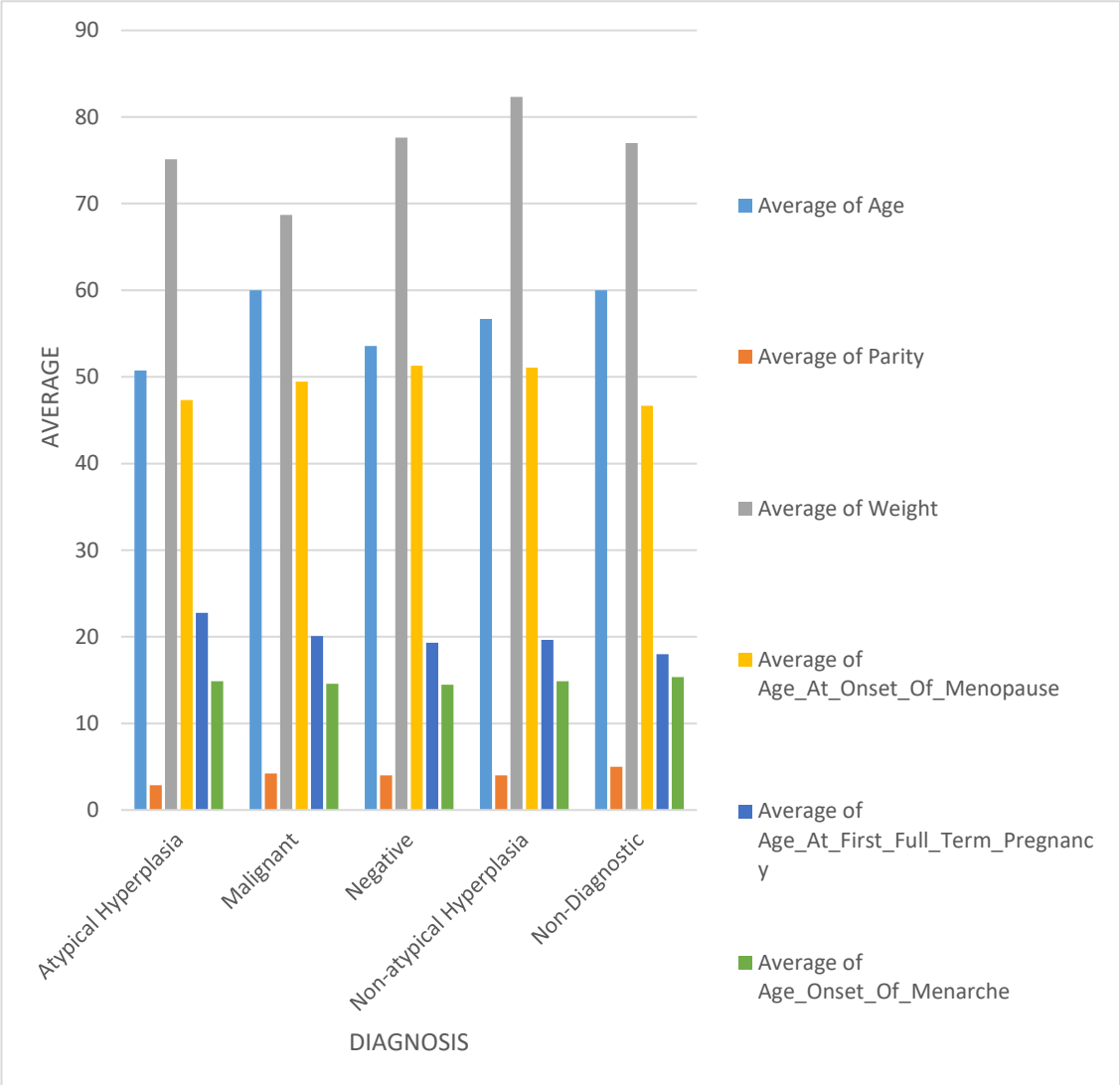

Figure 4.1: The Average Age, Parity, Weight, Age at Onset of Menopause, Age at First Full Term Pregnancy and Age on Onset of Menarche

From the data collected from patients who were on oral contraceptives, 9 patients who reported to have used the combined oral contraceptives were diagnosed with endometrial cancer while 5 patients were diagnosed with endometrial cancer and they had never used the oral contraceptives. Five patients were diagnosed with atypical hyperplasia and they had used oral contraceptives while 3 patients were diagnosed with atypical hyperplasia and they had never used the oral contraceptives as shown in Figure 4.2 below.

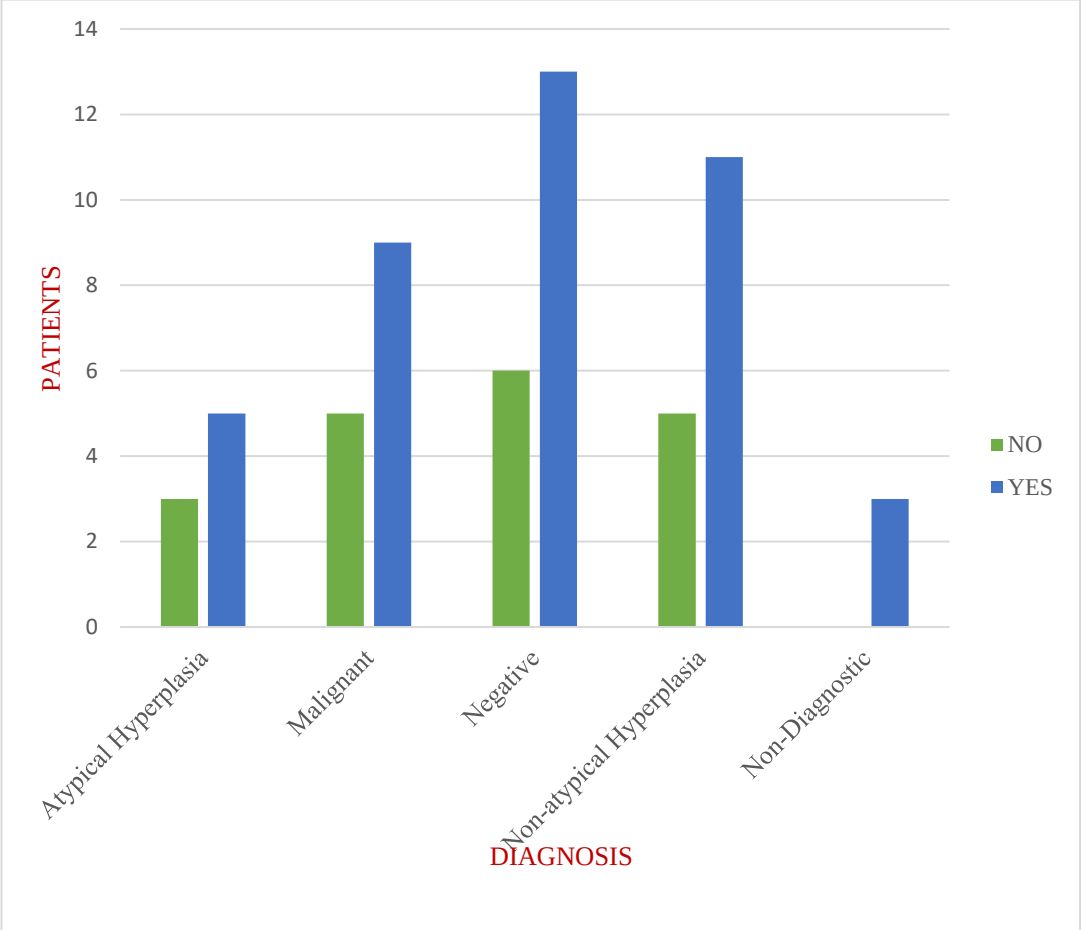

Figure 4.2: Oral Contraceptive Use

On other contraceptives use, the data was as follows; 1(Norplant), 4(3 months Injection), 2(Both 3months injection and IUD), 1(IUD), 1(IUD & Tubal ligation), 4(None) and 1(Tubal ligation) were diagnosed with the endometrial cancer. 3(3 months Injection), 1(3months injection, IUD & Norplant), 1(IUD), 1(IUD & Tubal ligation) and 2(None) were diagnosed with atypical hyperplasia as shown in Figure 4.3 below.

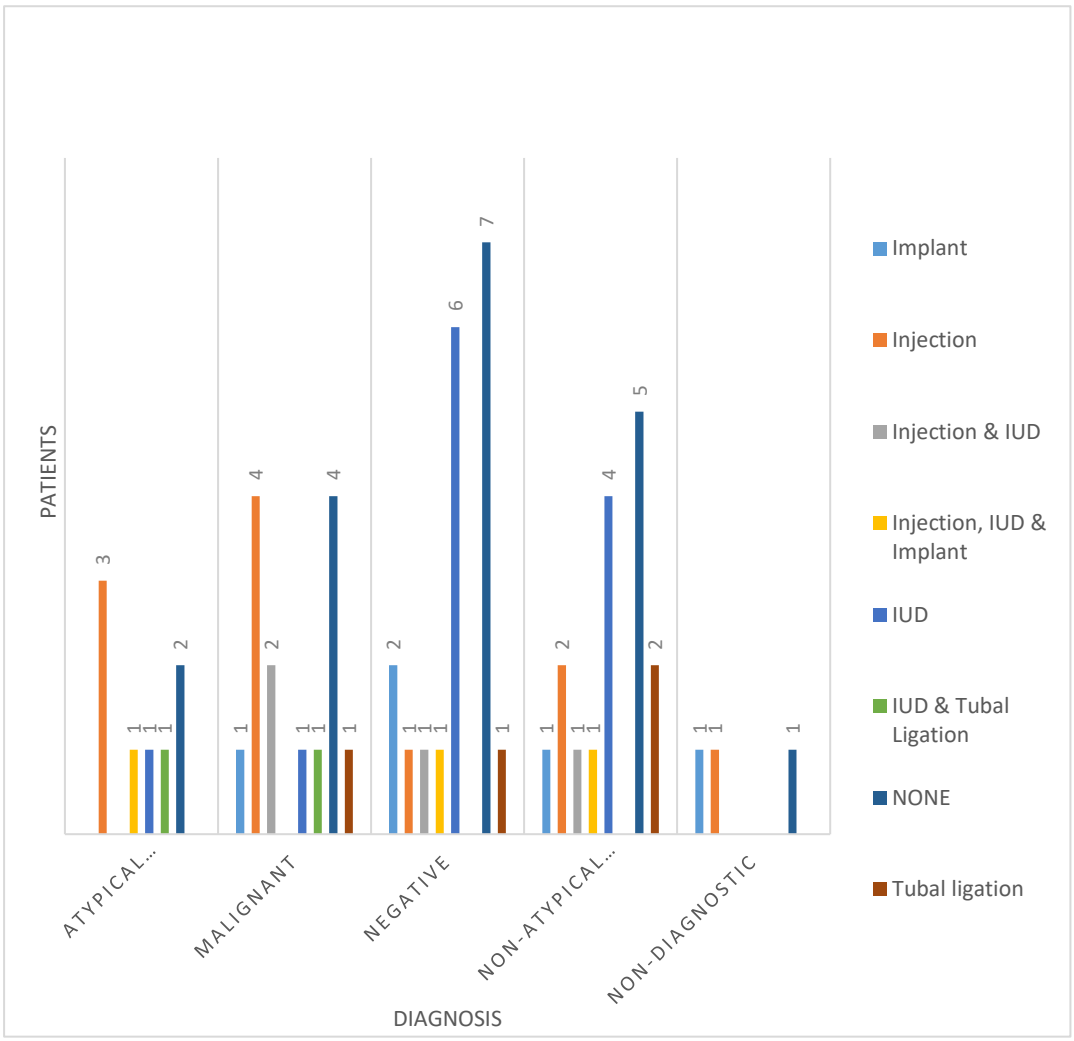

Figure 4.3: Other Contraceptives Use

Blood pressure was also assessed as one of the risk factors for Endometrial cancer, 5 patients with high blood pressure were diagnosed with endometrial cancer while nine patients with a normal blood pressure were also diagnosed with endometrial cancer. Six patients with high blood pressure were diagnosed with atypical hyperplasia while 2 patients with normal blood pressure were also diagnosed with atypical hyperplasia as shown by Figure 4.4 below.

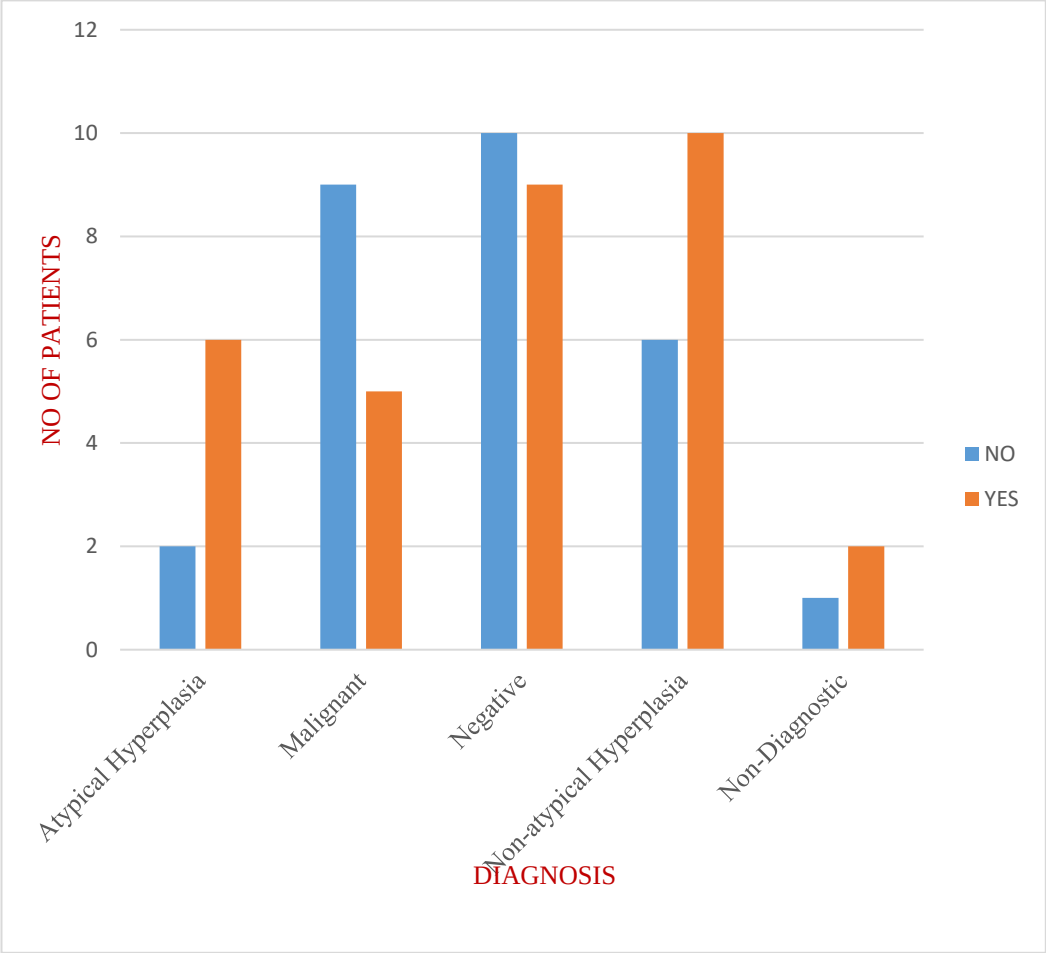

Figure 4.4: Blood Pressure

4.2 Comparison of Conventional and Manual Liquid-Based Specimens Collected with Tao Brush.

The current study included a total of 60 specimens comprising; 14 (23%) (Fig.4.5, C2; Table 4.2) patients with Histopathologic diagnoses of Endometrial Cancers, 24 (40%) (Fig.4.5, B2; Table 4.2) with hyperplasia, 3 (5%) (Table 4.2) Non-diagnostic, and 19 (32%) (Fig.4.5, A2: Table 4.2) patients with Normal Endometrial Histology. The 14 tumors which were malignant were categorized as adenocarcinoma of the endometrioid type (7 patients; 50%) (Fig.4.7, C3), papillary serous adenocarcinoma (4 patients; 29%) (Fig.4.7, B3), and squamous cell carcinoma (3 patients; 21 %) (Fig.4.7, A3). The 24 patients with hyperplasia comprised 8 (13%) with Complex hyperplasia with atypia, 16 (27%) with Simple, mild hyperplasia without atypia, and some with hyperplastic endometrial polyp. The 19 patients with normal endometrium were represented by 3(10%) patients with proliferative endometrium, 4 (13%) with atrophic endometrium, 1 (3%) with secretory endometrium, 1(3%) with hormonal imbalance, 2 (6%) with leiomyoma and 1(3%) with Endometritis.

Numerous common features were seen among the samples that were atypical by cytology and malignant on the Dilatation and Curettage specimens. There was a dominance of stroma which was benign but isolated clusters of glandular clusters that demonstrated slight to moderate atypia. The other feature was the presence of a moderately abundant group of cells with only mild features of atypia. During evaluation considerations included; Secretory endometrium, disordered endometrium which is proliferative, complex, or simple hyperplasia and possibly endometrial cancer subtypes which are well-differentiated.

For the histology samples, Simple cystic endometrial hyperplasia demonstrated fragments of endometrial tissues with hyperplastic cystically dilated endometrial glands in an edematous endometrial stroma. The glands were lined by stratified epithelium with no atypia. For the Papillary serous endometrial carcinoma, the sections showed fragments of endometrial tissues in which there was an invasive malignant epithelial tumor forming papillary patterns supported by central fibrovascular cores.

The papillae were lined by serous epithelial cells with moderate pleomorphic vesicular nuclei with increased mitotic activity noted.

In squamous cell carcinoma (an extension from the cervix), the bulk of the tissues were necrotic in which an invasive endometrial carcinoma disposed in solid anastomosing cords and nests infiltrating into the stroma. The tumor comprised of large malignant squamous cells with cytoplasmic keratin bearing moderately pleomorphic and hyperchromatic nuclei that have frequent mitotic activity. For the hyperplastic endometrial polyp, the sections showed fragments of a polyp with hyperplastic endometrial glands that are cystically dilated and lying back to back in a compact stroma. The endometrial glands were lined by stratified epithelial cells with no atypia.

Table 4.2: Manual Liquid Based Cytology versus Histopathology Cross-Tabulation

The Manual Liquid Based cytology and the equivalent histopathology findings from the 60 patients are presented in Table 4.2 below. Twenty percent of the 60 patients were diagnosed as malignant neoplasms (12) by cytology, 42% of the patients had hyperplasia; (9) (15%) were atypical while 16(27%) were hyperplastic without atypia, 5% were unsatisfactory for evaluation (3) whereas 33% (20) of the patients were diagnosed as negative for malignant tumors and precursors.

		Histopathology					**Total**
		Negative	E. hyperplasia without atypia	Atypical E. hyperplasia	Malignant tumors	Non-Diagnostic	
Manual Liquid Based Cytology	Negative	19	1	0	0	0	20
	E. hyperplasia without atypia	0	15	1	0	0	16
	Atypical E. hyperplasia	0	0	7	2	0	9
	Malignant neoplasms	0	0	0	12	0	12
	Unsatisfactory	0	0	0	0	3	3
Total		19	16	8	14	3	60

Key: *E. hyperplasia without atypia – Endometrial hyperplasia without atypia, *Atypical E. Hyperplasia- Atypical Endometrial hyperplasia

Table 4.3: Conventional Smear versus Histopathology Cross Tabulation

The Conventional cytology and the equivalent histopathology findings from the 60 patients are presented in Table 4.3 below. Twenty-five percent of the 60 patients were diagnosed as malignant neoplasms (15) by cytology, 35% of the patients had hyperplasia; (8) (13%) were atypical while 13(22%) were hyperplastic without atypia, 10% were unsatisfactory for evaluation (6) whereas 30% (18) of the patients were diagnosed as negative for malignant tumors and precursors.

		Histopathology					**Total**
		Negative	E. hyperplasia without atypia	Atypical E. hyperplasia	Malignant tumors	Non-Diagnostic	
Conventional Smear	Negative	18	0	0	0	0	18
	E. hyperplasia without atypia	0	13	0	0	0	13
	Atypical E. hyperplasia	0	1	7	0	0	8
	Malignant neoplasms	1	0	0	14	0	15
	Unsatisfactory	0	2	1	0	3	6
Total		19	16	8	14	3	60

Key: *E. hyperplasia without atypia – Endometrial hyperplasia without atypia, *Atypical E. Hyperplasia- Atypical Endometrial hyperplasia

Fig.4.5 (A1) Negative/Normal endometrium, Conventional Smear: The endometrial cells were regularly arranged and the nucleus was round to oval. The spaces between the nuclei were regular with fine granular chromatin. Presence of blood interfered with staining and Microscopy. **Fig. 4.5(A2).** Negative endometrium, MLBC Smear: The endometrial cells were regularly arranged and the nucleus was round to oval. The spaces between the nuclei were regular with fine granular chromatin. The slide was clear with no obscuring material. **Fig. 4.5(B1).** Atypical Hyperplasia, Convectional smear: The endometrial cells were increased in number. The cells were also overcrowded with coarse chromatin and lying back to back/overlapping. Presence of blood interfered with staining and Microscopy. **Fig. 4.5(B2).** Atypical Hyperplasia, MLBC smear: The endometrial cells were increased in number. The cells were also overcrowded with coarse chromatin and lying back to back/overlapping. The slide was clear with no obscuring material. **Fig. 4.5(C1).** Malignant Neoplasm, Conventional Smear: The cells were of variable sizes with an increased nucleus-cytoplasmic ratio. **Fig. 4.5(C2).** Malignant Neoplasm, MLBC Smear: The cells were of variable sizes with an increased nucleus-cytoplasmic ratio. Nuclear pleomorphism was evident with Orangeophilic and irregular cytoplasm as demonstrated in the figure 4.5 below.

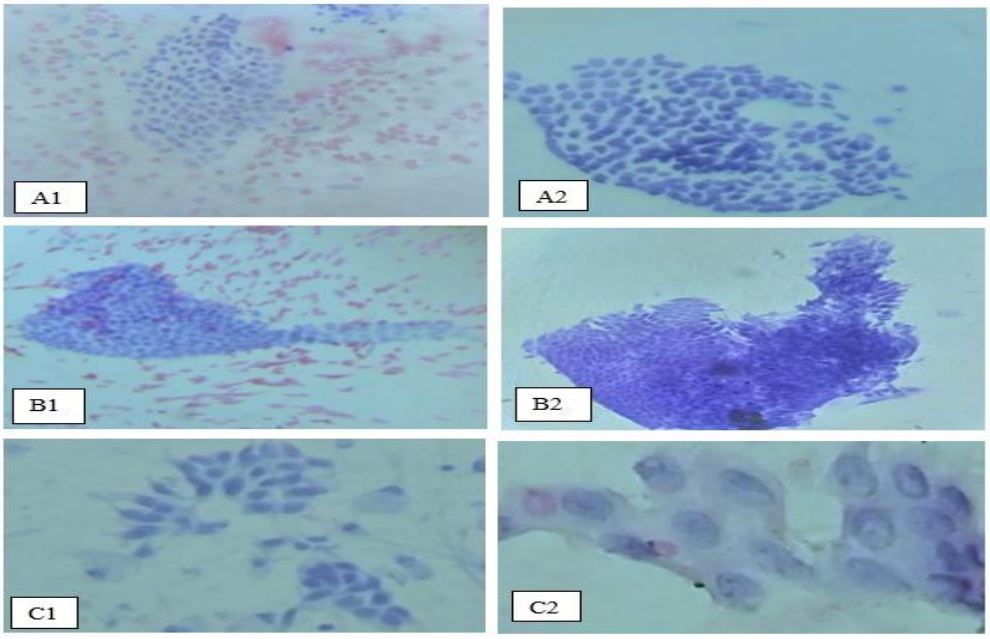

Figure 4.5: Conventional vs Manual Liquid Based Cytology (Papanicolaou Staining; Original Magnification ×40)

Fig.4.6 (A2). Negative/Normal endometrium: This was a representation of a secretory phase of the endometrium with a uniform glandular to stromal ratio. There was secretion material within the glands and the vacuoles were lined towards the surface of the glands. The nuclei were round and larger than those in the proliferative phase and the glandular structure was well preserved. **Fig.4.6 (B2).** Atypical Hyperplasia: There was increased proliferation of the glands irrespective of the stroma. Swiss cheese pattern was evident and there were no prominent epithelial atypical features. The cystically dilated and bulbous three-dimensional glands were evident. **Fig.4.6 (C2).** Malignant: The endometrial glands were irregular and complex. The stroma was slightly stratified. The chromatin and the nuclei were irregular. There was a distortion of the glandular and nuclei arrangement. Pleomorphism and stromal invasion were also evident as shown in the figure 4.6 below.

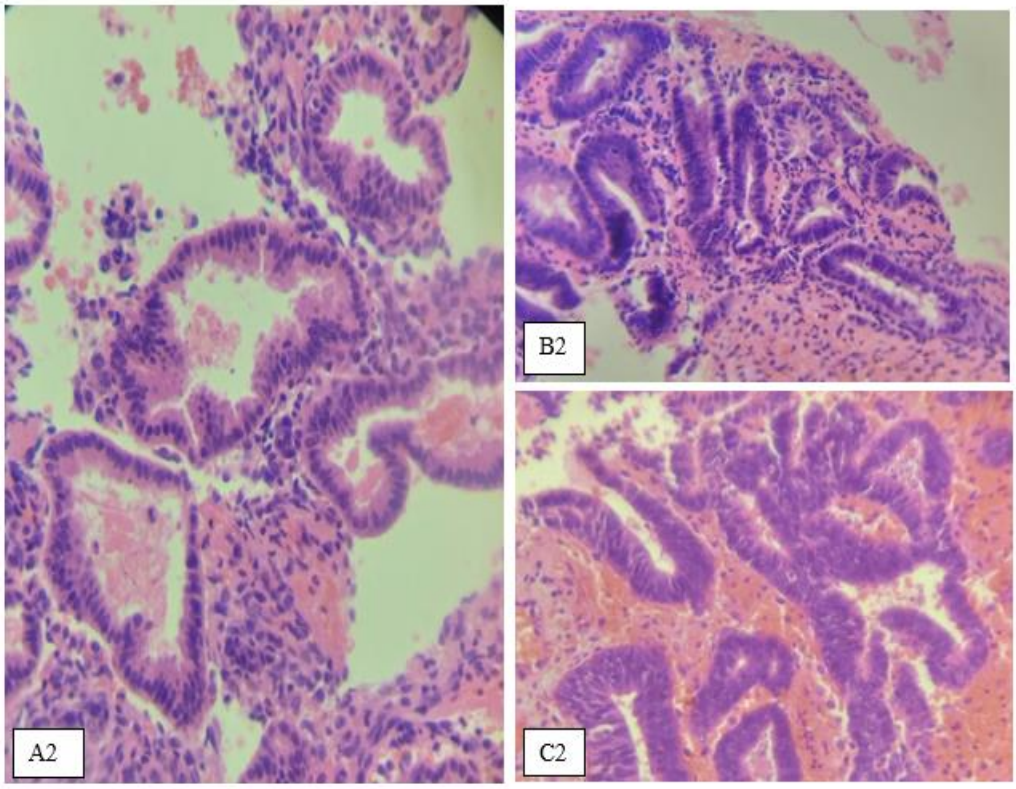

Figure 4.6: Histopathology (Hematoxylin and Eosin Stain, Original Magnification ×40)

Fig.4.7 (A3). Squamous Cell Carcinoma: Necrotic tissues with an invasive endometrial carcinoma disposed in solid anastomosing cords and nests were infiltrating into the stroma. Large malignant squamous cells with cytoplasmic keratin bearing moderately pleomorphic and hyperchromatic nuclei that have frequent mitotic activity were also evident. **Fig. 4.7(B3).** Papillary Serous Carcinoma**:** Fragments of endometrial tissues with invasive malignant epithelial tumor forming papillary patterns supported by central fibrovascular cores. The papillae were lined by serous epithelial cells with moderate pleomorphic vesicular nuclei with increased mitotic activity. **Fig. 4.7(C3).** Endometrioid Adenocarcinoma: There was back to back glandular arrangement. There was no intervening stroma since it was invaded by the tumor cells. Increased nuclear to cytoplasmic ratio was present and the villi glandular pattern was also evident as shown in figure 4.7 below.

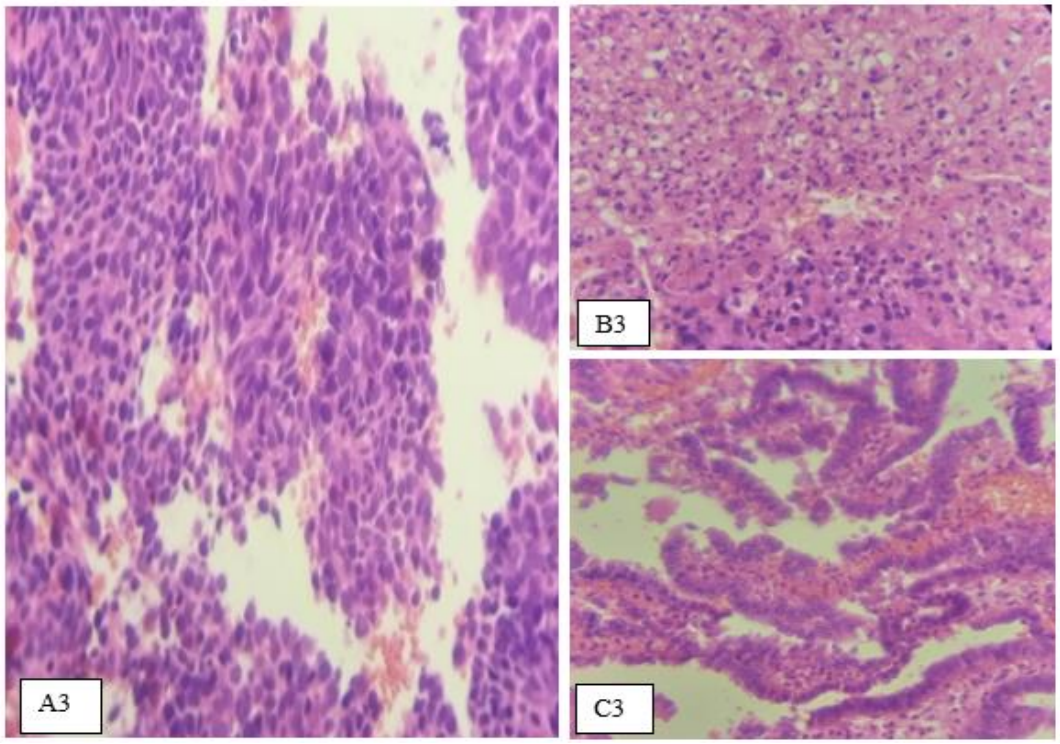

Figure 4.7: Endometrial Cancer Subtypes (Hematoxylin and Eosin Stain, Original Magnification ×40)

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4.3 The Performance Characteristics of Endometrial Cytology Using Tao Brush

Overall specificity and sensitivity and specificity of Manual liquid-based cytology and Conventional smear in the detection of atypical hyperplasia and endometrial carcinoma depended on the classification of atypical endometrial cytology and atypical histopathology results. The sensitivity of MLBC was 95.45% and the specificity was 100% while the Conventional Smear had a sensitivity of 90.91% and a specificity of 95% respectively when both malignant neoplasms and hyperplasia with atypia cytology and atypical/malignant biopsy diagnoses were considered as evidence of pre-malignant/malignant changes. The Tao brush had a negative predictive value (NPV) of 97.44% and a positive predictive value (PPV) of 100% for MLBC and a negative predictive value (NPV) of 95% and a positive predictive value (PPV) of 90.91% for the Conventional smear as shown in Table 4.4 & 4.5 below.

Table 4.4: Diagnostic Accuracy of Tao Brush (MLBC vs Histopathology)

	Sen (%)	**Spec (%)**	**PPV (%)**	**NPV (%)**
Tao Brush	95.45	100	100	97.44

Key: Sen-sensitivity; Spec- specificity; PPV-positive predictive value; NPV-negative predictive value.

Table 4.5: Diagnostic Accuracy of Tao Brush (Conventional vs Histopathology)

	Sen (%)	**Spec (%)**	**PPV (%)**	**NPV (%)**
Tao Brush	90.91	95	90.91	95

Key: Sen-sensitivity; Spec- specificity; PPV-positive predictive value; NPV-negative predictive value.

4.4 The Discordance and Diagnostic Level of Agreement between Tao Brush and Histopathological Finding (Gold Standard)

The Manual Liquid cytology had 3 samples which were discordant and 57 samples which were concordant with the Histopathology results while the Conventional smears had 6 samples which were discordant and 54 samples which were concordant with the Histopathology results as displayed in Table 4.6 and 4.7 respectively. This shows that MLBC had increased sample concordance as compared to the Conventional samples. This was possible because the slides displayed unclamped, monolayered, and even, random cell spreads and they gave a clear demonstration of normal and atypical cells.

Table 4.6: Correlation; MLBC vs Histopathology

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Concordant	57	95.0	95.0	95.0
	Discordant	3	5.0	5.0	100.0
	Total	60	100.0	100.0	

Table 4.7: Correlation; CS vs Histopathology

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	Concordant	54	90.0	90.0	90.0
	Discordant	6	10.0	10.0	100.0
	Total	60	100.0	100.0	

The Cohen's kappa value between MLBC and Histopathology was .912 while the Cohen's kappa value between conventional cytology and Histopathology was .891 which denotes nearly perfect agreement between cytology specimens and histopathology biopsies which were the gold standard as shown in Table 4.8 and 4.9. This depicts a very high agreement value between the endometrial samples collected using the endometrial Tao brush and the biopsy samples collected by Dilatation and Curettage.

Table 4.8: Measurement of Agreement (MLBC vs Histopathology)

		Value	**Asymp. Error**	**Std.Approx. T[b]**	**Approx. Sig.**
Measure of Agreement	Kappa	.912	.042	12.782	.000
N of Valid Cases		60			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis

Table 4.9: Measurement of Agreement (Conventional vs Histopathology)

		Value	**Asymp. Error[a]**	**Std.Approx. T[b]**	**Approx. Sig.**
Measure of Agreement	Kappa	.891	.046	12.888	.000
N of Valid Cases		60			

a. Not assuming the null hypothesis.

b. Using the asymptotic standard error assuming the null hypothesis.

The results did not support the null hypothesis; hence the null hypothesis was rejected and the alternative hypothesis was confirmed.

CHAPTER FIVE

DISCUSSION

Dilatation and curettage or Endometrial curetting have been the gold standard method of diagnosis or treatment for assessing endometrial lesions which are suspicious (Han *et al*., 2019). However, it has numerous shortcomings; apart from it being expensive, it is possible to cause perforation, and at times it results in false-negative diagnoses since it can only assess less than 1/2 of the endometrium in roughly sixty percent of dilatation and curettage procedures (Campion & Clayton, 2008). Tao brush can sample a representative section of the endometrial cavity and can be done in an outpatient setting with the least anguish and distress to the patient (Campion & Clayton, 2008).

Endometrial carcinoma stands as the fifth most frequent cancer in females globally and its incidence is rising in countries that are developing (Clarke *et al*., 2022), and it accounts for nearly fifty percent of all new cases of gynecological cancer (Braun et al., 2016). The likely outcome, the chance of recovery or recurrence of uterine cancer is reliant on factors such as the histological stage, patient's age, grade, depth of invasion into the myometrial and the cervix, and the existence of metastases to the lymph nodes (Narice *et al*., 2018). Unlike cervical carcinoma, in which screening and evaluation can be done routinely, only women with signs and symptoms of cancer of the endometrium usually look for medical assistance for them to be evaluated, diagnosed and treated (Raffone *et al*., 2022).

In this study most patients recruited were between 52- 61 years old with mean age of 56 years at the time of diagnosis. This agrees with a previous study by Ueda *et al* in 2008 who demonstrated that the prevalence of endometrial malignancy in the United States is 25.7 in 100,000 women per year and the peak ages at the time of diagnosis were between (55- 64) years (Ueda *et al*., 2008). The average age at the time of diagnosis was higher when compared with a study done by Soliman et al in 2005 who demonstrated that 12% (188/1531) of patients with cancer of the endometrial were younger than 50 years of age and the average age at diagnosis was 41 years (range 21-49 years) (Soliman *et al*., 2005).

The Manual liquid-based cytology specimens had a nearly perfect agreement with the histology biopsies. Manual liquid-based samples were easier to screen compared to conventional ones, due to a smaller screening area and an excellent quality of cell preparations. This corroborates with a study done by Manoli *et al* in 2017 who demonstrated that Manual liquid-based cytology on endometrial samples is more accurate and precise than conventional smears in the diagnosis of cancer. The contingency coefficient or the agreement value for Conventional smear/Histopathology v/s Liquid-based cytology/Histopathology was 0.556 v/s 0.572 (Manoli *et al*., 2017).

This study demonstrated that endometrial specimens sampled directly using Tao brush can detect carcinoma of the endometrium and hyperplasia with atypia with very high diagnostic accuracy. The endometrial Tao brush had a sensitivity of 95.45% and a Specificity of 100% with the histological biopsies results as the gold standard. This study corroborates with a study done by Raffone *et al*., 2022 which demonstrated a sensitivity of 95% Confidence Interval and a specificity of 92% Confidence Interval after assessing the diagnostic accuracy of Tao brush sampling cytological diagnoses (Raffone *et al*., 2022).This also agrees with a study done by Maksem et al which demonstrated that Specimen satisfaction with the endometrial Tao brush was 89.9 to 100 %, while its accuracy pathologically was 91 to 96 % (Maksem, 2000).

The sensitivity and specificity was slightly higher when compared with a previous study by Fujiwara *et al*.,2015 who demonstrated that cytology of the Endometria detected cancer in 1,279 (363 suspicious and 916 positives) cases had a sensitivity (inclusion of suspicious and positive cases) of 88.8% and a specificity of 98.5% (Fujiwara *et al*., 2015) and slightly lower when compared with a study by Wu et al., 2000 also showed that the sensitivity was 100% and specificity was 100% for detecting hyperplasia with atypia and endometrial carcinoma with Tao Brush in two hundred cases of sampling the endometrium using the endometrial Tao brush and correlated with histopathological results (Wu *et al*., 2000).

SAP-1 devices, Endometrial Tao brush, Aspiration devices, and pipelle are among the tools used to screen the Endometrial lining. Out of these screening tools, the Endometrial Tao brush is the utmost auspicious uterine sampler for screening for malignancies in the endometrium (Du *et al*., 2016). The Hysteroscopic guided biopsy is the most consistent method of evaluating lesions from the endometrium and hyperplasia since it has high diagnostic accuracy, especially when using the "grasp technique" but the major disadvantage is that the procedure is very costly (Gkrozou *et al*., 2015). Gkrozou et al.,2020 reported that endometrial cancer had a specificity and sensitivity of 99.7% and 82.6% respectively. For endometrial hyperplasia, the sensitivity was 75.2% while the specificity was 91.5% (Di Spiezio Sardo *et al*., 2020).

The results of this study also indicated a high level of diagnostic agreement between the endometrial cytology and histological diagnosis in evaluating hyperplasia with atypia and endometrial lesions. This agrees with a study done by Taraboanta *et al.,2020* who demonstrated that Diagnoses from endometrial cytology had a high agreement value with histopathological biopsy results and hence may be used to screen for endometrial malignancies and their precursors (Taraboanta *et al*., 2020).

CHAPTER SIX
CONCLUSION AND RECOMENDATIONS

6.1 Conclusion

This study demonstrated that;

a) Manual Liquid based cytology improved the detection of abnormal lesions and atypical disorders of the endometrium when compared with the Conventional cytology smears.
b) Direct sampling of the endometrium using the Tao brush is a sensitive and specific method for detecting endometrial lesions and hyperplasia with atypia.
c) The endometrial Tao brush samples has exhibited a nearly perfect agreement with the Histopathology samples.

6.2 Recommendations

a) Manual Liquid based cytology to be adopted when processing endometrial cytology samples.
b) Endometrial Tao brush to be used in Kenyan Hospitals to screen for endometrial cancer and its precursors.
c) Further ancillary and comparison studies with techniques such as the Pipelle biopsy and the Li-brush with Endometrial Tao brush could be considered in the future to improve the diagnosis of Endometrial Cancer.

REFERENCES

Ã, J. J. Z. L. (2010). *Sample size calculation for an agreement study*. *132*(June 2009), 125–132. https://doi.org/10.1002/pst

Agarwal, S. K., Chapron, C., Giudice, L. C., Laufer, M. R., Leyland, N., Missmer, S. A., Singh, S. S., & Taylor, H. S. (2019). Clinical diagnosis of endometriosis: a call to action. *American Journal of Obstetrics and Gynecology*, *220*(4). https://doi.org/10.1016/j.ajog.2018.12.039

Audebert, A. (2018). Endometriosis. In *Encyclopedia of Endocrine Diseases*. https://doi.org/10.1016/B978-0-12-801238-3.95837-9

Aue-Aungkul, A., Kleebkaow, P., & Kietpeerakool, C. (2018). Incidence and risk factors for insufficient endometrial tissue from endometrial sampling. *International Journal of Women's Health*, *10*. https://doi.org/10.2147/IJWH.S172696

Bianchi, I., Reventós, J., Capellà, G., Diaz, M., Vidal, A., & Piulats, J. M. (2019). *New perspectives on screening and early detection of endometrial cancer*. *May*. https://doi.org/10.1002/ijc.32514

Braun, M. M., Army, M., Overbeek-wager, W. E. A., Army, E., Hospital, C., Carson, F., Grumbo, C. R. J., & Army, M. (2016). *Diagnosis and Management of Endometrial Cancer*.

Bulun, S. E., Yilmaz, B. D., Sison, C., Miyazaki, K., Bernardi, L., Liu, S., Kohlmeier, A., Yin, P., Milad, M., & Wei, J. J. (2019). Endometriosis. In *Endocrine Reviews* (Vol. 40, Issue 4). https://doi.org/10.1210/er.2018-00242

Cakmak, Y., & Oge, T. (2020). *The effect of age on prediction of concurrent endometrial cancer in patients with atypical endometrial hyperplasia*. https://doi.org/10.31083/j.ejgo.2020.04.5351

Campion, M. B., & Clayton, A. C. (2008). *Direct Uterine Sampling With the Tao*

Brush Sampler Using a Liquid-based Preparation Method for the Detection of Endometrial Cancer and Atypical Hyperplasia. *June*, 228–235. https://doi.org/10.1002/cncr.23636

Chandra, V., Kim, J. J., Benbrook, D. M., Dwivedi, A., & Rai, R. (2016). *Therapeutic options for management of endometrial hyperplasia*. *27*(1), 1–25.

Clarke, M. A., Ph, D., Long, B. J., Sherman, M. E., Lemens, A., Podratz, K. C., Hopkins, M. R., Ahlberg, L. J., Guire, L. J. M., & Laughlin-tommaso, S. K. (2022). *A prospective clinical cohort study of women at increased risk for endometrial cancer*. *156*(1), 169–177. https://doi.org/10.1016/j.ygyno.2019.09.014.A

Colombo, N., Creutzberg, C., Amant, F., Bosse, T., Ledermann, J., Marth, C., Nout, R., Querleu, D., Mirza, M. R., Sessa, C., Consensus, E., & Working, C. (2016). *ESMO-ESGO-ESTRO Consensus Conference on Endometrial Cancer : diagnosis , treatment*. 16–41. https://doi.org/10.1093/annonc/mdv484

Di Spiezio Sardo, A., De Angelis, M. C., Della Corte, L., Carugno, J., Zizolfi, B., Guadagno, E., Gencarelli, A., Cecchi, E., Simoncini, T., Bifulco, G., Zullo, F., & Insabato, L. (2020). Should endometrial biopsy under direct hysteroscopic visualization using the grasp technique become the new gold standard for the preoperative evaluation of the patient with endometrial cancer? *Gynecologic Oncology*, *158*(2), 347–353. https://doi.org/https://doi.org/10.1016/j.ygyno.2020.05.012

Doll, K. M., Hempstead, B., Alson, J., Sage, L., & Lavallee, D. (2020). *Assessment of Prediagnostic Experiences of Black Women With Endometrial Cancer in the United States*. *3*(5), 1–12. https://doi.org/10.1001/jamanetworkopen.2020.4954

Du, J., Li, Y., Lv, S., Wang, Q., Sun, C., Dong, X., He, M., Ulain, Q., Yuan, Y., Tuo, X., Batchu, N., Song, Q., & Li, Q. (2016). Endometrial sampling devices for early diagnosis of endometrial lesions. In *Journal of Cancer Research and Clinical Oncology* (Vol. 142, Issue 12). https://doi.org/10.1007/s00432-016-2215-3

Fujiwara, H., Takahashi, Y., Takano, M., Miyamoto, M., Nakamura, K., Kaneta, Y., Hanaoka, T., Ohwada, M., Sakamoto, T., Hirakawa, T., Fujiwara, K., & Suzuki, M. (2015). Evaluation of endometrial cytology: cytohistological correlations in 1,441 cancer patients. *Oncology*, *88*(2), 86–94. https://doi.org/10.1159/000368162

Fulciniti, F., Yanoh, K., Karakitsos, P., Watanabe, J., Di, A., Niki, L., Yoshinobu, M., Maki, M., Norimatsu, Y., Kobayashi, T. K., & Hirai, Y. (2018). *The Yokohama system for reporting directly sampled endometrial cytology : The quest to develop a standardized terminology*. *January*, 400–412. https://doi.org/10.1002/dc.23916

Gkrozou, F., Dimakopoulos, G., Vrekoussis, T., Lavasidis, L., Koutlas, A., Navrozoglou, I., Stefos, T., & Paschopoulos, M. (2015). Hysteroscopy in women with abnormal uterine bleeding: a meta-analysis on four major endometrial pathologies. *Archives of Gynecology and Obstetrics*, *291*(6), 1347–1354. https://doi.org/10.1007/s00404-014-3585-x

Gupta, P., De Peralta-Venturino, M. N., Jane Purslow, M., & Kini, S. R. (1995). Endometrial cells of the "Lower uterine segment" (LUS) in cervical smears obtained by endocervical brushings: A source of potential diagnostic pitfall. *Diagnostic Cytopathology*, *12*(3). https://doi.org/10.1002/dc.2840120315

Han, L., Du, J., Zhao, L., Sun, C., Wang, Q., & Tuo, X. (2019). *An Efficacious Endometrial Sampler for Screening Endometrial Cancer*. *9*(February), 1–7. https://doi.org/10.3389/fonc.2019.00067

Horta, M., & Cunha, T. M. (2019a). Endometrial cancer. In *Medical Radiology* (pp. 179–208). Springer Verlag. https://doi.org/10.1007/174_2016_84

Horta, M., & Cunha, T. M. (2019b). Endometrial cancer. In *Medical Radiology*. https://doi.org/10.1007/174_2016_84

Johnston, K. M., Lakzadeh, P., Donato, B. M. K., & Szabo, S. M. (2019). Methods of sample size calculation in descriptive retrospective burden of illness studies. *BMC Medical Research Methodology*, *19*(1), 1–7.

https://doi.org/10.1186/s12874-018-0657-9

Koninckx, P. R., Ussia, A., Adamyan, L., Wattiez, A., Gomel, V., & Martin, D. C. (2019). Pathogenesis of endometriosis: the genetic/epigenetic theory. *Fertility and Sterility*, *111*(2). https://doi.org/10.1016/j.fertnstert.2018.10.013

Levy-Zauberman, Y., Pourcelot, A. G.Levy-Zauberman, Y., Pourcelot, A. G., Capmas, P., & Fernandez, H. (2017). Update on the management of abnormal uterine bleeding. Journal of Gynecology Obstetrics and Human Reproduction, 46(8). https://doi.org/10.1016/j.jogoh.2017.07.005Lev, 46(8). https://doi.org/10.1016/j.jogoh.2017.07.005, Capmas, P., & Fernandez, H. (2017). Update on the management of abnormal uterine bleeding. *Journal of Gynecology Obstetrics and Human Reproduction*, *46*(8). https://doi.org/10.1016/j.jogoh.2017.07.005

Lv, S., Wang, Q., Li, Y., Zhao, L., Wang, Y., Feng, X., Han, L., Zhang, K., Yin, P., Hou, H., Shi, G., & Li, Q. (2020). A clinical comparative study of two different endometrial cell samplers for evaluation of endometrial lesions by cytopathological diagnosis. *Cancer Management and Research*, *12*. https://doi.org/10.2147/CMAR.S272755

Maksem, J. A. (2000). *Performance Characteristics of the Indiana University Medical Center Endometrial Sampler (Tao Brush) in an Outpatient Office Setting , First Year ' s Outcomes : Recognizing Histological Patterns in Cytology Preparations of Endometrial Brushings*. *22*(3).

Manoli, N. N., Manoli, N. S., Kakoti, L., Kamath, A., & Kulkarni, S. (2017). *Manual Liquid Based Cytology in Diagnosis of Gynecologic Pathology Manual Liquid Based Cytology in Diagnosis of Gynecologic Pathology*. *May*. https://doi.org/10.11648/j.crj.20160404.11

Me, F., Sabadell, J., Jm, M., & Na, C. P. (2018). *Fertility-sparing treatment for atypical endometrial hyperplasia and endometrial cancer (Protocol)*. *8*. https://doi.org/10.1002/14651858.CD013111.www.cochranelibrary.com

Mittal, K. R., Schwartz, P. E., & Barwick, K. W. (1988). *Architectural (FIGO) Grading, Nuclear Grading, and Other Prognostic Indicators in Stage I Endometrial Adenocarcinoma With Identification of High-Risk and Low-Risk Groups*. *3*, 538–545.

Narice, B. F., Delaney, B., & Dickson, J. M. (2018). *Endometrial sampling in low-risk patients with abnormal uterine bleeding : a systematic review and meta-synthesis*. 1–13.

Njoku, K., Chiasserini, D., Whetton, A. D., & Crosbie, E. J. (2019). Proteomic biomarkers for the detection of endometrial cancer. In *Cancers* (Vol. 11, Issue 10). https://doi.org/10.3390/cancers11101572

Ohgami, T. (2014). *About the Authors*. 18–32. https://doi.org/10.2217/FMEB2013.13.320

Raffone, A., Raimondo, D., Raspollini, A., Oliviero, A., Travaglino, A., Santoro, A., Renzulli, F., Lopez, G., Michele, C., Maio, D., Casadio, P., Franco, G., Renato, Z., & Mollo, A. (2022). *Accuracy of cytological examination of Tao brush endometrial sampling in diagnosing endometrial premalignancy and malignancy*. *February*, 615–621. https://doi.org/10.1002/ijgo.14204

Ranjana, H., & Sadhna, S. (2016). *Comparison of conventional pap smear versus liquid based cytology in a diagnostic centre of central Madhya Pradesh*. *3*(March), 1–6. https://doi.org/10.5958/2394-6792.2016.00010.7

Rodriguez, A. C., Blanchard, Z., Maurer, K. A., & Gertz, J. (2019). Estrogen Signaling in Endometrial Cancer: a Key Oncogenic Pathway with Several Open Questions. In *Hormones and Cancer* (Vol. 10, Issues 2–3). https://doi.org/10.1007/s12672-019-0358-9

Soliman, P. T., Oh, J. C., Schmeler, K. M., Sun, C. C., Slomovitz, B. M., Gershenson, D. M., Burke, T. W., & Lu, K. H. (2005). Risk factors for young premenopausal women with endometrial cancer. *Obstetrics and Gynecology*, *105*(3), 575–580. https://doi.org/10.1097/01.AOG.0000154151.14516.f7

Taraboanta, C., Britton, H., Plotkin, A., Azordegan, N., Clement, P. B., & Gilks, C. B. (2020). Performance Characteristics of Endometrial Sampling in Diagnosis of Endometrial Carcinoma. *International Journal of Gynecological Pathology*, *39*(1). https://doi.org/10.1097/PGP.0000000000000569

Tran, A. Q., & Gehrig, P. (2017). Recent Advances in Endometrial Cancer. In *F1000Research* (Vol. 6). https://doi.org/10.12688/f1000research.10020.1

Tsai, M. C., & Goldstein, S. R. (2012). Office diagnosis and management of abnormal uterine bleeding. *Clinical Obstetrics and Gynecology*, *55*(3). https://doi.org/10.1097/GRF.0b013e31825d3cec

Ueda, S. M., Kapp, D. S., Cheung, M. K., Shin, J. Y., Osann, K., Husain, A., Teng, N. N., Berek, J. S., & Chan, J. K. (2008). Trends in demographic and clinical characteristics in women diagnosed with corpus cancer and their potential impact on the increasing number of deaths. *American Journal of Obstetrics and Gynecology*, *198*(2), 218.e1-218.e6. https://doi.org/https://doi.org/10.1016/j.ajog.2007.08.075

Van Hanegem, N., Prins, M. M. C., Bongers, M. Y., Opmeer, B. C., Sahota, D. S., Mol, B. W. J., & Timmermans, A. (2016). The accuracy of endometrial sampling in women with postmenopausal bleeding: A systematic review and meta-analysis. In *European Journal of Obstetrics and Gynecology and Reproductive Biology* (Vol. 197). https://doi.org/10.1016/j.ejogrb.2015.12.008

Vermij, L. (2020). *Incorporation of molecular characteristics into endometrial cancer management*. *i*, 52–63. https://doi.org/10.1111/his.14015

Visser, N. C. M., Reijnen, C., Massuger, L. F. A. G., Nagtegaal, I. D., Bulten, J., & Pijnenborg, J. M. A. (2017a). Accuracy of endometrial sampling in endometrial carcinoma: A systematic review and meta-analysis. In *Obstetrics and Gynecology* (Vol. 130, Issue 4). https://doi.org/10.1097/AOG.0000000000002261

Visser, N. C. M., Reijnen, C., Massuger, L. F. A. G., Nagtegaal, I. D., Bulten, J., & Pijnenborg, J. M. A. (2017b). Accuracy of Endometrial Sampling in Endometrial

Carcinoma. *Obstetrics & Gynecology*, *130*(4). https://doi.org/10.1097/aog.0000000000002261

Williams, A. R. W., Brechin, S., Porter, A. J. L., Warner, P., & Critchley, H. O. D. (2008). Factors affecting adequacy of Pipelle and Tao Brush endometrial sampling. *BJOG: An International Journal of Obstetrics and Gynaecology*, *115*(8). https://doi.org/10.1111/j.1471-0528.2008.01773.x

Woolderink, J. M., De Bock, G. H., Van Hemel, B. M., Geuken, E., Hollema, H., Werner, N., & Mourits, M. J. (2020). Feasibility of endometrial sampling by vaginal tampons in women with Lynch syndrome. *BMC Women's Health*, *20*(1). https://doi.org/10.1186/s12905-020-00920-y

Wu, H. H., Harshbarger, K. E., Berner, H. W., & Elsheikh, T. M. (2000). Endometrial brush biopsy (Tao brush). Histologic diagnosis of 200 cases with complementary cytology: an accurate sampling technique for the detection of endometrial abnormalities. *American Journal of Clinical Pathology*, *114*(3), 412–418. https://doi.org/10.1093/ajcp/114.3.412

Wyckoff, E. T., Gabrilovich, S., & Castagno, J. (2016). A Rare Case Presentation of Endometrial Clear-Cell Carcinoma. *Journal of Gynecologic Surgery*, *32*(4). https://doi.org/10.1089/gyn.2016.0011

Yang, B., Xu, Y., Zhu, Q., Xie, L., Shan, W., Ning, C., Xie, B., Shi, Y., Luo, X., Zhang, H., & Chen, X. (2019). Gynecologic Oncology Treatment ef fi ciency of comprehensive hysteroscopic evaluation and lesion resection combined with progestin therapy in young women with endometrial atypical hyperplasia and endometrial cancer. *Gynecologic Oncology*, *153*(1), 55–62. https://doi.org/10.1016/j.ygyno.2019.01.014

APPENDICES

Appendix Ia: Patient Informed Consent Form

Title of Study: **DETERMINATION OF PERFORMANCE CHARACTERISTICS OF ENDOMETRIAL TAO BRUSH IN THE DETECTION OF ENDOMETRIAL CANCER AND ATYPICAL HYPERPLASIA**

Investigators

Investigator	Position	Institution
1. Eudia Jepkoech Kemei	Principal Investigator	JKUAT
2. Prof. Mutinda Kyama	Supervisor	JKUAT
3. Dr. Mary Mungania	Supervisor	KNH
4. Prof. Eunice Cheserem	Supervisor	UON

Investigator statement

I would like to tell you about a study being conducted by the above listed investigators. The purpose of this consent form is to give you the information you will need to help you decide whether or not to be a participant in the study. Feel free to ask any questions about the purpose of the research, what happens if you participate in the study, the possible risks and benefits, your rights as a volunteer, and anything else about the research or this form that is not clear. When we have answered all your questions to your satisfaction, you may decide to be in the study or not. This process is called 'informed consent'. Once you understand and agree to be in the study, I will request you to sign your name on this form. You should understand the general principles which apply to all participants in a medical research: i) Your decision to participate is entirely voluntary

ii) You may withdraw from the study at any time without necessarily giving a reason for your withdrawal iii) Refusal to participate in the research will not affect the

services you are entitled to in this health facility or other facilities. We will give you a copy of this form for your records.

May I continue? YES / NO

Background Information

a) Study Objectives

The Main objective of the study is to Determine the Performance Characteristics of Endometrial Tao brush in Detection of Endometrial Cancer and Atypical Hyperplasia using the Endometrial Tao brush and Dilatation and curettage samples of selected patients with endometrial cancer in KNH.

Specific objective(s) of the research:

i. To compare Conventional and Manual liquid-based specimens collected with Tao brush in the detection of endometrial lesions.
ii. To evaluate the performance characteristics of endometrial cytology using Tao brush for the detection of endometrial malignancies.
iii. To determine the discordance and diagnostic level of agreement between endometrial Brush cytological sampler (Tao Brush) with Histopathological finding (Gold Standard).

b) Purpose of the Study

This study is expected to assess the clinical utility of the Endometrial Tao Brush that will improve the screening and evaluation of Endometrial cancer patients in Kenya since it can be used in an outpatient setting without the need for anesthesia and hospitalization. It is expected that the use of Endometrial Tao Brush for screening of endometrial cancer and its precursors will facilitate early detection of cancer and therefore improve treatment outcomes for patients with Endometrial cancer. This may increase the survival of Endometrial cancer patients and thus improve their health condition and quality of life in Kenya.

c) Study procedures

The study population will be the symptomatic women undergoing Dilatation and Curettage procedure of age 45 years and above. The Subjects will be examined using the questionnaire clinically and with the relevant imaging findings. After obtaining a signed informed consent, all the women will sequentially proceed to endometrial cytology using Tao Brush and then Dilatation and curettage (biopsy) endometrial sampling. The samples will then be taken to the laboratory for analysis.

Benefits and Risks of the study to you

Potential Benefits;

i. There is a direct benefit to the participant since an endometrial biopsy is the most common and accurate test used to diagnose endometrial cancer.
ii. The results will be out in about a weeks' time and it will guide the clinician on the best treatment strategy of managing your endometrial cancer subtype.
iii. The diagnostic accuracy of Endometrial Tao brush compares favorably with that of D&C (dilatation and curettage) and hence it can be adopted as a future screening tool for endometrial cancer cases.
iv. The procedure is not painful.
v. Endometrial sampling is a safe and effective method for histological assessment of the endometrium.

Potential Risks;

i. Perforation of the uterus with hemorrhage is possible, but occurs rarely.
ii. There may be a risk of infection
iii. One may experience nausea, weakness, dizziness during the procedure but, the symptoms usually disappear within 10-15 minutes.

I kindly request you to join the study and allow the use of the specimen to Determine the Performance Characteristics of Endometrial Tao brush in Detection of Endometrial Cancer and Atypical Hyperplasia using the Endometrial Tao brush and Dilatation and curettage in the detection of Endometrial Malignancies and its precursors.

Cost

Being in this study will not cost you any extra payments. It will be free.

Confidentially

i. Names will not be required in the study, since you will be identified by the study number.
ii. Questionnaire will be kept under key and lock and only the principal investigator will access it.
iii. Questionnaires will be kept for one year then destroyed. Any information given to us will remain confidential and will be for your benefit. You will get your results in the usual manner during your next visit.

Withdrawal from study

Participation in this study will be voluntary and it is part of your routine evaluation and you are free to withdraw at any time without losing the benefits to which you are entitled in this institution.

What if you have Questions in the future?

Contact information:

If you have any questions regarding the study please contact me Eudia Kemei, Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62,000 – 00200 Nairobi, Kenya on Mobile no 0719389086.

Supervisor: Prof. Dr. Mutinda C. Kyama, Jomo Kenyatta University of Agriculture and Technology (JKUAT), P.O. Box 62,000 – 00200 Nairobi, on mobile number 0711169526.

Supervisor: Dr. Mary Mungania, Consultant Pathologist, UON, P.O BOX 19676-00202 Nairobi, on Mobile No;0722820155.

Supervisor: Prof. Eunice Cheserem, Gynaecologist, UON, P.O BOX 19676-00202 Nairobi, on Mobile No;0722722440.

Consent Form (Statement of Consent)

Participant's statement

I ……. after reading and being explained the study purpose do hereby give informed consent to participate in the study fully aware of the benefits and risks. I have had the chance to discuss this research study with a study counselor and I have had my questions answered in a language that I understand. I have not been pressurized to participate in this study in any way. I understand that participation in this study is completely voluntary and that I may withdraw from it at any time and without loss of any benefit or quality of management to which I am entitled. I am fully aware that the results of this study will be used for scientific purposes and may be published. I understand that all efforts will be made to keep information regarding my personal identity confidential. By signing this consent form, I have not given up any of the legal rights that I have as a participant in a research study.

Participant Signature: ……. Date…….

Witness Signature……. Date…….

Doctor/Nurse Signature…….……Date …….

Researcher's Statement

I, the undersigned, have fully explained the relevant details of this research study to the participant named above and believe that the participant has understood and has willingly and freely given her consent.

Principal investigator Signature……...Date …….

Appendix Ib: Fomu ya Ridhaa ya Mgonjwa

Kichwa cha Utafiti: UAMUZI WA SIFA ZA UTENDAJI WA ENDOMETRIAL TAO BRUSH KATIKA UGUNDUZI WA KANSA YA UTI WA UKOO NA HYPERPLASIA YA ATIPICAL.

Wachunguzi

Mpelelezi	**Nafasi**	**Taasisi**
1. Eudia Jepkoech Kemei	Mpelelezi Mkuu	wa JKUAT
2. Prof. Mutinda Kyama	Msimamizi	wa JKUAT
3. Dk. Mary Mungania	Msimamizi	wa KNH
4. Prof. Eunice Cheserem	Msimamizi	wa UON

Kauli ya mpelelezi

Ningependa kukuambia kuhusu utafiti unaofanywa na wachunguzi walioorodheshwa hapo juu. Madhumuni ya fomu hii ya idhini ni kukupa taarifa utakayohitaji ili kukusaidia kuamua kama kuwa mshiriki au la katika utafiti. Jisikie huru kuuliza maswali yoyote kuhusu madhumuni ya utafiti, nini kitatokea ukishiriki katika utafiti, hatari na manufaa yanayoweza kutokea, haki zako kama mtu wa kujitolea, na jambo lingine lolote kuhusu utafiti au fomu hii ambalo haliko wazi. Wakati tumejibu maswali yako yote kwa kuridhika kwako, unaweza kuamua kuwa katika utafiti au la. Utaratibu huu unaitwa 'kibali cha taarifa'. Ukishaelewa na kukubali kuwa katika utafiti, nitakuomba utie sahihi jina lako kwenye fomu hii. Unapaswa kuelewa kanuni za jumla zinazotumika kwa washiriki wote katika utafiti wa matibabu: i) Uamuzi wako wa kushiriki ni wa hiari kabisa.ii) Unaweza kujiondoa kwenye utafiti wakati wowote bila kutoa sababu ya kujitoa iii) Kukataa kushiriki katika utafiti hakutaathiri huduma unazostahili kupata katika kituo hiki cha afya au vituo vingine. Tutakupa nakala ya fomu hii kwa rekodi zako.

Naweza kuendelea? NDIO au LA

Maelezo ya Usuli

a) Malengo ya Utafiti

Lengo Kuu la utafiti huu ni Kubainisha Sifa za Utendaji kazi wa brashi ya Endometrial Tao katika Kugundua Saratani ya Endometrial na Hyperplasia ya Atypical kwa kutumia brashi ya Endometrial Tao na Upanuzi na sampuli za tiba za wagonjwa waliochaguliwa walio na saratani ya endometriamu katika KNH

Malengo mahususi ya utafiti:

i. Ili kulinganisha Vielelezo vya Kawaida na vya Mwongozo vya kioevu vilivyokusanywa na brashi ya Tao katika ugunduzi wa vidonda vya endometriamu.
ii. Kutathmini sifa za utendakazi wa saitologi ya endometria kwa kutumia brashi ya Tao ili kugundua kasoro za endometriamu
iii. Kubainisha kiwango cha kutofautiana na uchunguzi wa makubaliano kati ya sampuli ya saitolojia ya Brashi ya endometria (Tao Brashi) yenye ugunduzi wa Histopathological (Gold Standard).

b) Madhumuni ya Utafiti

Utafiti huu unatarajiwa kutathmini manufaa ya kliniki ya Endometrial Tao Brush ambayo itaboresha uchunguzi na tathmini ya wagonjwa wa saratani ya Endometrial nchini Kenya kwa kuwa inaweza kutumika katika hali ya wagonjwa wa nje bila kuhitaji ganzi na kulazwa hospitalini. Inatarajiwa kuwa matumizi ya Endometrial Tao Brush kwa ajili ya uchunguzi wa saratani ya endometriamu na vitangulizi vyake vitarahisisha utambuzi wa mapema wa saratani na hivyo kuboresha matokeo ya matibabu kwa wagonjwa wa saratani ya Endometrial. Hii inaweza kuongeza maisha ya wagonjwa wa saratani ya Endometrial na hivyo kuboresha hali yao ya afya na ubora wa maisha nchini Kenya.

c) Taratibu za masomo

Idadi ya watafitiwa watakuwa wanawake wenye dalili wanaopitia utaratibu wa Upanuzi na Uponyaji wa umri wa miaka 45 na zaidi. Wahusika watachunguzwa kwa kutumia dodoso kimatibabu na matokeo ya taswira husika. Baada ya kupata kibali kilichotiwa saini, wanawake wote watafuata saitologi ya endometriamu kwa kutumia Tao Brashi na kisha Upanuzi na uponyaji (biopsy) sampuli za endometriamu. Kisha sampuli zitapelekwa kwenye maabara kwa uchunguzi.

Faida na Hatari za utafiti kwako

Faida Zinazowezekana;

i. Kuna manufaa ya moja kwa moja kwa mshiriki kwani biopsy ya endometriamu ndicho kipimo cha kawaida na sahihi kinachotumika kutambua saratani ya endometriamu.

ii. Matokeo yatatoka baada ya wiki moja na yatamwongoza daktari juu ya mkakati bora wa matibabu wa kudhibiti aina ndogo ya saratani ya endometriamu.

iii. Usahihi wa uchunguzi wa brashi ya Endometrial Tao inalinganishwa vyema na ile ya D&C (kupanua na kuponya) na kwa hivyo inaweza kupitishwa kama zana ya uchunguzi wa baadaye wa visa vya saratani ya endometriamu.

iv. Utaratibu hauna uchungu.

v. Sampuli ya endometriamu ni njia salama na madhubuti ya tathmini ya kihistoria ya endometriamu.

Hatari zinazowezekana;

i. Kutoboka kwa uterasi na kutokwa na damu kunawezekana, lakini hutokea mara chache.

ii. Kunaweza kuwa na hatari ya kuambukizwa

iii. Mtu anaweza kupata kichefuchefu, udhaifu, kizunguzungu wakati wa utaratibu lakini, dalili kawaida hupotea ndani ya dakika 10-15.

Ninakuomba ujiunge na utafiti huu na uruhusu utumizi wa sampuli Kubainisha Sifa za Utendaji za Burashi ya Endometrial Tao katika Kugundua Saratani ya Endometrial na Hyperplasia ya Atypical kwa kutumia brashi ya Endometrial Tao na Upanuzi na tiba katika kugundua Ugonjwa wa Endometrial na vitangulizi vyake. .

Gharama

Kuwa katika utafiti huu hakutakugharimu malipo yoyote ya ziada. Itakuwa bure.

Kwa siri

i. Majina hayatahitajika katika utafiti, kwa kuwa utatambuliwa kwa nambari ya utafiti.

ii. Hojaji itawekwa chini ya ufunguo na kufuli na mpelelezi mkuu pekee ndiye atakayeifikia.

iii. Hojaji zitawekwa kwa mwaka mmoja kisha kuharibiwa. Taarifa zozote tulizopewa zitaendelea kuwa siri na zitakuwa kwa manufaa yako. Utapata matokeo yako kwa njia ya kawaida wakati wa ziara yako ijayo.

Kujiondoa kutoka kwa masomo

Kushiriki katika utafiti huu kutakuwa kwa hiari na ni sehemu ya tathmini yako ya kawaida na uko huru kujiondoa wakati wowote bila kupoteza manufaa ambayo unastahili kupata katika taasisi hii.

Je, ikiwa una Maswali katika siku zijazo?

Maelezo ya mawasiliano:

Ikiwa una maswali yoyote kuhusu utafiti huu tafadhali wasiliana nami Eudia Kemei, Chuo Kikuu cha Kilimo na Teknolojia cha Jomo Kenyatta (JKUAT), P.O. Box 62,000 – 00200 Nairobi, Kenya kwenye Simu ya Mkononi no **0719389086.**

Msimamizi: Prof. Dk. Mutinda C. Kyama, Chuo Kikuu cha Kilimo na Teknolojia cha Jomo Kenyatta (JKUAT), P.O. Box 62,000 - 00200 Nairobi, kwenye nambari ya simu **0711169526**.

Msimamizi: Dk. Mary Mungania, Mtaalamu Mshauri wa Patholojia, UON, P.O BOX 19676-00202 Nairobi, kwenye Simu ya Mkononi No**;0722820155**.

Msimamizi: Prof. Eunice Cheserem, Daktari Binakolojia, UON, P.O BOX 19676-00202 Nairobi, kwenye Simu ya Mkononi No;**0722722440**

FOMU YA RIDHAA (TAARIFA YA RIDHAA)

Kauli ya mshiriki

Mimi ……. baada ya kusoma na kuelezwa madhumuni ya utafiti, toa kibali cha kushiriki katika utafiti huku ukifahamu kikamilifu manufaa na hatari. Nimepata nafasi ya kujadili utafiti huu na mshauri wa utafiti na nimejibiwa maswali yangu katika lugha ninayoielewa. Sijashinikizwa kushiriki katika utafiti huu kwa njia yoyote ile. Ninaelewa kuwa kushiriki katika utafiti huu ni kwa hiari kabisa na kwamba ninaweza kujiondoa kutoka kwa utafiti huu wakati wowote na bila kupoteza manufaa yoyote au ubora wa usimamizi ninaostahiki. I

Ninafahamu kikamilifu kwamba matokeo ya utafiti huu yatatumika kwa madhumuni ya kisayansi na yanaweza kuchapishwa. Ninaelewa kuwa juhudi zote zitafanywa ili kuweka taarifa kuhusu utambulisho wangu wa kibinafsi kuwa siri. Kwa kutia saini fomu hii ya idhini, sijaacha haki zozote za kisheria nilizo nazo kama mshiriki katika utafiti wa utafiti.

Sahihi ya Mshiriki: ……. Tarehe…….

Sahihi ya Shahidi……. Tarehe…….

Sahihi ya Daktari/Muuguzi…….…….

Kauli ya Mtafiti

Mimi, aliyetia sahihi hapa chini, nimeeleza kikamilifu maelezo muhimu ya utafiti huu kwa mshiriki aliyetajwa hapo juu na ninaamini kuwa mshiriki ameelewa na ametoa ridhaa yake kwa hiari na kwa uhuru.

Sahihi ya mpelelezi mkuu…….

Appendix IIa: Study Questionnaire

Project title: Determination of Performance characteristics of Endometrial Tao Brush in the detection of Endometrial Cancer and atypical hyperplasia in a symptomatic spectrum of women attending Obstetrics and Gynaecology clinic 66 at Kenyatta National Hospital.

Study number----------

Demographic data

1) Age ----------
2) Weight----------
3) Age at onset of menarche----------
4) Age at first full-term pregnancy----------
5) Breast feeding Yes ☐ No ☐
6) Age at onset of menopause----------
7) Parity----------
8) Physical activity Yes ☐ No ☐
9) History of Endometrial cancer Yes ☐ No ☐
10) Family history of cancer Yes ☐ No ☐
11) High Blood Pressure Yes ☐ No ☐
12) Oral contraceptive use? Yes ☐ No ☐
13) (a) Any other hormonal therapy/ use Yes ☐ No ☐

(b) Type used ----------

14) Education level None ☐ primary ☐

Secondary Post-secondary ☐ ☐

15) Marital status single ☐ Married ☐

Widowed ☐ other (specify) …….

16) What is your occupation? Housewife ☐ Farmer ☐

Business ☐ other specify…….

Appendix IIb: Dodoso la Kujifunza

Kichwa cha mradi: Tathmini ya Endometrial Cytology katika Kugundua Saratani ya Endometria na Hyperplasia ya Atypical katika masafa ya dalili za wanawake wanaohudhuria kliniki za Uzazi katika Hospitali ya Kitaifa ya Kenyatta.

Nambari ya masomo---------- ----------

Data ya idadi ya watu

1) Umri ----------

2) Uzito----------

3) Umri mwanzoni mwa hedhi----------

4) Umri wa ujauzito wa kwanza kabisa----------

5) Kunyonyesha Ndiyo ☐ Hapana ☐

6) Umri wa mwanzo wa kukoma hedhi----------

7) Usawa----------

8) Shughuli za kimwili Ndiyo ☐ Hapana ☐

9) Historia ya saratani ya Endometrial Ndiyo ☐ Hapana ☐

10) Historia ya familia ya saratani Ndiyo ☐ Hapana ☐

11) Shinikizo la Juu la Damu Ndiyo ☐ Hapana ☐

12) Matumizi ya uzazi wa mpango kwa mdomo? Ndiyo ☐ Hapana ☐

13) (a) Tiba nyingine yoyote ya homoni/ tumia Ndiyo ☐ Hapana ☐

(b) Aina iliyotumika ----------

14) Ngazi ya elimu Hakuna ☐ Msingi ☐

Sekondari ☐ Baada ya sekondari ☐

15) Hali ya ndoa Bado kuoleka ☐ Kuwa mtu aliyeolewa ☐

Mjane ☐ Mwingine (taja) …….

16) Kazi yako ni gani? Mama wa nyumbani ☐ Mkulima ☐

Mwanabiashara ☐ Nyingine bayana.....

Appendix III: Papanicolaou Staining Method

A conventional smear will be stained using PAP stain and examined microscopically by PI and the pathologists to sign out.

Principle of the stain

Hematoxylin stains the nuclei blue by dye lake formation and it has a high affinity for chromatin. The eosin azure solution being acidic stains the cytoplasm. The eosin Y stains the mature squamous cells pink while light green stains the young cells blue. Orange G stains the cytoplasm of mature and keratinized cells (Austin R.M. et al).

Staining technique

1. Fix the smear in 95% ethanol

2. Hydrate smears through ethanol grades of 80%, 70% and then 50%

3. Rinse in distilled water	10 dips
4. Stain in Harris haematoxylin for	4 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	2 minutes
10. Rinse in 95% ethanol	10dips
11. Stain in E.A.50 for	4 minutes
12. Rinse in 95% ethanol	10 dips

13. Dehydrate in three changes of absolute ethanol	10 dips each
14. Clear in 3 changes of xylene	10 dips each
15. Mount in D.P.X	cover-slip

Appendix IV: Reporting System for Endometrial Cytology

a) Specimen adequacy

- Satisfactory for evaluation
- Unsatisfactory for evaluation

b) Interpretation/result

- Negative for malignant tumors and precursors
- Endometrial hyperplasia without atypia
- Atypical endometrial hyperplasia
- Malignant Neoplasms

Appendix V: Haematoxylin and Eosin(H&E) Staining Method

A conventional smear and /or tissue section of about four microns will be cut and labeled with the patient's identification number. It will be stained using H/E stain and examined microscopically by PI and the pathologists. The available tissue section from D&C of about four microns will be cut and used in the histological classification of Endometrial cancer and other malignancies (Ada T Feldman et al).

Staining technique

1. Bring sections to distilled water
2. Stain nuclei with the alum hematoxylin for 3-4 minutes
3. Rinse in running tap water
4. Differentiate with 0.3% acid alcohol
5. Rinse in running tap water
6. Rinse in Scott's tap water substitute
7. Rinse in tap water
8. Stain with eosin for 2 minutes
9. Dehydrate, clear and mount.

Appendix VI: Reporting System for Endometrial Biopsy

i. Non-diagnostic/Unsatisfactory

ii. Negative for malignancy

- Atrophic endometrium
- Benign reactive Changes
- Proliferative endometrium
- Secretory endometrium
- Endometrial polyp

- Endometrial glandular and stromal breakdown

iii. Endometrial Hyperplasia with atypia

- Hyperplastic polyp

iv. Atypical endometrial hyperplasia

v. Malignant tumors

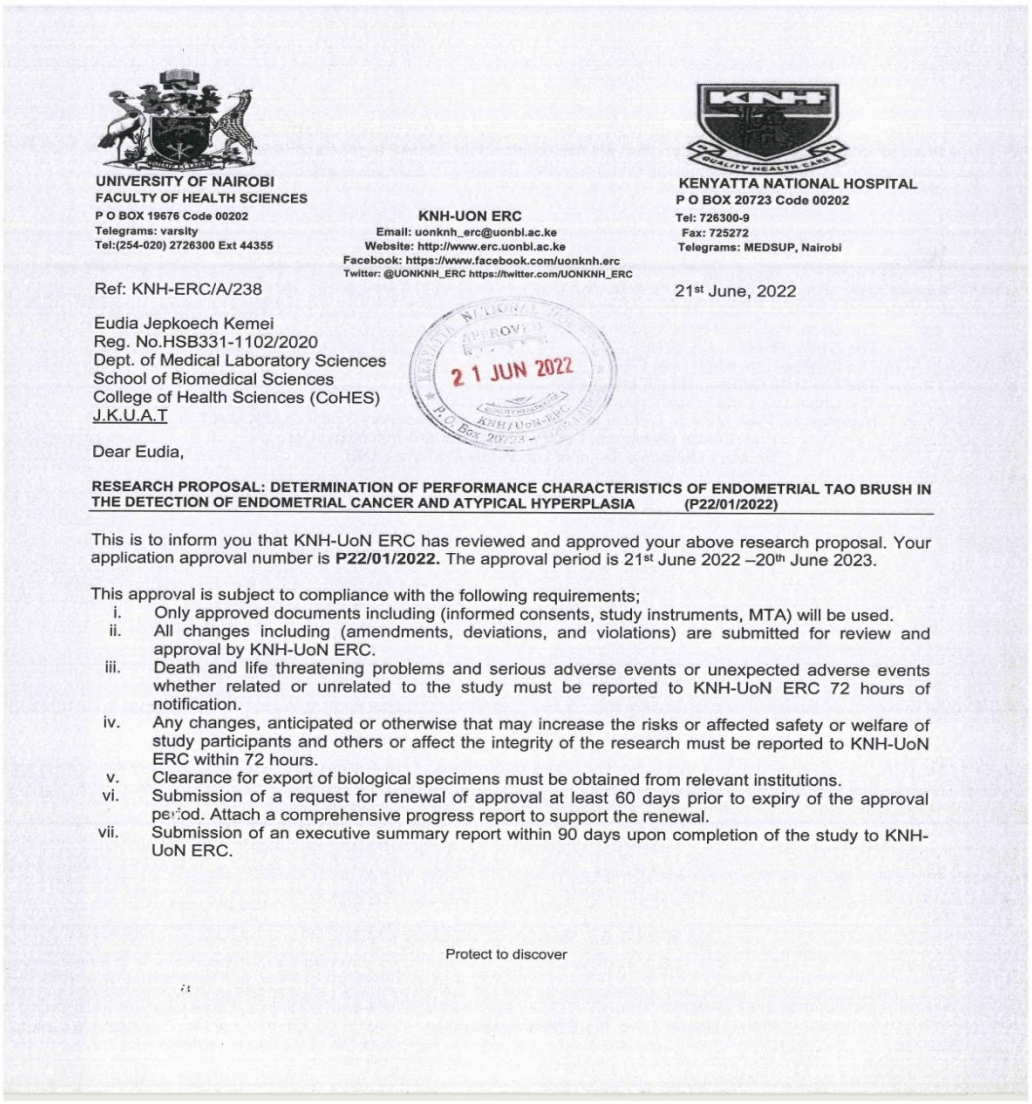
✓ Endometrioid adenocarcinoma

- Grade 1
- Grade 2

✓ Non -Endometrioid adenocarcinoma

- Papillary serous carcinoma
- Serous carcinoma
- Squamous cell carcinoma
- Undifferentiated

Appendix VII: KNH-UON ERC Ethical Approval



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Ref: KNH-ERC/A/238

21st June, 2022

Eudia Jepkoech Kemei
Reg. No.HSB331-1102/2020
Dept. of Medical Laboratory Sciences
School of Biomedical Sciences
College of Health Sciences (CoHES)
J.K.U.A.T

APPROVED
21 JUN 2022
KNH/UoN-ERC
P.O. Box 20723

Dear Eudia,

RESEARCH PROPOSAL: DETERMINATION OF PERFORMANCE CHARACTERISTICS OF ENDOMETRIAL TAO BRUSH IN THE DETECTION OF ENDOMETRIAL CANCER AND ATYPICAL HYPERPLASIA (P22/01/2022)

This is to inform you that KNH-UoN ERC has reviewed and approved your above research proposal. Your application approval number is **P22/01/2022.** The approval period is 21st June 2022 –20th June 2023.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used.
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by KNH-UoN ERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to KNH-UoN ERC 72 hours of notification.
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH-UoN ERC within 72 hours.
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to KNH-UoN ERC.

Protect to discover

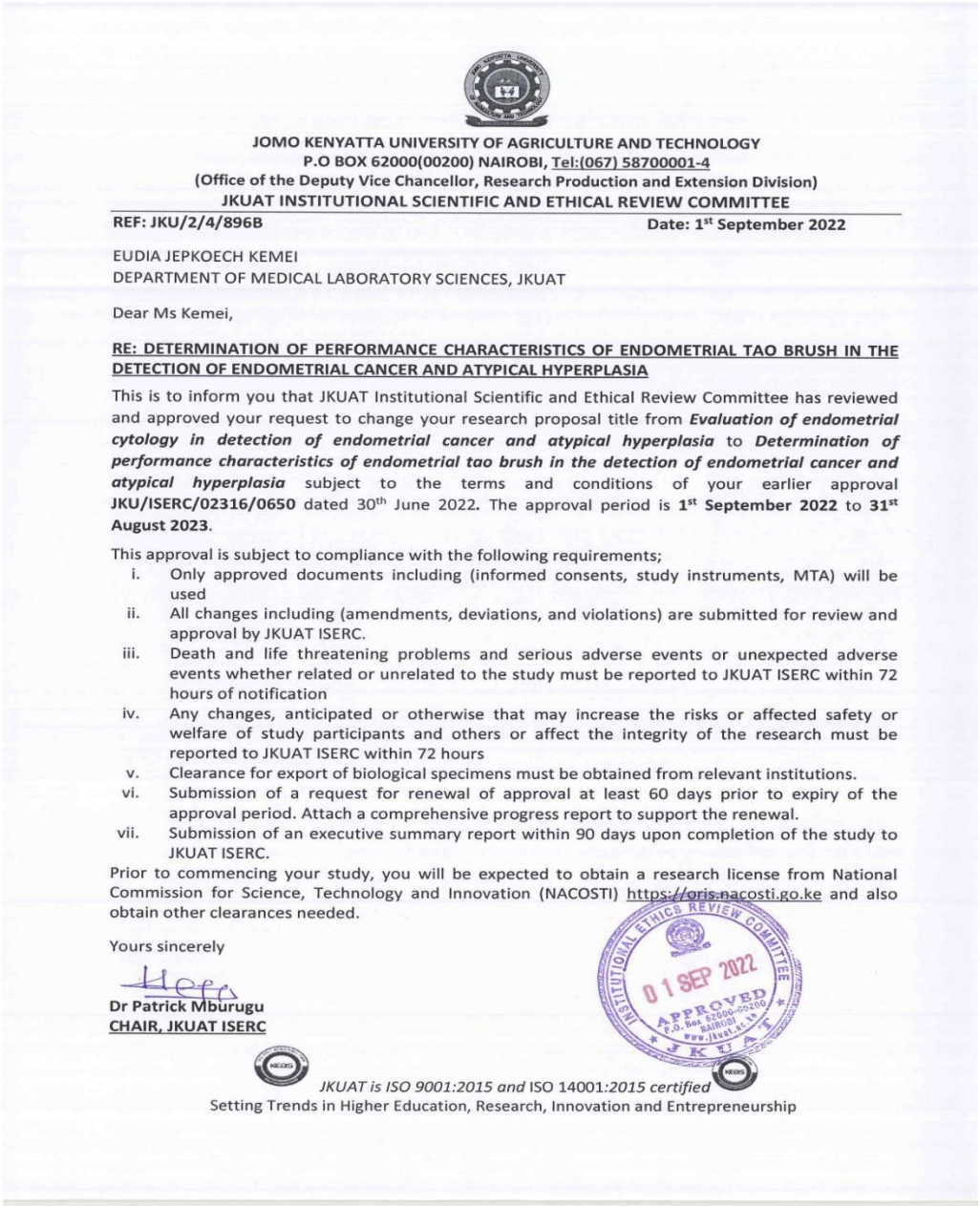
Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://research-portal.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely,

DR. BEATRICE K.M. AMUGUNE
SECRETARY, KNH-UoN ERC

c.c. The Dean, Faculty of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information Dept., KNH
The Chair, Dept. of Medical Laboratory Sciences, J.K.U.A.T
Supervisors: Prof. Mutinda C. Kyama, Dept. of Medical Laboratory Sciences, J.K.U.A.T
Prof. Eunice Chesarem, Dept. of Obstetrics & Gynecology, UoN
Dr. Mary Mungania, Dept. of Laboratory Medicine, KNH

Appendix VIII: JKUAT ISERC Ethical Approval



JOMO KENYATTA UNIVERSITY OF AGRICULTURE AND TECHNOLOGY
P.O BOX 62000(00200) NAIROBI, Tel:(067) 58700001-4
(Office of the Deputy Vice Chancellor, Research Production and Extension Division)
JKUAT INSTITUTIONAL SCIENTIFIC AND ETHICAL REVIEW COMMITTEE

REF: JKU/2/4/896B **Date: 1st September 2022**

EUDIA JEPKOECH KEMEI
DEPARTMENT OF MEDICAL LABORATORY SCIENCES, JKUAT

Dear Ms Kemei,

RE: DETERMINATION OF PERFORMANCE CHARACTERISTICS OF ENDOMETRIAL TAO BRUSH IN THE DETECTION OF ENDOMETRIAL CANCER AND ATYPICAL HYPERPLASIA

This is to inform you that JKUAT Institutional Scientific and Ethical Review Committee has reviewed and approved your request to change your research proposal title from ***Evaluation of endometrial cytology in detection of endometrial cancer and atypical hyperplasia*** to ***Determination of performance characteristics of endometrial tao brush in the detection of endometrial cancer and atypical hyperplasia*** subject to the terms and conditions of your earlier approval **JKU/ISERC/02316/0650** dated 30th June 2022. The approval period is **1st September 2022** to **31st August 2023**.

This approval is subject to compliance with the following requirements;

i. Only approved documents including (informed consents, study instruments, MTA) will be used
ii. All changes including (amendments, deviations, and violations) are submitted for review and approval by JKUAT ISERC.
iii. Death and life threatening problems and serious adverse events or unexpected adverse events whether related or unrelated to the study must be reported to JKUAT ISERC within 72 hours of notification
iv. Any changes, anticipated or otherwise that may increase the risks or affected safety or welfare of study participants and others or affect the integrity of the research must be reported to JKUAT ISERC within 72 hours
v. Clearance for export of biological specimens must be obtained from relevant institutions.
vi. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. Attach a comprehensive progress report to support the renewal.
vii. Submission of an executive summary report within 90 days upon completion of the study to JKUAT ISERC.

Prior to commencing your study, you will be expected to obtain a research license from National Commission for Science, Technology and Innovation (NACOSTI) https://oris.nacosti.go.ke and also obtain other clearances needed.

Yours sincerely

Dr Patrick Mburugu
CHAIR, JKUAT ISERC

INSTITUTIONAL ETHICS REVIEW COMMITTEE
01 SEP 2022
APPROVED
P.O. Box 62000-00200 NAIROBI www.jkuat.ac.ke
JKUAT

JKUAT is ISO 9001:2015 and ISO 14001:*2015 certified*
Setting Trends in Higher Education, Research, Innovation and Entrepreneurship

Appendix IX: NACOSTI Research License

REPUBLIC OF KENYA

NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Ref No: **879311**

Date of Issue: **04/July/2022**

RESEARCH LICENSE

This is to Certify that Ms.. Eudia Jepkoech Kemei of Jomo Kenyatta University of Agriculture and Technology, has been licensed to conduct research in Nairobi on the topic: Determination of Performance Characteristics of Endometrial Tao Brush in the Detection of Endometrial Cancer and Atypical Hyperplasia for the period ending : 04/July/2023.

License No: **NACOSTI/P/22/18677**

879311

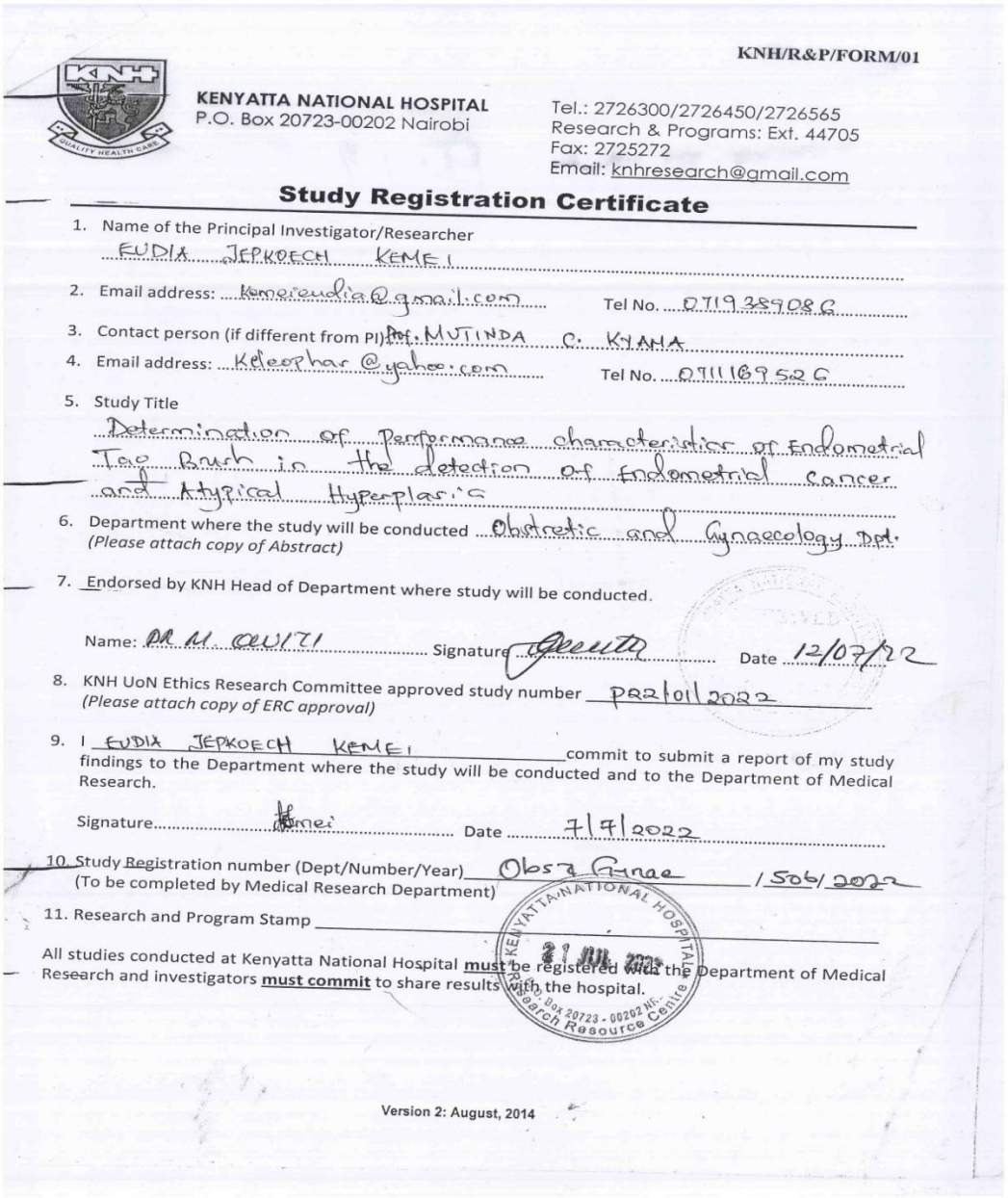
Applicant Identification Number

Director General
NATIONAL COMMISSION FOR SCIENCE,TECHNOLOGY & INNOVATION

Verification QR Code

NOTE: This is a computer generated License. To verify the authenticity of this document, Scan the QR Code using QR scanner application.

Appendix X: KNH Study Registration Certificate



KNH/R&P/FORM/01

KENYATTA NATIONAL HOSPITAL
P.O. Box 20723-00202 Nairobi

Tel.: 2726300/2726450/2726565
Research & Programs: Ext. 44705
Fax: 2725272
Email: knhresearch@gmail.com

Study Registration Certificate

1. Name of the Principal Investigator/Researcher
   EUDIA JEPKOECH KEMEI
2. Email address: kemeieudia@gmail.com Tel No. 0719389080
3. Contact person (if different from PI) Prof. MUTINDA C. KYAMA
4. Email address: Kcleophar@yahoo.com Tel No. 0711169526
5. Study Title
   Determination of Performance characteristics of Endometrial Tao Brush in the detection of Endometrial Cancer and Atypical Hyperplasia
6. Department where the study will be conducted Obstetric and Gynaecology Dpt.
   (Please attach copy of Abstract)
7. Endorsed by KNH Head of Department where study will be conducted.
   Name: DR M. OUTI Signature [signature] Date 12/07/22
8. KNH UoN Ethics Research Committee approved study number P82/01/2022
   (Please attach copy of ERC approval)
9. I EUDIA JEPKOECH KEMEI commit to submit a report of my study findings to the Department where the study will be conducted and to the Department of Medical Research.
   Signature [signature] Date 7/7/2022
10. Study Registration number (Dept/Number/Year) Obst Gynae /506/2022
    (To be completed by Medical Research Department)
11. Research and Program Stamp KENYATTA NATIONAL HOSPITAL P.O. Box 20723 - 00202 Nairobi Research Resource Centre 21 JUL 2022

All studies conducted at Kenyatta National Hospital **must** be registered with the Department of Medical Research and investigators **must commit** to share results with the hospital.

Version 2: August, 2014

Appendix XI: Publication

East African Medical Journal Vol. 100 No. 9 September 2023

DETERMINATION OF THE UTILITY OF ENDOMETRIAL TAO BRUSH IN THE DETECTION OF ENDOMETRIAL CANCER AND ATYPICAL HYPERPLASIA

Eudia Jepkoech Kemei, Department of Medical Laboratory Sciences, School of Biomedical Sciences, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya, Eunice Jeptoo Cheserem Ph.D., Department of Obstetrics and Gynaecology, School of Medicine, University of Nairobi, P.O. Box 19676-00202, Nairobi, Kenya, Mary Mungania MBChB, MMed, FC Path ECSA, Department of Laboratory Medicine, Kenyatta National Hospital, P.O. Box 20723-00202, Nairobi, Kenya, James Mutua Ph.D., Department of Mechanical Engineering, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya, Cleophas Mutinda Kyama Ph.D., Department of Medical Laboratory Sciences, School of Biomedical Sciences, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya

Corresponding author: Eudia Jepkoech Kemei, Department of Medical Laboratory Sciences, School of Biomedical Sciences, College of Health Sciences, Jomo Kenyatta University of Agriculture and Technology, P.O. Box 62000-00200, Nairobi, Kenya. Email: kemeieudia@gmail.com

DETERMINATION OF THE UTILITY OF ENDOMETRIAL TAO BRUSH IN THE DETECTION OF ENDOMETRIAL CANCER AND ATYPICAL HYPERPLASIA

E. J. Kemei, E. J. Cheserem, M. Mungania, J. Mutua and C. M. Kyama

ABSTRACT

Background: **Endometrial cancer is among the most common gynecologic malignancies in Kenya as it is in other developing countries. Screening using the Tao brush for direct sampling of the endometrium is a reliable technique for detecting lesions and can be done in an outpatient setting. These attributes enable early detection which is crucial for favorable outcomes, as over 90% of patients with early stages of endometrial cancer can be cured with treatment.**

Objective: **To determine the Utility of Endometrial Tao brush in the detection of endometrial malignancies.**

Design: **Prospective Cross-sectional Study.**

Setting: **Department of Laboratory Medicine, Kenyatta National Hospital and Department of Obstetrics and Gynaecology, Kenyatta National Hospital.**

Study setting: **Kenyatta National Hospital (KNH) gynecology Clinic.**

Measurable Variables: **Independent variables included demographic and clinical data including; age, early menarche, late menopause, obesity, parity, and hypertension. Dependent variables included histopathological parameters of lesions; tumor size, type, tumor invasion, and metastasis.**

Results: **Sixty women fulfilled the study entry criteria and were evaluated. Histopathological diagnoses comprised of; 14 (23%) Endometrial Cancers, 8 (13%) Complex hyperplasia with atypia, 16 (27%) Simple and mild hyperplasia without atypia, 3 (5%) Non-Diagnostic, and 19 (32%) patients with Negative Endometrial**