HISTOPATHOLOGICAL ASSESSMENT OF ENDOMETRIAL BIOPSIES, THEIR INDICATION AND ASSOCIATED FACTORS AMONG WOMEN ATTENDING MOI TEACHING AND REFERRAL HOSPITAL, KENYA

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DECLARATION

I declare that this thesis is my original work and has not been presented to any other University or Institution for a degree or any other award

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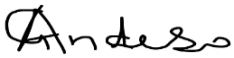
Signed  Date 23rd May 2021

We confirm that the work reported in this thesis was carried out by the candidate under our supervision. This thesis has been submitted for examination with our approval as University Supervisors

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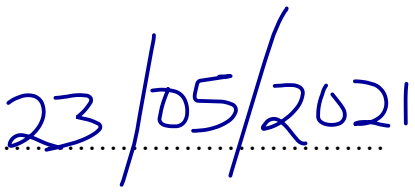
Signed  Date 

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DEDICATION

This thesis is dedicated to my family for the support they gave me during my study period.

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OPERATIONAL DEFINITIONS OF TERMS

Carcinoma: A type of cancer that develops from epithelial cells

Developing Country: A country having a standard of living or level of industrial production well below that possible with financial or technical aid.

Indication: Reason for endometrial sampling.

Malignant: Cancerous cells that can spread to other sites in the body (metastasize) or to invade and destroy tissues

Morbidity: A diseased state or symptom

Mortality: Death rate

Parity: Number of pregnancies that have had a gestational age of 28 weeks or more.

Pathognomic: Characteristic or symptomatic of a disease or condition.

Radiology: The use of medical imaging to diagnose and treat diseases within the body

Retrospective study: A study that looks backward in time using pre-existing data.

Products of conception; Presence of chorionic villi in the endometrium.

Decidualization: Change in the endometrial lining onto which blastocyst implants.

Atrophy: Endometrial cells are flattened with no mitotic activity

ABSTRACT

Background: Endometrial diseases are among the most common gynecological disorders affecting women both globally and locally; accounting for 60% of global maternal deaths. Kenya is ranked thirteenth out of 181 countries with the highest maternal mortality globally. Endometrial disorders such as hyperplasia's, neoplastic, inflammatory and pregnancy related conditions of the endometrium have been reported to increase with demographic traits such as maternal age and parity. Other risk factors for endometrial disorders include changes in lifestyle, increase in lifespan and the use of hormonal replacement therapy among post-menopausal women. Histopathological evaluation of endometrial samples is a vital tool for early diagnosis of endometrial disorders.

Objectives: The current study aimed to determine histopathological patterns of endometrial biopsies, their indication and associated factors among women attending Moi Teaching and Referral Hospital, Kenya.

Methodology: This was a retrospective laboratory-based study where 121 banked endometrial biopsy blocks collected from participants aged 19 to 70 years between August 2014 and August 2016 were retrieved, re-sectioned and stained using routine histological (Hematoxylin and Eosin) stains in the histology laboratory before being sent to the pathologist for examination. Sociodemographic and reproductive history data were collected from the medical records. Descriptive statistical techniques such as cross-tabulation were used to determine variations in proportions between age-groups, parity, indication and endometrial disorders. Pearson chi-square test was used to test association between predictor and outcome variables. The median age of all the study participants was 44 years.

Results: Majority (52.9%) of these participants had a parity of 1 - 4 followed by those with a parity of 5 - 9 (37.2%). About one-quarter (24.2%) had a history of miscarriage. Most of the study participants (39.2%; n=38) presented with simple endometrial hyperplasia. Age group was significantly associated with endometrial patterns (P =0.012).

The age group which had various endometrial patterns was 41-60 years, accounting for 51.2% of all the study participants. Parity was not associated with endometrial patterns (P=0.0847). The most common endometrial patterns among women with a parity of 1-4 was simple endometrial hyperplasia (n=21). Pelvic bleeding (88.3%) was the major condition that made women present for endometrial sampling.

Conclusions: Majority of the study participants presented with simple endometrial hyperplasia followed by complex hyperplasia and adenocarcinoma, respectively. The age group which had numerous varied endometrial patterns were those between 41-60 years. Nulliparous women only presented with complex hyperplasia, which is a precursor to endometrial carcinoma. Pelvic bleeding was the most common indication for endometrial sampling.

Recommendations: Endometrial sampling should be recommended for women who are 40 years and above presenting with bleeding and backache. A parity of five or more could reduce the risk of endometrial disorders. All post-menopausal bleeding should be investigated, especially if they present with risk factors for endometrial hyperplasia or cancer.

CHAPTER ONE: INTRODUCTION

1.1 Background

Endometrial biopsies and curetting are among the most common tissue specimens received in the pathology laboratory and most important sampling methods for definitive diagnosis of the endometrial lesions (Trimble et al., 2012). Endometrial diseases are ranked among the most common gynecological disorders that affect women globally and locally (Yu et al., 2015). These diseases cut across all age groups and contribute significantly to increased maternal morbidity and mortality.

The histopathological patterns of the endometrium are classified into various categories including: preneoplastic condition: endometrial hyperplasia, inflammatory conditions: chronic endometritis, Pregnancy related conditions: products of conception and molar pregnancy, benign condition: endometrial polyp and malignant conditions (Abdullahi et al., 2016). Due to the wide range of histopathological patterns, the need for urgent diagnosis and treatment cannot be overemphasized. Most of these lesions can only be diagnosed by sampling the endometrium and they range from simple endometrial hyperplasia to more complex disorders including endometrial carcinoma (Abdullahi et al., 2016).

Histological characteristics of endometrial curettages and biopsy material as assessed by light microscopy remain the diagnostic standard for the clinical diagnosis of endometrial pathology (Baydar et al., 2005). Indeed, the initial diagnosis is made by endometrial biopsy or by curettage, which may be therapeutic (Helpman et al., 2014; McCluggage, 2006). A group of gynaecologists have shown that histopathological patterns of diagnosis vary with respect to the age of patients (Obstetricians & Gynecologists, 1998) . Most young women of reproductive age present more

commonly with changes associated with hormonal imbalance (Abid et al., 2014). However, older women of premenopausal and postmenopausal age group present more commonly with endometrial hyperplasia and endometrial carcinoma (Lacey Jr et al., 2012; Lacey Jr et al., 2010; Trimble et al., 2012). Endometrial and ovarian cancers occur later in reproductive life than carcinoma of the cervix and choriocarcinoma, which are seen commonly in premenopausal or peri-menopausal women (Nicolae Bacalbasa, Balescu, & Filipescu, 2018). Previous studies have indicated that women of high parity have relatively low risk of developing endometrial cancer; thus, age and parity are known to affect the incidence of gynecological cancers (Brunette, Katzir, Amneus, Aoyama, & Holschneider, 2012; Setiawan et al., 2013; Wu et al., 2015) .

A normal endometrium undergoes a variety of morphologic changes, especially during the reproductive years, when cyclical hormonal influences and pregnancy affect uterine growth (Hecht, Dolinski, Gardner, Violette, & Weinreb, 2008; Vaidya et al., 2013). Biopsy induced artifacts confound this heterogeneous group of morphologic changes. Whether the biopsy is limited or a thorough curettage, the procedure usually is "blind," with no visualization of the tissue sampled (Van Doorn et al., 2007). The final specimen contains multiple, irregularly oriented tissue fragments mixed with blood and contaminating cervical tissue and mucus (Li, Sanchez, Patel, Quenby, & Rajpoot, 2015). Interpreting the biopsy material demands a logical approach that considers many factors, including patient history; the specific requests of the clinician performing the biopsy; and an appreciation of the limitations, potential pitfalls, and complex array of patterns encountered in the microscopic sections (Bagnasco, 2018; McCluggage, 2006). As in evaluation of any pathologic specimen, proper interpretation requires appropriate fixation, processing, and sectioning of the tissue (Braun, Overbeek-Wager, & Grumbo, 2016; McCluggage, 2006). These lesions range from simple endometrial hyperplasia to more complex disorders including

endometrial carcinoma (Nicolae Bacalbasa et al., 2018; Chang, Baker, & Landen, 2016). Ninety-seven percent of all cancers of the uterus arise from the glands of the endometrium and are known as endometrial carcinomas. Its annual incidence is estimated at 10–20 per 100,000 women and it is increasing globally (Arnett, Soisson, Ducatman, & Zhang, 2003; Nijkang, Anderson, Markham, Fraser, & Manconi, 2018). Approximately, 75% of cases are diagnosed at an early stage, with a tumor confined to the uterine corpus (Kanyilmaz, Aktan, Koc, & Findik, 2016; Platz & Benda, 1995).

Globally, endometrial carcinoma is the fourth most common cancer in women after carcinomas of breast, colorectal, and lung (Braun et al., 2016; Pessoa et al., 2014). Corpus cancer is commoner in developed countries than in developing countries (Kanyilmaz et al., 2016). In 2009, there were 236, 643 cases worldwide, out of which 113,486 occurred in developing countries, representing approximately 48% of the global burden (Fader, Arriba, Frasure, & von Gruenigen, 2009). Low incidences less than 4/100 000 are found in South Asia and Africa. More than 90% occur in women aged 50 years and above (Binesh, Akhavan, Behniafard, & Jalilian, 2014; Sinawat & Chiyabutra, 2004). Gynecological cancers continue to be important health problems worldwide (Hentze, Hogdall, Kjaer, Blaakaer, & Hogdall, 2017). The proportion of cancers in the female which are of genital tract origin range from 31.6% to 35.0% in sub-Saharan Africa, 12.7% to 13.4% in North America, 13.9% to 16.8% in France and the Scandinavian countries, 15.5% to 43.1% in South America and 22.4% to 55.8% in India. One major problem in developing countries, and especially in sub-Saharan Africa, is the absence of accurate population and health statistics. While in developed countries endometrial carcinoma is the commonest gynaecological cancer, in African countries carcinoma of the cervix has been reported in many series to be the commonest, with most of the patients presenting in late stages of the disease. In Kenya, the true prevalence of endometrial

cancer remains largely unknown. However, data from hospitals show that for every 30 cases of cervical cancer reported one case of endometrial cancer will be documented (Odongo et al., 2013). Indeed, the risk of developing endometrial cancer in a lifetime is approximately 2% (Odongo et al., 2013). Although it is not possible to reliably calculate incidence rates for the various cancers, data (January 2008 – December 2012) from a recent retrospective cross-sectional study conducted at Kenyatta National Hospital (KNH) and Moi Teaching and Referral Hospital (MTRH) investigating the types of cancers and infections attributed to them among adult population revealed that 30.8% and 48.2% of the total cancer cases tested in KNH and MTRH, respectively were associated with infectious agents, while 27.6% and 44.4% were linked to infections in the two hospitals, respectively (Macharia, Mureithi, & Anzala, 2018). Additionally, the study showed that the 5 most common cancers among female (n=300) tested at KNH were cervical, breast, ovarian, chronic leukemia, endometrial and stomach, while those registered at MTRH (n=282) presented with breast, cervical, Kaposi's sarcoma, non-Hodgkin's lymphoma and cancer of the ovary (Macharia et al., 2018). The data presented in the study above was collected from randomly selected hospital records of patients.

Unlike other malignancies affecting women, endometrial cancer often presents at an early stage, with the possibility of curative treatment by hysterectomy. Prognosis is increasingly bleak the more advanced the disease presents. As there have been no recent advances in the treatment of endometrial cancer that can be expected to increase survival, the importance of accurate and timely diagnosis of endometrial cancer is paramount in order to reduce mortality further. There is need for detailed study of the epidemiology of cervical and endometrial cancers in women to determine whether they share other common antecedents in addition to age and parity, since if these exist, they would have implications for public education on and the prevention of both cancers. It is

against this background that the study seeks to determine the histopathological evaluation of endometrial biopsies in patients attending Moi Teaching and Referral Hospital (MTRH) in Uasin Gishu County, Kenya. A recent study from 2 largest referral hospitals (KNH and MTRH) in Kenya report that 48.2% of cancers associated with infectious agents were documented at MTRH, presenting the highest frequency. Although endometrial cancer recorded at MTRH was only 1% compared to 3% at KNH of the types of cancers, the low numbers at MTRH could be attributed to: (i) convenient sampling bias of files (500 from a total of 4,304 files) compared to 500 files from a total of 17,584 files from KNH (Macharia et al., 2018), and (ii) and decision to sample randomly selected files rather than investigate all the files available. Irrespective of these limitations, MTRH recorded the highest number of types of cancers investigated and therefore appropriate for investigation of endometrial cancers.

1.2 Statement of the problem

Endometrial diseases are among the most common gynecological disorders seen in women of all ages. Although the actual prevalence of endometrial disorders is unknown in Kenya, their prevalence and associated mortality remain high both globally and in Kenya. This rising prevalence rate could be attributed to its risk factors such as changes in lifestyle of the affected women, an increased lifespan which also portends the likelihood of contracting it over time and the use of hormonal replacement therapy among post-menopausal women. Because of this, most women who present to health facilities with complains of bleeding and back pain often come with advanced stage of the disease. This could be verified by either conducting ultrasonography of the pelvis or endometrial sampling for histopathology. Although ultrasonography is non-invasive with a rapid turn-around time, there has been cases of misdiagnosis due to interuser variability in reporting the findings as well as inability to detect more salient endometrial disorders. This

misdiagnosis further compounds the suffering of the patients and delays treatment. On the other hand, histopathological evaluation of endometrial samples has been considered a gold standard and vital diagnostic tool for early detection of endometrial disorders for it sub-classifies the disorders leading to proper and timely treatment intervention. However, there are limited local studies addressing the reasons for endometrial sampling, the age group and reproductive characteristics of women who have this test requested for. Therefore, there is need to determine histopathological outcomes and their associated factors to better personalize diagnosis and treatment for women with endometrial disorders.

1.3 Significance of the study

Because of the high disease burden and poor quality of life for women with endometrial disorders, there is need for early diagnosis, immediate onset of treatment and identification of patient factors that are associated with various histopathological findings. This study employed the latest histopathological techniques to identify endometrial disorders patterns among women attending a national teaching hospital (MTRH) in Kenya. It provides the basis of determining the patient characteristics and associated histopathological outcomes to inform both differential and definitive diagnosis for endometrial disorders.

Furthermore, the findings of this study will inform clinical healthcare professionals involved in gynae-oncology to better institute proper and patient targeted interventions for women presenting with either uterine bleeding and lower backpains indicative of endometrial abnormality. The study could be utilized by health policy makers to better invest in histopathological equipment and personnel across various levels of healthcare service delivery in both the public and private sector. Further appreciation of histopathology may lead to increased investment in complementary services such as immunohistochemistry to better assay for the endometrial disorders based on their

endocrine aetiology. Put together, there will be an ultimate reduction in the disease burden and improved quality of life for women presenting with endometrial disorders in Kenya.

1.4 Study objectives

1.4.1 Broad objective

To determine histopathological patterns of endometrial biopsies, their indication and associated factors among women attending Moi Teaching and Referral Hospital (MTRH).

1.4.2 Specific objectives

i. To determine endometrial biopsies patterns among patients attending MTRH.
ii. To relate age and endometrial patterns in patients attending MTRH.
iii. To find out the association between parity and endometrial patterns among patients attending MTRH.
iv. To determine the common indications for endometrial sampling at MTRH.

1.5 Research questions

i. What are the endometrial patterns in biopsies among patients attending MTRH?
ii. How does age and endometrial biopsy pattern relate in these patients attending MTRH?
iii. What is the relationship between parity and endometrial disorders among patients attending MTRH?
iv. What are the indications of endometrial biopsy sampling in patients' disorders at MTRH?

CHAPTER TWO: LITERATURE REVIEW

2.1 The uterine endometrium

The uterus is a pear-shaped muscular organ situated in the pelvis between the bladder anteriorly and rectum posteriorly (Morice, Leary, Creutzberg, Abu-Rustum, & Darai, 2016). It is partially covered by the peritoneum of the pelvis floor (Slater, 2013; Susan, 2015). It is divided into the body and the cervix (Susan, 2015). The body is lined by the endometrium whose thickness varies at different ages and stages of the menstrual cycle (Abdullahi et al., 2016). The endometrium is composed of endometrial glands and mesenchymal stromal cells, both of which are very sensitive to the action of female sex hormones (Nijkang, Anderson, Markham, & Manconi, 2019). At the interval Os, the endometrium becomes continuous with the endocervical canal, which is lined by columnar epithelium and contains mucous glands (Arenas et al., 2015).

The endometrium is the mucosal lining of the uterus and is divided into the stratum basalis and the stratum functionalis (Pessoa et al., 2014). The stratum basale gives rise to the stratum functionalis that grows and is then shed each month (Oats & Abraham, 2015). The basalis is the deepest region of the endometrium and does not undergo significant monthly proliferation instead; it is the layer from which the endometrium regenerates after shedding (Susan, 2015). Overlaying functionalis is the superficial two thirds of the endometrium that proliferates and is ultimately shed with each cycle if pregnancy doesn't occur (Oats & Abraham, 2015). The monthly changes that occur in the endometrium correspond to the hormonal changes of the follicular and luteal phases of the ovarian cycle. The endometrial tissue that lines the uterine cavity is made up of simple columnar epithelium (N. Bacalbasa et al., 2016). The supporting stroma contains simple tubular glands. The endometrium is 1-2 mm thick at the beginning of a cycle and the glands are straight, non-secreting, and lined with columnar epithelium (N. Bacalbasa et al., 2016; van Hanegem et al., 2016). The

proliferative phase is maintained until ovulation by the increasing synthesis and secretion of estrogen from the developing ovarian follicles. The secretory phase of the uterine cycle occurs after ovulation and is characterized by progesterone and estrogen induced endometrial changes (Trussell & Guthrie, 2007). In the absence of fertilization, the menstrual phase of the uterine cycle begins (Chen et al., 2011). Without progesterone, the endometrium can no longer be maintained and is shed during menstruation. Blood and necrotic endometrium appear in the uterine lumen, to be discharged from the uterus and out through the vagina as menstruation (Trimble et al., 2012). During this time, FSH secretion is induced, causing a new cycle of follicle development and production of estrogen, which begins the process of resurfacing. Although the process of menstruation represents the endpoint of the cycle of endometrial changes, the first day of menstruation also marks the beginning of a new proliferative phase (Jetley, Rana, & Jairajpuri, 2014).

2.2 The common endometrial disorders

The uterine endometrium undergoes monthly cyclical changes under the influence of hormonal stimuli during the reproductive years, except during pregnancy (Dueholm & Hjorth, 2017). Before menarche (onset of menses) and after menopause; the endometrial glands and stroma are compact and inactive (Nijkang et al., 2019). Excessive or uncoordinated hormonal stimulation of the endometrium may produce diffuse endometrial hyperplasia; some variants of which are pre-malignant (Dueholm & Hjorth, 2017; Vaidya et al., 2013). The most common malignant tumor of the endometrium is endometrial carcinoma (an adenocarcinoma derived from endometrial glands) (Brinton et al., 2013; Sri, Steren, & Stratton, 2015).

2.2.1 Inflammatory lesions of the endometrium

Endometriosis is a female reproductive disorder present in approximately 15% of adult women between the ages of 25-35 (Ballweg, 2004). This disorder occurs when the endometrial tissue (cells that line the uterus) grows in other areas of the body. This abnormal growth of endometrial tissue is referred to as ectopic endometrium and can occur anywhere in the body, but is most observed in the pelvis-on the outer surface of the ovaries, fallopian tubes, or the uterus (Nijkang et al., 2018). The cyclic shedding of ectopic endometrium within the abdomen can cause irritation; lower back, intestinal, or pelvic pain, heavy menstrual periods or spotting between periods, dysmenorrhea, dyspareunia, and infertility (Abid et al., 2014). Endometriosis is characterized mainly by the presence of ectopic endometrial glands outside the uterine cavity. Carl von Rokitansky in 1860 was the first person to report it and named the condition ‘adenomyoma’. The pathogenesis of endometriosis is ascribed to one (or more) of three mechanisms: the retrograde regurgitation of endometrium through the oviducts during menstruation, Müllerian metaplasia of coelomic epithelium, or lymphatic and venous dissemination of endometrial tissue to distant sites with implantation. Extra-pelvic (that is, non-gynecologic) endometriosis has received special attention primarily because of the diversity of affected sites and its unusual symptomatology. Extra-pelvic endometriosis may occur in any of four anatomical regions: the lungs, bowel-omentum, urinary tract, and all other sites inclusive (Varma, Mascarenhas, & James, 2003). Explanations for the spread of endometrial tissues to distant sites rest on hypotheses of venous or lymphatic circulation, or analogies to the metastatic spread of neoplasms. Therefore, the hypothesis is that endometriosis does not result solely from retrograde bleeding, but also from endometrial cells that are shed in the pelvic cavity and which have a tendency to implant and proliferate (Kama et al., 2001). Endometriosis, an estrogen dependent disease occurs when estrogen levels increase during the

menstrual cycle the ectopic tissue grows and then regresses in the absence of estrogen similar to the activity of normal uterine endometrium (Yadav, Singla, Sidana, Suneja, & Vaid, 2017).

Endometriosis, also known as endometrioma is a benign endometriotic ovarian cysts or Deeply infiltrating endometriosis (DIE); is a disease characterized by the presence of endometrium like epithelial and stromal tissue outside (commonly the ovaries and peritoneum) their normal location on the uterus (Kanyilmaz et al., 2016). Its symptoms include pelvic pain, dysmenorrhea (painful menses) and subfertility; however, it could also be asymptomatic. The main pathological processes associated with endometriosis are peritoneal inflammation, fibrosis and the formation of adhesions and endometriomas (benign ovarian cysts containing endometrium like cells) (Kvaskoff et al., 2015; Nalaboff, Pellerito, & Ben-Levi, 2001). It is estrogen hormone dependent and is more common in women with low parity (number of pregnancies a woman has had beyond 24-week gestation (Pfeiffer et al., 2013; Setiawan et al., 2013).

Previous studies have found the pathogenesis of endometriosis to be unclear; however, the theory of retrograde menstruation (whereby endometrial tissue spills out of the fallopian tube during menstruation and implants on the ovaries and peritoneum) has been used to explain endometriosis pathogenesis (Amant et al., 2005). Endometrial lesions can also be found outside the peritoneum, leading to alternative theories such as epithelial metaplasia of tissue endometrium and lymphovascular spread (Chang et al., 2016; Clark & Stevenson, 2017). Endometriosis has thus been shown to have similar characteristics as tumorigenesis. Endometriosis may also invade tissue, traversing the basement membrane and later metastasize to other locations (Uccella et al., 2013). Endometrial tumors may also show self-sufficiency of growth and intensity to anti-proliferative signals. Endometriosis can also increase local estrogen production and its own responsiveness to

estrogen (López et al., 2014); and the disease is associated with progesterone resistance related to an overall reduction in the progesterone receptors and the lack of specific progesterone receptor isoforms – Progesterone receptor β (Carneiro, Lamaita, Ferreira, & Silva-Filho, 2016). These aberrant mechanisms set up a positive feedback cycle of growth. Tumors are often formed by the somatic acquisition of multiple genetic alterations producing a clone with selective advantage. Endometriosis is therefore expected to show some of these alterations if it is a benign neoplasm (Baral & Pudasaini, 2011). Endometriosis can also be monoclonal in nature, a key feature in cancer. Therefore, endometriosis increases the risk of various cancers such as breast cancer, ovarian cancer, non-Hodgkin lymphoma and melanoma (Kvaskoff et al., 2015). Exposure to drugs with anti-estrogenic effect following endometriosis diagnosis increases the risk of breast cancer (Cintolo-Gonzalez et al., 2017). There is therefore a complex interplay of hormonal factors in the development of endometrial and breast carcinoma.

Acute endometriosis occurs as a result of a post-partum or post-abortion infection where the usual organisms are streptococci and as an ascending gonococcal infection (Kanyilmaz et al., 2016). The acute inflammation of the endometrium could also result due to an obstruction to the outflow of the uterus at the cervical or either by neoplasm or fibrosis. This often leads to the accumulation of blood in the endometrial cavity, which may be followed by infection and accumulation of pus.

Chronic (non-specific) endometriosis often occurs among individuals harboring foreign material in their uterine cavity, specifically intra-uterine contraceptive device or retained products of conception (Svirsky et al., 2008). It is often less associated with chronic salpingitis and bacteriologic studies rarely produce a positive culture (Kvaskoff et al., 2015). Chronic endometriosis interferes with the cyclic development of the endometrium (Arnett et al., 2003).

2.2.2 Endometrial hyperplasia

Endometrial hyperplasia is a proliferation of glands of irregular size and shape with an increase in the gland stroma ratio compared with proliferative endometrium (N. Bacalbasa, Stoica, Popa, Mirea, & Balescu, 2015). Hyperplasia is classified as simple or complex based on the absence or presence of architectural abnormalities such as glandular complexity and crowding. If left untreated, approximately 8% of patient with simple atypical hyperplasia will progress to carcinoma (Abdullahi et al., 2016; Svirsky et al., 2008). Whereas the progression rate in women with complex atypical hyperplasia was almost 30% in one study, and as high as 52% in another that found lesions with varying degrees of complexity and presence of atypia, when left untreated, progressed to adenocarcinoma at different rates (Abdullahi et al., 2016; Svirsky et al., 2008). Simple hyperplasia was associated with a 1% rate of progression to cancer, complex hyperplasia 3% rate of progression, simple atypical hyperplasia 8% rate of progression, whereas complex atypical hyperplasia had a 29% rate of progression to cancer (Baral & Pudasaini, 2011; Byun et al., 2015). A recent study found that 306 patients with preoperative biopsies that diagnosed atypical endometrial hyperplasia had concurrent invasive adenocarcinoma in 42.6% of hysterectomy specimen. Studies report only 40-69% inter observer agreement for hyperplasia or cancer (Pollock, Fernandes, & Hartling, 2017).

This is a premalignant lesion caused by unopposed estrogen stimulation (Vaidya et al., 2013). It is most common around or after menopause and is often associated with excessive and irregular uterine bleeding. The risk of malignancy (advancement to endometrial carcinoma) correlates with the severity of hyperplasia classified as: Simple Hyperplasia, Complex Hyperplasia with and without atypia (Abdullahi et al., 2016). Although the glands are crowded, they are separated by densely cellular stroma and are of varying sizes. There is a low risk of endometrial carcinoma in

this group of patients (Chang et al., 2016). Complex Hyperplasia without atypia (Moderate Hyperplasia) have a greater increase in gland number with crowding (Pessoa et al., 2014). The epithelial lining is stratified and shows numerous mitotic figures. The lining cells maintain normal polarity and do not show pleomorphic or cytologic atypia. Densely cellular stroma is still present between glands. Complex Hyperplasia with atypia (Severe Hyperplasia) are characterized with gland crowding with back-to-back glands and marked cytologic atypia characterized by pleomorphism, hyperchromatic and abnormal nuclear chromatin pattern. It carries a high risk of endometrial carcinoma (Pessoa et al., 2014; Sri et al., 2015).

2.2.3 Neoplasm of the endometrium

Neoplasms of the endometrium are either benign or malignant (Abdullahi et al., 2016; Amant et al., 2005; Byun et al., 2015; Jetley et al., 2014). The frequently seen benign endometrial neoplasm in the clinical setting is the endometrial polyp (Parkar & Thagana, 2004; Whitaker & Critchley, 2016), while adenocarcinoma of the endometrium (Arnett et al., 2003; Byun et al., 2015; Chang et al., 2016) is the most common malignant endometrial neoplasm.

Endometrial polyps are common particularly around menopause (Clark & Stevenson, 2017; López et al., 2014). They vary in size from 0.5 – 3 cm and are covered by endometrial epithelium. They may be asymptomatic or cause excessive uterine bleeding. They rarely transform to carcinoma. Endometrial polyps are often problematic to be diagnosed on endometrial biopsy as depicted by a previous study in which 16% cases were detected (Chang et al., 2016; Frey, David-West, Mittal, Muggia, & Pothuri, 2016). A study conducted in Dhaka-Bangladesh revealed presence of 8.95% cases of endometrial polyp on endometrial biopsy (Abid et al., 2014). Endometrial polyps are a localized intra-uterine growth that may be single or multiple, may measure from a few millimeters to a few centimeters, and may be sessile or pedunculated. They are a common gynecologic disorder

whose incidence is unknown because many polyps are asymptomatic and rarely contain foci of neoplastic growth. In one large series of 509 consecutive women with endometrial polyps removed by operative hysteroscopy, histology was benign in 70%, and showed hyperplasia without atypia in 26%, hyperplasia with atypia in 3%, and cancer in 0.8%. The usual histological pattern of endometrial polyps is characterized by irregular proliferative glands, with a fibrotic stroma containing thick-walled blood vessels. Previous studies identified endometrial polyps in 12–34% of uteri containing endometrial carcinoma (Nijkang et al., 2018). In another case-control study examining previous pathology in women diagnosed with endometrial carcinoma, endometrial polyps were twice more likely to be detected than in the control group (Reslova, Tosner, Resl, Kugler, & Vavrova, 1999).

In a study conducted at the Peking Union Medical College in China (Jia et al., 2018), there was no pathological review of specimens done confounding the study finding. To mitigate this challenge, this study ensured that all the tissue blocks sampled were processed using automatic tissue processors, stained and reviewed by a specialist anatomical pathologist. Furthermore, in the Chinese study (Jia et al., 2018), not all the women enrolled underwent endometrial biopsies. This study overcame the challenge by ensuring that the eligibility criteria was endometrial sampling. A retrospective study conducted at the Stanford University in California – United States of America determined that selecting women based on ultrasonographic suspicion of endometrial polyps could lead to a high prevalence of the disorder which could not be reflective of the general population seen in clinical practice (Kamaya et al., 2016). The study (Kamaya et al., 2016) further stated that there are limited published studies on the true sensitivity and specificity of ultrasonography in the diagnosis of endometrial polyps alongside other endometrial disorders. Therefore, this study opted to use histopathological assay to identify endometrial polyps and other

endometrial women among women complaining of lower backpain and pelvic bleeding seen at Moi Teaching and Referral Hospital in Eldoret-Kenya.

Endometrial carcinoma is among the common malignancies of the female genital tract. It is more prevalent than ovarian cancer. Its risk factors include extended exposure to unopposed estrogen action such as null-parity, late menopause, obesity, diabetes mellitus, estrogen replacement therapy and tamoxifen treatment. Although tamoxifen treatment is an effective therapy for Estrogen Receptor positive (ER+) breast cancer; it has a partial estrogen agonist effect in the human uterus. Among post-menopausal women, estrogen agonistic activity of tamoxifen results in an increased risk of endometrial hyperplasia and endometrial cancer development. This was demonstrated by the National Surgical Adjuvant Breast and Bowel Project – P1 study where tamoxifen treatment increased endometrial cancer risk among post-menopausal women by four to five-fold (Hedden et al., 2012; Perez et al., 2014). It has also been demonstrated that the increased risk of endometrial cancer continues for years after the termination of tamoxifen therapy.

Endometrial cancer is classified into two major types based on biological and histopathological variables. Type I tumors are well differentiated and endometrial in histology and are associated with a history of unopposed estrogen exposure or other hyperestrogenic risk factors such as obesity (Brinton et al., 2013; Purdie, 2003; Uccella et al., 2013). Type II tumors are poorly differentiated non-endometrioid and are not associated with hyper-estrogenic factors (Brinton et al., 2013; Purdie, 2003). These tumors are more likely to be metastatic and can recur even after clinical intervention.

Although the pathogenesis of endometrial cancer is not well understood; accumulation of genetic abnormalities and epigenetic alterations are thought to cause the transformation of normal endometrium to cancerous tissue. Malignant Mixed Mesodermal (Mullerian) Tumor are rare

neoplasms that occur in women older than 55 years of age (Freeman, Sammel, Lin, & Gracia, 2012). These mixed mesodermal tumors present with uterine bleeding, which is usually post-menopausal. Endometrial Stromal Neoplasm although rare, they include: Benign stromal nodule (cells resemble normal endometrial cells with a low mitotic rate); Endolymphatic stromal meiosis (behaves like a low grade malignant neoplasm); stromal sarcoma (malignant proliferation of stromal cells characterized by cytologic atypia and a high mitotic rate of 10 mitoses per 10 high power fields) (Jetley et al., 2014). A previous study on chronic endometritis showed 1.2% cases of were detected on endometrial biopsy (Abid et al., 2014).

A woman's lifetime risk of developing endometrial cancer is about 2.6%, though the incidence is rising in developed countries (Morice et al., 2016). The likelihood of surviving endometrial cancer is significantly affected by age; older patients and diabetic patients have a decreased overall survival and are more likely to have a higher stage of disease at presentation. Histological evaluation of the endometrium is of paramount importance when a pre-malignant or malignant lesion is suspected. Endometrial cancer is commonly grouped into 2 different profiles with distinct risk factors. Type 1 endometrial cancer, which is more common, consists of tumors of endometrioid histology (typical endometrial adenocarcinoma) (Yang et al., 2013). Type I is believed to be hormone-related and to be significantly associated with both unopposed estrogen therapy and obesity. Type 2 endometrial cancer, which is less common, consists of less common histological subtypes such as papillary serous, clear cell, mucinous, and carcinosarcoma. This second group is usually not associated with excess estrogen exposure. Endometrial cancers are most frequently diagnosed in the peri/postmenopausal age group. However, 10% to 15% of cancers can occur in premenopausal patients, of whom up to 2% to 5% will be under the age of 40 years. Women aged 70 or more with postmenopausal bleeding have an estimated cancer risk of 50%

(Braun et al., 2016; Van Doorn et al., 2007) documented that endometrial cancer is the most common gynecologic malignancy in western women with 41,000 new cases projected in the United States for 2006 whereas rates in developing countries and Japan are four to five times lower. A previous study found that approximately, 75% of cases are diagnosed at an early stage with a tumor confined to the uterine corpus and it is the fourth most common cancer in women after carcinomas of breast, colorectum, and lung (Kanyilmaz et al., 2016).

2.2.5 Pregnancy related disorders

Gestational trophoblastic disease (GTD) includes disorders of placental development (hydatidiform mole) and neoplasms of the trophoblast (Nalaboff et al., 2001). Hydatidiform mole, either partial or complete, is the most common form of GTD, and this also is the trophoblastic lesion most commonly encountered in endometrial curetting (Vaidya et al., 2013), while choriocarcinoma is infrequent. In our local setting research has not been done on endometrial pathologies and yet it is a major cause of maternal morbidity and mortality. Thus, this study seeks to determine the most common endometrial disorders in MTRH.

2.3 Relation between age and endometrial patterns

Endometrial diseases cut across all age groups and contribute significantly to increased maternal morbidity and mortality. These diseases significantly affect quality of life, result in time off work, lead to surgical intervention including hysterectomy, and ultimately have a significant impact on the health care system.

A group of gynaecologists (Obstetricians & Gynecologists, 1998) did a study that showed that histopathological patterns of diagnosis varies with respect to the age of patients. Most young women of reproductive age present more commonly with changes associated with hormonal

imbalance. However, older women of premenopausal and postmenopausal age group present more commonly with endometrial hyperplasia and endometrial carcinoma.

In an American study, it was reported that the median age of patients at the diagnosis of endometrial carcinoma is 63 years (Yang et al., 2013). The incidence of endometrial carcinoma is highly dependent on age; there are 12 cases per 100,000 women at 40 years of age and 84 per 100,000 at 60 years. Five percent of women with endometrial cancer are less than 40 years of age. Seventy-five percent of women with endometrial carcinoma are postmenopausal. In the Czech Republic, a study found that among women undergoing endometrial biopsy or hysterectomy, the prevalence of endometrial polyps is 10-24%; the incidence rises with increasing age, peaks in the fifth decade of life and gradually declines after menopause (N. Bacalbasa et al., 2015). Endometrial polyps appear to increase by age during the reproductive years (Kamaya et al., 2016). Age and parity are known to affect the incidence of gynecological cancers. Endometrial and ovarian cancers occur later in reproductive life than carcinoma of the cervix and choriocarcinoma which are seen commonly in premenopausal or peri-menopausal women. Although studies (Setiawan et al., 2013) examining age as a risk factor for endometrial disorders have focused on women older than 35 years, this study enrolled women of all age groups to assess whether a younger age could increase the likelihood of abnormal endometrial finding.

2.4 Association between parity and endometrial patterns.

Parity is the state of having given birth to an infant alive or dead with 20 or more weeks of completed gestation (Zervoudakis et al., 2011). The gestation age is calculated from the first day of the last menstrual period (LMP). In the event a woman has a multiple birth, this is considered a single parous experience.

Cancer of the corpus uteri (endometrial carcinoma) is the sixth most commonly diagnosed cancer and the fourteenth cause of cancer related death globally (Kanyilmaz et al., 2016). High parity, late age at first or last birth amongst other factors have been associated the endometrial cancer risk reduction (L. A. Brinton et al., 2005; Eaton et al., 1994). Changes in reproductive factors have been found to increase the number of endometrial cancer cases (Jia et al., 2018). Although high parity protects from endometrial cancer, this has been found to decrease across countries due socioeconomic transitions that lead to either null parity or low parity (Wu et al., 2015). The increase in nulliparity and infertility rates in Austria, Japan, Spain and Thailand have put more women at risk to endometrial cancer (Braun et al., 2016). These reproductive patterns in low fertility countries (with the highest nulliparity rates) in Europe and East Asia (Singapore having the highest nulliparity rate) could be attributed to the rapid increase in endometrial cancer incidence rate (Disease, Injury, & Prevalence, 2017). In a retrospective study conducted at the MD Anderson Cancer Center in Texas-United States of America (Soliman et al., 2004), the study did not review all factors that are associated with endometrial carcinoma. The authors primary focused on nulliparity as a risk factor and did not look at other forms of parity. The current study collected data on all forms of parity to mitigate this previously reported research gap.

2.5 The most common indication for endometrial sampling

A normal endometrium often undergoes a variety of morphologic changes (during the reproductive years of a woman) where cyclic hormonal changes and pregnancy influence uterine growth (Maybin & Critchley, 2015). Endometrial sampling could involve hysteroscopy with curettage (the gold standard) and a "*blind*" biopsy with no visualization to the tissue sampled.

The main indications for endometrial sampling (curettage) are: to determine the causes of abnormal bleeding; to evaluate the status of the endometrium among women suffering from

infertility; to evaluate products of conception that could be a result of spontaneous abortion, termination of pregnancy or retained tissue; and to assess the response of the endometrium to hormonal therapy (Arora & Quinn, 2012; Trimble et al., 2012). Hormonal therapy is often estrogen replacement therapy in pre- and postmenopausal women or progesterone therapy among women of reproductive age with endometrial hyperplasia or endometrial carcinoma (Arora & Quinn, 2012).

Other indications for endometrial sampling are: Patients with atypical (abnormal glandular of undetermined significance - AGUS) in cervical-vaginal cytologic specimens that require endometrial sampling to exclude hyperplasia or carcinoma (Yu et al., 2015). On some occasions, endometrial sampling could be performed prior to hysterectomy to exclude significant pathology when there is a history of abnormal bleeding. On other occasions, complications of pregnancy such as a missed abortion or trophoblastic disease that is accompanied by abnormal uterine bleeding could warrant endometrial sampling.

2.5.1 Abnormal uterine bleeding

Abnormal uterine bleeding has been reported as the most common indication for endometrial sampling. According to the international federation of gynecology and obstetrics (FIGO), the classification system of abnormal uterine bleeding is based on the acronym PALM-COEIN (Polyps, Adenomyosis, Leiyoma, Malignancy and Hyperplasia- Coagulopathy, Ovulatory dysfunction, Endometrial, Iatrogenic and Not-yet specified). The structural lesions are Polyps, Adenomyosis, Leiyoma, Malignancy and Hysperplasia (table 2.1). COEIN on the other hand describes causes that are not defined by imaging or histopathology and were previously described as dysfunctional uterine bleeding (Dueholm & Hjorth, 2017; Whitaker & Critchley, 2016). This

abnormal uterine bleeding could be a sign of one or more uterine disorders that could either be structural lesions or non-structural abnormalities of the endometrium.

Causes of abnormal uterine bleeding vary significantly according to the age and menstrual status of the patient, thus age and menstrual/ menopausal status are important data (N. Bacalbasa et al., 2016). Abnormal uterine bleeding may be the common presenting complaint in patients with malignant or pre-malignant endometrial lesion (Whitaker & Critchley, 2016).

Table 2.1: Clinical terms for abnormal uterine bleeding (AUB)

Abnormal uterine bleeding (AUB) (PALM)	Abnormal uterine bleeding caused by structural lesions	
	Polyps	(AUB-P)
	Adenomyosis	(AUB-A)
	Leiomyoma	(AUB-L)
	Malignancy and hyperplasia	(AUB-M)
Abnormal uterine bleeding (AUB) (COEIN)	Abnormal uterine bleeding with no structural cause	
	Coagulopathies	(AUB-C)
	Abnormalities in ovulation	(AUB-O)
	Primary disorders of the endometrium	(AUB-E)
	Iatrogenic	(AUB-I)
	Other causes not yet specified	(AUB-N)
Acute AUB	Non-gravid, reproductive-aged women with bleeding of sufficient quantity to require immediate intervention to prevent further blood loss	
Chronic AUB	Bleeding that is abnormal in duration, volume, and/or frequency and has been present for the majority of the last 6 months.	

Adapted from (Vaidya et al., 2013).

Abnormal uterine bleeding is one of the most frequent problems in life of an adult female and the common causes of abnormal uterine bleeding include chronic endometritis, endometrial polyp, endometrial hyperplasia or carcinoma (Nijkang et al., 2018). Most patients present with abnormal uterine bleeding, while a few patients are asymptomatic (N. Bacalbasa et al., 2016). Due to lack of health education most patients are not able to seek medical attention on time and thus seek

medical attention when it is late (Whitaker & Critchley, 2016). In a clinical expert series (Goldstein, 2010), the author suggested that although sampling the endometrium blindly is becoming a common phenomenon, there is little clinical validation conducted for this approach. Therefore, this study ensured that participants who had undergone endometrial sampling were identified based on their symptomatology.

The most common warning sign for uterine endometrial cancer is abnormal vaginal bleeding. Recognition of this symptom often affords an opportunity for early diagnosis and treatment. In older women, any bleeding after menopause may be a symptom of endometrial cancer. Younger women are also at risk and should note irregular or heavy vaginal bleeding as this can be symptoms of endometrial cancer (N. Bacalbasa et al., 2016; Jia et al., 2018; Pereira, Cabar, Raiza, Roncaglia, & Zugaib, 2005). Most cases develop in women aged in their 50s and 60s. It rarely develops in women under the age of 50 years. It is more common in women who are older, obese and of low parity. Hypertension and diabetes mellitus are also predisposing factors (Kaaks, Lukanova, & Kurzer, 2002). Symptoms of some of the disorders present late and people are not sensitized early enough to know the presenting symptoms thus seek medical attention in time. Information concerning these common endometrial disorders can be disseminated through health education because there is lack of knowledge concerning the same (Abdullahi et al., 2016). This study sought to evaluate the most common indication for endometrial sampling in patients attending MTRH.

CHAPTER THREE: MATERIALS AND METHODS

3.1 Study area

The retrospective study was conducted at the Department of Histopathology of Moi Teaching and Referral Hospital (MTRH), Eldoret, Uasin Gishu County (figure 3.1). The hospital is situated on the eastern part of the town along Nandi road. It serves as a teaching hospital for Moi University College of health sciences, University of Eastern Africa, Baraton, Kenya Medical Training College, MTRH Training Center and many other tertiary institutions undertaking relevant health sciences courses across the country. MTRH is the second largest Referral Hospital in Kenya and serves the entire western region of the nation. Majority of patients in need for endometrial sampling in Western Kenya seek care in this facility. The Hospital serves all people of different economic status, but more so those of low economic standards due to the subsidized charges. A recent study at MTRH investigating the types of cancer and their associated infectious agents at observed that majority of the cancer patients presenting at MTRH were from the Rift valley (66.8%), Western (24.8%) and Nyanza (6.4%) Provinces. Specifically, patients were mainly from Uasin Gishu (22.8%), Nandi (8.4%), Lugari (8.4%), Trans Nzoia (7.4%) and Bungoma (6%). Overall, 41% of all cancer cases were referrals from other neighboring hospitals (Macharia et al., 2018). MTRH is 0°30'51.2"N 35°16'51.2"E (Appendix I).

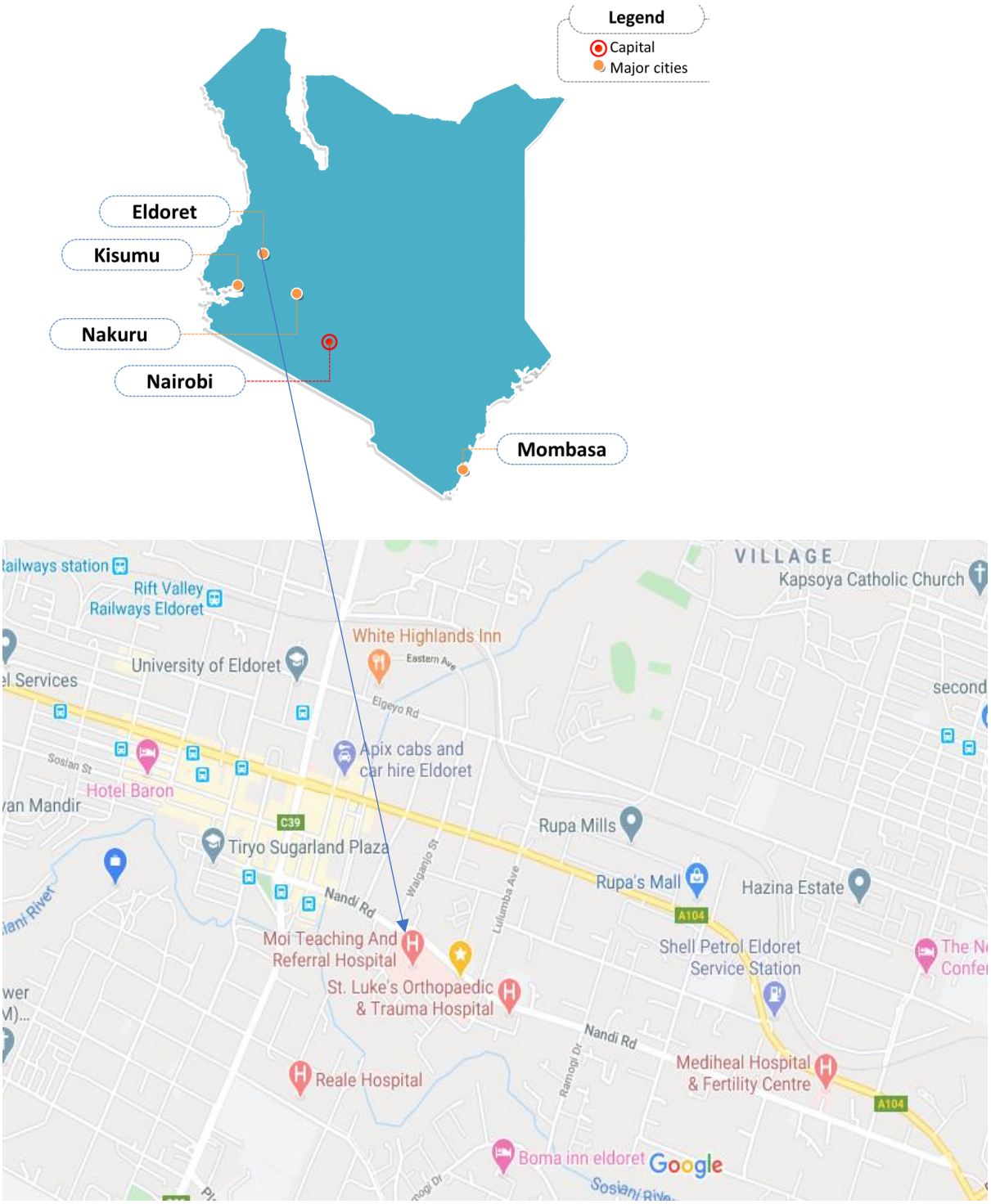

Figure 3.1: Map of the study area

3.2 Study design

The study adopted a retrospective laboratory-based design.

3.3 Study population

Endometrial specimen (endometrial biopsies and curettages) that had been collected from women age between 19 to 70 years who presented with pelvic bleeding and back-pain complaints were analyzed. The respective blocks (n=390) prepared between August, 2014 to August, 2016 were retrieved, re-sectioned, stained and then examined microscopically.

3.3.1 Eligibility criteria

Inclusion

i. All endometrial curettage and biopsies received at MTRH histopathology laboratory between August 2014 to August, 2016.This was informed by IREC approval.

Exclusion

i. Biopsies missing the required information such as age, indication for endometrial sampling and parity.

3.3.2 Sample size determination

The sample size (n) is calculated according to the Fisher's formula: $n = [z^2 * p * (1 - p) / e^2] / [1 + (z^2 * p * (1 - p) / e^2 * N)]$ (Whitehead, 1993). Where: $z = 1.96$ for a confidence level (α) of 95%, p = proportion (expressed as a decimal), N = population size, e = margin of error.

$z = 1.96$, $p = 0.13$, $N = 390$, $e = 0.05$

$n = [1.96^2 * 0.13 * (1 - 0.13) / 0.05^2] / [1 + (1.96^2 * 0.13 * (1 - 0.13) / 0.05^2 * 390)]$

$n = 173.794 / 1.4456 = 120.221$

The sample size (with finite population correction) is equal to 121.

3.4 Sampling technique

Simple random sampling was applied in the selection of the tissue samples using statistical package for social sciences (SPSS). As such, 121 tissue samples were selected from a pool target of 390 tissue biopsies.

3.5 Data collection techniques

3.5.1 Histological methods

Specimen harvesting and fixing

The specimens were harvested and immediately fixed in 10% buffered formalin saline containing, as final concentrations, 40% farmaldehyde, 0.4gms sodium dihydrogen phosphate, 0.65gms disodium dihydrogen phosphate and appropriate volume of water (total 100mLs).

Specimen trimming

Following fixation, the tissues were trimmed to a thickness of 3mm to enhance proper processing, and thereafter placed in plastic tissues-teks.

Automated specimen processing

The tissues were then mounted on an automatic tissue processor that performed the following functions: (1). **Dehydration** that removes water completely from the tissue. This is an important step because the paraffin wax that is used (embedding media) is water immiscible. The process of dehydration was facilitated by passing the tissue through ascending grades of alcohol usually ranging from 70% to 100% Ethyl alcohol until all the water had been completely removed from the specimen. The tissue was allowed to remain in each strength of alcohol for as long as it is necessary (2 hrs) for complete saturation, (2). **Clearing** (or de-alcoholization) which involved removal of alcohol from tissues by immersing them in xylene because it is miscible in both dehydrating and embedding media, (3). **Tissue impregnation** involved saturation the specimen

with embedding medium to remove the clearing agent from the tissues by diffusion into the surrounding melted wax and the wax in turn diffused into the tissues to replace it. One change of wax was adequate to remove the clearing agent that had been displaced from the tissue and ensured its replacement with pure wax.

Blocking/embedding/casting out

Embedding involved the process of placing the impregnated tissue in a precisely arranged position into a mould containing the embedding medium (paraffin wax) and causing this medium to solidify. To achieve this, the metal mould was smeared lightly with glycerin, and thereafter filled with molten paraffin wax. Using a warm pair of blunt nosed forceps, the tissue was aseptically transferred from the paraffin wax to the mould. For this purpose, electrically heated forceps were used. The forceps was warmed again and used to orientate the tissue until it is lying in the desired plane. The surface to be sectioned was placed face down against the base of the mould. The warm forceps was run round the tissue to ensure that any wax which may have solidified during the transferring from the paraffin bath to the mould was melted. The mould was then transferred to a refrigerated surface, and later transferred into a freezer for further cooling.

Tissue sectioning and drying

The tissue block was sectioned at 5 microns using a rotary microtome to produce tissue ribbons. The ribbons were thereafter picked from the microtome using a camel hairbrush and transferred to a floating out water bath to straighten them out and remove folds. Once straight, the ribbons were removed from the water bath using a clean labeled slide and planed in a hot air oven preheated to 58^0C for drying.

Staining

The slides were then removed from the oven and stained using Haematoxylin and Eosin staining method. Briefly, the tissue sections were deparaffinized in three changes of xylene and then hydrated by passing through decreasing concentration of alcohol (100%, 90%, 80%, 70%) baths and water. This was followed by staining in hematoxylin dye for 20 minutes and washed in running tap water for 2 minutes. The slides were then differentiated in in 1% acid alcohol (1% HCl in 70% alcohol) for 1 minute, washed running tap water for 1 minute then dipped in ammoniated water (an alkaline solution) until the sections turned blue and then washed by tap water. The sections were then stained in 1% Eosin Y for 3 minutes and washed in tap water for 2 minutes. They were then dehydrated in increasing concentration of ethyl alcohol. The slides were cleared in 3 changes of xylene, mounted using DPX (mounting media) and examined under a light microscope with the guidance of a pathologist.

Principle of the hematoxylin and eosin staining method

Alum acts as mordant and hematoxylin containing alum stains the nucleus light blue. This turns red in presence of acid, as differentiation is achieved by treating the tissue with acid solution. Staining the sections blue converts the initial soluble red color within the nucleus to an insoluble blue color. The counterstaining is done by using eosin which imparts pink color to the cytoplasm. As such, the results were read as follows: nuclei – Blue; Cytoplasm – shades of pink.

3.5.2 Collection of patients characteristics data

Secondary data on patients sociodemographic characteristics (age and parity) were collected from the existing medical records between 2014 and 2016. Approval to obtain this information was sought from the hospital administration.

3.6 Data analysis

The data was entered on a data collection form designed to collect the variables of interest, namely age, parity, pathology and indication for endometrial sampling. The data was entered into Microsoft excel, cleaned and coded. The data was the exported to the SPSS Version 23 for analysis. Frequencies, means and standard deviations were determined for variables being investigated. Fischer's exact test of association was used to determine variations in proportions between and across age groups, indication for endometrial sampling and endometrial disorders.

3.7 Ethical considerations

Ethical approval to conduct the study was obtained from MTRH / Moi University Institutional Research Ethics Committee (IREC). Since this was a retrospective study that utilized archived tissue specimen, an informed consent waiver was sought from IREC and granted (Appendix III). Confidentiality was ensured by not including any potential patient identifiers on the data collection tool, which was kept under key and lock. Electronic data was encrypted on password protected computers.

CHAPTER FOUR: RESULTS

4.1 General characteristics of study participants

This study sampled 121 endometrial tissue blocks banked at Moi Teaching and Referral Hospital's histopathology laboratory. The general characteristics of the study participants are shown in Table 4.1. The women from whom the tissue blocks were sampled were aged between 19 and 70 years (with a median age of 44 years); majority (52.5%; n=63) had conceived between 1 and 4 children followed by those with 5 to 9 children (38.3%; n=46). Those with a history of miscarriage were 24.2% (n=29).

Table 4.1: General characteristics of study participants

Participant Characteristics	n (%)
Age (Years)	
≤20 years	1 (0.8%)
21-40 years	48 (39.7%)
41-60 years	62 (51.2%)
>61 years	10 (8.3%)
Parity Category	
History of Miscarriage	29 (24%)
Never had a child	3 (2.5%)
All live births	89 (73.5%)
Parity (number of children)	
No child	3 (2.5%)
1-4 children	64 (52.9%)
5-9 children	45 (37.2%)
>= 10 children	9 (7.4%)

Data was presented as absolute number (n) and percentages (%). Frequency distribution of participant characteristics of age (19-70 years; median 44 years), parity category and number of children (parity) were analyzed.

4.2 Identification of endometrial patterns presenting at MTRH

Endometrial disorder types were determined on endometrial curettage and biopsies sectioned and stained using the routine Hematoxylin and Eosin (H&E) staining methods and examined on a light

microscope. Micrographs of stained section blocks identified normal endometrium, with visible normal glands and stoma when examined at both ×100 and ×400 magnification under a light microscope (figure 4.1).

A. B.

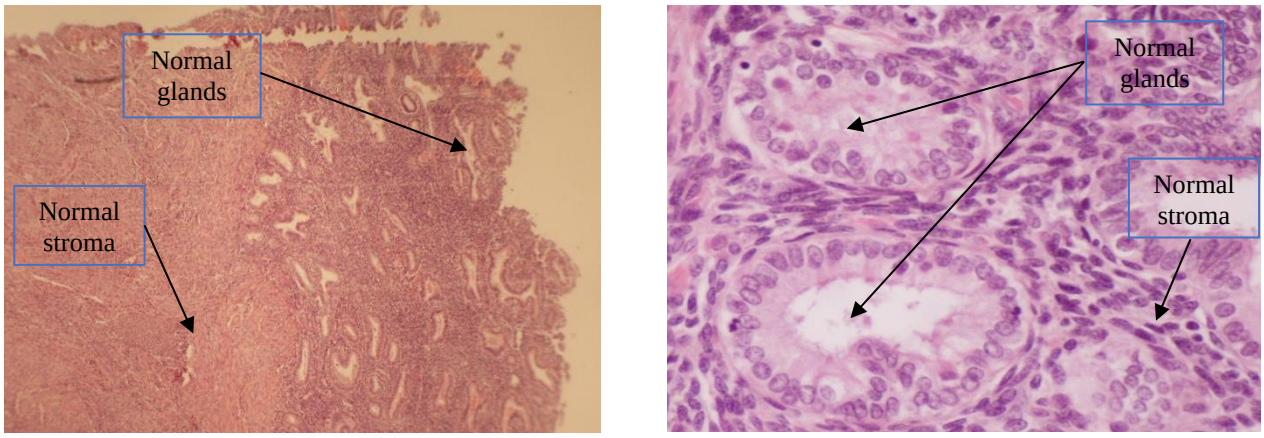

Figure 4.2: Normal endometrium
Endometrium sections stained with routine Hematoxylin and Eosin (H&E) and examined with a light microscope. Micrographs of normal glands and stroma identified under; A. ×100 magnification and B. ×400 magnification.

Additional analysis on stained blocks identified neoplastic disorders, with endometrial polyps with benign endometrial glands and stroma visible on sectioned tissues. Similarly, endometrial carcinoma (with malignant cells) were positively identified on infected tissue sections (figure 4.2).

A. B.

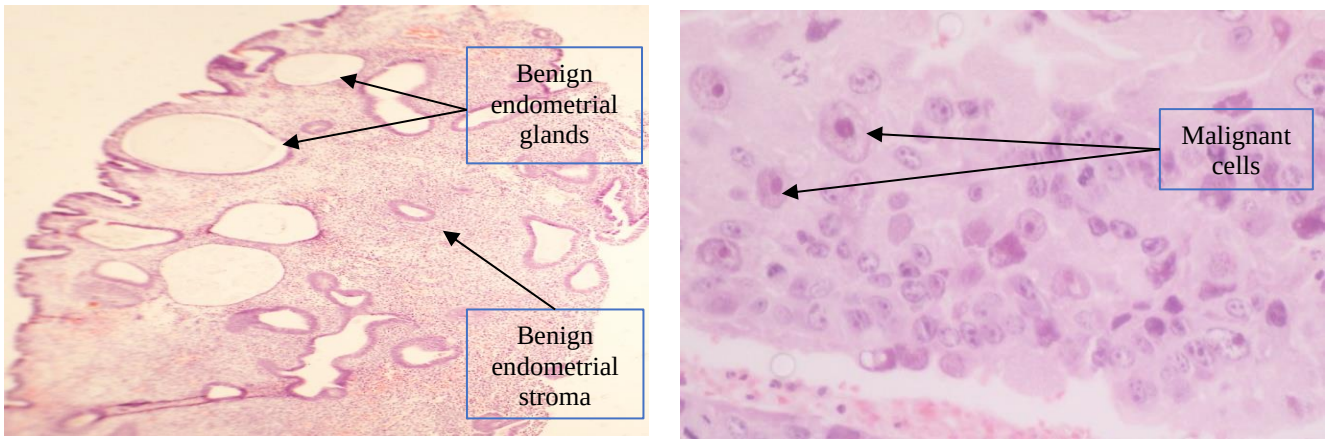

Figure 4.3: Neoplastic disorders

Endometrial sections were stained with routine Hematoxylin and Eosin (H&E) stain then examined under a light microscope. Micrographs of; A). Endometrial polyp ×100 and B). Endometrial carcinoma×100.

Further examination of the stained block identified simple and complex hyperplasia, with former showing cystically dilated glands (figure 4.3).

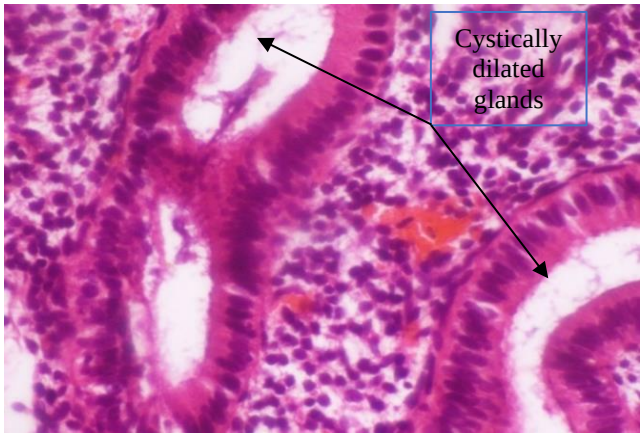

Figure 4.4: Hyperplasia

Endometrium sections were stained with routine Hematoxylin and Eosin (H&E) stain then examined under a light microscope. Micrograph of simple endometrial hyperplasia with cystically dilated glands×100.

Pregnancy related disorders were positively identified on stained sectioned tissues samples examined under a light microscope (figure 4.4). Indeed, products of conception were identified, with visible degenerating chorionic villi signs of haemorrhage (figure 4.4). Furthermore, large avascular edematous chorionic villi and extensive haemorrhage were observed for Hydatiform mole (figure 4.4)

A. B.

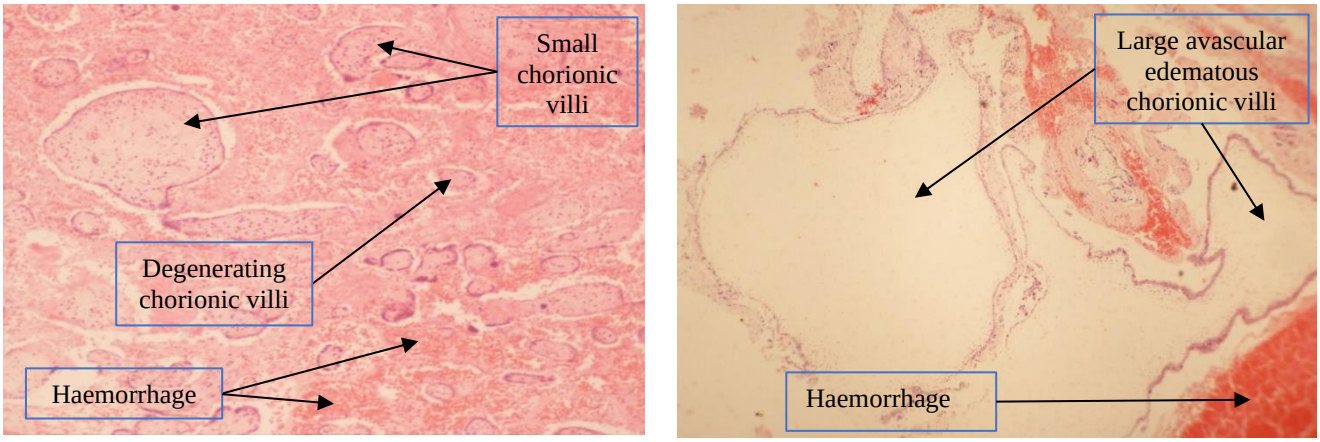

Figure 4.5: Pregnancy related disorders
Endometrium sections were stained with routine Hematoxylin and Eosin (H&E) stain then examined under a light microscope. Micrographs of; A. Products of conception×100 B. Hydatiform Mole×100

Other endometrial disorders that included atrophic endometritis presenting with chronic inflammatory cells was identified on tissue samples sectioned, stained and observed under a light microscope (figure 4.5).

A.

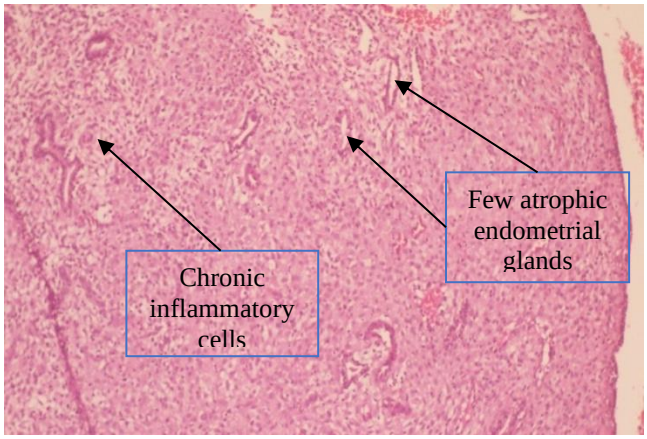

Figure 4.6: Endometritis

Endometrial sections were stained with routine Hematoxylin and Eosin (H&E) stain then examined under a light microscope. Micrograph of an Atrophic endometritis with chronic inflammatory cells. ×100

4.3 Endometrial patterns presenting at MTRH

One hundred and twenty-one (121) participants were included in this study. The endometrial disorders were categorized as Hyperplasias (50.4%; n=61), Inflammatory disorders (7.4%; n=9), Neoplastic Disorders (14.9%; n=18), Pregnancy related disorders (20.7%; n=25) and Other Disorders (6.6%; n=8) (Figure 4.6).

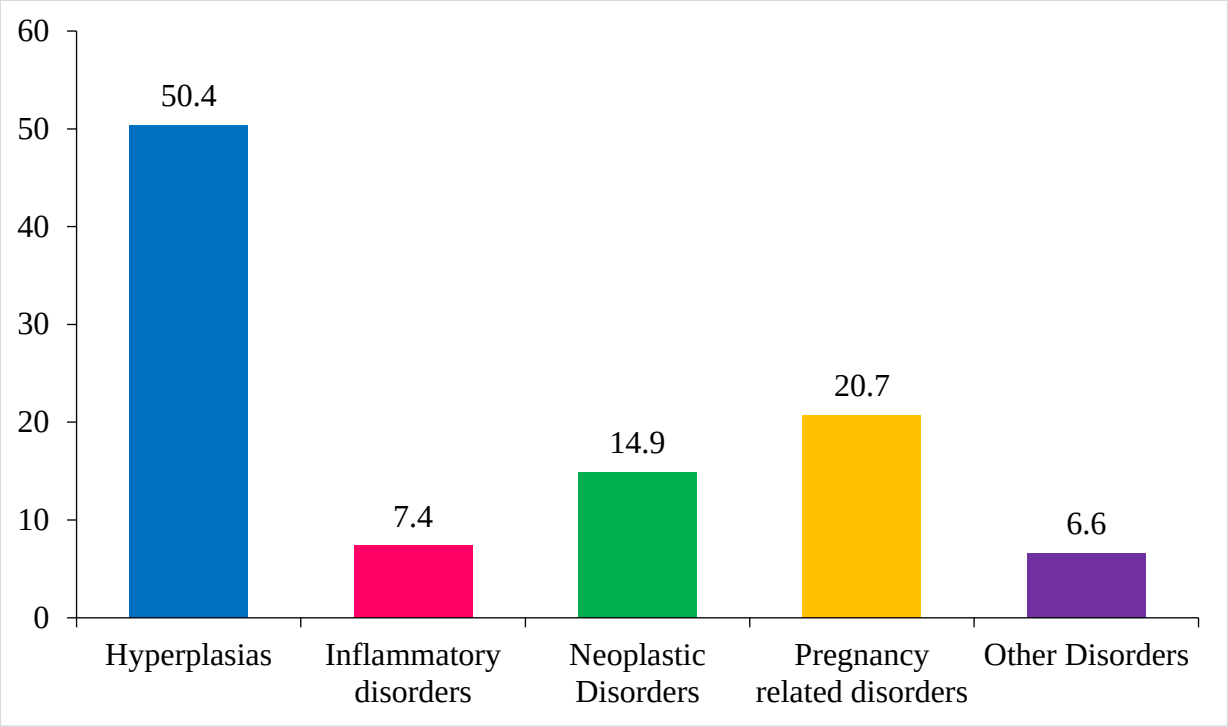

Figure 4.7: Categories of endometrial patterns
Frequency distribution of endometrial disorders identified among the 121 sampled tissues.

The categories were assigned to specific endometrial patterns. Hyperplasias were either simple (n=48) or complex hyperplasias (n=13). Inflammatory disorder was mainly made up of endometritis (n=9). Neoplastic disorders were either adenocarcinomas (n=14) or polyps (n=4). Pregnancy related disorders were made up of Hydatid-form Mole (n=8); Products of Conception (n=15) and Decidualization (n=2). Other endometrial (physiological) patterns were atrophic (n=3) and proliferative endometrium (n=5) as shown in figure 4.7.

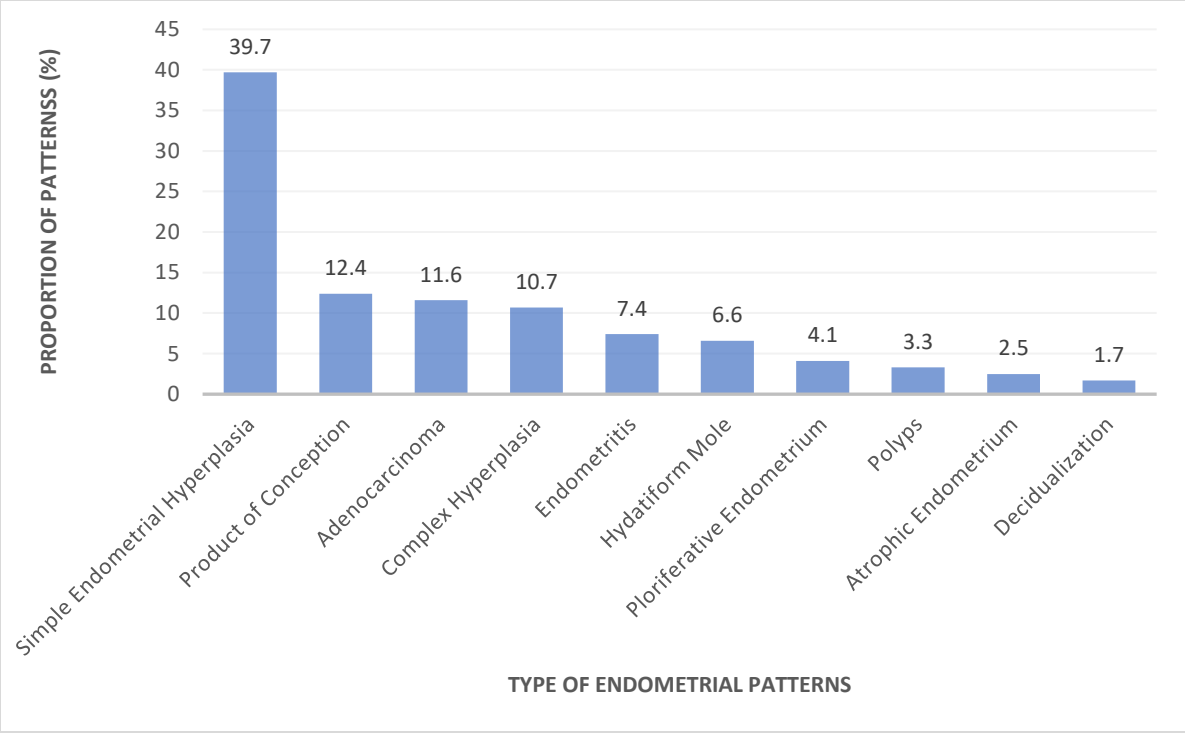

Figure 4.8: Types of endometrial disorders.
The distribution of specific endometrial disorders under the Hyperplasias, Inflammatory disorders, Neoplastic Disorders, Pregnancy related disorders and Other Disorders.

4.4 Relationship between age and endometrial patterns among women presenting with back pain and endometrial bleeding

The age groups were categorized as those less or equal 20 years, those aged between 21-40 years, 41-60 years and greater or equal 61 years (table 4.2). The most frequent age group were those aged 41 – 60 years at 51.2% (n=62), followed by those aged between 21-40 years (39.7%; n=48). The most frequent endometrial disorders by age group were: Hyperplasias at 50.4% (n=61) mostly affecting those aged 41-60 years at 60.7% (n=37) of those with hyperplasias. The second most frequent endometrial were pregnancy related at 20.7% (n=25), of which 84% (n=21) were aged 21-40 years. Neoplastic disorders were third most frequent disorders at 14.9% (n=18) mainly affecting those aged 41-60 year at 61.1% (n=11) and older than 60 years (16.7%; n=3). Other disorders (atrophic and proliferative) were the least prevalent at 6.6% (n=8) of which the majority

were those aged 41-60 years (75%; n=6), followed by those aged 21-40 years (25%; n=2) as shown on table 4.2. When a fishers exact test of association was conducted to compare age groups and endometrial disorders, a significant p-value of 0.012 was obtained.

Table 4.2: Distribution of endometrial pattern categories in age group categories

Endometrial disorders	**Age in years**				**Total, n (%)**
	≤20	**21-40**	**41-60**	**≥61**	
Hyperplasias, n (%)	0(0.0)	17(27.9)	37(60.7)	7(11.5)	61 (50.4%)
Inflammatory, n (%)	1(11.1)	4(44.4)	4(44.4)	0(0.0)	9 (7.4%)
Neoplastic disorders, n (%)	0(0.0)	4(22.2)	11(61.1)	3(16.7)	18 (14.9%)
Pregnancy related, n (%)	0(0.0)	21(84.0)	4(16.0)	0(0.0)	25 (20.7%)
Others, n (%)	0(0.0)	2(25.0)	6(75.0)	0(0.0)	8 (6.6%)
Total, n (%)	1 (0.8)	48 (39.7)	62 (51.2)	10 (8.3)	121 (100%)

Data are presented as absolute counts (n) and percentages (%). The distribution of various categories of endometrial disorders across participant age groups. Fishers exact test P=0.012.

4.5 Parity mostly affected by endometrial disorders at MRTH

Parity was categorized as those women with no child (2.5%; n=3), those with 1-4 children (52.9 %; n=64), 5-9 children (37.2%; n=45) and those with 10 or more children at 7.4% (figure 4.8).

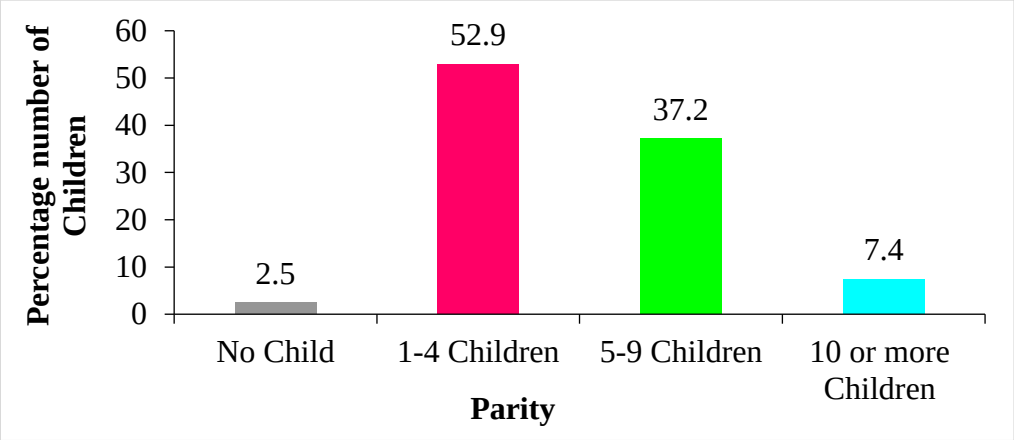

Figure 4.9: Categorization of parity of women of whose samples were analyzed.
Frequency distribution of the women parity.

As shown previously, the most frequent endometrial pattern by age group were hyperplasias at 50.4% (n=61) of the entire population. Most women (49.2%; n=30) with hyperplasias had 1-4 children followed by those with 5-9 children (44.3%, n=27). The second most frequent endometrial pattern were pregnancy related (20.7%; n=25), mainly affecting those with 1-4 children (64%; n=16) and 5-9 children (24%; n=6). Neoplastic disorders were third most frequent disorders at 14.9% (n=18), mainly affecting those with 1-4 children (50%, n=9), with near equal prevalence among those with 5-9 children (27.8%; n=5) and those with 10 or more children (22.2%; n=4). Other disorders (atrophic and proliferative) were the least prevalent at 6.6% (n=8) and only affected those with 1-4 children (62.5%; n=5) and 5-9 children (37.5%; n=3) (table 4.3). When a test of association was conducted, there was no significant association between parity (number of children carried to term) and occurrence of endometrial disorders (*Fishers exact test P=0.0847*).

Table 4.3: Histology disorder category versus parity

Endometrial patterns	**Parity**				**Total**
	0	**1-4**	**5-9**	**>=10**	
Hyperplasias, n (%)	2 (3.3)	30(49.2)	27 (44.3%)	2 (3.3%)	61 (50.4%)
Inflammatory, n (%)	1 (11.1)	4 (44.4)	4 (44.4%)	0 (0%)	9 (7.4%)
Neoplastic disorders, n (%)	0 (0)	9 (50.0)	5 (27.8%)	4 (22.2%)	18 (14.9%)
Others, n (%)	0 (0)	5 (62.5)	3 (37.5%)	0 (0%)	25 (20.7%)
Pregnancy related, n (%)	0 (0)	16 (64.0)	6 (24%)	3 (12 %)	8 (6.6%)
Total	3 (2.5)	64 (52.9)	45 (37.2%)	9 (7.4 %)	121 (100%)

Data are presented as absolute counts (n) and percentages (%). The distribution of various categories of endometrial disorders across participant parity. Fishers exact test P=0.0847

4.6 Common indication for endometrial sampling

Pelvic bleeding 88.3% (n=106) and back pains 11.7% (n=14) were the main causes that made women present for endometrial sampling. When these indications were matched with endometrial disorders; majority (84.2% n=32) of the women presenting with simple endometrial hyperplasia complained of pelvic bleeding, while 15.8% (n=6) of these women had back pain. All women with products of conception (n=10), Decidualization (n=1), autolytic (n=2), massive Decidualization (n=1), non-specific endometritis (n=1), complex endometrial hyperplasia (n=13), acute endometritis (n=1) and atrophic endometrium (n=1) complained of pelvic bleeding (Table 4.4). When a test of significance was conducted, there was no significance (*P*=0.309) seen on the effect of indication on endometrial disorders.

Table 4.4: Endometrial disorders versus Indication

Histology categories		**Indication**		**Total**
		Pelvic Bleeding	**Back pain**	
Neoplastic Disorders	Polyps	3 (2.9%)	1 (7.1%)	4 (3.3%)
	Adenocarcinoma of the Endometrium	13 (12.2%)	1 (7.1%)	14 (11.5%)
Hyperplasias	Simple Endometrial Hyperplasia	41 (38.4%)	7 (50%)	48 (39.7%)
	Complex Hyperplasia	13 (12.2%)	0	13 (10.7%)
Inflammatory Disorders	Chronic Endometritis	6 (5.7%)	0	6 (5%)
	Non-specific Endometritis	1 (0.9%)	0	1 (0.8 %)
	Acute Endometritis	2 (1.8%)	0	2 (1.7 %)
Pregnancy related disorders	Decidualization	2 (1.8%)	0	2 (1.7 %)
	Product of Conception	15 (14%)	0	15 (12.4 %)
	Hydatiform Mole	5 (4.6%)	3 (21.4%)	8 (6.6 %)
Others (Normal, Proliferative and Atrophic Endometrium)	Normal Endometrium	4 (3.7%)	1 (7.1%)	5 (4.1 %)
	Proliferative Endometrium	1 (0.9%)	1 (7.1)	2 (1.7 %)
	Atrophic Endometrium	1 (0.9%)	0	1 (0.8 %)
Total		107 (88.3%)	14 (11.7%)	121 (100%)

Data are presented as absolute counts (n) and percentages (%). The frequency distribution of indicators of endometrial disorders. Fishers exact test P=0.933.

CHAPTER FIVE: DISCUSSION

5.1 Endometrial biopsies patterns among women presenting with back pain and endometrial bleeding

The study set out to investigate histopathological endometrial patterns on archived specimen isolated from women presenting at Moi Teaching and Referral Hospital in Uasin Gishu County, Kenya. Various patterns were identified on stained tissue sections examined under a light microscope. The study found out that majority of the study participants (31.7%; n=38) presented with simple endometrial hyperplasia. This finding differ from a Nigerian study that investigated the morphological patterns of endometrial biopsies in South-West Nigeria and found that the frequency of endometrial hyperplasia was 6.7% of all the endometrial biopsies reviewed in the study (Abdullahi et al., 2016). The second and third most common endometrial patterns were complex hyperplasia at 10.7% (n=13) and adenocarcinoma at 11.6% (n=14). Complex hyperplasia is a precursor of adenocarcinoma of the endometrium (L. A. Brinton et al., 2005). Several studies have shown atypical endometriosis precede endometrioid ovarian cancers, suggesting that these forms of endometriosis may act as pre-cancerous lesions, as has been shown in the relationship between atypical endometrial hyperplasia and endometrial cancer (Clement et al., 2017; Kurman, Kaminski, & Norris, 1985; Lacey Jr et al., 2010).

The less frequently occurring endometrial patterns were: Hydatidiform mole chronic endometritis, polyps, proliferative endometrium, decidualization and atrophic endometrium. The low frequency of these endometrial disorders could be attributed to the fact that, despite the sensitivity of endometrial biopsy to allow accurate diagnosis of endometrial hyperplasia and or carcinomas; endometrial biopsy may fail to detect other uterine pathologies such as submucous leiomyomas. The study also found that a large proportion of the histopathologic samples were products of

conception. Endometritis or endometrial polyp are benign causes for abnormal uterine bleeding among endometrial disorders.

The majority of the of endometrial hyperplasia in the present study were classified as simple endometrial hyperplasia, accounting for 78.7% of the hyperplasias. These results are in tandem with reports from different parts of Kenya and Africa that utilized similar study designs and target populations (Dawodu, Ikeri, & Banjo, 2017). In previous studies, endometrial hyperplasia in patients investigated for infertility accounted for 3% of cases, with all cases reported being simple endometrial hyperplasia (Louise A Brinton et al., 2005; Shen et al., 2011).

5.2 Relationship between age and endometrial patterns among women presenting with back pain and endometrial bleeding

Results from this study show that the age group were 41 - 60 years had various different patterns followed by those aged 21-40 years. This is consistent with the findings of an Indian study which found that the most affected age group was perimenopausal women between 41-50 years (Shukla, Fonseca, Kharat, & Tekale, 2017). Results from this study showed that hyperplasias presenting as simple endometrial hyperplasia were common among those aged 41-60 years. This finding is consistent with results from a recent study that showed simple endometrial hyperplasia without atypia was most common among perimenopausal women aged 41-50 years (Shukla et al., 2017). Neoplastic disorders such as adenocarcinoma of the endometrium was the second most frequent among those aged 41-60 years, followed by those aged 60-69 years. This finding is consistent with a retrospective study by (Reed et al., 2009) conducted between the year 1985 and 2003. The study determined that the peak incidence of simple endometrial hyperplasia (142 per 100,000) and

complex endometrial hyperplasia (213 per 100,000) were among women in their early fifties (Reed et al., 2009). Results from another previous study revealed that endometrial cancer risk was directly associated with age (OR = 1.8, 95% CI = 1.1–2.7, for ≥55 vs. <50 years) (Zucchetto et al., 2009). This is similar to the current study's findings that indicated that complex hyperplasia of the endometrium as the second most common hyperplasia among those aged 41-60 years (4.2%; n=5). Previous African studies have found peak age incidence of endometrial carcinoma in the fifth decade of life (Abdullahi et al., 2016). Abdullahi et al found that eighty seven percent (87%) of cases were seen within the age bracket 50-70 years; this is in conformity to the fact that endometrial carcinoma predominantly develops after menopause (Abdullahi et al., 2016). These studies clearly indicate that endometrial carcinomas are associated with advancement of age, with being older than 50 years increasing the risks, suggesting a relationship between age group and the occurrence of endometrial disorders. The finding from the study presented here are consistent with those or another recent investigation that described that more than 90% of case-notifications of endometrial cancer occurred in women aged more than 50 years of age, with a median age at diagnosis of 63 years (Colombo et al., 2016). A retrospective study in New York found a significant ($p<0.001$) number of endometrial cancer cases occurring in women aged 50 years and above, with a median age of 56.3 years (Nevadunsky et al., 2014). Therefore, being 50 years or more is a risk factor for developing endometrial cancer.

5.3 Association between parity and endometrial disorders

In this study, nulliparous women (with no child) only presented with complex endometrial hyperplasia. This condition often has gland crowding with back-to-back glands and marked cytologic atypia characterized by pleomorphism, hyperchromatin and abnormal nuclear chromatin

pattern. Complex endometrial hyperplasia has been found to carry a high risk of endometrial carcinoma. Previous studies have determined that nulliparity, unopposed exposure of the endometrium to estrogen, including unopposed estrogen therapy, early menarche, late menopause and tamoxifen therapy increase the risk of endometrial cancer (Adami et al., 1994; Pfeiffer et al., 2013; Wu et al., 2015).

The most common endometrial pattern among women with a parity of 1-4 was simple endometrial hyperplasia, followed by adenocarcinoma of the endometrium, hydatid mole and proliferative endometrium. Simple endometrial hyperplasia was characterized by increased number of proliferative glands without cytologic atypia. Although the glands are crowded, they were separated by densely cellular stroma and were of varying sizes. There is a low risk of endometrial carcinoma in this group of patients (Clement et al., 2017; Dawodu et al., 2017; Lacey Jr et al., 2010). However, analyses of parity-specific risks of endometrial cancer showed highest risks for nulliparous women and women who had given birth to one child (Wu et al., 2015). Furthermore, studies have shown that the risk decreased if the woman gave birth to more than one child, although the trend was not statistically significant (Adami et al., 1994; Pfeiffer et al., 2013; Wu et al., 2015). This could explain the proportion of women with adenocarcinoma of the endometrium being higher among women with only one child and decreasing with the advancement in parity. A study done by Kurman and Silverberg indicated that gestational trophoblastic disease (GTD) includes disorders of placental development (hydatidiform mole) and neoplasms of the trophoblast (Abdullahi et al., 2016; Kurman et al., 1985). Results from the current study presented here show that the second most frequent endometrial patterns were pregnancy related, mainly affecting those with a parity of 1-4 followed by those with 5-9. The retained products of conception could be as a result of previous miscarriage (Nalaboff et al., 2001).

Further, results presented here show that among women with a parity of 5-9, simple endometrial hyperplasia was the most frequent, followed by complex endometrial hyperplasia, adenocarcinoma and endometritis. For women with a parity of ten or more, majority presented with products of conception, followed by simple endometrial hyperplasia and decidualization in equal frequencies. Endometritis is a histopathological diagnosis characterized by endometrial inflammation rich in lymphocytic cell infiltrates. While the diagnostic criteria for endometritis remains controversial, it is generally agreed upon that the presence of plasma cells within the substance of the stroma is the most useful criteria for diagnosis (Mautner et al., 2015). Hydatidiform mole, either partial or complete, is the most common form of gestational trophoblastic disease (GTD), and this also is the trophoblastic lesion most encountered in endometrial sampling (Nalaboff et al., 2001), while choriocarcinoma, is infrequent. In Western Kenya, limited research has been conducted on endometrial pathologies and yet it is a major cause of maternal morbidity and mortality.

In the current study, there was no significant association between parity (number of children carried to term) and occurrence of endometrial disorders. This finding is like those of other studies that did not find any association between parity and endometrial disorders (Dawodu et al., 2017; Maybin & Critchley, 2015; Whitaker & Critchley, 2016). However, previous studies reported that nulliparity is a risk factor of endometrial disorders, while multi-parity is protective of endometrial disorders (Abdullahi et al., 2016). This variation could be attributed to differences in study design adopted by this study compared to the previous studies. These previous associations were reported based on retrospective reviews of National Swedish Cancer Register, Pathology database of University of California Los Angeles and America's National Institute of Health databases respectively (Brunette et al., 2012; Melin, Sparen, & Bergqvist, 2007; Pfeiffer et al., 2013). On the

other hand, (Setiawan et al., 2013) and (Wu et al., 2015) adopted a mixed method approach (10 cohort and 14 case-control) and systematic reviews.

5.4 Common indication for endometrial sampling

In many histopathology laboratories, endometrial specimens account for a major proportion of the workload. Most specimens are taken because of abnormal uterine bleeding or other related symptoms, and the pathologist is expected to exclude an endometrial cancer or a precancerous lesion. This study determined that pelvic bleeding and back pains were major indications for endometrial sampling. This is consistent with a United Kingdom NICE study which recommend endometrial sampling in women with persistent inter-menstrual bleeding or for those aged greater than 45 years following treatment failure (Maybin & Critchley, 2015). The findings from the NICE study supports the current study's results that pelvic bleeding is the most common symptom that leads to endometrial sampling. When these indications were matched with endometrial disorders; majority of the women presenting with simple endometrial hyperplasia complained of pelvic bleeding, with more than of these women presenting with back pain. In a study that correlated histopathological findings in patients with uterine bleeding; (Shukla et al., 2017) sampled 120 women all with uterine bleeding aged between 22-79 years and found that abnormal uterine bleeding was most common among perimenopausal women aged 41-50 years (Shukla et al., 2017). More than half of all study participants in the Shukla et al. study had heavy menstrual bleeding as a presenting symptom (Shukla et al., 2017). Shukla found proliferative endometrium as the most common histopathological finding in 27% patients, followed by endometrial hyperplasia in 13.5% patients, secretory endometrium (12.7%) and disordered proliferative endometrium in 10.9% patients. Malignancy was detected in 1.7% of cases and endometrial carcinoma was the most

common lesion. Although the sample size of this study was similar to that of (Shukla et al., 2017), the difference in the proportion of women with abnormal uterine bleeding across different endometrial histopathological findings could be attributed to sampling technique of using bleeding as an eligibility criterion. The other differences between Shukla et al study and the current study could be attributed to differences in the target populations and geographic settings.

Results from the study presented here showed that all women with products of conception, Decidualization, non-specific endometritis, complex endometrial hyperplasia, acute endometritis and atrophic endometrium complained of pelvic bleeding. Furthermore, nearly all women diagnosed with endometrial carcinoma complained of pelvic bleeding compared to those who complained of back pain. This is consistent with previous studies that have reported that pelvic (vaginal) bleeding is the most common clinical presentation of endometrial cancer in postmenopausal women (Nijkang et al., 2019). (Vaidya et al., 2013) further stated that approximately 75% of postmenopausal women who are diagnosed with endometrial cancer are diagnosed at an early stage, which improves the chances of successful treatment (Vaidya et al., 2013). However, only 10% to 20% of postmenopausal women who are evaluated for uterine bleeding are diagnosed with endometrial cancer because the most common cause of postmenopausal bleeding is endometrial atrophy (N. Bacalbasa et al., 2016; Nijkang et al., 2019; Whitaker & Critchley, 2016). Abnormal uterine bleeding can also be a sign of endometrial cancer in premenopausal women, who comprise 20% of cases of endometrial cancer.

CHAPTER SIX: SUMMARY, CONCLUSIONS AND RECOMMENDATIONS

6.1 Summary

Banked tissue blocks from 121 study participants were retrospectively sampled and tested for endometrial disorders using histopathology techniques. The participants were aged between 19 to 70 years with a median age of 44 years; with majority being aged 40-49 years. Most (74.2%, n=89) of the study participants had all live births with over half of all study participants having a parity of 1 to 4. Simple endometrial hyperplasia was the most common endometrial pattern. The age groups which many varied patterns were 40-49 years and 50-59 years. Majority of the women with a parity of 1-4 presented with simple endometrial hyperplasia. Majority of the women sought endometrial sampling due to pelvic bleeding.

6.2 Conclusions

1. Majority of the study participants presented with simple endometrial hyperplasia followed by complex hyperplasia and adenocarcinoma, respectively.
2. The age group which had varied endometrial patterns was those between 41-60 years.
3. Nulliparous women only presented with complex hyperplasia which is a precursor to endometrial carcinoma.
4. Pelvic bleeding was the most common indication for endometrial sampling.

6.3 Recommendations from the study

1. Awareness campaigns on general endometrial health should conducted
2. Endometrial sampling should be recommended for women who are 40 years and above presenting with bleeding and backache.
3. A parity of five or more could reduce the risk of endometrial disorders

4. All post-menopausal bleeding should be investigated, especially if they present with risk factors for endometrial pathology.

6.4 Limitations

Some of the disorders could have required other confirmatory diagnostic tests which were not assayed in the current study.

The study adopted a retrospective study design so this prolonged the study duration due to the incomplete data for some samples.

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APPENDICES

Appendix I: Endometrial histology techniques

1. The specimens after harvesting were immediately fixed in 10 % buffered formalin saline

40% formaldehyde ----------10mls

Sodium dihyhrogen Phosphate ----------0.4gms

Disodium dihyhrogen Phosphate----------0.65gms

Tap water----------90mls

2. After fixation the tissues were trimmed to a thickness of 3 mm to enhance proper processing then placed in plastic tissue-teks.

3. They were then processed in an automatic tissue processor

Figure 1: Automatic tissue processor

a) Dehydration -This is the complete removal of water from the tissues. It's necessary because the paraffin wax that is used (embedding media) is water immiscible. Dehydration is facilitated by passing the tissue through ascending grades of alcohol usually ranging from 70% to 100% Ethyl alcohol until all the water has been completely removed from the specimen. The tissue is allowed to remain in each strength of alcohol for as long as it is necessary (2 hrs) for complete saturation.

b) Clearing

Clearing or de-alcoholization is the term applied to the removal of alcohol from tissues by immersing them in xylene because it is miscible in both dehydrating and embedding media.

c) Impregnation

Is the process of saturating the tissue specimen with the medium to be used for embedding? Its purpose is to remove the clearing agent from the tissue. The clearing agent is eliminated from the tissue by diffusion into the surrounding melted wax and the wax in turn diffuses into the tissues to replace it. One change of wax was given to remove the clearing agent that has been displaced from the tissue and to ensure its replacement with pure wax.

4. **Blocking/ embedding/casting out**

Embedding is the process of placing the impregnated tissue in a precisely arranged position into a mould containing the embedding medium (paraffin wax) and causing this medium to solidify

Embedding is achieved by rapidly cooling the block on a cold plate at -5^0 c as soon as a firm film has formed on the wax surface. When the block has solidified, it is removed from the base mould.

Technique

1. The metal mould was smeared lightly with glycerin.
2. The mould was then filled with molten paraffin wax.
3. A warm pair of blunt nosed forceps was used to transfer the tissue from the paraffin wax to the mould. (Electrically heated forceps are ideal for this purpose).
4. The forceps was warmed again and used to orientate the tissue until it is lying in the desired plane. (Surface to be sectioned should face down) against the base of the mould.
5. The warm forceps is run round the tissue to ensure that any wax which may have solidified during the transferring from the paraffin bath to the mould is melted.

6. Transfer the mould to a refrigerated surface (cold plate)

The tissue block was then put in a freezer for further cooling.

Figure 2: Tissue block

MICROTOMY

The tissue block was sectioned on a microtome at 5microns to produce tissue sections (ribbons).

Figure 3: Microtome

Figure 4: Picking of tissue ribbons from the microtome

The ribbons were picked from the microtome using a camel hairbrush and transferred to a floating out water bath for them to straighten out (remove folds).

Figure 5: Floating out water bath

They were then fished out using a clean labeled slide and put in a hot air oven (58^0C) for drying purposes.

Figure 6 : Fishing-out from a floating out water bath

Figure 7: Hot air oven

STAINING

The slides were then removed from the oven and stained using Haematoxylin and Eosin staining method

Principle

Alum acts as mordant and hematoxylin containing alum stains the nucleus light blue. This turns red in presence of acid, as differentiation is achieved by treating the tissue with acid solution. Bluing step converts the initial soluble red color within the nucleus to an insoluble blue color. The counterstaining is done by using eosin which imparts pink color to the cytoplasm.

Reagents

1 Harris Hematoxylin stain

A = 1 gm hematoxylin in 10 ml ethanol

B = 20 gm ammonium alum in hot distilled water

Mix A & B, boil and add 0.5 gm of mercuric oxide and filter.

2 Eosin solution

Eosin yellow = 1 gm

Distilled water = 80 ml

Ethanol = 320 ml

Glacial Acetic Acid = 2 drops

3 0.5% HCl

5 mls = Concentrated Hydrochloric acid

100 mls = Distilled water

4 Dilute ammonia water

Procedure

1 The tissue sections were deparaffinized in three changes of xylene (bring sections to water).

2 They were then hydrated by passing through decreasing concentration of alcohol (100%, 90%, 80%, 70%) baths and water.

3 Stained in hematoxylin for 20 minutes

4 Washed in running tap water for 2 minutes.

5 Differentiate in 1% acid alcohol (1% HCl in 70% alcohol) for 1 minute.

6 Washed in running tap water for 1 minute then dipped in ammoniated water (an alkaline solution) until the sections are blue followed by tap water wash.

7 Stained in 1% Eosin Y for 3 minutes

8 Washed in tap water for 2 minutes

9 Dehydrated in increasing concentration of ethyl alcohol then cleared in 3 changes of xylene

10 Mounted using DPX (mounting media) then observed under a microscope.

Figure 8: Staining jars

Appendix II: Ethical approval

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471//2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2015/28
Approval Number: 0001378

17th March, 2015

Andeso Grace,
Maseno University,
School of Public Health & community Development,
MASEMO-KENYA.

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
17 MAR 2015
APPROVED
P. O. Box 4606-30100 ELDORET

Dear Ms. Andeso,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"Histopathological Patterns of Endometrial Biopsies received at Moi Teaching and Referral Hospital."

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1378*** on 17th March, 2015. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 16th March, 2016. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

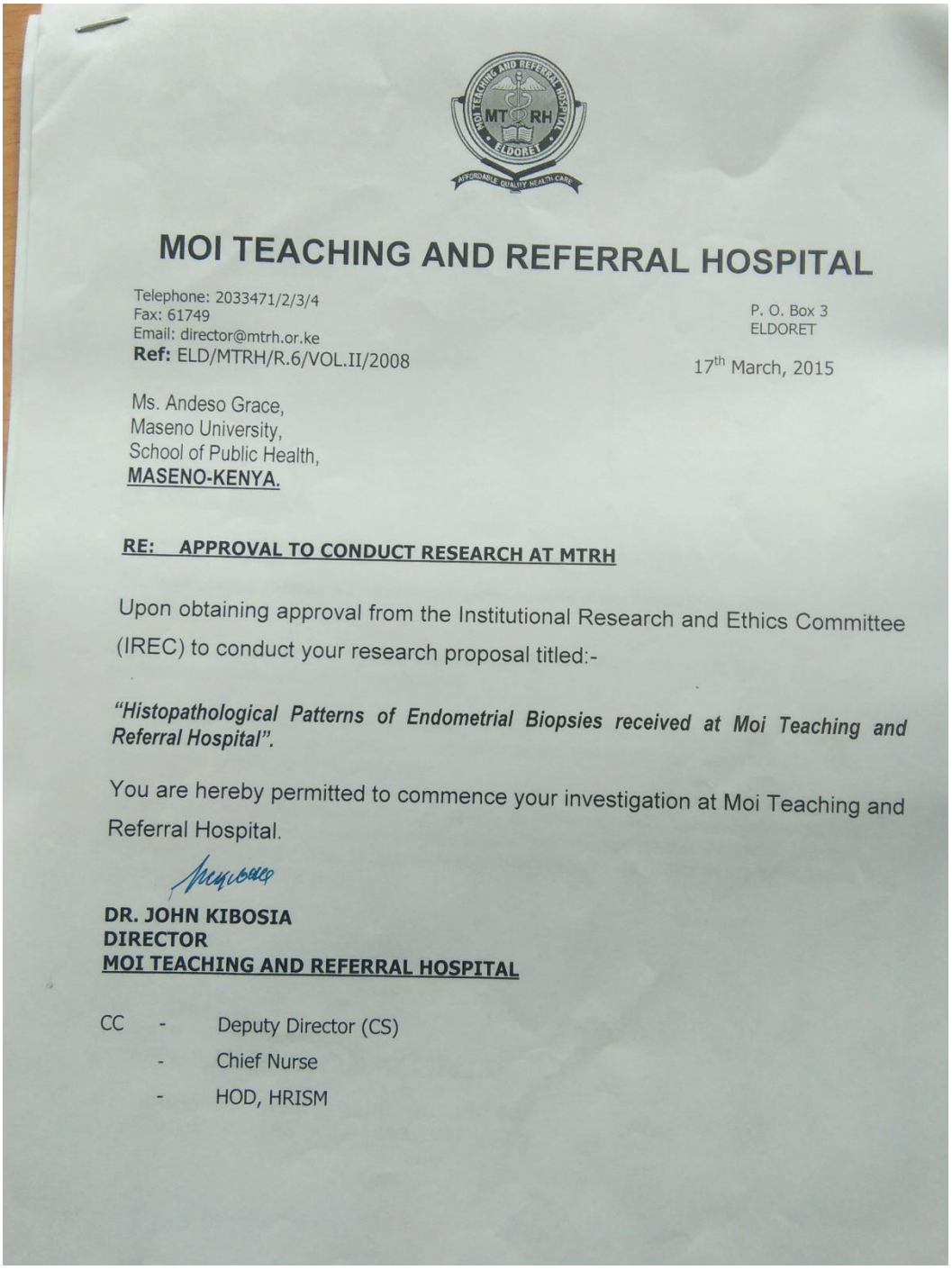
You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc Director - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD

Appendix III: MTRH approval



MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
Ref: ELD/MTRH/R.6/VOL.II/2008

P. O. Box 3
ELDORET

17th March, 2015

Ms. Andeso Grace,
Maseno University,
School of Public Health,
MASENO-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"Histopathological Patterns of Endometrial Biopsies received at Moi Teaching and Referral Hospital".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

DR. JOHN KIBOSIA
DIRECTOR
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

Appendix IV: University approval

MASENO UNIVERSITY
SCHOOL OF GRADUATE STUDIES

Office of the Dean

Our Ref: PG/MSC/00033/2013

Private Bag, MASENO, KENYA
Tel:(057)351 22/351008/351011
FAX: 254-057-351153/351221
Email: sgs@maseno.ac.ke

Date: 22nd June, 2017

TO WHOM IT MAY CONCERN

RE: PROPOSAL APPROVAL FOR ANDESO GRACE—PG/MSC/00033/2013

The above named is registered in the Master of Science Programme of the School of Public Health & Community Development, Maseno University. This is to confirm that her research proposal titled "Histopathological Evaluation of Endometrial Biopsies Received at Moi Teaching and Referral Hospital" has been approved for conduct of research subject to obtaining all other permissions/clearances that may be required beforehand.

MASENO UNIVERSITY
12 JUL 2017
DEAN SCHOOL OF GRADUATE STUDIES P.O. BAG, MASENO

Prof. J.O. Agure
DEAN, SCHOOL OF GRADUATE STUDIES