Chapter One
Introduction

1.1 Introduction:

Endometrial carcinomas originate in the endometrial lining, and constitute the majority of the cancers that affect the body of the uterus, while uterine sarcomas arise in the muscle layer or supporting connective tissue of the uterus (Shalini, *et al*. 2015).

From a histological and molecular pathology perspective, at least two major types of endometrial tumors can be distinguished. Type I tumors are mostly endometrioid carcinomas and are generally associated with endometrial hyperplasia. Type II tumors are more often serous papillary, clear cell, or adenosquamous carcinomas; generally develop from atrophic endometrial tissues in older women; and are associated with a much more aggressive phenotype (David, *et al*. 2006).

Globally, endometrial cancer is the sixth most common cancer among women. It has been estimated that 319,605 new cases and 76,160 deaths due to endometrial cancers among women during 2012 (Shalini, *et al* .2015).

Risk Factors which increases the incidence of getting type I adenocarcinomas are associated with states related to excess estrogen stimulation e.g. obesity, Polycystic ovarian syndrome, diabetes mellitus, unopposed estrogen replacement therapy, tamoxifen, hereditary nonpolyposis colorectal cancer syndrome. Type II adenocarcinomas have no generally accepted risk factors associated with its diagnosis (Dizon, *et al*. 2011).

Methods of endometrial cancer diagnosis include physical examination for woman with abnormal uterine bleeding, endometrial biopsy,

hysteroscopy, radiological procedure, cytologic and histologic immunohistochemistry evaluation (Shalini, *et al* .2015).

The primary treatment of endometrial cancer is surgical. Other adjuvant management includes the use of radiation therapy, chemotherapy and hormonal therapy (Dizon, *et al*. 2011).

E-cadherin is a transmembrane glycoprotein of the cadherin family, which promotes and maintains cell adhesion. Cadherins are tissue specific and are required for the assembly of cells into solid tissues. Epithelial cells express E-cadherin and its levels are reduced in many carcinomas including breast, lung, prostate, and also endometrial. Reduced E-cadherin expression was more seen in type 2 endometrial cancers and also in those carcinomas with advanced stage. Fifty seven percent of type2 endometrial cancers demonstrated loss of heterozygosity of E-cadherin gene at 16q22.1 compared to 22 % of type 1 carcinomas (Shalini, *et al*. 2015).

E-cadherin applied on cell block section of benignant and malignant endometrial lesions. The result show significant association between reduced E-Cadherin expression and malignancy (Zheng, *et al.* 2015).

1.2 Objectives:

1.2.1 General objective:

To study the expression of E-cadherin in endometrial tumors.

1.2.2 Specific objective:

1- To detect the expression of E-cadherin in endometrial tumors using immunohistochemistry.

2- To correlate E-Cadherin expression with histological diagnosis and grade.

Chapter Two
Literature Review

2.1 Scientific background:

Endometrial carcinomas are broadly categorized as types 1 and 2 carcinomas. Type 1 carcinoma occurs in younger premenopausal women and is associated with unopposed estrogenic stimulation, coexistent and preceding endometrial hyperplasia, and the histology is typically low-grade, low-stage endometrioid carcinoma. The prototypic type 2 carcinoma is serous carcinoma. These cancers occur in older postmenopausal women and are not associated with estrogen stimulation, arising on a background atrophic endometrium (David, *et al*, 2006).

2.2 Structure of the endometrium:

Uterus comprised of the corpus (uterine body), which terminates at the cervix. Comprised of two layers: the endometrium and the Myometrium. Endometrium thickens in response to estrogen; in absence of pregnancy, is shed in response to progesterone, constituting the menstrual cycle. (Dizon and Susana. 2011).

The endometrium of the corpus is composed of two layers: the basalis (the layer from which the endometrium regenerates after menstrual shedding) and the overlying functionalis. In the second half of the menstrual cycle, the functionalis may be differentiated into the superficial compacta and the underlying spongiosa, which extends to the basalis. During the menstrual cycle the endometrium varies from 1 mm

(postmenstrual) to about 8 mm at the end of the 3rd week. Every layer consists of two major structures: the epithelial component, either as glands or as superficial epithelium, and the mesenchymal component of stromal cells. Both cellular components are pluripotential and can undergo various metaplastic changes (Gisela.1987).

2.3 Disorders of the endometrium:

2.3.1 Benign disorders:

2.3.1.1 Endometrial hyperplasia:

Endometrial hyperplasia is a common clinical problem that all gynecologists will encounter in their practice. By definition, endometrial hyperplasia is an abnormal proliferation of both the glandular and stromal elements of the endometrium, with the glandular component being the most prominent. Endometrial hyperplasia is a result of estrogen excess in the absence of adequate progesterone. The most widely used classification of endometrial hyperplasia is the WHO classification, and divides hyperplasia into: simple hyperplasia with or without atypia, complex hyperplasia with or without atypia, based on configuration and number of glands, degree of glandular crowding, and presence or absence of nuclear atypia. Only those patients with cytologic atypia are at significant risk of developing endometrial cancer (Daniel, *et al*. 2010).

2.3.1.2 Endometritis:

Inflammatory cells normally comprise 10%–20% of endometrial cells, and their nature varies with the phases of the menstrual cycle. Endometritis exhibits increased numbers and/or an abnormal distribution of endometrial inflammatory cells, frequently accompanied by morphological abnormalities of endometrial glands and stroma.

Endometritis traditionally classified as either acute or chronic. More than 70% of chronic endometritis cases result from nongonococcal, nonchlamydial infections. The common bacteria along with *Mycoplasma* are the most frequent etiological agents of chronic endometritis. Other commonly encountered forms of endometritis include intrauterine device (IUD)-associated granulomatous endometritis (John, *et al*. 2009).

2.3.1.3 Endometrial polyps:

It is a uterine lesion that takes up space within the uterine cavity found in 10% of women. Endometrial polyps are biphasic growths of endometrial glands and stroma that arise as monoclonal overgrowths of genetically altered endometrial stromal cells with secondary induction of polyclonal benign glands. The common polyp is nonfunctional and lacks the cyclical changes seen in the adjacent normal endometrium (John, *et al*. 2009).

2.3.2 Malignant disorders:

2.3.2.1 Type 1 (endometrioid carcinoma):

Endometrioid adenocarcinoma is the most common type of endometrial carcinoma (approximately 85%). They are considered type I endometrial cancers according to the Bokhman classification because of their epidemiologic association with estrogen excess. By definition, it should resemble, at least focally, proliferative- type endometrium showing tubular glands with smooth luminal surfaces, lined by mitotically active columnar cells. Endometrioid adenocarcinomas may include mucinous carcinoma, ciliated carcinoma, secretory carcinoma and villoglandular carcinoma (Franco, *et al*. 2009).

2.3.2.2 Type 2 (non- endometrioid carcinoma):

2.3.2.2.1 Serous carcinoma:

Pure serous carcinomas comprise about 10% of endometrial cancers. In contrast to the endometrioid (type 1) group of endometrial carcinomas, serous carcinoma of the endometrium is an aggressive tumor with a high recurrence and low survival rate (John, *et al*. 2009).

It is characterized by branching complex papillae, formed of fibrovascular cores, covered by one or more layers of cuboidal cells with high-grade nuclei and a scalloped apical border (Antonio, *et al*. 2007).

2.3.2.2.2 Clear cell carcinoma:

Clear cell carcinoma is the third most common endometrial carcinoma subtype, even though it represents < 5% of cases. It occurs in women with a similar clinical profile to that of serous carcinoma. The tumor is characterized by clear cells, hobnail cells, or both (David, *et al*, 2006).

2.3.2.2.3 Endometrial carcinosarcoma:

Carcinosarcoma is rare; it represents < 5% of all malignant uterine tumors. Also referred to as malignant mixed mullerian tumors. They are epithelial malignancies with a malignant mesenchymal component that may include homologous or heterologous sarcomatous elements (John, *et al*, 2009).

2.4 Epidemiology:

Endometrial cancer is the fourth most commonly diagnosed cancer among women, with nearly 50,000 cases diagnosed in the United States in 2013 (Michele, *et al*. 2015).

Incidence of endometrial cancer has been shown to vary by race and ethnicity, adjusted estimation show that black women actually have higher incidence rates of endometrial cancer than white women. (Patricia, *et al*. 2015).

The annual mortality rate per 100,000 people from uterine cancer in Sudan has decreased by 4.6% since 1990, an average of 0.2% a year (health grove, 2013).

2.5 Risk factors:

2.5.1 Age:

The risk of endometrial cancer increases as a woman gets older. Most cases of endometrial cancers are found in women over 55 years of age. A few cases may occur before age 45(Shalini, *et al*. 2015).

2.5.2 Risk factors related to reproduction:

2.5.2.1 Menstrual factors:

Early menarche is associated with 1.5–4 fold increased risk of endometrial cancer. Late menarche was identified as a protective factor. Also, a reduction in endometrial cancer risk was observed in women with early menopause. Menstrual span of more than 39 years was associated with 4.2 times higher risk than one with less than 25 years (Shalini, *et al*. 2015).

2.5.2.2 Parity:

A woman's probability of developing endometrial cancer is inversely related to the number of children she has borne. In addition to nullparity, impaired fertility has also been associated with an increased risk of endometrial cancer (Earl and David. 1989).

2.5.2.3 Pregnancy:

Decreased risk of endometrial cancer was associated with cumulative duration of full-term pregnancy. An increased risk of endometrial cancer associated with induced abortions (David, *et al*. 2006).

2.5.3 Estrogen-related risk factors:

2.5.3.1 Polycystic ovarian syndrome (PCOS):

Women who have polycystic ovaries secrete abnormally large quantities of androstenedione, resulting in persistent estrogen levels characteristic of the peak of the normal ovulatory cycle. Prolonged endometrial exposure to unopposed estrogen due to anovulation and endometrial progesterone resistance increase the risk for developing endometrial cancer (Earl and David. 1989).

2.5.3.2 Unopposed estrogen replacement therapy:

There is strong evidence suggesting estrogen therapy unopposed by progesterone therapy is a major risk factor for endometrial cancer in women with an intact uterus, with the risk substantially increasing with current, long duration use (Shalini, *et al*.2015).

2.5.3.3 Tamoxifen therapy:

Tamoxifen is one of the selective estrogen receptor modulators (SERMs). It also has modest estrogenic activity and hence is found to be associated with endometrial carcinoma. There is an increasing risk of endometrial cancer associated with longer tamoxifen treatment, extending well beyond 5 years which does not diminish in follow-up to at least 5 years after the end of the last treatment (Shalini, *et al*. 2015).

2.5.4 Endometrial hyperplasia:

The overall progression risk for endometrial hyperplasia is three times higher than the average population risk of endometrial carcinoma. Fewer than 5 % of women with non-atypical or simple endometrial hyperplasia will experience progression to carcinoma, but 28 % of women with atypical hyperplasia will progress to carcinoma during 20 years (Shalini, *et al*. 2015).

2.5.5 Breast or ovarian cancer:

Women diagnosed with breast cancer have a higher incidence of second primary cancers, particularly of endometrial cancer in women over 50 at diagnosis. The granulosa–theca cell tumor of the ovary secretes estrogen, which is uncontrolled, and this can sometimes lead to high estrogen levels, leading to stimulation of the endometrium and resulting in endometrial cancer (Shalini, *et al*. 2015).

2.5.6 Lynch syndrome:

This disorder is commonly caused by a defect in either the gene *MLH1* or the gene *MSH2*. Defects in other genes can also cause HNPCC, namely *MLH3, MSH6, TGBR2, PMS1* and *PMS2,* and increase the risk of endometrial cancer (Shalini, *et al*. 2015).

2.5.7 Lifestyle factors:

2.5.7.1 Obesity:

Large body mass in general and obesity in particular, has been linked to increased risk of endometrial cancer. Relative risks associated with obesity range from 2 to 10. An association with obesity is biologically plausible. Postmenopausal obese women are known to have higher endogenous estrogen levels than thin women, due to the aromatization of androstenedione in adipose tissue (David, *et al*. 2006).

2.5.7.2 Sedentary behavior:

Excessive sitting time is associated with an increased endometrial cancer risk. Physical activity was clearly associated with reduced risk of endometrial cancer, with active women having an approximately 30–33 % lower risk than inactive women (Shalini, *et al*. 2015).

2.5.8Hypertension and hypertension:

Endometrial cancer risk in women with a history of hypertension to be 30 to 100% greater than that among normotensives. Also diabetes mellitus is long known to be associated with endometrial cancer. Hyperinsulinemia and higher levels of insulin-like growth factor I (IGF-I) are proposed to play a role in the diabetes–endometrial cancer association (David, *et al*. 2006).

2.5.9 Genetic risk factors:

Risk of endometrial cancer is increased in women reporting endometrial or ovarian cancer in a mother or sister (Earl and David. 1989).

2.6 Diagnosis of endometrial cancer:
2.6.1 Physical examination:
A patient with suspected endometrial carcinoma should be subjected to a thorough physical and pelvic examination. The size and mobility of the uterus and the presence or absence of gross cervical involvement, extra uterine mass, or ascites should all be assessed. The supraclavicular area must be examined to rule out enlarged nodes (Shalini, *et al*. 2015).

2.6.2 Endometrial biopsy:

Endometrial biopsy is the gold standard test, which can help to confirm the presence of endometrial carcinoma. It can be done as an outpatient/office procedure without anesthesia or under local anesthesia, by using Pipelle sampling device. This is the least invasive technique for obtaining an endometrial biopsy and has a sensitivity of 73 % and specificity of 100 % for detecting endometrial disease. A dilatation and curettage (D&C) needs to be performed only if the office endometrial biopsy shows insufficient endometrial cells for evaluation (Shalini, *et al*, 2015).

2.6.3 Cytological sampling:

Direct cytological sampling and examination of the endometrium is not generally practiced, which is surprising as the endometrium is exceedingly easy to sample. Endometrial cytology found to be an effective method both for ensuring endometrial normalcy and for discovering and diagnosing malignant and premalignant states. Cytological sampling of the endometrium is reliable and well-tolerated method of detecting uterine pathology. Tao brush method uses a small, flexible brush to gently brush the inside of the uterus. Thus, the Tao brush gathers a complete sampling of the uterine lining, removes tissue in a less traumatic fashion, and, as there is no need for continuous movement of the device across the cervical canal, is less painful than suction biopsy (John, *et al*, 2009).

2.6.4 Hysteroscopy:

The addition of hysteroscopy offers any improvement in the accuracy of the diagnosis, or in the preoperative staging, is unclear. Fears that hysteroscopy might increase the incidence of disseminated intraperitoneal disease by washing malignant cells through the fallopian tubes have proven unfounded (David, *et al*, 2006).

2.6.2 Radiological procedures:

2.6.2.1 Transvaginal ultrasound:

Transvaginal US is performed with the patient's urinary bladder empty so provides superior resolution of pelvic structures and greater detail of the characteristics of pelvic masses. The main disadvantage of transvaginal US is the limited field of view allowed by the transducer, making large masses or masses high in the pelvis difficult to evaluate (Patricia, 2006).

2.6.2.2 Sonohysterogram:

Sonohysterogram was recommended in the evaluation of women on tamoxifen. Sterile saline is instilled into the endometrial cavity and then a transvaginal ultrasound is performed. The saline will reveal subtle irregularities such as small polyps and it will reduce the error in measuring the stripe thickness (Franco, 2009).

2.6.2.3 Magnetic resonance imaging (MRI):

Magnetic resonance imaging (MRI) provides high spatial resolution and excellent soft tissue contrast. The contrast of tumors to uterine cavity and myometrium can be further improved with the use of contrast agents and the enhancement features of tumors at different stages can be analyzed quantitatively and dynamically (Salvador, 2012).

2.6.2.4 Positron-emitting tomography (PET):

Increased endometrial fluoro-deoxyglucose (FDG) uptake is seen in both physiologic and malignant conditions of the ovaries, cervix, and uterus (Dizon, 2011).

2.6.3 Immunohistochemistry (IHC):

Various available IHC markers that are useful in substantiating tumor subtyping in endometrial adenocarcinomas include estrogen receptor (ER), progesterone receptor (PR), β-catenin, p16, etc. It is important to note that expression of IHC markers should be interpreted in a clinicopathological context (Shalini, *et al*. 2015).

2.7 Treatment of endometrial cancer:

2.7.1 Surgery:

Surgery plays the central role in the treatment of uterine cancer, the exact nature and extent of the surgery is the subject of ongoing debate. Even with advanced or recurrent disease, removal of the uterus, tubes, and ovaries, if feasible, will improve the quality of life for most patients. Particularly with endometrial cancer there is a role for non-oncologists to treat relatively fit women with well-differentiated tumors in the absence of metastatic disease (David, *et al*. 2006).

2.7.2 Radiation Therapy:

Whole pelvic radiation therapy (WPRT) reduces the rate of pelvic disease recurrence in patients who undergone hysterectomy for endometrial cancer with high risk of recurrence. Radiotherapy is generally administered as external irradiation alone and/or vaginal brachytherapy. The delivery technique is a critical part of the success of radiotherapy for patients with endometrial cancer. Many modern external beam radiotherapy techniques available for endometrial cancer, including 3D-CRT (Three-dimensional Conformal Radiation Therapy), IMRT (Intensity-modulated Radiation Therapy), HT (Helical Tomotherapy) and VMAT (Volumetric Modulated Arc Therapy) (Salvador, 2012).

2.7.3 Hormonal therapy:

Hormonal therapy use for the purpose of preserving the corpus and future fertility. This treatment obviously pertains to premenopausal women. An occasional postmenopausal woman who is not a candidate for surgery may benefit from similar approaches (Franco, 2009).

2.7.4 Chemotherapy:

Chemotherapy not routinely administer for adjuvant therapy in endometrial cancer patients. In high-risk patients there may be a slight survival advantage. Chemotherapy use to treat patients with UPSC or

clear cell carcinoma. The rationale for this treatment approach is that the high risk for peritoneal spread with these histologic types (Patricia, 2006).

2.8 E-cadherin and its relation with endometrial cancer:

E-cadherin has been shown to play a central role in the organization and maintenance of epithelial tissue structure. Decreased cell-to-cell contact in epithelial cells has been shown to be largely attributable to down-regulation in the expression of E-cadherin. *In vitro* studies have demonstrated that expression of E-cadherin may preclude the invasiveness of epithelial tumor cell lines, suggesting that the E-cadherin gene may primarily act as an invasion-suppressor gene. Decreased E-cadherin expression is a crucial step in the progression to a more malignant phenotype, and has also been associated with decreased cell-to-cell adhesion and increased invasive and metastatic potential in endometrial and other carcinomas (Yalta, *et al*. 2009).

Chapter Three

3. Materials and Methods

3.1 Materials:

Archived tissue blocks of endometrial tumors were selected for this study.

3.2 Methods:

3.2.1 Study design:

This is hospital based analytical retrospective case control study aimed to detect the expression of E-Cadherin in endometrial tumors.

3.2.2 Study sample:

Forty paraffin blocks were collected from patient previously diagnosed as endometrium tumors, 20 (50%) were malignant and 20 (50%) were benign. (Age, histopathological diagnosis, malignant tumor grade) were obtained from patient's files.

3.2.3 Study area:

This study conducted in Ribat university hospital, Soba university Hospital, the Military Hospital and Sudan University of science and technology – college of medical laboratory science, during the period from July to October 2016.

3.2.4 Sample processing:

Tissue sections to be stained were cut at (3μm) thickness by rotary microtome, mounted in positively charged slides and baked at 60°C for 30 minutes.

3.2.5 Immunohistochemical staining:

Sections of 3μm thickness were cut and placed on slides and then mounted in positively charged slide. Following de-paraffinization in xylene, slides were rehydrated through a graded series of alcohol (50%, 70%, 90%, and 100%) and were placed in running water. Samples were steamed for antigen retrieval for E-cadherin using PT link (Dako An Agilent Technologies Company). Briefly, slides were placed in slide tank containing tris EDTA buffer (pH 9.0), then heated for 20 minutes, then sections were cooled at RT. Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 15 minutes, then slides were incubated with 100 μl of primary antibodies for 20 min at room temperature in a moisture chamber, and then were rinsed in phosphate buffer saline. After washing with PBS for 3 min, binding of antibodies were detected by incubating for 20 minutes with dextrin labeled polymer. Finally, the sections were washed in three changes of PBS, Followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen for 5 min. Slides were counterstained with Mayer's haematoxylin for one minute and washed in water and blued in 0.05% ammoniated water for 16 seconds, then washed in tap water, dehydrated through ascending grades of ethanol (50%, 70%,90%, 100%) two minutes for each then cleared in two change of xylene two minutes for each, and mounted in (DPX) mounting media.

3.2.6 Result interpretation:

All quality control measures were adopted; Immunoreaction was assessed as positive (strong) or negative (weak or absent), and the internal positive control was represented by the neighbouring normal structures. Negative expression was stated when the immunohistochemical reaction was negative or less than 70% of the tumor cells show positive reaction with membrane pattern. Positive expression was stated when $\geq$70% of the tumor cells showed positive reaction with membrane pattern (Suciu, *et al.*, 2008).

3.2.7 Data analysis:

Data was analysed using SPSS 20 computer program. Frequency, mean, standard division and Chi-square test values were calculated.

3.2.8 Ethical consideration:

Samples were collected after taking ethical approval from each hospital to use the tissue blocks for research purposes.

Chapter Four

4. Results

The study include forty sample, 20 (50%) of them were malignant and 20 (50%) sample were benign.

The age of study population range between 25 and 85 with mean age of 56.35($\pm$14.856).

Most of patients were more than 60 years representing 22 (55%) and the remaining 18 (45%) were younger than 60 years as indicated in table (4.1).

The diagnosis of study population includes adenocarcinoma in 8 (40%) samples, papillary carcinoma in 6 (30%) samples, squamous cell carcinoma in 5 (25%) samples, mixed mullerian tumor in 1 (5%), atypical hyperplasia 4 (20%), hyperplasia without atypia 16 (80%) as indicated in table (4.2).

The grade of study population includes grade I in 3 (15%) samples, grade II 6 (30%), and grade III 3 (15%), not graded 8 (40%) as indicated in table (4.3).

E-Cadherin positive expression was found in **17 (42.5%)** samples, and 3 (7.5%) samples showed negative expression in both malignant and benign tumors. There was insignificant differences between E cadherin expression and endometrial tumors (p. value = 1.00) as indicated in table (4.4).

Table (4.1): Distribution of age group among the study population

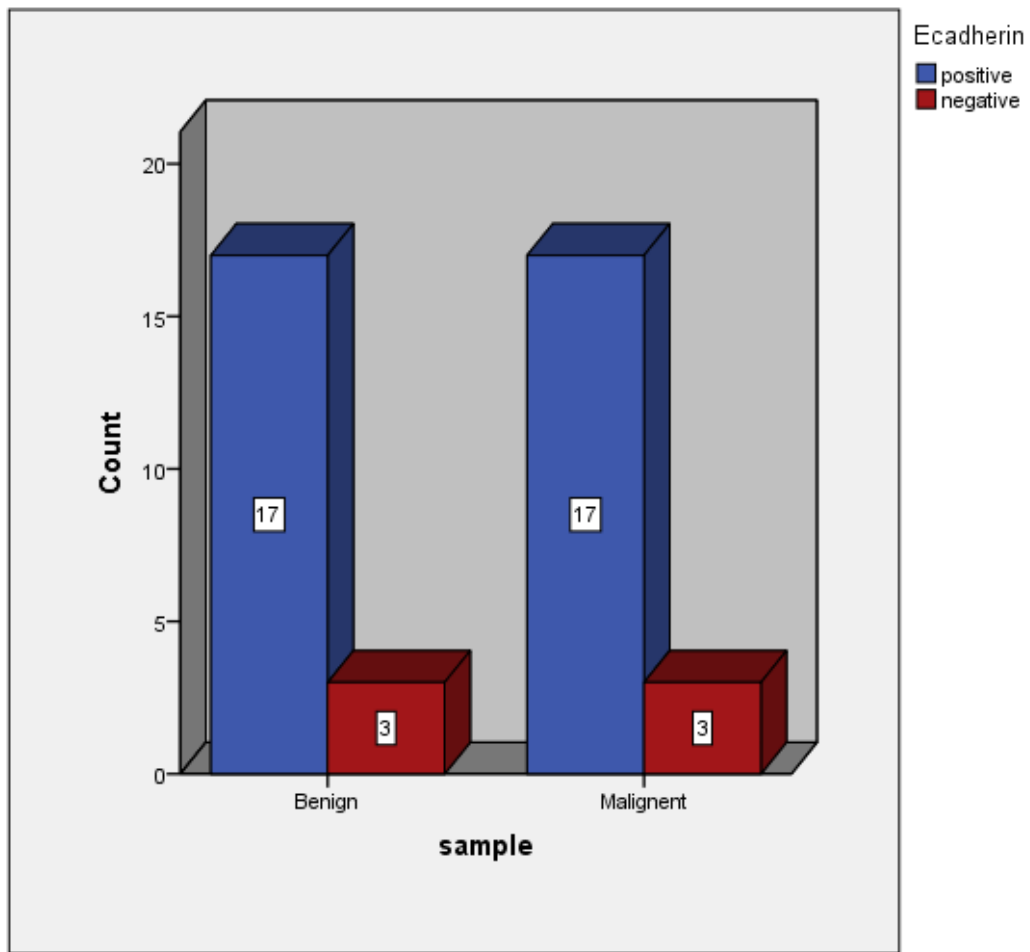| Age group	Frequency	Percentage
60 years≤	22	55%
60 years>	18	45%
Total	40	100%

Figure (4.1): Frequency of E cadherin expression among the study population.

Table (4.2): Distribution of histopathological diagnosis among the study population

Histopathological diagnosis		Ferquency	Percentage
Malignant	Adenocarcinoma	8	20%
	Papillary adenocarcinoma	6	15%
	Squamous carcinoma	5	12.5%
	Mixed mullerun carcinoma	1	2.5%
Benign	Atypical hyperplasia	16	80%
	Typical hyperplasia	4	20%
Total		40	100%

Table (4.3): Distribution of tumor grade among the study population

Grade	Frequency	percentage
Grade I	3	15%
Grade II	6	30%
Grade III	3	15%
Not graded	8	40%
Total	20	100%

Table (4.4): Relation between E cadherin expression and histopahological diagnosis

| Histological diagnosis | E-cadherin Expression | | P. value |
	Positive	Negative	
Benign	17 (42.5%)	3 (7.5%)	1.000
Malignant	17 (42.5%)	3 (7.5%)	
Total	34 (85%)	6 (15%)	
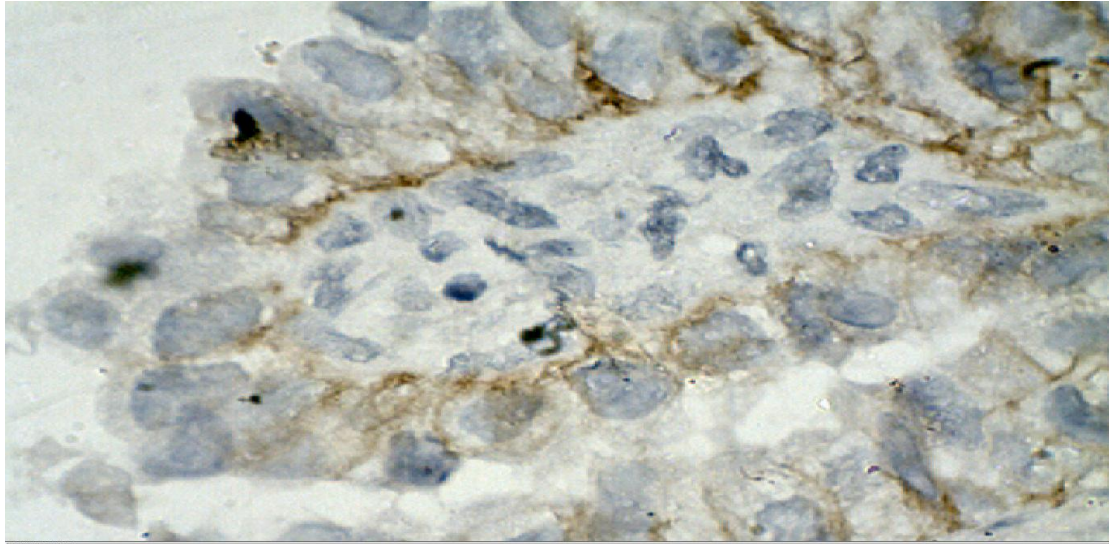
Microphotograph (4.1): Papillary Adenocarcinoma showed positive membranous expression of E-Cadherin (100X)
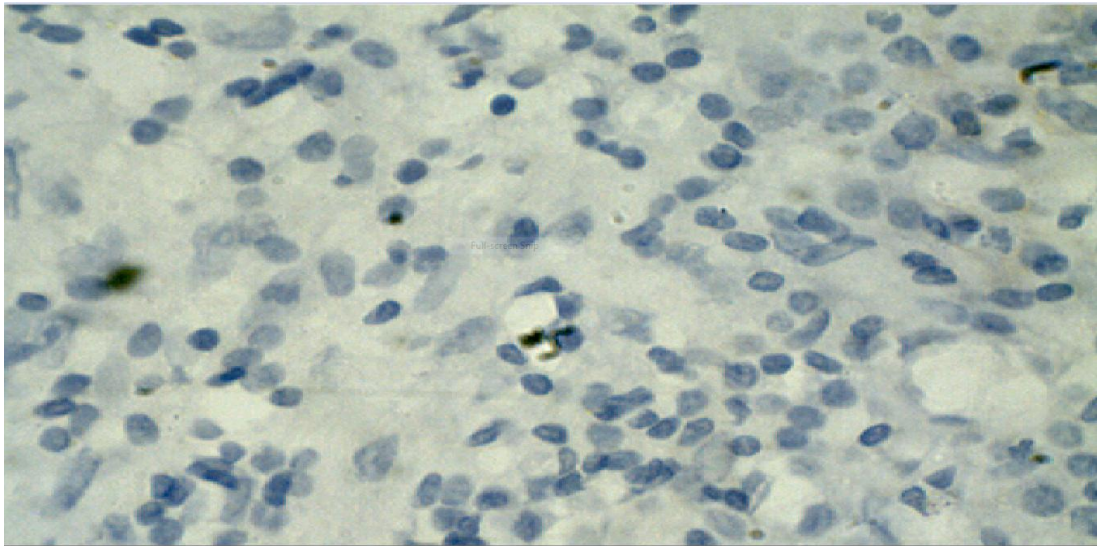
Microphotograph (4.2): Malignant Mixed Müllerian Tumor showed negative expression of E-Cadherin (100X)

Chapter Five

5. Discussion

The present study involves 40 cases of endometrial lesions, stained immunohistochemicaly by E-Cadherin. Regarding the age of study population, the study revealed most of patients were more than 60 years indicating that older women are more susceptible to endometrial cancer due to endogenous hormones level, genetic factors and obesity. This result compatible with Doorn *et al. (2007),* who reported the risk of developing endometrial cancer increases considerably until 55 years. Also agree with Karsten *et al. (2004),* who reported that endometrial carcinoma occurs in advanced age (postmenopausal). This result also agree with La Vecchia *et al.(1984),* who reported that endometrial cancer risk elevated in older women(greater than or equal 65 years).

The histopathological diagnosis revealed that endometrial adenocarcinoma is the most frequent type. This result is compatible with Karsten *et al. (2004),* who reported that endometroid adenocarcinoma is the most common type (60 – 80%). Also agree with Montalto *et al. (*2009*),* who reported that 73.4% were referred with endometrioid adenocarcinoma.

Regarding the available data, endometrial cancer grade II is the most common type. These results agreed with Creasman, *et al,* (1987) who reported that grade II moderate differentiation is the most frequent type.

The expression of E-Cadherin revealed there was no significant difference between marker expression in malignant and benign endometrial tumors. To our knowledge, there are no studies agree with this result, may be due to small sample size. This result disagrees with Zheng *et al,* (2015) who reported the significant association between reduced E-Cadherin expression and malignancy. Also disagree with Shih *et al, (2004)* who reported the loss of E-Cadherin expression associate with aggressive biological behavior, especially in high grade tumor.

Chapter Six

6. Conclusion and Recommendations

6.1Conclusion:

On the bases of this study we conclude that: ●

The age of the endometrial cancer patient in Sudan is commonly more than 60 years.

● Most histological type of endometrial cancer is adenocarcinoma.

●There is no association between E-cadherin expression and endometrial tumors.

6.2 Recommendations:

On the bases of this study we recommended that:

● Further study should be done on expression of E-Cadherin in endometrial tumors tissues with large sample size.

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Appendices

Appendix1:

Materials and instruments used for processing and staining of the specimens include:

●Disposable gloves.

●Rotary microtome.

●Microtome knife.

●Positively charged slides (Thermo).

●Cover glasses

●Dry oven.

●Water path (Dako water path).

●Coplin jars.

●Humidity chamber.

●Ethanol(100%, 90%, 70%, 50%).

●Xylene.

●Mayer`s haematoxlin

(Haematoxlin, DW, K or ammonium alum, sodium iodate, citric acid, chloral hydrate).

●tris EDTA buffer (pH 9.0).

●Phosphate buffer (PH 6.7).

●0.3 Hydrogen peroxidase.

●Primary antibody (E-cadherin).

●Secondary antibody.

●Dextrin labeled polymer.

●Substrate Chromogen.

●DBX.

Appendix2:

Dako

CE

**Monoclonal Mouse
Anti-Human
E-cadherin
Clone NCH-38**

ENGLISH
Code M3612

Intended use
For In Vitro Diagnostic Use.

Monoclonal Mouse Anti-Human E-cadherin, clone NCH-38 (Anti-E-cadherin, NCH-38) is intended for laboratory use to identify qualitatively by light microscopy E-cadherin positive cells in normal and neoplastic tissues using immunohistochemical (IHC) test methods. The clinical interpretation of any positive staining or its absence should be complemented by morphological and histological studies with proper controls. Evaluations should be made within the context of the patient's clinical history and other diagnostic tests by a qualified individual.

Synonyms
E-CD, uvomorulin, L-CAM, Arc-1, or cell-CAM 120/180[1-3]

Summary and explanation
E-cadherin is a 120 kD transmembrane cell adhesion molecule. The gene has been localized on chromosome 16q22.1. In its extracellular domain, E-cadherin is involved in cell-cell adhesion through calcium-regulated homophilic interactions, whereas in its intracellular domain, E-cadherin connects to the actin cytoskeleton via catenins. E-cadherin has a significant function in intercellular adhesion of epithelial cells, the establishment of epithelial polarization, glandular differentiation, and stratification. It is localized mainly in the adherens junctions and concentrates the urokinase plasminogen and the epidermal growth factor receptor to cell contact sites.[4,5] Down-regulation of E-cadherin expression has been observed in a number of carcinomas and is usually associated with advanced stage and progression.[6-8]

Refer to Dako's *General Instructions for Immunohistochemical Staining* or the detection system instructions of IHC procedures for: 1) Principle of Procedure, 2) Materials Required, Not Supplied, 3) Storage, 4) Specimen Preparation, 5) Staining Procedure, 6) Quality Control, 7) Troubleshooting, 8) Interpretation of Staining, 9) General Limitations.

Reagent provided
Monoclonal Mouse antibody provided in liquid form as tissue culture supernatant in 0.05 mol/L Tris-HCl, pH 7.2 and 0.015 mol/L sodium azide. This product contains stabilizing protein.

Clone: NCH-38[4]   Isotype: IgG_1, kappa
Mouse IgG concentration mg/L: See label on vial.

M3612 may be used at a dilution range of 1:50 to 1:100 when performing IHC using the LSAB2, HRP, Liquid DAB detection system. These are guidelines only. Optimal antibody concentrations may vary depending specimen and preparation method and should be determined by each individual laboratory.

Immunogen
E-cadherin (uvomorulin) and GST recombinant protein[4]

Specificity
Anti-E-cadherin, NCH-38 recognizes the 120 kD mature form and 82 kD fragment of E-cadherin in Western blots of A431 cells lysates.[4]

Materials required, but not supplied
Refer to the "General Instructions for Immunohistochemical Staining" and/or the Detection System "Instructions." Suggested diluent for IHC procedures:
     Antibody Diluent (code S0809)
The following negative control is recommended for IHC procedures:
     Mouse IgG_1 (code X0931)

Precautions
1. For professional users.
2. This product contains sodium azide (NaN_3), a chemical highly toxic in pure form. At product concentrations, though not classified as hazardous, NaN_3 may react with lead and copper plumbing to form highly explosive build-ups of metal azides. Upon disposal, flush with large volumes of water to prevent metal azide build-up in plumbing.[9]
3. As with any product derived from biological sources, proper handling procedures should be used.
4. Wear appropriate Personal Protective Equipment to avoid contact with eyes and skin.
5. Unused reagents should be disposed of according to local, State, and Federal regulations.

Storage
Store at 2–8 °C. Do not use after expiration date stamped on vial. If reagents are stored under any conditions other than those specified, the conditions must be verified by the user. There are no obvious signs to indicate instability of this product. Therefore, positive and negative controls should be run simultaneously with patient specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Dako Technical Support.

Specimen preparation
Paraffin Sections

Anti-E-cadherin, NCH-38 can be used on formalin-fixed, paraffin-embedded tissue sections. The deparaffinized tissue sections must be treated with heat prior to the IHC staining procedure. Target retrieval involves immersion of tissue sections in a pre-heated buffer solution and maintaining heat, either in a water bath (95–99 °C), a steamer (95–99 °C) or an autoclave (121 °C) . For greater adherence of tissue sections to glass slides, the use of Silanized Slides (code S3003) is recommended. Target Retrieval Solution (code S1700) or 10x Concentrate (code S1699) is recommended using a 20-minute heating protocol.

Cryostat Sections and Cell Smears

Anti-E-cadherin, NCH-38 can be used for labeling acetone-fixed cryostat sections or fixed cell smears. Target or antigen retrieval is not required.

Staining procedure
Follow the procedure for the detection system selected.

Staining interpretation
The cellular staining pattern for anti-E-cadherin, NCH-38 is membranous and/or cytoplasmic.

Performance characteristics
Normal Tissues

E-cadherin expression has been demonstrated by immunohistochemistry in (40/40) normal urothelium specimens (frozen and paraffin).[8] Normal human mammary gland has been found to strongly express E-cadherin in the intercellular borders of the luminal cells of both the interlobular ducts and the intralobular terminal ducts and ductules but expression was much weaker in myoepithelial cells of ducts and ductules (frozen and paraffin).[10] Squamous epithelial cells in esophagus were found to be strongly immunoreactive (15/15) on cell-cell boundaries, except in the most superficial keratinizing layer. Also, E-cadherin was immunolocalized in normal gastric mucosa at the cell-cell boundaries of the foveolar epithelia as well as in gastric crypts and deep gastric glands (frozen and paraffin).[1, 2] In normal endometrium, almost all the glands revealed strong E-cadherin expression (frozen).[11] E-cadherin immunoreactivity has been localized in normal skin along the lateral and upper surfaces of basal keratinocytes at intercellular borders but was absent at the basal cell surface. In the suprabasal layers of skin, E-cadherin expression was localized uniformly around the periphery of the cells, however no expression was seen in the superficial corneal layer. The adnexal structures of skin also demonstrated E-cadherin immunoreactivity including membrane staining of the outer root sheath cells (the inner sheath cells were negative), acinar germinative cells in the sebaceous glands and sweat gland cells of skin. No E-cadherin expression was demonstrated in the dermis of normal skin (paraffin).[5] E-cadherin was also strongly expressed in epithelial cells of normal prostate, especially in areas of cell-cell contact (frozen).[3]

Abnormal Tissues

E-cadherin immunoreactivity has been demonstrated in a variety of abnormal cell types.[1,3,5-6,10,11] Table 1 summarizes expression of E-cadherin in abnormal tissues.

Table 1. Abnormal Tissue Reactivity

Tissue Type	Positive and Negative Tissue Element Staining and Staining Pattern
Bladder: Primary transitional cell carcinoma of the bladder[A,B]	13/40 positive, homogeneous (membranous)
	13/40 positive, heterogeneous
	14/40 negative
	High Grade (grade IIb and III): 10/24 positive
	Low Grade (grade I and IIa): 16/16 positive
	Superficial stage (Ta and Ti): 21/22 positive
	Invasive stage (T2, T3 and T4): 5/18 positive
Breast cancer: Node negative[6] (without chemotherapy or hormonal therapy)	136/168 positive
Breast carcinoma: Ductal[3,5]	55/87[3] strong positive, majority of cells
	29/87[3] weaker positive, heterogeneous
	14/24[5] strong positive, majority of cells
	10/24[5] weaker positive, heterogeneous
Breast carcinoma: Lobular[3,5]	3/21[3] positive focal—very sparse intercellular membrane—or weak cytoplasmic
	3/14[5] positive, focal—very sparse intercellular membrane—or weak cytoplasmic
Esophagus: Squamous cell carcinoma[1]	4/15 positive
	10/15 positive, heterogeneous or weak
Gastric carcinoma[1]	108/413 positive, homogeneous linear expression and comparable to normal gastric mucosa
	95/413 positive, moderately reduced linear or dotted intercellular staining in 20–60% tumor cells
	86/413 positive, highly reduced finely dotted intercellular staining in <20% cells
	124/413 negative or weak dotted immunoreactivity <5% cells. Special pattern of E-cadherin expression was present in a small percentage of signet ring-cell carcinomas and of undifferentiated carcinomas, where a strong intracytoplasmic "plaque-like" expression of E-cadherin could be demonstrated, sometimes in combination with a very weak immunoreactivity at tumor-cell membrane.
Endometriosis[5]	3/9 positive
	6/9 positive, heterogeneous

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