Sudan University of Science and Technology
College of Graduate Studies

Immunohistochemical Detection of Estrogen Receptors in Ovarian Tumors in Sudanese Women

الكشف المناعي النسيجي الكيميائي عن مستقبلات الإستروجين في أورام المبيض لدى النساء السودانيات

A dissertation submitted in partial fulfillment for the requirements of master degree in medical laboratory science (Histopathology and Cytology)

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الآية

قال تعالى : (وَمَا يَعْلَمُ تَأْوِيلَهُ إِلَّا اللَّهُ وَالرَّاسِخُونَ فِي الْعِلْمِ يَقُولُونَ آمَنَّا بِهِ كُلٌّ مِّنْ عِندِ رَبِّنَا وَمَا يَذَّكَّرُ إِلَّا أُولُو الْأَلْبَابِ)

ال عمران الآيه7

Dedication

I dedicate this work to:-

My father

My mother

My brother

My husband

My family

My teachers

My colleagues and friends

With love and respect

Acknowledgment

First of all, I am grateful and thankful to Almighty Allah who gives us mind to thinkand health and power to move and enabling me to complete this research.

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Abstract

This is analytical retrospective case controlhospital based study was conducted at Alamal Hospital and Altayseer-2 labrotary in Khartoum state during the period from April 2018 to August 2018.

The studyaimed to detect estrogen receptor expression in ovarian tumors using immunohistochemistry.

Fifty seven paraffin blocks were collected from patients samples previously diagnosed as ovarian tumors, 40 (66.6%) samples were malignant and 17 (33.4%) samples were benign. The histopathological subtype of malignant samples includes 24 (40%) serous adenocarcinoma, 5 (8.3%) mucinous adenocarcinoma, 8 (13.3%) granulosa cell tumors and 3 (5%) non epithelial tumor.

One section of 3µm was cut from each paraffin block, then stained by immunohistochemical method (new indirect technique) for detection of ER expression. The data obtained was analyzed using SPSS program version 16.

The age of studypopulation range between 28 and 70 years with mean age of 49 years. Most of patients were younger than 50 years representing 32(56%) and the remaining 25(44%) were older than 50 years.

ER expression was revealed positive result in 3 (7.5%) samples in malignant tumor while 37 (92.5%) samples showed negative expression, In benign ovarian tumors positive expression was found in 2 (11.7%) samples and 15(88.3%) samples showed negative expression, this result showed insignificant association between ER expression and malignant ovarian tumors (P. value =0.27).

The study conclude that no association between ER expression and types of ovarian tumors.

المستخلص

أجريت هذه الدراسة المستشفوية التحليلية الاسترجاعية الحالة والحالة الضابطة في مستشفى الأمل ومعمل التيسير-2 في ولاية الخرطوم، في الفترة من ابريل 2018 إلى اغسطس 2018 ، هدفت الدراسة للكشف عن مستقبلات الاستروجين في اورام المبيض باستخدام كيمياء الانسجة المناعية .

جمع 57 قالب شمعي من عينات مرضي كانوا مشخصين مسبقا باورام المبيض ، 40 (66.6%) عينة كانت اورام مبيض خبيثة و 17(33.4%) عينة كانت اورام مبيض حميدة. الاورام الخبيثة كانتموزعة كالاتي 24 (40%) من العينات كانت ورم غدي مصلي ،و 5 (8.3%) من العينات ورم غدي مخاطي و 8 (13.3%) من العينات كانت ورم الخلايا الجرثومية ، 3 (5%) من العينات كانت اورام غير الظهارية .

تم قطع مقطع نسيجي واحد من كل عينة بسمك 3 مايكرون من كل قالب شمعي و صبغت بواسطه طرق كيمياء الانسجة المناعية (الطريقة الجديدة غير المباشرة) للكشف عن ظهور مستقبلات الاستروجين في اورام المبيض باستخدام كيمياء الانسجة المناعية .

استخدم برنامج الحزم الاحصائية للعلوم الاجتماعية، النسخة 16 لتحليل البينات .

تراوحت اعمار المرضي ما بين 28 – 70 سنة بمتوسط عمر 49 سنة ، اظهرت الدراسة ان معظم المرضي كانت اعمارهم اقل من 50 سنة، و كان عددهم 32 مريضا بنسبة (56%) و25 مريضا بنسبة (44%) كانت اعمارهم اكثر من 50 سنة .

اظهرت الدراسة ان ظهور مستقبلات الاستروجين كان موجب الظهور في 3 (7.5%) عينات فقط من اورام المبيض الخبيثة و سالب الظهور في 37 (92.5%) المتبقية ،بينما في اورام المبيض الحميدة كان موجب الظهور في عينتين (5.8%) و سالب الظهور في 15 (94.2%) عينة مع عدم وجود علاقة ذات دلالة احصائية بين ظهور مستقبلات الاستروجين واورام المبيض (القيمة الاحتمالية =0.27).

خلصت الدراسة الي انه لا توجد علاقة بين ظهور مستقبلات الاستروجين و نوع اورام المبيض .

List of contents

List of tables

List of Microphotographs

Chapter one

Introduction

Chapter One

Introduction

1.1 Introduction

Ovarian cancer is any malignant tumor that develops in the ovarian tissues; ovarian cancer is commonly classified as epithelial ovarian carcinoma (EOC), ovarian germ cell tumor and sex cord-stromal tumor (Zhang and Zhang, 2016).

Ovarian cancer is one of the most killing gynecological malignancies due to late presentation, poor response to therapy and high recurrent rate (Mohammed, *etal*.2014).

Ovarian cancer is the 8thmost common cancer among women, according to global estimates 225,000 new cases were detected each year and 140,000 people annually die from the disease (Ferlay, *et al*. 2010).

In Sudanese population ovarian cancer was the third most commonly diagnosed cancer among women after breast and cervix (Saeed, *etal*. 2016).

The exact causes of ovarian cancer is still unclear however a number of risk factors for developing ovarian cancer such as early menarche and menopause, null parity, age atmenopause, obesity,hormone replacement treatment during menopause, and ethnicityhave been identified(Dossus, *et al*. 2010;Setiawan, *et al.* 2012).

Most of these risk factors are associated with the changes in levels of sex hormones during women's lifetime. Of these sex hormones, estrogen has an effect on ovarian cell proliferation, which is shown by studies suggesting that women who have postmenopausal hormone replacement therapy (HRT) with estrogen for 10 years or longer have an increased risk of developing ovarian cancer(Vo, *et al*. 2007).

Symptoms of ovarian cancer in early stage disease are often vague and ill–defined. Advanced disease can present with pain in the pelvis, pain in lower stomach, back pain, nausea, weight loss and tiredness (Soumit, *et al*. 2016).

Methods of diagnosis of ovarian cancer are imaging tests (ultrasonography, computed tomography and magnetic resonance imaging), serum tumor biomarker (CA125, HE4), fine needle aspiration (FNA) and immunohistochemistry (IHC) (Melissa, 2015; Soumit, *et al*. 2016).

Treatments for ovarian cancer are surgery, chemotherapy, hormone therapy, targeted therapy (Michelle, *et al*. 2009).

Estrogen receptors are located in the tissues of the female reproductive tract and breast as one would expect, but also in tissues as diverse as bone, brain, liver, colon, skin, and salivary gland. In evolutionary terms, the estrogen receptor is an ancient protein that is expressed in all vertebrates and a few invertebrates(Eyster, *et al*. 2016).

Estrogen acts via two nuclear receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) which are coded from two separate genes, ERα and ERβ, located on chromosomes 6q25.1 and 14q22-24, respectively (Lindgren, *et al*. 2004).

Estrogenreceptors mediate the effects of female steroid hormones on proliferation and apoptosis of ovarian cancer cells (Modugno, *et al*.2012).

The expression of ER was more in malignant tumors than borderline and benign (Sylvia, *et al*. 2012).

1.2 Objective:

To detect estrogen receptor expression in ovarian tumors, byimmunohistochemical method and its correlation with histopathological diagnosis and tumor grades.

Chapter two

Literature review

Chapter Two

LiteratureReview

2.1 Scientific background:

Ovarian cancer is the most lethal gynecologic cancer. Less than one-half of patients survive for more than five years after diagnosis. Ovarian cancer affects women of all ages but is most commonly diagnosed after menopause. More than 75% of affected women are diagnosed at an advanced stage because early-stage disease is usually asymptomatic, the majority of cases are sporadic, and only 5% to 10% of ovarian cancers are familial (Christine, *et al*. 2000, Chyke, *etal*. 2016).

2.2 Structure of the ovary:

The ovaries are paired sex glands or gonads in female and are concerned with germ cell maturation, storage and its release. The ovary is covered by a single layer of cuboidal cells known as germinal epithelium. The substance of the gland consists ofouter cortex which shows the structural changes during ovular cycle. The medulla consists of connective tissue, some unstrapped muscles, blood vessels and nerves.Medulla also has hilus cells (Shivaji and Panchaksharayya, 2016).

The body of the ovary consists of spindle-shaped cell; fine collagen fibers and ground substance which together constitute the ovarian stroma which contain cells resemble fibroblasts and smooth muscle cells. In the peripheral zone of the stroma (cortex) are numerous follicles which contain female gametes in various stages of development.In addition, there may also be post-ovulatory follicles of various kinds, namely corpora lutea (responsible for estrogen and progesterone production), degenerate and former corpora lutea (corpora albicantes) and degenerate (atretic) follicles (Barbara and John, 2000).

2.3 Disorders of the ovary:

2.3.1 Benign disorders:

2.3.1.1 Serous cystadenoma:

Serous cystadenomas are common and account for approximately 25% of benign ovarian neoplasm, bilaterally in 12–23% of cases, ovarian serous cystadenomaarises from the surface epithelium of the ovary depending on the amount of the fibrous tissue and are usually oval, about 3–10 cm in diameter, with a glistening surface and cystic fluid is clear to yellowish (Ahmad, *etal*. 2016).

2.3.1.2 Mucinous cystadenomas:

Mucinous cystadenoma are benign epithelial tumors that are typically multilocular, thin walled cysts with smooth external surface containing mucinous fluid. These are the largest tumors of ovary, of all ovarian tumors, mucinous tumors comprise 12% to 15% and 75% of all mucinous tumours are benign (Humera, *etal*. 2011).

2.3.1.3 Ovarian cystadenofibroma:

Ovarian cystadenofibroma is a relatively rare benign ovarian tumor that contains both epithelial and fibrous stromal components, that is seen in women aged 15–65 years. The routine imaging features of this tumor may mimic a malignant neoplasm, but the presence of the fibrous component often gives a specific/characteristic MRI appearance that may help differentiate it from malignant ovarian tumors (Ashishand Khaled, 2010).

2.3.1.4 Struma ovarii:

Struma ovarii is a very rare and usually benign ovarian tumor. It accounts for 0.3–1% of all ovarian tumors and for 3% of all mature teratomas. Struma ovarii is the most common form of monodermalteratoma and is characterized by the presence of macroscopically and histologically detectable thyroid tissue

containing variablesized follicles with colloid material, strumaovarii presents as a multi-cystic mass with a peak incidence in the 5th decade of life and peak age at presentation of50 years (Martine, *etal*. 2014).

2.3.1.5 Benign cystic teratomas:

Mature cystic teratoma is the most common ovarian neoplasm and affects mostly young patients. It is a benign germ cell tumor consisting of at least two of the three embryogenic germ cell layers, and usually contains ectodermal (skin, brain), mesodermal (fat, bone) and/or endodermal (thyroid tissue, gastrointestinal and bronchial epithelium) mature tissue (Pietro, *etal*. 2016).

2.3.1.6 Benign brenner tumor:

Brenner tumors are composed of epithelial elements histologically resembling urothelium. Brenner tumors comprise less than 5% of all benign epithelial tumors. Most of them are found incidentally and are of less than 2cm in size. These tumors were seen in all age groups and 3.6% cases were seen in the pediatric age group. Microscopically, they show well defined small nests of transitional cells with intervening fibromatousstroma. Nuclei show typical grooved coffee bean appearance (Ghartimagar, *etal*. 2013).

2.3.1.7 Ovarian fibromas andfibrothecomas:

These are benign tumors of stromal origin. Fibromas originate from spindle cells producing collagen and can be associated with ascites. Fibrothecomas originate from both spindle and theca cells and may produce a small amount of estrogens. Fibromas and fibrothecomas can also show cystic areas, due to hemorrhage, edema or necrosis within the stromal tissue; frequently the lesions show little peripheral vascularity(Ahmad, *etal*. 2015).

2.3.2 Malignant disorders:

2.3.2.1 Serous cystadenocarcinoma:

Serous carcinomas are the most common form of ovarian carcinoma and make up 30-70% of all diagnoses; it is classified as low grade or high grade on the basis of the extent of nuclear atypia and mitosis morphologically, low -grade serous carcinoma has minimal nuclear atypia, and mitoses are rare, high -grade serous carcinoma, on the other hand, is characterized by marked nuclear atypia and more mitoses. Low-grade and high-grade carcinomas are different at the genomic and molecular levels (Daniel, *etal*. 2009, Elham, 2013).

2.3.2.2 Mucinous cystadenocarcinoma:

The mucinous cystadenocarcinoma of the ovary accounts for 5-10% of all ovarian mucinous tumors even in adults. The mucinous tumors are filled with mucus -like material, this mucus is produced by mucus-secreting goblet cells very similar to the cells lining normal intestine. These tumors may become verylarge. The cystadenocarcinomas contain a more solid growth pattern with the hallmarks of malignancy cellular atypia and stratification, loss of the normal architecture of the tissue, and necrosis. The appearance can look similar to colonic cancer. The mucinous ovarian cancer sometimes associated with pseudomyxomaperitonei(Jitendra, *etal*. 2015).

2.3.2.3 Clear cell adenocarcinoma:

Ovarian clear-cell adenocarcinoma (CCA) accounts for 10% of all epithelial ovarian cancers. It typically occurs at a younger age and diagnosed at an early-stage disease, clear cell carcinoma has also been associated with endometriosis and displays theFollowing architectural and cytological features papillary, tubulocystic or solid architecture; hobnail tumor cells with clear cytoplasm (Catherine, *et al*. 2015,Lauren, *et al*. 2015).

2.3.2.4 Endometrioid adenocarcinomas:

Ovarian endometrioid carcinoma (ECs) comprises 10–20% of all epithelial ovarian cancer cases; highly resemble endometrioid carcinomas of the uterus in morphology,occur most frequently in women of premenopausal age, and most are found at an early stage . Most ECs are low-grade adenocarcinomas and seem to arise from endometriotic cysts, up to 42% of cases have evidence of endometriosis (Prat,*et al*. 2012).

2.3.2.5 Malignant brennertumors:

Malignant Brenner tumors are very rare; they are large, friable tumors, partly cystic, which show histologically continuity between benign mesonephric-type nests and malignant epithelial tumor tissue that is of transitional, squamous, or glandular type (Albert, *et al*. 2003).

2.3.2.6 Malignant germ cell tumors:

Ovarian malignant germ cell tumors (OMGCTs) are heterogeneous tumors that are derived from the primitive germ cells of the embryonic gonad. OMGCTs are rare, accounting for about 2.6% of all ovarian malignancies, and typically manifest in adolescence. There are different histological subtypes of OMGCTs as:Dysgerminoma, immature teratomas, yolk sac tumors andembryonal carcinomas. Malignant ovarian germ cell tumors spread in the same manner as epithelial ovarian neoplasms but are more likely to involve regional lymph nodes. Suspicious areas may be sampled during surgery. Because OMGCTs are almost always unilateral and are chemosensitive, fertility-sparing surgery is the standard of care (Shaaban, *etal*. 2014).

2.3.2.7 Sex cord stromal tumor:

Sex cord stromal tumors of the ovary are rare, making up approximately 8% of all ovarian neoplasms derived from the sex cords and specialized stroma of the developing gonad. Stromal tumors may arise from female-type

(granulosa, theca) and male-type cells (Sertoli, Leydig) as well as other indifferent sex cord derivatives. Sex cord stromal tumors of the ovary affect all age groups and account for most of the hormonally active ovarian tumors that show estrogenic effects or virilization. The most common stromal tumor is the granulosa cell tumor (Seung, *etal*. 2005, Melissa and Daniel, 2010).

2.4 Epidemiology of ovarian cancer:

Ovarian cancer is the eighth most common cancer among women, and it includes about 4% of all women's cancers this disease has high morbidity and mortality rates among cancers of the reproductive system according to global estimates 225,000 new cases were detected each year, and 140,000 people annually die from the disease (Ferlay, *etal*. 2010).

In a more recent data set (2009-2010) from the national cancer registry for Khartoum state alone, ovarian cancer was the fourth most common cancer in women, with an estimated incidence rate of 188 per 100,000 populations, a gender specific rate of 8.0 per 100,000 population, and an age-standardized rate (ASR) of 7.0 per 100,000 population furthermore, neither the morality rate for ovarian cancer nor the survival rate in Sudan has previously been described, the majority of patients presenting with advanced stage disease were not thoroughly investigated or treated symptomatically(Saeed, *et al*. 2014).

2.5 Risk factors of ovarian cancer:

2.5.1 Age:

The risk for ovarian cancer increases with age. The majority of patients are postmenopausal, with 80% of cases diagnosed being older than 50 years, and a peak incidence of 61.8 per 100,000 women is observed in the 60-64 year old age group(Jermaine, *et al*. 2015).

2.5.2 Family history:

The greatest risk factors of ovarian cancer are a family history and associated genetic syndromes, women with a strong family history of breast and/or ovarian cancer are a high-risk group who may carry a mutation of the BRCA1 and BRCA2 genes. These women have a risk of ovarian malignancy of up to 50% (Cleola and Michael, 2003).

2.5.3 Hormone replacementtherapy:

Application of hormone replacement therapy (HRT) was found to be a risk factor for ovarian cancer. An approximately 22% increased risk of ovarian cancer over 5 years was seen in postmenopausal women using unopposed estrogen as HRT. The risk was still significantly increased (by approximately 10%) by the application of a combination of estrogen and progestin, the incidence of ovarian cancer increased with longer duration of HRT therapy (Felicitas and Theresia,*etal*. 2014).

2.5.4 Obesity:

Increased body weight was associated with an increased risk of ovarian cancer; particular, in the premenopausal period (Liu, *et al*. 2015).

2.5.5 Chronic inflammation:

Chronic inflammation has been proposed as the possible causal mechanism that explains the observed association between certain risk factors, such as the use of talcum powder in the pelvic region and epithelial ovarian cancer, other factors potentially associated with ovarian inflammation (pelvic inflammatory disease, human papilloma virus infection) found an increased risk of endometrioid and clear cell ovarian cancer only among women with a history of endometriosis (Merritt,*et al*. 2008).

2.5.6 Dietary factor:

Epidemiologic studies have suggested that some dietary factors may play a role in the etiology of ovarian cancer, suggested that ovarian cancer risk was positively associated with higher consumption of dietary cholesterol and eggs and inversely associated with higher intake of total vegetables and cruciferous vegetables and supplementation of vitamin E (Pan, *et al*. 2004).

2.5.7 Smoking:

Cigarette smoking regarding as a potential risk factor for ovarian cancer ,The strongest association appears to be with mucinous ovarian tumors, while the association with other histological types is less certain (Mette, *et al*. 2013).

2.5.8 Parity:

Parity refers to number of births in women lifetime. Parous women have a lower risk for ovarian cancer than nulliparous women. Women with term pregnancies versus failed pregnancies have lower odds ratios, with a risk reduction of about 14% for each subsequent pregnancy after the first. Data suggest a 40% decrease in risk for epithelial ovarian cancer with the first birth. This data support theories that suggest that the hormonal changes associated with pregnancy provide a respite from continuous ovarian exposure to estrogen, known mitogen. (Pike, *et al*. 2004)

2.6 Diagnosis of ovarian cancer:

2.6.1 Imaging tests:

2.6.1.1 Ultrasonography:

Transvaginal ultrasonography (TVS) has been shown to be the most effective means to screen for ovarian cancer. TVS is accurate in detecting abnormalities in ovarian volume and morphology, but is less reliable in differentiating benign

from malignant ovarian tumors, TVS is sensitive, but has a low positive predictive value (John and John, *et al*. 2014).

2.6.1.2 Computedtomography (CT):

Is the preferred technique in the pretreatment evaluation of ovarian cancer to define the extent of disease and assess the likelihood of optimal surgical cytoreduction. CT has been shown to predict suboptimal cytoreduction with sensitivity of 79% and specificity of 75%. However, accuracy varies considerably among institutions, CT is also particularly helpful in the identification of fat components in mature cystic teratomas (Veena and Susanna,*et al*. 2010).

2.6.1.3 Magnetic resonance imaging (MRI):

MRI is an essential problem-solving tool to determine the site of origin of a pelvic mass, and is effective for the diagnosis and accurate characterization of a wide spectrum of ovarian masses as non-neoplastic or neoplastic (Pietro, *et al*. 2016).

2.6.2 Fine needle aspiration cytology (FNAC):

Image guided FNAC from ovarian lesions are being increasingly used for diagnosis of ovarian lesions. FNAC can reliably distinguish between benign and malignant ovarian lesions. FNAC helps in reaching an early diagnosis in advanced ovarian cancers (Soumit, *et al*. 2016).

2.6.3 Serum tumor biomarker:

2.6.3.1 Cancer antigen 125 (CA125):

Cancer antigen 125 (CA-125) is a high molecular weight glycoprotein that is expressed by a large proportion of epithelial ovarian cancers. Elevated CA 125 values most often are associated with epithelial ovarian cancer, so CA-125 has become well established as a tumor marker for epithelial ovarian cancer. However, the sensitivity and specificity of CA-125 is known

tobe poor. It is only raised in approximately 50% of stage I epithelial ovarian cancers and in 75% to 90% of patients with advanced disease and false-positive results have been noted in many medical disorders, both malignant and benign (Jose, *et al*. 2011).

2.6.3.2 Human epididymisprotein 4 (HE4):

HE4 is a glycoprotein secreted by müllerianepithelia of the female reproductive tract as well as male epididymis, its role as a potential biomarker for ovarian cancer and HE4 may serve as a useful prognostic biomarker for ovarian. Elevated levels ofHE4 are associated with increases stage, grade, and is strongly expressed by the most common ovarian tumor subtypes (serous and endometrioid). HE4 is both more sensitive and specific than CA-125 in detecting early-stage ovarian cancer and is not associated with benign conditions to the same degree, enabling HE4 to distinguish malignant ovarian tumors from benign cystic lesions (Alison and Ronny, 2010,Archana, *et al*. 2013).

2.6.4 Immunohistochemistry:

Immunohistochemistry (IHC), which are useful in the diagnosis of ovarian tumors (mostly neoplasms but also a few tumor-like lesions), each of the three main categories of ovarian tumors has distinctive immunohistochemical features and stains can be used to suggest or confirm a diagnosis. IHC is often useful to differentiate between primary ovarian adenocarcinoma and metastatic adenocarcinomas specially those of colorectal origin (Divya and Mandakini,*etal*. 2013).

2.7 Treatment of ovarian cancer:

2.7.1 Surgery:

Surgery is the primary treatment for ovarian cancer. It is used for staging and cytoreduction (debulking), surgical staging involves total abdominal

hysterectomy bilateral salpingo-oophorectomy (TAHBSO), and the removal of pelvic and para-aorticlymph nodes and omentum, as well as other supplemental procedures,unilateral salpingo-oophorectomy, preserving the uterus and contralateral ovary, is an option for women with early stage invasive epithelial ovarian cancers, lesions with low potential for malignancy (lesions with histologically abnormal cells that are judged to have a low likelihood of developing into cancer), germ cell tumors, or sex cord–stromal tumors (Michelle, *et al*. 2009, Chyke, *et al*. 2016).

2.7.2 Chemotherapy:

Chemotherapy is the main standard adjuvant treatment for ovarian carcinoma, chemotherapy-based platinum and paclitaxel is currently considered the standard treatment after surgical staging and resection of abdominal and pelvic disease.A high proportion of patients (60–80%) with advanced ovarian epithelial cancer respond to first-line chemotherapy, but most of these patients (about 70%) will later have disease progression and thus be candidates for second-line chemotherapy (Alberto, *et al*. 2010).

2.7.3 Targeted therapy:

The use of targeted biologic agents is the effective treatment of recurrent ovarian cancer, a variety of molecular targeted agents, the majority of which are monoclonal antibodies and small-molecule protein-kinase inhibitors, have been explored in the management of ovarian cancer. The antiangiogenic agent, bevacizumab, has been reported as the most effective targeted agent that target the vascular endothelial growth factor (VEGF) pathway (Hiroaki, 2010).

2.7.4 Hormone therapy:

Hormone therapy is classified under other drugs that are potentially effective as approved treatment for recurrent forms of epithelial ovarian cancer, which include tamoxifen (antiestrogen) (Yoshihito and Hideki, 2013).

2.8 Estrogen receptor and it relation with ovarian tumors:

Estrogen acts via two nuclear receptors, estrogen receptor alpha (ERα) and estrogen receptor beta (ERβ) which are coded from two separate genes, ERα and ERβ, located on chromosomes 6q25.1 and 14q22-24, respectively (Lindgre,*etal*. 2004). Another study has revealed lower levels of ERβin ovarian epithelial primary tumors, and only ERαin metastatic tumors (Rutheford, et al. 2000).

Steroid hormones are thought to play an important role in the process of carcinogenesis in ovarian carcinoma. Estrogen may contribute to initiation and/or promotion of ovarian carcinogenesis. It is thus logical to speculate that the overexpression of ER should be associated with a poor prognosis (Munstedt, *et al*. 2000) .

Substantially, many studies have demonstrated that ERβ is highly represented in normal epithelial ovarian cells or benign tumors (Cunat, *et al*. 2004, Brandenberger, *et al*. 1998), whereas ERαis the main form expressed in malignant tumors (Cunat, *et al*. 2004).

Chapter three

Materials and methods

Chapter three

Materials and Methods

3.1 Materials:

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

3.2 Methods:

3.2.1 Study design:

This is analytical retrospective case controlhospital basedstudy aimed to detect estrogen receptor expression in ovarian tumors.

3.2.2 Study sample:

Fifty seven paraffin blocks were collected from patients samples previously diagnosed as ovarian tumors, 40 (70%) of them were malignant and the remaining 17 (30%) were benign. Patient's identificationinformation and histopathological results were obtained from patient's records.

3.2.3 Study area:

This study conducted atAlamal Hospital and Altayseer-2 laboratory in Khartoum state.

3.2.4 Sample processing:

Section was cut at 3µm thickness by rotary microtome, mounted in positively charged slides.

3.2.5 Immunohistochemical staining:

Immunohistochemical staining was carried out using new indirect dextran polymer immune peroxidase technique. Tissue sections were deparaffinized in xylene and rehydrated through graded alcohol (100%, 90%, 70%, and 50%) to water. The antigens were retrieved using Dako water path with tris EDTA buffer (pH 9) for 5 minutes and then cooled down to room temperature for 20 min. Endogenous peroxidase activity was blocked by 3% peroxidase blocker

for 10 minutes. The slide then treated with anti-estrogen primary antibody for 20 min at room temperature in a humidity chamber, and then washed in phosphate buffer saline (pH 7.4) for 3 minutes. Then incubated in dextran polymer-HRB (horseradish peroxidase) secondary antibody for 15 minutes then washed in three changes of phosphate buffer saline, after that incubated in 3, 3 diaminobenzidinetetrahydrochloride substrate solution for 5 minutes, then washed in running water. Then counter stained in Mayer's haematoxylin stain for one minute. After that dehydrated, cleared and mounted in DPX mounting media (Bancroft, *et al*. 2013).

3.2.6 Result interpretation:

All quality control measures were adopted. A neg ative control slide was completed by omission of the primary antibodies. A known positive estrogen receptor section obtained from breast cancer block used as positive control during immunohistochemistry staining. Positive staining for estrogen receptor appeared as brown particles at the nucleus using X40 lens.Under microscopy, detection of more than 5 cells per one field considered positive result.

3.2.7 Data analysis:

Data was analyzed using SPSS 16 computer program. Frequency, mean, and chi-square test values were calculated.

3.2.8 Ethical consideration:

Samples were collected after taking ethical approval from Alamal Hospital and Altayseer-2 laboratory to use the tissue blocks for research purposes.

as

Chapter Four

Results

Chapter four

4. Results

The study includes 57 samples, 40(70%) samples were malignant tumors and 17(30%) samples were benign tumors.

The age of study population range between 28 and 70 years with mean age of 49 years. Most patients were younger than 50 years representing 32(56%) and the remaining 25(44%) were older than 50 years as indicated in table (4.1).

The histopathological diagnosis of study samples includes 24(40%) serous adenocarcinoma, 5(8.3%) mucinous adenocarcinoma, 8 (13.3%) granulosa cell tumors, 3(5%) non epithelial tumor and 17(33.4%) benign tumor as showed in table (4.2).

The tumor grade of study samples revealed 13(35%) sample were grade I, 14(38%) sample were grade II, and 10(27%) sample were grade III,as showed in table (4.3).

ER positive expression was found in 3/40(7.5%) samples of malignant tumors and 37/40(92.5%) samples showed negative expression,while in benign ovarian tumors positive expression was found in 2/17(5.8%) samples and 15/17(94.2%) samples showed negative expression for ER. This result revealed in significant association (P .value = 0.27), as indicated in table (4.4).

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

Age group	Frequency	Percentage
Less than or equal 50 years	32	56%
More than 50 years	25	44%
Total	57	100%

Table (4.2): Frequency of histopathological diagnosis among the study samples:

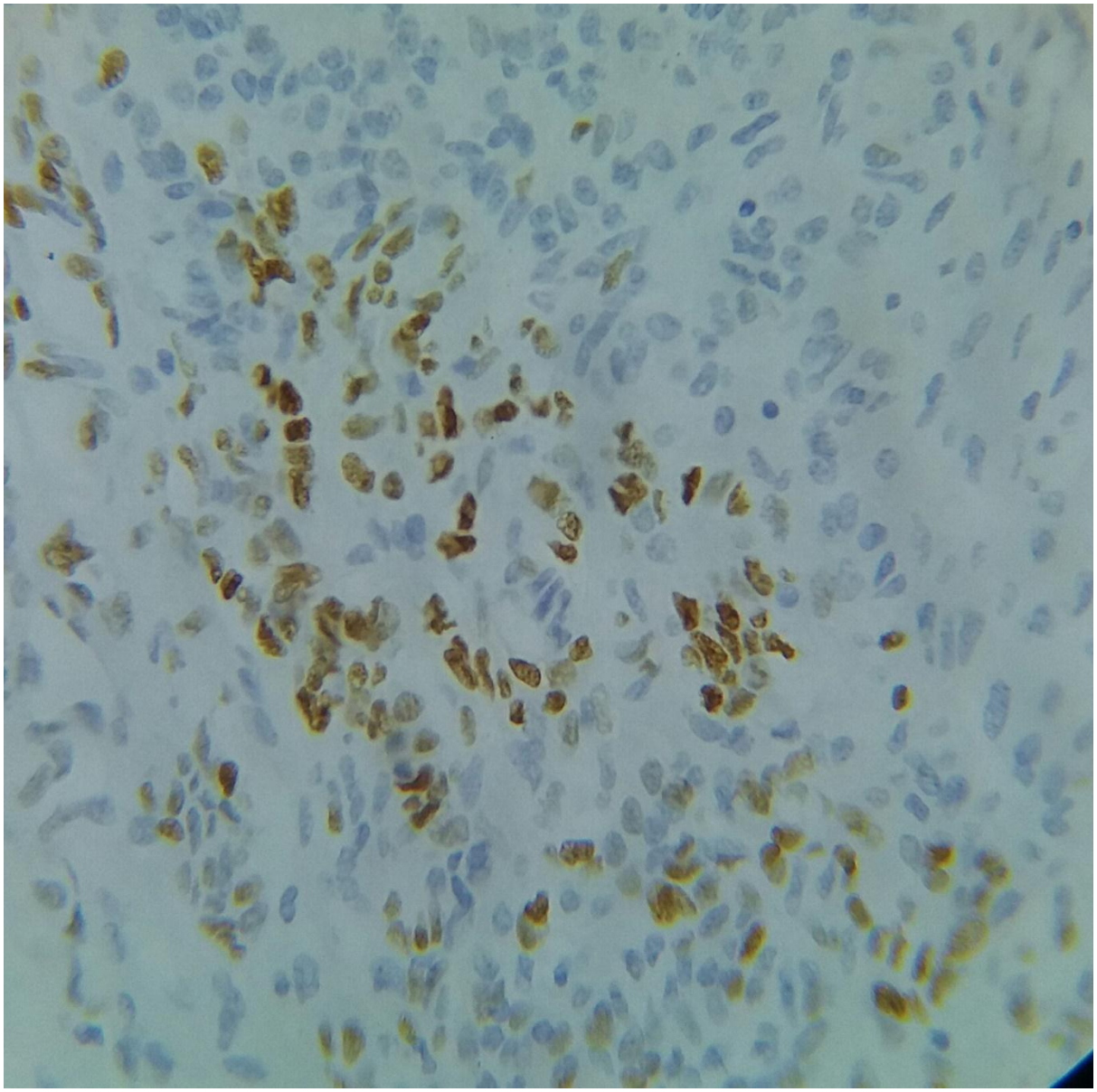
Histological diagnosis	Frequency	Percentage
Serous adenocarcinoma	24	40%
Mucinous adenocarcinoma	5	8.3%
Granulose cell tumor	8	13.3%
Non epithelial tumor	3	5%
Benign tumor	17	33.4%
Total	57	100%

Table (4.3): Distribution of malignant tumor grades:

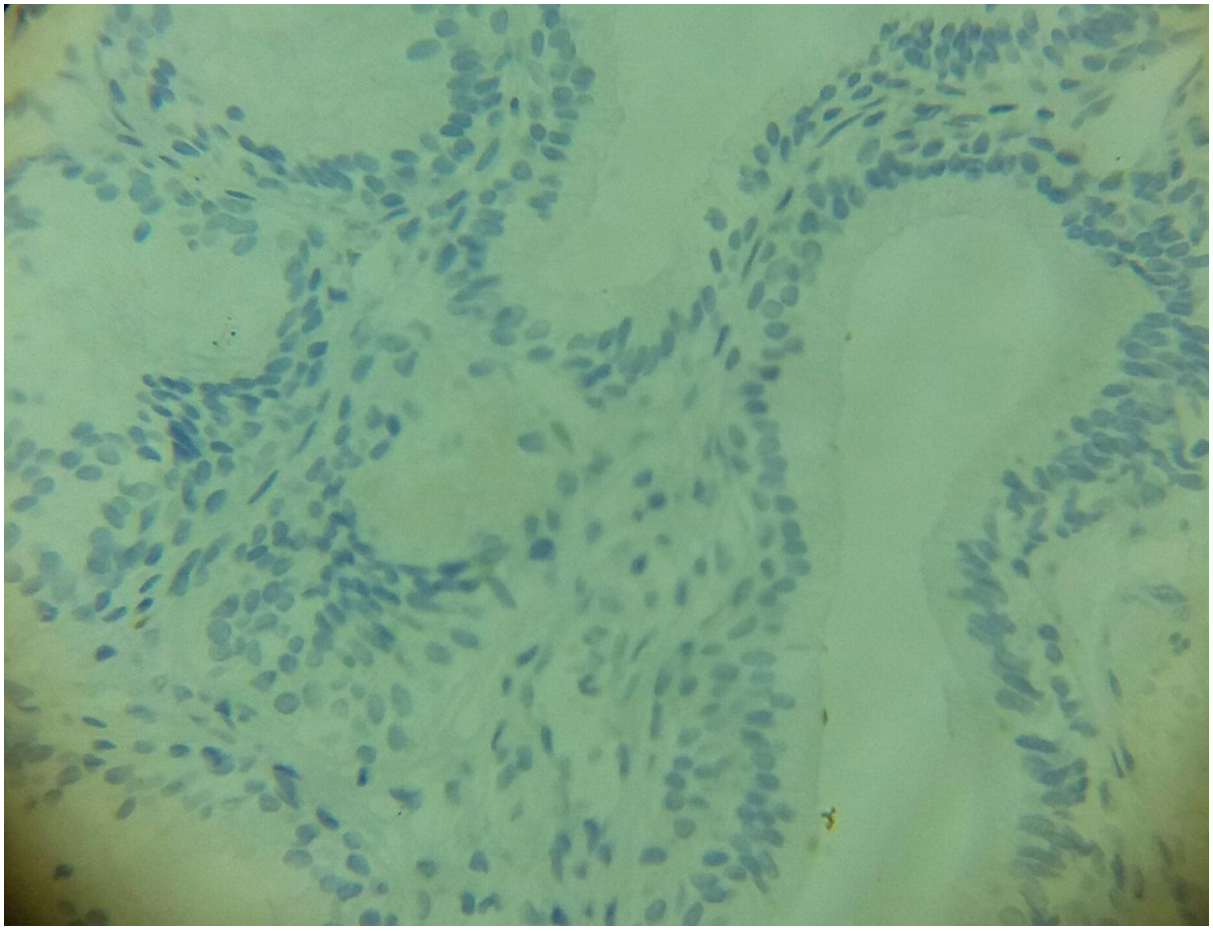
Grade	Frequency	Percentage
GI	13	35%
GII	14	38%
GIII	10	27%
Total	37	100%

Table (4.4): Relation between the expression of ER and histopathological diagnosis of ovarian tumor:

Histological Type	ER expression		total	P. value
	Positive	Negative		
Malignant	3(7.5%)	37(92.5%)	40(100%)	0.27
Benign	2(5.8%)	15(94.2%)	17(100%)	
Total	5	52	57	



Microphotograph (4.1): Serous adenocarcinoma showed positive expression of ERin the nucleus (40X).



Microphotograph(4.2): Serous adenocarcinoma showed negative expression of ER in the nucleus (40X).

Chapter Five

Discussion, conclusion and recommendations

Chapter Five

5. Discussion, Conclusion and Recommendations

5.1 Discussion

The presentstudy include 57 samples of ovarian lesions stained by immunohistochemistry for detection of Estrogen Receptor. Concerning the age group of the study population, the study revealed that most of patients were less than 50 years indicating that women less than 50 years are more affected with ovarian tumor. This result is compatible with Mohammed, *et al*. (2014), who reported that involved age by ovarian carcinoma was the age group of 30-40 years. While disagree with Jermaine *et al*. (2015), who reported that risk of developing ovarian cancer increases with age. Also disagree with Dominic, *et al*. (2013), who reported that 80% of all ovarian cancer cases are diagnosed after the age of 50 years.

The histopathological diagnosis of the study samples revealed that more frequent type of ovarian cancer was epithelial ovarian cancer. This result is compatible with Robert, *et al*. (2011), who reported that epithelial ovarian cancer comprises most malignant ovarian neoplasms. Also agree with Colombo, *et al*. (2010), who reported that about 90% of primary malignant ovarian tumors are epithelial carcinomas.

ER expression Positive expression of ERwas found in 7.5% of malignant condition compared to 5.8% in benign condition with (p=0.27) which suggest that the ER expression was in significant associated between ER expression and histological diagnosis. This result is agree with Halon, *et al*. (2011), who reported that expression of ERα in tissue specimens did not reveal any correlations between histopathologicalparameters, such as histologictype of tumors and ovarian cancer grading. Also disagree with Sylvia , *et al*.

(2012),who reported that expression of ERα was more in malignant than border line and benign. Which give significant result, Also disagree with Sieh, *et al*. (2013), who reported that estrogen receptoralpha (ERα)isexpressedin approximately 70% of the epithelial ovarian cancer patients andpresents a potential drug target for these tumors.

5.2 Conclusion:

From this study we concluded that:

The age of ovarian cancer patients in our samples is commonly less than 50 years.

Most histological type of ovarian cancer is epithelial ovarian cancer.

No association betweenER expression and ovarian tumor.

5.3 Recommendations:

From this study we recommended that:

Further study should be done for expression of ER in ovarian tumors with large sample size.

Carry out further study to assess the expression of ER isoform (alpha & beta) in ovarian tumor and analyze it association with histologic type of tumors and ovarian cancer grading.

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Appendices

Appendices

Appendix 1:

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

- Disposable gloves.
- Rotary microtome
- Microtome knives.
- Positively charged slides (thermo).
- Cover glasses.
- Dry oven.
- Water path (Dako water path)
- Coplin jars.
- Humidity chamber.
- Ethanol (100%, 90%, 70%, 50%)
- Xylene.
- Mayers haematoxylin
- (Haematoxylin, DW, K or ammonium alum, sodium iodate, citric acid, chloral hydrate).
- Tris EDTA buffer (PH 9).
- Phosphate buffer saline (PH 7.4).
- Peroxidase blocker
- (0.3% hydrogen peroxide in methanol).
- Primary antibody (anti- human β- catenin).
- Secondary antibody (dextran polymer conjugated secondary antibodyHRP).
- DAB (3, 3 diaminobenzidinetetrahydrochloride) substrate solution.
- DPX

CE IVD

Rabbit anti-Estrogen Receptor

Cat. No.: BRB053 (16 ml Ready-to-use)

Instructions for use

Intended Use
This antibody is designed for the specific localization of Estrogen Receptor antigen in formalin-fixed, paraffin-embedded tissue sections.
Anti-Estrogen receptor antibody is intended for in vitro diagnostic use.

Specifications

Specificity:	Human Estrogen Receptor (alpha)
Immunogen:	Synthetic peptide according to the C-terminal of human estrogen receptor
Clone:	SP1
Isotype:	Rabbit IgG
Species reactivity:	Human +, pig (predicted because of sequence homology), others not tested

Summary and explanation
The antibody recognises a 67 kDa protein which is known as estrogen receptor (ER) alpha.
The determination of steroid hormone receptors is of great importance for therapy of patients with hormone receptor expressing tumours. Estrogen and progesterone receptor positive breast carcinoma patients have demonstrated a better response to endocrine therapy than receptor negative patients.

Reagent provided
Rabbit monoclonal antibody in TBS with carrier protein and preservative for stabilisation in the following format:
Ready-to-use: 16 ml (Cat. No. BRB053)

Dilution of primary antibody
None

Storage and handling
The antibody should be stored at 2-8°C without further dilution.
If necessary, dilutions of the antibody should be done with a suitable antibody dilution buffer (e.g. ZUC025 from Zytomed Systems). The diluted antibody should be stored at 2-8°C after use. Stability of this working solution depends on various parameters and has to be confirmed by appropriate controls.
The antibody provided is stable until the expiry date indicated on the label, if stored at 2-8°C. Do not use product after the expiry date. Positive and negative controls should be run simultaneously with all specimens. If unexpected staining is observed which cannot be explained by variations in laboratory procedures and a problem with the antibody is suspected, contact Zytomed Systems' technical support or your local distributor.

Precautions
Use through qualified personnel only. Wear protective clothing to avoid contact of reagents and specimens with eye, skin and mucous membranes. If reagents or specimens come in contact with sensitive area, wash with large amounts of water.
Microbial contamination of the reagent must be avoided, since otherwise non-specific staining may occur.
ProClin300 and sodium azide (NaN_3) are used for stabilisation. Reaction of sodium azide with lead or copper in drainage pipes can result in the formation of highly explosive metallic azides. Discard the antibody solution in a large volume of running water to avoid formation of deposits. A material safety data sheet (MSDS) for the pure substances is available upon request.

Staining procedure

Refer to the following table for conditions specifically recommended for this antibody. Also refer to detection system data sheets for guidance on specific staining protocols or other requirements.

Parameters	Zytomed Systems recommendations
*Pre-treatment:	Heat Induced Epitope Retrieval (for example in Citrate buffer pH 6.0 ZUC028)
*Control tissue	Breast carcinoma
*Working dilution	None
*Incubation time	30 - 60 minutes

Quality control

The recommended positive control tissue for this antibody is a breast carcinoma. Please refer to the instructions of the detection system for guidance on general quality control procedures.

Troubleshooting

If you observe unusual staining or other deviations from the expected results please read these instructions carefully, refer to the instructions of the detection system for relevant information or contact your local distributor.

Expected results

The antibody stains positive in nuclei of Estrogen Receptor positive cells in formalin-fixed, paraffin-embedded tissue. Further details about the expression pattern of ER can be found in the chapter 'Summary and Description'. The interpretation of the results is solely the responsibility of the user. Any experimental result should be confirmed by a medically established diagnostic procedure.

Limitations of the Procedure

Immunohistochemistry is a complex technique involving both histological and immunological detection methods Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (Nadji and Morales, 1983). Endogenous peroxidase, alkaline phosphatase or biotin may cause non-specific staining depending on the detection system used. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horse radish peroxidase) detection systems (Omata *et al*, 1980). Inadequate counterstaining and mounting can influence the interpretation of the results.

Zytomed Systems warrants that the product will meet all requirements described from its shipping date until the expiry date is reached, if the product is stored and utilised as recommended. No additional guarantees can be given. Under no circumstances shall Zytomed System be liable for any damages arising out of the use of the reagent provided.

Performance characteristics

Zytomed Systems has conducted studies to evaluate the performance of the antibody utilising a standard detection system. The product has been found to be sensitive and specific to the antigen of interest with minimal or no cross-reactivity.

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Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983

October 26, 2015 Rev: A1015 Doc: DBE_BRB053

Explanations of the symbols on the product label:

REF	Bestellnummer Catalog Number Reference du catalogue		Verwendbar bis Use By Utiliser jusque		Gebrauchsanweisung beachten Consult Instructions for use Consulter les instructions d'utilisation
LOT	Chargenbezeichnung Batch Code Code du lot		Lagerungstemperatur Temperature Limitation Limites de température	RUO	Nur für Forschungszwecke For Research Use Only Pour la recherche uniquement
IVD	In vitro Diagnostikum In Vitro Diagnostic Medical Device Dispositif médical de diagnostic in vitro		Achtung Warning Attention		Hersteller / Manufacturer / Fabricant Zytomed Systems GmbH • Anhaltinerstraße 16 14163 Berlin, Germany • Tel: (+49) 30-804 984 990 www.zytomed-systems.com