Chapter one

Introduction

1.1 Introduction

Ovarian cancer (OC) is defined as any malignant tumor that develops in the ovarian tissues (Zhang and Zhang, 2016). OC is uncommon and often advanced at the time of diagnosis and has a poor prognosis (Nomura, *et al*. 2017).

Worldwide, in 2015 about 251000 women developed OC, it caused 161000 deaths, and the probability of women to developed OC between birth and age 79 years about one in 130 at the global level (Fitzmaurice, *et al*. 2017).

In Sudan OC is the third most commonly diagnosed cancer among adult female after breast and cervical cancer (Saeed, *et al*. 2016).

The risk factors of OC include age, hormonal and reproductive factors, benign gynecologic conditions, hormone replacement therapy, obesity, family history, cigarette smoking and alcohol consumption (Reid, *et al*. 2017).

Methods of diagnosis of OC are transvaginal ultrasonography, computed tomography, magnetic resonance imaging, positron emission tomography, serum tumor biomarker (CA125 and HE4) and biopsy (Tewari and Monk, 2015, Dey, *et al*. 2016).

The treatment options for OC include surgery, chemotherapy, radiation therapy, hormonal therapy, targeted therapy and immunotherapy (Berek and Hacker, 2015).

Progesterone receptor (PR) is a member of the family of nuclear hormone receptor, it has been detected in variety of tissues, and there are three isoforms

of nuclear PRs are known, namely PR-A, PR-B and PR- C (Gadkar-Sable, *et al*. 2005).

PR expression showed protective role in survival of patients, its expression was higher in borderline, serous tumors as compared to malignant and mucinous tumors (Verma, *et al*. 2018). Also previous study demonstrated that the expression of PR is associated with favorable prognostic parameters such as early stage, histologic type and low tumor grade (Ayadi, *et al*. 2010). Data regarding the prognostic significance of PR expression in ovarian carcinoma among Sudanese female are unavailable.

1.2 Objectives

To detect PR expression in ovarian tumors by immunohistochemical method and its correlation with histopathological diagnosis.

Chapter Two

Literature Review

2.1 Scientific background

Ovarian cancer is the one of the most lethal gynecologic malignancies due to its advanced stage at the moment of diagnosis, OC presents with the highest mortality rate. About 90% are epithelial ovarian cancer (EOC), and unfortunately, 70% are late diagnosed with widespread metastasis (Ghuffa, *et al*. 2016).

2.2 Anatomy of the ovary

The ovaries are paired structures lying in the right and left iliac fossae, on either side of the uterus attached to the posterior aspect of the broad ligaments, typically the ovary is ovoid in shape. In the reproductive age group, each ovary measures about 3 cm long, 1.5 cm wide, and 1 cm thick, it contains an outer cortex and an inner medulla (Allen and Cameron, 2017).

The medulla is a core of fibrous connective tissue occupied by the principal arteries and veins of the ovary. The cortex is the site of the ovarian follicles, each of which consists of one developing ovum surrounded by numerous small follicular cells (Saladin, *et al*. 2017).

2.3 Diseases of the ovary

2.3.1 Benign tumors of the ovary

2.3.1.1 Serous cystadenoma

Benign serous tumors are bilateral in 10% to 20% of cases. Most are either partially or completely cystic. The glands, cysts, and broad-based papillae of benign serous tumors are lined with a mixture of secretory and ciliated cells similar to what is seen in the fallopian tube with rare mitotic figures. In some adeno the epithelium is cuboidal and without cilia, resembling the ovarian surface epithelium (Howitt, *et al*. 2017).

2.3.1.2 Mucinous cystadenoma

Almost 80% of all mucinous ovarian neoplasms are benign unilocular or multilocular cystadenomas. Benign mucinous tumors are typically unilateral, and can reach 30 cm or more in diameter. Microscopically, the tumors are composed of a columnar epithelial lining with abundant pale staining intracellular mucin. Goblet cells are uncommon in benign mucinous tumors (Kong, *et al*. 2015).

2.3.1.3 Benign endometrioid tumor

Benign endometrioid tumors are rare, accounting for fewer than 1% of all benign ovarian tumors, endometrioid tumor contain variably shaped glands and cysts lined predominantly by stratified columnar or cuboidal epithelial cells resembling endometrial cells. A minority of cells may have ciliated or mucinous cytoplasm (Howitt, *et al*. 2017).

2.3.1.4 Benign brenner tumor

The most common type of ovarian transitional cell tumor, they are often microscopic or incidental findings discovered at laparotomy for unrelated pelvic conditions. They are typically solid, unilateral tumors with small cysts on cut section (Kong, *et al*. 2015).

2.3.2 Malignant tumors of the ovary

2.3.2.1 Serous cystadenocarcinoma

Represent the vast majority of primary ovarian malignant tumors (75-80%) this carcinoma is bilateral in 60% of cases, characterized by very large cystic and solid tumor with frequent areas of hemorrhage and necrosis (Shisheboran, 2016).

2.3.2.2 Mucinous cystadenocarcinoma

Its account for 5-10% of epithelial ovarian malignancies, upon gross inspection, these tumors are typically large, unilocular, or multilocular cysts filled with mucoid liquid that becomes gelatinous at room temperature. The mean size at

diagnosis is 18 cm, but these tumors can be massive and fill the abdomen and pelvis (Brown and Frumovitz, 2017).

2.3.2.3 Ovarian endometrioid carcinoma (OEC)

Its bilateral in 28% of cases and coexist with nonmalignant lesions such as endometriosis or even endometrioid carcinoma of the endometrium. It has a mean size of 15 cm with mostly a smooth outer surface. Microscopically OEC shares the morphological features of its endometrial counterpart (Cock, *et al*. 2017).

2.3.2.4 Clear cell carcinoma (CCC)

A unique entity of adenocarcinoma of the ovary, the tumor cell of CCC resembled renal cell carcinoma. Most of CCC tumors are unilateral and the pathological characterized by clear cells growing in solid/tubular or glandular patterns as well as hobnail cells (Takano, *et al*. 2017).

2.3.2.5 Malignant brenner tumor

Malignant transitional cell tumors with benign or atypical proliferating transitional elements are designated as malignant Brenner tumors. Arise through metaplasia of the ovarian surface epithelium and are analogous to Walthard nests, which are transitional type epithelial inclusions occurring beneath the serosa of the fallopian tubes and in the hilar regions of the ovaries (Kong, *et al*. 2015).

2.3.2.6 Malignant ovarian germ cell tumors (GCTs)

Non-epithelial malignancies of the ovary account for around 10% of all ovarian malignancies, these cancers account for 58% of all ovarian tumors diagnosed in patients younger than 20 years, there is different histological subtypes for GCTs as: primitive germ cell tumors, dysgerminoma, yolk sac tumor, embryonal carcinoma, and mature teratoma (Alifrangis and Seck, 2017).

2.3.2.7 Ovarian sex cord tumors

Non-epithelial malignancies develop from the dividing cell population surrounding the oocytes, including the cells that produce ovarian hormones. Constitute a heterogeneous group of tumors and according to the World Health Organization, they are classified into: pure stromal tumors, granulosa cell tumor and Sertoli-Leydig cell tumors (Garbi, *et al*. 2017).

2.4 Epidemiology of ovarian cancer

OC ranks among the top ten most commonly diagnosed cancers and top five deadliest cancers in most countries. In 2015, OC was present in 1.2 million women and resulted in 161,100 deaths worldwide. And in the twenty-first century, a woman's overall lifetime risk of developing OC is around 1.6%, and her chance of dying of the disease is 1 in 100 (You, *et al*. 2018).

The incidence rate of OC in the entire Sudan has yet to be identified; however, in a hospital-based data set from the radiation and isotopes center in Khartoum (RICK), collected between 2009 and 2013, OC ranked the 9th most common cancer for both genders and the third most commonly diagnosed cancer among adult female (Saeed, *et al*. 2016).

2.5 Risk factors of ovarian cancer

2.5.1 Age:

The risk for OC 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 years old age group (Jermaine, *et al*. 2015).

2.5.2 Hormonal and reproductive factors

Hormones such as estrogen and androgens may stimulate ovarian cancer formation whereas progestins are protective. The number of ovulatory cycles increases the rate of cellular division associated with the repair of the surface

epithelium after each ovulation, thereby increasing the likelihood of spontaneous mutations that might promote carcinogenesis, also failure of the apoptosis of the granulose and theca cells after ovulation which continued producing steroid hormones, thereby stimulating the formation of cancer (Montes, *et al*. 2012).

2.5.3 Benign gynecologic conditions

Several gynecologic conditions have been examined as a risk factors for OC, including polycystic ovarian syndrome, endometriosis, and pelvic inflammatory disease, also inflammation occurring from vaginal or uterine contaminants that reach the ovary as talcum powder, epidemiological studies have indicated an association with talc use and increased OC risk (Reid, *et al*. 2017).

2.5.4 Hormone replacement therapy (HRT)

Some studies suggest that women using HRT after menopause have an increased risk of developing OC. The risk seems to be higher in women taking estrogen alone (without progesterone) for many years (at least 5 or 10), the increased risk is less certain for women taking both estrogen and progesterone (Masaka, *et al*. 2014).

2.5.5 Obesity

Possible biological mechanisms linking obesity with OC risk and progression include insulin resistance and hyperinsulinaemia, increased levels of circulating growth factors, chronic inflammation, and altered levels of sex hormones (Jochem, *et al*. 2018).

2.5.6 Family history

There are a number of inherited genetic risk factors emerging for OC. Most hereditary cancers are due to BRCA1 and BRCA2 mutations, less commonly, hereditary OC occurs due to the Lynch syndrome or hereditary non-polyposis colorectal cancer syndrome (Terry and Missmer, 2017).

2.5.7 Cigarette smoking

A cohort study of a more than 300000 women reported that there are association between smoking duration, pack-years, and number of cigarettes smoked per day, and both invasive and borderline mucinous OC, but not associated with serous or endometrioid OC (Licaj, *et al*. 2016).

2.5.8 Alcohol consumption

Epidemiological studies reported a direct correlation between alcohol consumption and an increased risk of developing OC. The most deleterious effects of chronic alcohol intake on the ovaries include alterations in estrogen and progesterone receptors, increases in estradiol levels, production of reactive oxygen species, and changes in ovary structure and size. Furthermore, ethanol and its metabolite inducing DNA adduct formation (Jammal, *et al*. 2017).

2.6 Diagnosis of ovarian cancer

2.6.1 Transvaginal ultrasonography (TVS)

A powerful tool that allows one to determine whether an adnexal mass is solid or cystic, simple or complex, and if complex, is the complexity due to septations, excrescences, or solid components. But it is less reliable in differentiating between benign and malignant ovarian tumors (Tewari and Monk, 2015).

2.6.2 Magnetic resonance imaging (MRI)

MRI may discriminate between benign and malignant pelvic tumors. Generally, after gadolinium administration, OC enhances earlier, more rapidly, and more avidly than benign lesions. Moreover, delayed images help in abdominal staging of ovarian cancer, increasing the detection of small peritoneal implants and omental infiltration (Fagotti, *et al*. 2017).

2.6.3 Computed tomography (CT)

CT is the first choice technique to study advanced OC, being rapid, highly accurate, and widely available. Moreover, it has lower incidence of breathing

artifacts and image distortion comparing with MRI, but has a limited role in the primary detection and characterization of OC, since the low soft tissue contrast of the CT may affect its reliability to discriminate the benignity or malignancy of the lesion (Pujade-Lauraine, *et al*. 2017).

2.6.4 Positron emission tomography (PET)

PET integrated with CT and fludeoxyglucose 18 (PET/CT) is a hybrid technique combining metabolic and morphologic tomographic images, it has a high accuracy in differentiating OC from benign tumor but suboptimal accuracy in discriminating borderline from benign tumors, PET has demonstrated a better sensitivity and specificity in detecting OC recurrence compared with serum CA-125 and CT (Marzola, *et al*. 2017).

2.6.5 Serum tumor biomarker (CA-125 and HE4)

Increase in serum CA-125 may be an indicator of ovarian malignancy. However, elevation of serum CA-125 may occur in many non-neoplastic diseases or non-ovarian neoplastic diseases (Allen and Cameron, 2017).

Human epididymis protein 4 (HE4) has gained interest as a biomarker of recurrence. HE4 was shown to be elevated several months before CA-125, implying a potential role for early detection of recurrent disease (Eisenhauer, *et al*. 2018).

Although HE4 has been shown to be a better predictor of complete cyto-reduction than CA-125 in naive patients, CA-125 remains the principal marker used in clinical practice so far (Braicu, *et al*. 2013).

2.6.6 Biopsy

Fine needle aspiration (FNA) specimens of ovarian cystic lesions may be performed under ultrasound guidance (transvaginal or transabdominal) or at laparoscopy or laparotomy, ovarian wedge biopsies are occasionally performed at diagnostic laparotomy for lower abdominal pain and core biopsies may be carried out when it is unclear whether an ovarian mass is benign or malignant,

and cystectomy with preservation and reconstruction of the residual ovary may be performed in young patients in whom benign cystic lesions are suspected clinically (Houghton and McCluggage, 2017).

2.7 Treatment of ovarian cancer

2.7.1 Surgery

The purpose of surgical exploration is diagnostic and therapeutic. the goal of primary surgery for advanced ovarian cancer should be to remove all visible tumor, removal of large ovarian masses and omental involvement may reduce the tumor burden by 80% to 99% (Eisenhauer, *et al*. 2018).

2.7.2 Chemotherapy

Most cancer chemotherapeutic agents target DNA in malignant and normal cells, DNA damage results in cancer cell death. Chemotherapy for treated OC include: cyclophosphamide, chlorambucil, melphalan, and dacarbazine (Friedlander and Markman, 2015).

2.7.3 Radiation therapy

Epithelial ovarian cancer is known to be a radiosensitive tumor, both intraperitoneal radioactive chromic phosphate suspension (^{32}P) and whole abdomen irradiation can be used (Vicus, *et al*. 2016).

2.7.4 Hormonal therapy

Patients with tumors that highly express ER or PR may have the best responses to hormonal treatment. Tamoxifen has been reported to have activity in some patients with recurrent ovarian cancer (Berek, *et al*. 2015).

2.7.5 Targeted therapy

Various clinical trials have demonstrated the efficacy of bevacizumab treatment that target the vascular endothelial growth factor signaling pathway, also erlotinib which is a potent reversible inhibitor of epidermal growth factor receptor has been used for the treatment of OC (Dorigo and Berek, 2015).

2.7.6 Immunotherapy

Include monoclonal antibodies against antigenic targets expressed by tumor cells or within the tumor microenvironment, immunotherapy for OC include cetuximab, amatuximab, and oregovomab (Makkouk, *et al*. 2017).

2.8 Progesterone receptor

Progesterone receptor is a member of nuclear receptor superfamily, plays a vital role for female reproductive tissue development, differentiation and maintenance (Zheng, *et al*. 2016).

PR gene is located at chromosome 11q22-23 (Gimenes, *et al*. 2010). Two distinct promoters in the PR gene provide for the generation of two major PR isoforms, PRB (114 kDa) and PRA (94 kDa), the two PR forms are identical in all regions except the N- terminal domain, which is truncated by 128-164 amino acids in PRA. Both PR isoforms are expressed at relatively equal levels in tissues, although differences in the ratio have been noted in certain tissues. A third isoform, termed PR-C, an N-terminally truncated form that lacks the full A/B domain and first zinc finger of the C domain, has been described, but its expression as a protein is controversial (Binder, *et al*. 2015).

PR expression was associated with favorable prognostic factors that included younger age, benign tumor and low FIGO stage (Garg, *et al*. 2014).

Previous study analyzed tissues from 2933 women with different tumor subtypes reported that PR expression improved survival rate for high-grade serous OC and endometrioid carcinoma. however, no correlation was observed for the other types (Sieh, *et al*. 2013). These effects may be attributed to the fact that PR stimulates apoptotic cell death in OC cells, which could ameliorate the survival rate in PR-positive tumors (Modugno, *et al*. 2012).

Naik, *et al*. (2015) found that the expression of PR was more in benign than borderline and malignant tumors. The expression was also more in low-grade

tumors than high-grade ones, and less in metastasizing tumors than non-metastasizing ones among epithelial ovarian tumors.

Chen, *et al*. (2017) reported that PR positivity was higher in high-grade, low-grade serous and endometrioid carcinoma, but lower in mucinous and clear cell carcinoma. Significantly higher PR positivity was seen in high grade serous carcinoma with peritoneal metastases. In addition, there was no significant difference in PR positivity between cases with and without lymph node metastasis in OC subtypes.

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 hospital based case study aimed to detect PR expression in ovarian tumors.

3.2.2 Study samples

Forty-nine paraffin block samples were collected from patients previously diagnosed as ovarian tumor, 32 (65.3%) of them were malignant and the remaining 17 (34.7%) were benign. Patient's identification information (age, histopathological diagnosis, malignant tumor grade) were obtained from patient's records.

3.2.3 Study area

This study conducted at Alamel hospital and Altayseer-2 laboratory in Khartoum state.

3.2.4 Sample processing

A tissue microarray (TMA) was constructed from paraffin-embedded malignant tumor specimens from 32 blocks. Hematoxylin and eosin stained full sections were reviewed to select representative areas of tumor in the center of an initial donor block from which core was acquired for the microarray. 1.0 mm tissue core was taken from each targeted lesion and placed into a recipient block. After construction, 3 micron section was cut and stained with hematoxylin and eosin on the initial slide to verify the histologic diagnosis. Then slides were cut from

the TMA block and the rest 17 blocks at 3µm thickness by rotary microtome, and mounted in positively charged slides for immunohistochemical staining.

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%, 50%) to DW. The antigens were retrieved using water path at 95°c with tris EDTA buffer (pH 9) for 20 minutes and then cooled down to room temperature for 5 min, then washed in phosphate buffer saline (pH 7.4), Endogenous peroxidase activity was blocked by 3% peroxidase blocker for 10 minutes, then washed in phosphate buffer saline for 3 minutes. The slide then treated with 50µl of anti-PR primary antibody for 20 min at room temperature in a humid chamber, then washed in phosphate buffer saline for 3 minutes. Then 50µl of dextran polymer-horseradish peroxidase secondary antibody were added to each sections and then incubated for 20 minutes then washed in three changes of phosphate buffer saline, after that 50µl of 3, 3 diaminobenzidine tetrahydrochloride substrate solution were added to each section and incubated for 5 minutes, then washed in running water. Then counter stained in Mayer's haematoxylin stain for one minute, then washed and blued in running tap water. After that dehydrated through ascending concentration of ethanol, cleared and mounted in DPX mounting media (Bancroft, *et al*. 2013).

3.2.6 Result interpretation:

All quality control measures were adopted. A negative control slide was completed by omission of the primary antibody. A known positive PR section obtained from breast cancer blocks used as positive control during immunohistochemical staining. Positive staining for PR appeared as brown

particles at the nucleus. Under microscopy, detection of more than 5 cells per one field using X40 lens considered as 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 each hospital to use the tissue blocks for research purposes.

Chapter four

Results

The study includes 49 samples, 32 (65.3%) samples were malignant tumors and 17 (34.7%) samples were benign tumors, as indicated in table (4.1).

The age of study population ranged between 28 and 70 years with mean age of 49 years. Patients with malignant ovarian tumor and equal or less than 50 years representing 14 (28.6%) and the remaining 18 (36.7%) were older than 50 years, while Patients with benign ovarian tumor and equal or less than 50 years representing 13 (26.5%) and the remaining 4 (8.2%) were older than 50 years. Women more than 50 years are more affected with malignant ovarian tumor, while benign tumor tend to occurs more in women less than 50 years, as indicated in table (4.2).

The histopathological diagnosis of study sample includes 22 (44.9%) epithelial ovarian tumors, 9 (18.4%) sex cord tumors, 1 (2%) germ cell tumor and 17 (34.7%) benign ovarian tumors, as showed in table (4.1).

The tumor grade of study samples revealed 12 (37.5%) grade I, 10 (31.2%) grade II and 10 (31.2%) grade III, as showed in table (4.3).

Malignant tumors revealed positive PR expression in 1 (2%) sample and negative expression in 31 (63.3%), while benign ovarian tumors showed positive expression in 5 (10.2%) samples and negative expression in 12 (24.5%) samples. This result showed significant association (P.value 0.008), as indicated in table (4.4).

Positive expression of PR seen in 4/27 (8.16%) among age group that less than or equal 50 years, and seen in 2/22 (4.08%) among age group more than 50

years. This result showed no significant statistical association between PR expression and age group (P-value 0.543), as showed in table (4.5).

Table (4.1): Distribution of histopathological diagnosis among the study sample

Histopathological diagnosis	Types	F	%	Total	
				F	%
Malignant	Epithelial tumors	22	44.9	32	65.3
	Sex cord tumors	9	18.4		
	Germ cell tumor	1	2		
Benign	Benign ovarian tumors			17	34.7
Total				49	100

Table (4.2): Distribution of age groups among the study sample

Age group	Histopathological diagnosis		Total
	Malignant	Benign	
	F (%)	F (%)	F (%)
Less than or equal 50 years	14 (28.6)	13 (26.5)	27 (55.1)
More than 50 years	18 (36.7)	4 (8.2)	22 (44.9)
Total	32 (65.3%)	17 (34.7%)	49 (100)

Table (4.3): Distribution of tumor grades among malignant ovarian tumors

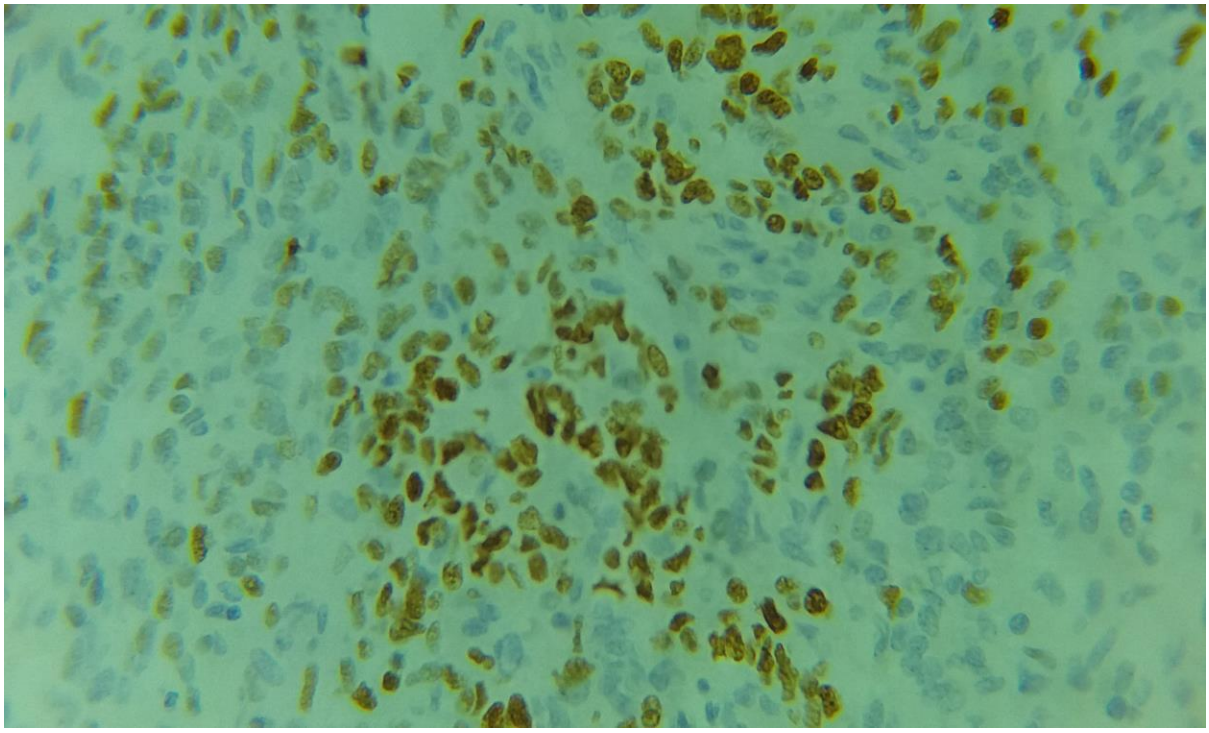
Tumor grades	Frequency	percent
Grade I	12	37.5
Grade II	10	31.2
Grade III	10	31.2
Total	32	100

Table (4.4): Relation between the expression of PR and histopathological diagnosis

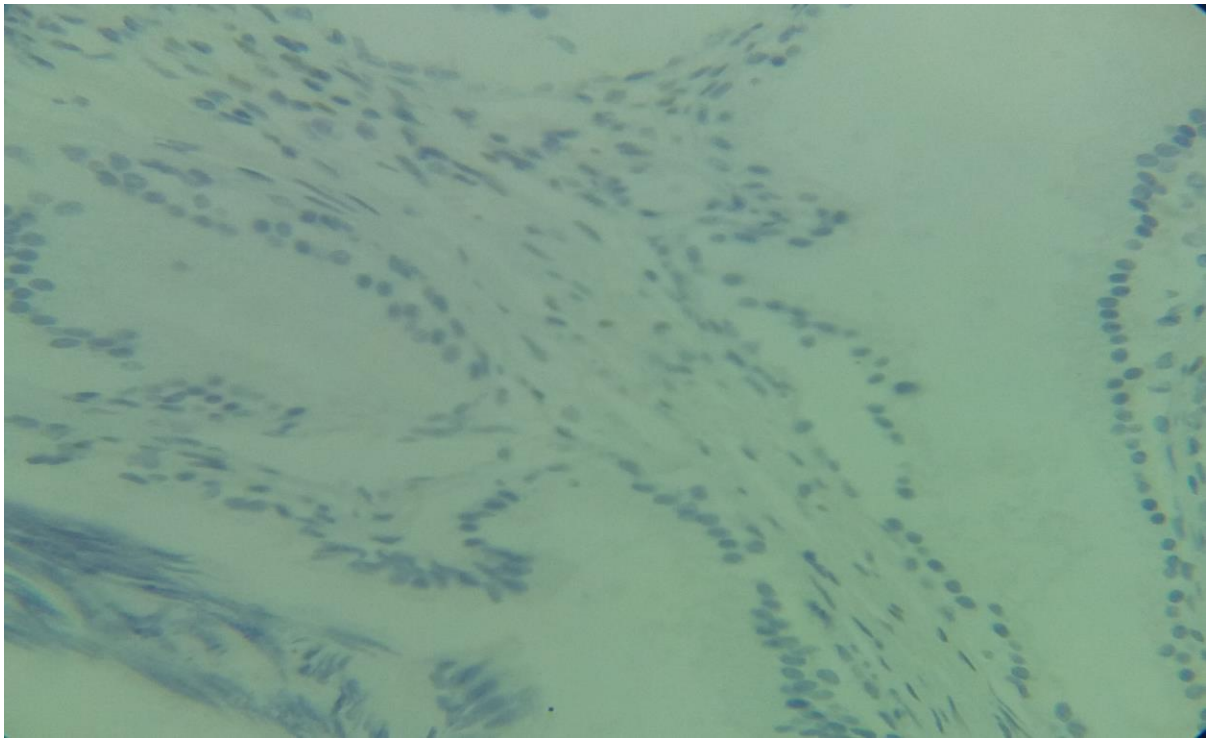
Histopathological diagnosis	PR expression		Total	P.value
	Positive	Negative		
	N (%)	N (%)	N (%)	0.008
Malignant	1 (2%)	31 (63.3%)	32 (65.3%)	
Benign	5 (10.2%)	12 (24.5%)	17 (34.7%)	
Total	6 (12.2%)	43 (87.8%)	49 (100%)	

Table (4.5): Shows the relation between age groups and PR expression

Age group	PR expression		Total	P-value
	Positive	Negative		
	N (%)	N (%)	N (%)	
Less than or equal 50 years	4(8.16%)	23(46.94%)	27(55.1%)	0.543
More than 50 years	2(4.08%)	20(40.82%)	22(44.9%)	
Total	6(12.24%)	43(87.76%)	49(100%)	



Micrograph (4.1): Serous cystadenocarcinoma G II shows positive expression of PR in the nucleus (X40).



Micrograph (4.2): Papillary serous cystadenocarcinoma G I shows negative nuclear expression of PR (X40).

Chapter five

Discussion, conclusion and recommendations

5.1 Discussion

The present study includes 49 samples of ovarian tumors stained by immunohistochemistry for PR. Concerning the age group of the study population, the study revealed that most of OC patients were more than 50 years. This result is compatible with Kheiri *et al.* (2018), who reported that almost two-thirds of OC patients were above 50 years of age, and only 14% of cases were < 40 years of age. Also the American cancer society reported that OC is rare in women less than 40 years of age. Typically, the diseases develop after menopause, and almost 50% of all OCs are found in women 63 years of age or older (Wentzensen, *et al.* 2016). While disagree with Mohammed, *et al.* (2013), who reported that common involved age by ovarian carcinoma was the age group of 30-40 years.

The histopathological diagnosis of the study population revealed that most frequent type of OC was epithelial ovarian cancer. This result is compatible with Kheiri, *et al.* (2018) and Adam, *et al.* (2017), both founded that the epithelial tumor is the commonest type of ovarian cancer among Sudanese female. Also agree with Shen, *et al.* (2017), who reported that epithelial ovarian cancer comprises most malignant ovarian neoplasm.

In this study PR expression was more frequent expressed in benign condition. Same result observed by Verma, *et al.* (2018) and Naik, *et al.* (2015), they reported that the expression of PR was more in benign tumors than borderline and malignant tumors.

The present study revealed no significant statistical association between PR expression and age group (P-value 0.543), a founding that disagree with

Scambia, *et al*. (1995), who demonstrated a higher incidence of PR positive in patients older than 60 years of age. However, this result was compatible with Ayadi, *et al*. (2010), who founded that there no significant association between PR expression and age group (P-value 0.537) and Ajani, *et al*. (2017), who also founded that there was no significant association between PR expression and age.

5.2 Conclusion

On basis of the result this study concluded that:

- The age of ovarian cancer patients in this study is commonly more than 50 years.
- Most histological type of ovarian cancer in this study is epithelial ovarian cancer.
- PR expression is more frequent expressed in benign tumors compared with malignant tumors of ovary.

5.3 Recommendations

According to the results, the study recommends:

- Further study should be done for expression of PR in ovarian tumors with large sample size and stratified by histological subtype.
- Carry out further study to assess the expression of PR isoforms (A & B) in OCs and analyze its association with histological subtypes, stage and tumor grade.
- Additional studies describing the relationship between sex hormones, PR in combination with other steroid receptors and the clinical aspects for each histological subtype of OCs are also needed to make a better decision during the application of an endocrine therapy.

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Appendices

Materials and instruments

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

Disposable gloves.

Rotary microtome.

Microtome knives.

Coated slides/ Microscope slides.

Cover glasses.

Water bath.

Dry oven.

Coplin jars.

Humidity chamber.

Xylene.

Ethanol (100%, 90%, 70%, 50%).

DW.

Mayer's haematoxylin.

Tris EDTA buffer (pH 9.0).

Phosphate buffer (pH 7.4).

Peroxidase blocker (0.3% hydrogen peroxide in methanol).

Primary antibody PR (anti- human PR).

Secondary antibody (dextran polymer conjugated secondary antibody- HRP).

Substrate.

Chromogen.