بسم الله الرحمن الرحيم

Sudan University of Sciences and Technology

College of Graduate Studies

Immunohistochemical Detection of Breast CancerType1 Mutant Gene and CancerAntigen125 in Ovarian Cancers among Sudanese Women

الكشف النسيجي الكيميائي المناعي عن الطفرة الجينية لسرطان الثدي من النوع الاول وانتجين السرطان 125 في سرطان المبيض لدى النساء السودانيات

A dissertation Submitted in Partial Fulfillment for the Requirement of M.Sc. Degree in Medical Laboratory Science (Histopathology and Cytology)

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2019

الآيـــــــــة

قال تعالى:

﴿أَلَمْ نَشْرَحْ لَكَ صَدْرَكَ (1) وَوَضَعْنَا عَنْكَ وِزْرَكَ (2) الَّذِي أَنْقَضَ ظَهْرَكَ (3) وَرَفَعْنَا لَكَ ذِكْرَكَ (4) فَإِنَّ مَعَ الْعُسْرِ يُسْرًا (5) إِنَّ مَعَ الْعُسْرِ يُسْرًا (6) فَإِذَا فَرَغْتَ فَانْصَبْ (7) وَإِلَى رَبِّكَ فَارْغَبْ (8)﴾

صدق الله العظيم

سورة الشرح، الآيات (1-8)

Dedication

To my mother

To the soul of my father and sister

To my husband

To my family

To all my colleagues and friends

With love and respect

Acknowledgements

I’m grateful to Allah for the care, insight, peaceful and pity in my life. I would like to express my profound thank to my supervisor, Dr. Abu Elgasim Abass, for his patience, guidance, unlimited assistance, encouragement and interest throughout the course of this work.

I wish to extend my warmest thanks to the staff of the histopathology and cytology department, college of medical laboratory sciences, Sudan University of science and technology for their continuous support and encouragement.

Finally, I would like to thank Miss. Nada Salih for her supported, help and encouragement.

Abstract

This a descriptive retrospective case hospital based study was conducted in Radiation and Isotope Center Khartoum (RICK), and AL-Amal hospital during the period from March 2017 to April 2018.The study aimed to detect BRCA1 mutant gene and CA125 in ovarian cancer using immunohistochemistry.

Thirty three paraffin embedded block previously diagnosed as ovarian cancer were collected. Samples included 23(69.7%) epithelial tumors, 9(27.3%) sex cord tumors, and 1(3.0%) germ cell tumors.

The patient's age range between 32and 65 years with mean age of 49 years, most of patients were more than 40 years, representing 26(78.8%) and the remaining 7(21.2%) patients were less than 40 years.

All samples prepred by microarray, two sections for microarray of 3 micrometer thickness were cut from each paraffin block by rotary microtome and stained by immunohistochemical method (Indirect dextran polymer immune peroxidase technique) for detection of BRCA1 mutant gene and CA125. Data collected from patient's files and results obtained were analyzed using SPSS computer program.

Immunohistochemical expression of BRCA1 mutant gene was not detected in all samples, while CA125 positive expression was observed in 27/33samples, and negative in 6/33 samples.

The positive result of CA125 in histopathologicl types of ovarian cancer revealed that, in epithelial tumor 5/18 samples, sex cord tumor 1/8 samples and germ cell tumor no samples, with insignificant correlation between CA125 expression and ovarian cancer subtypes (P-value 0.698).

The relation between CA125 positive expression and grading of malignant tumor as showed that, grade 1 in 2/9 samples, grade II in 4/10 samples and grade III no

positive sample, with insignificant correlation between CA125 expression and ovarian cancer grades (P-value 0.331).

This study concluded that there is no relation between BRCA1mutant gene expression and ovarian cancer, and no relation between CA125expression and histopathological types and grade.

المستخلص

أجريت هذه الدراسة الوصفية المستشفويه في المركز القومي للعلاج بالاشعة و الطب النووي و مستشفى الأمل خلال الفترة من مارس 2017 إلى ابريل 2018 هدفت الدراسة للكشف عن الطفرة في الجين المسبب لسرطان الثدي 1 CA125 في سرطان المبيض بإستخدام كيمياء الأنسجة المناعية.

جمع ثلاثة و ثلاثون قالب مطمور بشمع البارفين، لمرضي تم تشخصيهم مسبقاً بأورام المبيض. شملت العينات 23 (69.7%) عينة من الأورام الطلائية، 9 (27.3%) عينات من أورام الحبال الجنسية وعينه واحده (3.0%) من أورام الخلية الجرثومية.

تراوحت أعمار المرضى بين 32-65 سنة و متوسط العمر49 سنة، أغلب أعمار المرضى كانت أكثر من 40 سنه 26 (78.8%) وبقية اعمارالمرضي أقل من 40 سنة 7(21.2%).

تم تحضير جميع العينات بواسطه تقنيه المصفوصه الرقيقه ثم قطع مقطعين من عينه المصفوفة الرقيقة بسمك 3 ماكرون بواسطة جهاز المشراح الدوار. ثم صبغت العينات بواسطة كيمياء الأنسجه المناعية (طريقه تقينة بلوميرز ديكستريس المناعي غير المباشرة) للكشف عن الطفرة في الجين المسبب لسرطان الثدي1 CA125 . تم جمع البيانات من ملفات المرضى . ثم إستخدام برنامج الحزمة الاحصائية للعلوم ألاجتماعيه لتحليل البيانات.

أظهرت الدراسة ان التعبير المناعي للكشف عن الطفرة في الجين المسبب لسرطان الثدي 1 أنها كانت سالبة الظهور في كل العينات بينما كان التعبير المناعي لCA125 أنها موجبة الظهور في 33/27 عينة.

النتيجه الايجابيه للتعبير المناعي ل CA125 في انواع الورم الخبيث كانت كالاتي18/5 في سرطان الخلايا الطلائية و 8/1 سرطان خلايا الحبال الجنسية ولا توجد نتيجه ايجابيه في عينة سرطان الخلية الجرثومية، مع عدم وجود علاقة بين CA125 و نوع الورم الخبيث للمبيض (القيمة الاحتمالية تساوي 0.698).

أظهرت العلاقة بين التعبير المناعي الموجب لCA 125 ومرحلة الورم الآتي 9/2 في المرحلة الأولي و10/4 في المرحله الثانية ولا يوجد في المرحلة الثالثة مع عدم و جود علاقة بينCA125 ومرحله الورم (القيميه الإحتمالية تساوي 0.331).

خلصت هذه الدراسة إلي عدم وجود علاقة بين الطفرة الجينية المسبب لسرطان الثدي 1 و سرطان المبيض و إلى عدم وجود علاقة بين تعبير CA125 و أنواع و مرحلة الورم الخبيث بالمبيض.

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CHAPTER ONE
INTRODUCTION

Chapter One

Introduction

1.1 Introduction:

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

Ovarian cancer is fifth-leading cause of cancer related death in women. It estimated that in the 2016, there are more than 22.200 new case of ovarian cancer. And more than 14.200 death from ovarian cancer in united states (Doubeni, *et al*. 2016).

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

Risk factors of ovarian cancer include family history, age, childbirth menopause, genetics, pervious gynecological problem and lifestyle (Permuth and Seller, 2009).

Method of diagnosis of ovarian cancer is imaging techniques (ultrasongrophy, computed tomography and magnetic resonance imaging), serum tumor marker (CA 125, HE4), fine needle aspiration and immunohistochemistery (Dey, *et al.* 2016).

Method of treatment of ovarian cancer includes surgery, chemotherapy, radiation and combination of surgery and chemotherapy (Holschneider and Berak, 2000).

BRCA1gene on human chromosome 17q21is responsible for an outosomal dominant syndrome of inherited early onset breast/ovarian cancer (Merajver, *et al.*1995).

BRCA1 gene mutation is eastimed to confirm an ovarian cancer risk of 30% by age 60 years (Easton, *et al*. 1995). It believed that 24-40% of ovariens cancer have dysfunction in the BRCA1gene (Skytte, *et al*. 2011). BRCA 1 loss expression using immuohistochemical technique was observed by Meisel *et al*. in 48/135 of cases (Meisel, *et al*. 2014).

BRCA1expression was also observed by Sirisabya *et al.* In 12% of 99 epithelial ovarian tumor section. There was no association between clincopathological out come and BRCA1 expressions (Sirisabya, e*t al.* 2007).

CA 125 (cancer antigen 125) also known asmuc 16, is protein that in humans is encoded by asmuc 16 gene and it is a member of family glycoprotein's (Duraisamy, *et al.* 2009). CA 125 was observed by Mouhamed in 70% of 20 cases of undifferentiated ovarian cancer (Mouhamed, 2017).

1.2 Objectives:

1.2.1 General Objective:

To study the expression of BRCA1 and CA 125 in ovarian cancer among Sudanese patients.

1.2.2 Specific Objective:

1-To detect BRCA1, and CA 125 in ovarian cancer tissue using immunohistochem-istery.

2-To correlate BRCA1and CA 125 expression with cancer subtype and grade.

CHAPTER TWO
LITERATURE REVIEW

Chapter Two

Literature Review

2.1 Scientific background:

Ovarian cancer is most lethal malignant tumor of the female reproductive system. And metastasis is on factor that contributes in poor outcome of patient with ovarian cancer (Zhu, *et al.*2018).

Ovarian cancer in 2018 , approximately 22,240 new cases and 14,070 ovarian cancer deaths in the United States (Torre, *et al*. 2018).The majority of ovarian malignancies (95%) are derived from epithelial cell; the reminders arise from other ovarian cell type (germ cell tumor, sex cord-stromal tumors) (Markman, *et al*. 2001).

2.2 Structure of the ovary:

The ovaries are set of paired gland resembling unshelled almonds set in pelvic cavity below and to either side of umbilicus. They are pearl-colored and oblong. Each ovary weighs from 2 to 5 grams and about 4 *cm* long, and 1 *cm* thick (Speroff and Fritz, 2005). The surface layer of ovary is formed by simple cuboidal epithelium. Surface cell are fragile and invisible in resected ovaries (Simmon, *et al.*2013). The ovary consist of cortical region and medullary area. The cortical region consists of follicles (oocytes) that embedded in the stroma. The medullary region contains a rich vascular bed within a loose connective tissue (Junqnerira and Carneiro, 2005).

2.3 Disorders of the ovary:

2.3.1 Benign disorders:

2.3.1.1 Cystadenoma:

Cyst adenoma is account for 37-50% of benign of ovarian tumor in reproductive age. Their frequency tends to increase with age (Jung, *et al*. 2002). It is thin walled

unlocular or multi locular cystic lesion filled with serous, mucinous and sometimes hemorrhagic content (Hassen, *et al.* 2011).

2.3.1.2 Serous cyst adenoma:

These arise from the cuboidal epithelium covering the ovary. This type of benign ovarian tumors is the most common diagnosed in women age over 40years, and its presence gives the scarred ovarian surface smooth appearance (Karlan, *et al*. 2012).

2.3.1.3 Cystadenofibroma:

Cystadenofibroma account of 1-7% of ovarian tumor. They are compose of epithelial and various amounts of solid stromal elements. The margin tends to be well defined and smooth (Jung, *et al*. 2002).

2.3.1.4 Mature cystic teratoma:

Mature cystic teratoma or dermiod cysts are compose of mature tissue from at least two of three germ cell layers: ectoderm and endoderm. They typically un lateral lesion, it is filled with sebaceous martial (Kurman, *et al*. 2011).

2.3.2 Malignant disorders:

2.3.2.1 Epithelial carcinoma:

Epithelial carcinoma is most common type of ovarian cancer, apporexmetaly 90-95% of all ovarian cancers. Are thought to arise from the simple cuboidal surface epithelium of the ovary (Lee-Jones, 2004).

2.3.2.2 Serous carcinoma:

Serous carcinomas are thought to be beginning in the fallopian tube. Histologically, serous adenocarcinoma has psammoma bodies. Low –grade serous adenocarcinoma resemble fallopian tube epithelium, where high grade serous adenocarcinoma show anaplasia and nuclearlatypia (Vang, *et al.*2009).

2.3.2.3 Mucinous carcinoma:

Mucinous carcinoma of the ovary represent aspectrum of neoplastic disorders, usually occur as a large, mltioculated cystic mass with mucus-containing fluid. The mean size at presentation is 18 *cm* (Brown and Frumovitz, 2014).

2.3.2.4 Endomertrioid carcinomas:

Endomertriod carcinomas account for 10%of all ovarian carcinomas and are generally unilateral solid masses with a smooth outer surface. These tumors are compose of gland resembling endometrial and may be associated with ovarian or pelvic endometriosis (Geyer, *et al.* 2009).

2.3.2.5 Clear-cell cancers:

Clear cell carcinoma account for 5% of all ovarian cancer, although the incidence various worldwide. The prognosis for stage 1 clear cell carcinoma is relatively good. Clear cell carcinomas are also strong associated with endometriosis (Chen, *et al*. 2003).

2.3.2.6 Sex cord –stromal tumor:

Ovarian sex cord-stromal tumors are infrequent and represent approximately 7% of all primary ovarian tumors (Horta and Cunha, 2015).

2.4 Epidemiology of ovarian cancer:

The incidence of ovarian cancer varies widely among different populations worldwide with the highest rates reported in Scandinavia, Eastern Europe, Canada, and Africa. The lowest rates have been reported from Asia, with exception of Japan. The rate of ovarian cancer in Iran has been reported as 3.9per 100,000 (Haem, *et al*. 2015). In Sudan, according to reports derived (2009-2010) from the national cancer registry for Khartoum State alone, ovarian cancer was the fourth most common cancer in women, with an estimated rate of 188 per 100,000 population, and gender specific rate of 8.0 per 100,000 population (Abuidris, *et al.* 2016).

2.5 Risk factors of ovarian cancer:

The risk factors of ovarian cancer include:

2.5.1 HRT (Hormone replacement therapy):

Hormone replacement therapy it slightly increases women's risk of developing ovarian cancer. Experts says that the risk increases the longer the HRT continues, and returns to normal as soon as treatment stops, being obese or overweight increases of developing many cancer (Permuth, *et al*. 2009).

2.5.2 Genetic factors:

Family history of ovarian cancer is most significant risk factor. Approximately 10% of all ovarian cancer can be associated with familial genetic predisposition (Ozols, *et al*. 2005).

2.5.3 Smoking:

Smoking doesn't increase the risk of ovarian cancer over all, but it is linked to increase of the mucinous type (Faber, 2013).

2.5.4 Oral contraception:

Several studies have demonstrated that oral contraception decreases the risk of ovarian cancer due to reduction in ovulatory cycle (Whittemore, *et al.*1992).

2.5.5 Age:

The incidence rate of ovarian cancer is increase with aging .Women over 50year have the highest risk of developing ovarian cancer (Roett and Evans, 2009).

2.5.6 Infertility:

Infertile women and those with condition called endometriosis and those who use postmenopausal estrogen replacement therapy are increased risk for ovarian cancer (Pearce, *et al.* 2012).

2.6 Diagnosis of ovarian cancer:

Diagnosis of ovarian cancer start with taking history and physical examination, including pelvic examination of external and internal female pelvic organ and pelvic ultra sound. Blood test for marker molecule called CA 125 is useful in differential diagnosis of tumor, also HE4a, B-HCG Alpha–fetoprotein and lactate dehydrogenated CT scanning is used to assess the extent of the tumor in the abdominal-pelvic cavity (Doubeni and Myers, 2016).

2.7 Treatment of ovarian cancer:

2.7.1 Surgery:

Surgery is used to remove as much of tumor as possible. This known as debulking surgery or cytoreduction (Gubbles, *et al.* 2010).

2.7.2 Chemotherapy:

Chemotherapy after surgery is referred to as front-line treatment and involves a combination of platinum and taxane-based chemotherapy (usually carboplatin and paclitaxel). Patients with advanced ovarian cancer that isn't initially able to undergo surgery due to large ascites or invasive tumors, can be treated with chemotherapy before being considered for surgery (neoadjuvant treatment) (Hennessy, *et al*. 2009).

2.7.3 Radiation:

Radiation is most often used for palliative purposes and for localized persistent disease after chemotherapy (Roett and Evans, 2009).

2.8 BRCA1gene and it is relation with ovarian cancer:

The BRCA1 gene is an onco-suppresor gene located on chromosome 17q 21. It was first identified in 1994 and contain small deletion of insertion that result in per mature stop codons shorten (truncate) it is protein product. This gene participates in chromatin remodeling process and when mutation occur cellular control are un checked resulting in over growing. Alteration in this gene are found in 75% of

families with hereditary breast and ovarian cancer (Carroll, *et al*.2008).The average cumulative risk in BRCA1 gene mutation carriers by age 70 were 39% (18%-45%) for ovarian cancer (Antoniou, *et al.*2003).

Risch *et al.* reported that women with BRCA1mutation are diagnosed at younger age than no carriers, and most tumors in the mutation carriers are of serous histology (Risch, *et al.* 2001).

In hospital –based cohort study done on 218 women with invasive epithelial ovarian cancer, they found that BRAC1mutation in 8 % (17cases) (Hasmed, *et al.* 2016).

2.9 CA 125 and it is relation with ovarian cancer:

CA 125 is an antigenic determinant on a high-molecular –weight glycoprotein, recognized by monoclonal antibody which was raised using on ovarian cancer cell line as immunogen (Jacobs and Bast, 1989).

Torons *et al.* reported that metastic breast carcinoma to ovary is difficult to differentiate from primary ovarian carcinoma. They used CA 125 as tumor marker in differential diagnosis in study done on thirty- nine cases of metastic breast cancer to ovary, 36 primary breast carcinoma 42 primary ovarian carcinoma were examined. Thirty-eight (90%) ovarian carcinoma were positive to CA 125 most of breast carcinoma were negative to CA 125 (Tornos, *et al*. 2005).

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 hospital based descriptive retrospective case study aimed to detect BRCA1mutant gene and CA 125 in ovarian cancer.

3.2.2 Study samples:

Tissue blocks obtain from thirty three samples previously diagnosed as malignant ovarian tissue. Patient's data (age, histopathological diagnosis) were obtained from patient's files.

3.2.3 Study area:

This study was held in Radiation and Isotope Center Khartoum (RICK) and Al-Amal tower hospital during period from March 2017to Aprial2018.

3.2.4 Sample processing:

Two sections tissue microarray (TMA) was cut from each blocks. Heamatoxlin and eosin stained full sections were reviewed to select representative areas of tumor in center of an initial donor block from which core was acquired for microarray.1.0mmtissue core was taken from each targeted lesion and placed into a recipient block. After construction, 3 micron thickness was cut by rotary microtome, mounted in positively charged slide and put at 60˚ C oven for 30 minutes.

3.2.5 Immune histochemical staining:

Immunohistochemical staining was carried out using indirect dextran polymer immune peroxidase. Tissue sections were deparaffinized in xylene and rehydrated though graded alcohol (100%, 90%, 70%, 50%) and water. The antigens were retrieved using Dako water bath with tris EDTA buffer (pH9) for 5minutes and then cooled at room temperature for 20 min. Endogenous peroxidase activity was blocked by 3% peroxidase blocker for10 minutes. The slides then treated with anti BRCA 1, CA 125 primery antibody for 20 min at room temperature in a humid chamber, then washed in phosphate buffer saline (pH 7.4) for 3 minutes. Then sections were incubated in dextran polymer-HRB (horseradish peroxidase) secondary antibody for 15 minutes then wash in three change of phosphate buffer saline, after that were incubated in 3,3 diaminobenzidine tetra hydrochloride substrate solution for 5 minutes, then washed in running water. Then counter stained in Mayer's heamatoxylin stain for one minute.Then dehydrated, cleared and mounted in DPX mounting mounting media (Bancroft, *et al*. 2013).

3.2.6 Result interpretation:

All quality control measures were adopted. Positive and negative control slides were used during immunohistochemical staining. Positive staining for BRCA 1 appeared as brown particles at the nucleus. And positive staining for CA 125 appeared as brown particles at cytoplasm and cell membrane. Under microscopy, 5 representative high –power flied (X 40 mangnification) per tissue section were randomly selected and evaluated. Detection of more than positive cell per on field 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 acceptance from hospitals administration.

CHAPTER FOUR
RESULTS

Chapter Four

4. Results

The age of study population range between 32 and 65 years with mean age of 49 years, and stander deviation 8.8. Most patients were more than 40 years representing 26 (78.8%) and the remaining 7 (21.2%) were less than 40 years as indicated in table (4.1).

The study include thirty three malignant samples. The diagnosis of samples according to WHO classification include epithelial tumor in 23 (69.7%) samples, sex cord tumor in 9 (27.3%) samples, and germ cell tumor in 1 (3.0%) sample as indicated in table (4.2). The grade of malignant tumor include, II (33.3) were grade I, 14 (42.4) were grade II, 6 (18.2) were grade III and 2(6.1) were not graded, as indicated in table (4.3).

BRCA1 gene mutant expression was found negative in all samples as indicated in table (4.4). The relation between CA 125 expression and tumor malignant types as follow are, in epithelial tumor (5/18) samples, sex cord tumor (1/8) samples and germ cell tumor no sample, with insignificant correlation between CA 125 and ovarian cancer subtypes (P-value 0.698) as indicate in table (4.5). The relation between CA 125 expression and grading of malignant tumor as follow are (2/9) samples were grade I, (4/10) sample were grade II and no sample were grade III, with insignificant correlation between CA 125 and ovarian cancer grading (P-value 0.331) as indicate in table (4.6).

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

Age group	Frequency	Percentage
Less or equal 40 years	7	21.2%
More than 40 years	26	78.8
Total	33	100%

Table (4.2): Distribution of histopathological diagnosis of ovarian cancers among the study samples:

Histopathological diagnosis	Frequency	Percentage
Epithelial tumor	23	69.7%
Sex cord tumor	9	27.3%
Germ cell tumor	1	3.0%
Total	33	100%

Table (4.3): Grading of ovarian malignant tumor among the study samples:

Grading	Frequency	percentage
Grade I	11	33.3
Grade II	14	42.4
Grade III	6	18.2
Not graded	2	6.1
Total	33	100%

Table (4.4): BRCA1 mutant gene expression among ovarian cancer samples:

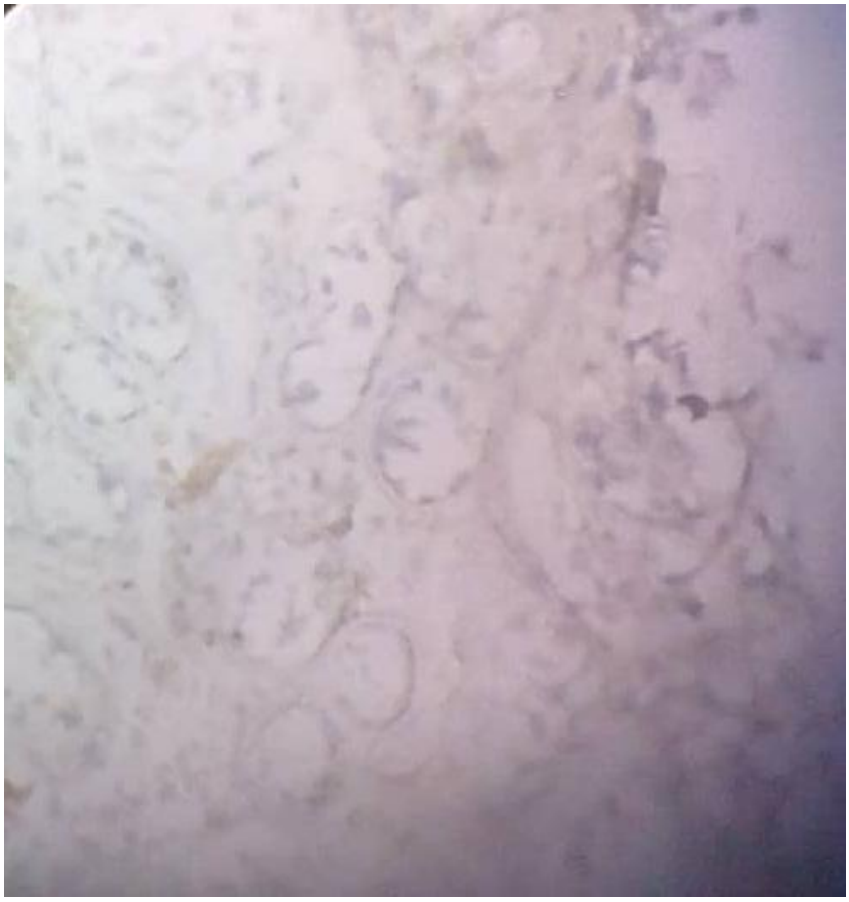
BRCA1 expression	Frequency	percentage
Positive	0	0
Negative	33	100%

Table (4.5): Relation between CA 125 expression and histopathological types of ovarian cancer among study samples:

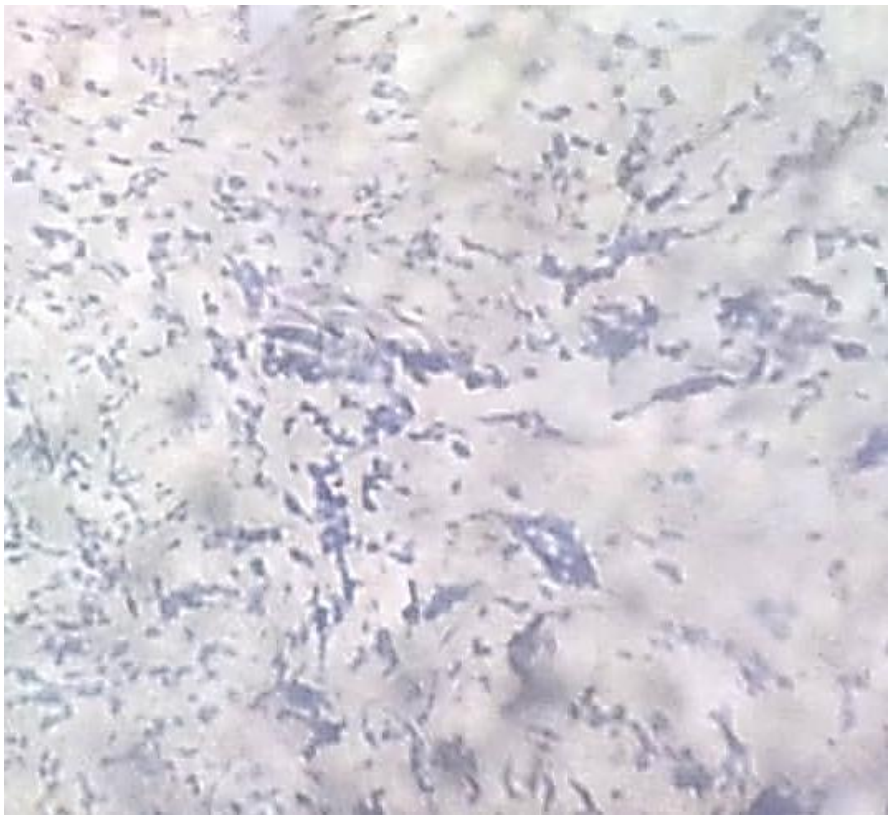
Histopathological types.	Expression of CA 125		P-value
	Positive N(%)	Negative N(%)	
Epithelial tumor	18(66.7%)	5(18.5%)	0.698
Sex cord tumor	8(29.6%)	1(3.7%)	
Germ cell tumor	1(3.7%)	0	
Total	27	6	

Table (4.6): Relation between CA 125 expression and ovarian cancer grade among study population:

Grade	Expression of CA 125		Total	P-value
	Positive N(%)	Negative N(%)		
Grade I	9 (29%)	2(6.5%)	11	0.331
Grade II	10 (32.3%)	4(12.9%)	14	
Grade III	6 (19.4%)	0	6	
Total	25	6	31	



Photograph (4.1): Epithelial ovarian cancer shows positive membranous CA125expression (40×)



Photograph (4.2): Epithelial ovarian cancer show negative expression (40×).

CHAPTER FIVE

DISCUSSION

Conclusion and Recommendation

Chapter Five

5.1 Discussion

The present study includes 33 cases of ovarian cancer, stained immunohistochemically for CA 125 and BRCA1 mutant gene. Regarding the age of patient's, the study revealed that most patients were more than 40 years, indicating that older women are more susceptible to ovarian cancer. This result is compatible with Booth *et al.* (1989), they proved that there was direct correlation between the age and ovarian cancer. Also compatible with Abuidris *et al*. (2016), who reported that the incidence rate of ovarian cancer increased greatly in women aged 55years or older.

The histopathological diagnosis of study population revealed that more frequent type of ovarian was epithelial ovarian cancer. This result is compatible with Adam *et al*. (2017), they reported that epithelial ovarian cancer comprises most malignant ovarian neoplasm. Also agree with Colombo, *et al*. (2010), who reported that about 90% of primary malignant ovarian tumors are epithelial carcinomas.

Regarding BRCA1 mutant gene expression the study found that all ovarian cancer tissues showed negative expression. This result is disagree with Sirisabya, *et al*. (2007), they reported that 12.1% (12/99) of ovarian cancer was positive.This result is compatible with Shawky, *et al*. (2014), they reported that there was no association between BRCA1expression ovarian cancer grade and type.

CA-125 expression in the study found that (27/33) of ovarian cancer showed positive expression. This result is compatible with Zhu and Michael (2007), they reported that 100% (20/20) of ovarian cancer showed positive expression for CA125.

Regarding the correlation between expression of CA 125 and ovarian cancer histological type, the study found that insignificant correlation between CA 125

expression and ovarian cancer histological type (P-value 0.698), this result is incompatible with Dela cuesta *et al*. (1999), they reported there are significant correlation between histological CA 125 and histological subtype (P<0.001). Also incompatible with Devan *et al*. (2013).They reported that CA 125 useful marker in diagnosis of epithelial ovarian cancer.

Regrinding the correlation between expressions of CA 125 and ovarian cancer grade, the study revealed that insignificant correlation between CA 125and ovarian cancer grade (P value 0.425). This result is compatible with Sparholt *et al*. (2013), they reported that there are no significant correlation between CA 125 expression and histological types and grade.

Conclusion and Recommendation

5.2 Conclusion:

From this study we conclude that:

- The most common age of ovarian cancer among our study population is more than 40years.
- Most histological type of ovarian cancer is epithelial carcinoma.
- BRCA1mutant gene expression is not associated with ovarian cancer.
- CA 125 expression is not associated with ovarian cancer histological types and grade.

5.3 Recommendations:

From this study we recommended that:

Further research should be done on expression of BRCA1 gene mutation and CA 125 in ovarian cancer tissue with large sample size.

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Appendix 1

Instrument:

Disposable gloves.

Puncher 0.5 cm.

Microarrays Mold.

Rotary microtome.

Microtome Knives.

Positively charged slides (Thermo).

Cover glasses.

Dry oven.

Water path (Dako water path).

Coplin jars. Humididity chamber.

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

Xylene.

Mayer's haematoxylin.

Citrate buffer (PH6.8).

Phosphate buffer (PH7.4).

0.3 Hydrogen peroxidase.

Primary antibody (BRCA1,CA 125).

Secondery antibo (Dextrease polymerase secondary antibody).

Substrate chromogen (DAB).

DPX.

Mouse anti-CA125
Cat. No.: MSG110 (6 ml Ready-to-use)

Instructions for use

Intended Use
This antibody is designed for the specific localisation of the CA125 ovarian cancer antigen in formalin-fixed, paraffin-embedded tissue sections.
Anti-CA125 antibody is intended for research use only.

Specifications

Specificity:	CA125
Clone:	Ov185:1
Isotype:	Mouse IgG1
Species reactivity:	Human +, others not tested

Summary and explanation
This antibody reacts specifically with CA125 ovarian cancer antigen. Several studies have shown the CA125 is a useful tumor marker for ovarian epithelial malignancies. However, CA125 has been described in other neoplasms such as seminal vesicle and anaplastic lymphomas.

Reagent provided
Monoclonal mouse antibody in TBS with carrier protein and preservative for stabilisation in the following format:
Ready-to-use: 6 ml (Cat. No. MSG110)

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	Ovarian carcinoma
*Working dilution	None
*Incubation time	30 - 60 minutes

Quality control
The recommended positive control tissue for this antibody is ovarian 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 the cytoplasm and cell membrane in formalin-fixed, paraffin-embedded tissue. Further details about the expression pattern of CA125 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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August 11, 2017 Rev: A0817 Doc: DBE_MSG110

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