بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of higher education and scientific research

Shendi University

Faculty of Graduate Studies and Scientific Research

The Frequency of Survivin expression among Sudanese Women with Ovarian Cancer

A dissertation Submitted in partial Fulfillment for the partial Requirement of MSc. in Medical laboratory (histopathology)

By:

Hafssa Bashir Mirghani Elmahi

B.S.c of Medical Laboratory Sciences

Hematology & histopathology

Shendi university (2011)

Supervisor:

D.r: Asma El-ameer Mohammed Zeen

PHD in histopathology (2018)

(2018)

الآيـــة

قال تعالى:

﴿وَوَصَّيْنَا الْإِنْسَانَ بِوَالِدَيْهِ حَمَلَتْهُ أُمُّهُ وَهْنًا عَلَى وَهْنٍ وَفِصَالُهُ فِي عَامَيْنِ أَنِ اشْكُرْ لِي وَلِوَالِدَيْكَ إِلَيَّ الْمَصِيرُ (١٤)﴾

سـورة لقـمان

Dedication

To my patient, lovely, kindness Mother who give me the life

To my greatest Father who thought me the alphabet of life

To my dear Husband who learn me the meaning of life

&

To my Precious sisters , brothers, colleagues and friends & to all who live with me and pray for me;

I dedicate this simple attempt.

Aknowledgement

Firstly the great praise and all thanks to God for giving me the power and well to complete this work.

I am gratefully acknowledging my supervisor

Dr. Asma El-ameer Mohammed Zeen

Who guide me throughout this research,,

Greatest thanks for my colleagues and friends especially my lovely sisters Suha Amer & Sufia Mohammed for their help.

Table of Contents

List of abbreviations

App	Means
ACTH	Adreno cortioco tropic hormone
AFP	Alpha fetoprotein
ASR	Age-standardized rate
ß- HCG	Beta-Human chorionic gonadotrophin
BIRC5	Baculoviral IAP Repeat Containing 5
BRCA1	Breast cancer type 1 susceptibility protein
CEA	Carcinoembryonicantigen
DAB	3, 3 di-amino benzidine tetra hydrochloride
DPX	Diesterin Plasticyzer Xylene
FSH	Follicle- stimulating hormone
IAP	Inhibitor of apoptosis protein
LH	Luteinizing hormone
OC	Ovarian cancer
POF	Premature ovarian failure
VTE	Venous thrompo embolism
WHO	World Health Organization

Abstract

This is a descriptive retrospective study conducted in El-Rahma medical center in Khartoum from march to august 2018, aimed to detect the frequency of survivin expression among Sudanese women with ovarian cancer. 36 paraffin blocks were selected, which are previously diagnosed as ovarian carcinoma, and the data were collected from records . the age of patient range between 32 and 65 years with mean age of 50 years, most of patients were 50 years and more representing 20 patients (55.6%) and the remaining 16 patients(44.4%) was less than 50 years. Histological types included :- stromal origin tumors (16.7%) germ cell tumors (2.8%), and epithelial cell origin (80.5%), it also divided to:- the differentiated ovarian neoplasm (72.2%), un differentiated ovarian neoplasms (27.8%) . All tumors were reviewed histopathologically and classified according to the WHO criteria.

A tissue microarray block were made and a section of 3µm was cut by rotary microtome and stained by Immunohistochemical method (modified in direct technique), the data obtained was analysed using SPSS program version 22.

In this study Survivin was highly expressed in the ovarian neoplasms, it was detected in (94.4%) of cases. The expression rate was 93% in epithelial neoplasms, stromal(100%), and germ cell (100%), 92.3% in differentiated tumors , and 100% for un differentiated tumors. The results, when analyzed statistically did not show any statistical significance between survivin and the origin, grade and type of ovarian tumor.

this study concluded that the cytoplasmic survivin expression is strongly associated to the ovarian tumor and recommend to be added to diagnostic marker panel of ovarian cancer.

مستخلص البحث

أجريت هذه الدراسة الوصفية الاسترجاعية في مركز الرحمة الطبي بمدينة الخرطوم في الفترة من مارس إلى أغسطس ٢٠١٨م، هدفت للكشف عن تكرار ظهور الـــSurvivin في أورام المبيض، تم اختيار ٣٦ قالب شمعي لعينات مرضى شخصت مسبقا على أنهم مصابون بسرطان المبيض وجمعت البيانات المتعلقة بها من السجلات، تراوحت أعمار المرضى بين ٣٢-٦٥ عام بمتوسط ٥٠ عاما معظمهم من ٥٠ سنة فما فوق، وقد كان عددهم٢٠ بنسبة (٥٥.٦%)، و١٦ مريضا بنسبة (٤٤.٤%) كانت أعمارهم أقل من ٥٠ عاما. وقد شملت العينات الأنواع النسيجية التالية :- الأورام اللحمية الأصل(١٦.٧%)، أورام خلية جرثومية الأصل(٢.٨%) والأورام ظهارية الأصل (٨٠.٥%)،والتي قسمت إلى :- أورام متميزة (٧٢.٢%)، وأورام غير متميزة (٢٧.٨%)، تمت مراجعة جميع الأورام المرضية وتصنيفها وفقاً لمعايير منظمة الصحة العالمية، تم عمل قالب شمعي واحد بطريقة مجموعات الأنسجة الصغيرة ثم قطع بسمك ٣ ميكروميتر بواسطة المشراح الدوار وصبغت بواسطة طريقة كيمياء الأنسجة المناعية (الطريقة المحسنة غير المباشرة)، واستخدم برنامج الحزم الإحصائية للعلوم الاجتماعية النسخة ٢٢ لتحليل البيانات. تم الكشف عن تعبير Survivin في ٩٤.٤ ٪ من حالات الأورام المبيضية الخبيثة وهذه النتيجة تظهر علاقة وطيدة بين ظهور الـــ Survivinوسرطان المبيض . كانت نسبة الظهور ٩٣ ٪ في الأورام الظهارية ،١٠٠% للأورام جرثومية الأصل ولحمية الأصل، ٩٢.٣% للأورام المتميزة ، ١٠٠% للأورام غير المتميزة . وهذه النتائج بعد التحليل الإحصائي لم تظهر أية دلالة إحصائية بين الـــ Survivin وأصل الورم، ودرجته، ونوعه. خلصت هذه الدراسة إلى أن ظهور الـــ

survivinفي سيتوبلازم الخلايا له علاقة وطيدة مع سرطان المبيض مع عدم وجود علاقة بين الـــ survivin وأصل الورم ودرجته ونوعه.

List of Tables

List of Photomicrographs

1.1. Introduction

Ovarian cancer posed the greatest challenge amongst gynecological malignancy. It is second most common malignancy in female reproductive system [1] and the seventh most common cancer in women (and the 18th most common cancer overall) worldwide. This cancer is usually fatal, and is the eighth most common cause of cancer death in women worldwide (14th overall) [2] In developed countries, ovarian cancer is the 2nd most frequent gynecological tumour and the 6th most frequent cause of cancer related death in women [1] . Survival is generally poor Approximately 90% of ovarian malignant tumors origin in the surface layer covering the ovary, called epithelial ovarian cancer.[3] In Sudan the incidence rate of ovarian cancer depend on a hospital-based data however, in recent data set (2009 to 2010) from the National Cancer Registry for Khartoum State alone, ovarian cancer was the fourth most common cancer in women [4].

Diagnosis of ovarian cancer is usually carried out by surgery followed by histopathology.[5] The introduction of prognostic and predictive markers in immunohistochemistry has made a tremendous beneficial impact on patient diagnosis and management. Many antibodies are now available to identify epitopes that survive the rigors of formalin fixation and processing to paraffin wax. In cases where morphology and clinical data alone do not allow a firm diagnosis, then immunohistochemistry is in valuable. The increasing use of prognostic and predictive markers permits the pathologist to make decisions [6] Amongst them cancer antigen (CA-125), Carcinoembryonic antigen (CEA), Alpha fetoprotein (AFP), & Beta-Human chorionic gonadotrophin (ß- HCG) are important and reliable serum biomarkers for early detection and prognostification of ovarian cancer .[1]

Survivin is a novel inhibitor of apoptosis commonly detected in tissues during fetal development and in cancer, but not usually in normal tissues. Expression of this protein may be of prognostic significance and therapeutically relevant in many cancers.[7]

1.2. Rationale (justification)

Ovarian cancer occupies the place among the leading causes of death from gynecological cancer. Recently the diagnosis, differentiation and prognosis of ovarian cancer is based on the expression of numerous tumour markers that have been produced by cancerous tissue, Survivin expression may be a useful diagnostic, prognostic, and predictive marker in certain malignancies. According to my knowledge there is no study done in Sudan to discuss the relation between survivin tumour marker and ovarian cancer, For this reason We need to analyzed survivin expression in ovarian neoplasms to evaluate its frequency in ovarian cancer in Sudan.

1.3. Objectives

1.3.1. General objective:

To detect the frequency of survivin expression among Sudanese women with ovarian cancer using immunohistochemical method.

1.3.2. Specific objectives:

- To examine the expression of survivin in ovarian cancer of different origin using immune histochemical method.
- To study survivin expression among different types and grades of ovarian cancer.

2.1. Literature review

2.1.1. The ovary: The human female reproductive system consists of paired internal ovaries, paired uterine (fallopian) tubes, and a single uterus. Inferior to the uterus and separated by the cervix is the vagina. [8] the ovaries arise from the genital ridge, a thickening in the mesothelium high on the posterior wall of the peritoneal cavity. [9]

2.1.1.1. Anatomy: Ovary is a flattened, ovoid structure located deep in the pelvic cavity. One section of the ovary is attached to the broad ligament by a peritoneal fold called the mesovarium and another section to the uterine wall by an ovarian ligament [8]. The size and appearance of the ovaries depends on both age and stage of the menstrual cycle. In a child, the ovaries are small structures approximately 1.5 cm long; however, they increase to adult size in puberty due to proliferation of stromal cells and commencing maturation of the ovarian follicles. In the young adult, they are almond-shaped and measure approximately 3 cm long, 1.5 cm wide and 1 cm thick. After the menopause, no active follicles are present and the ovary becomes smaller with a wrinkled surface. The ovary is the only intra-abdominal structure not to be covered by peritoneum.[9]

2.1.1.2. Histology: The body of the ovary consists of spindle-shaped cells, fine collagen fibers and ground substance that together constitute the ovarian stroma. The stromal cells resemble fibroblasts, but some contain lipid droplets. Bundles of smooth muscle cells are also scattered throughout the stroma [9]. The ovarian surface is covered by a single layer of cells called the germinal epithelium that overlies the dense irregular connective tissue tunica albuginea. Located below the tunica albuginea is the cortex of the ovary. The ovarian follicles are located in the connective tissue of the cortex. Deep to the cortex is the highly vascularized, connective tissue core of the ovary, the medulla. There is no distinct boundary line between the cortex and medulla, and these two regions blend together. In the peripheral zone of the stroma (cortex) there are numerous follicles that 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 oestrogen and progesterone production), degenerate and former corpora lutea (corpora albicantes) and degenerate (atretic) follicles . The superficial cortex is more fibrous than the deep cortex and is often called the tunica albuginea. However, unlike the testis, this is not an anatomically distinct capsule. On the surface of the ovary is an epithelial covering, misleadingly called germinal epithelium, which is a continuation of the peritoneum. The central zone of the ovarian stroma, the medulla M, is highly vascular and contains hilus cells, which are morphologically very similar to Leydig cells of the testis. [10]

2.1.1.3. Function:- The ovaries have two interrelated functions: gametogenesis (the production of gametes) and steroidogenesis (the production of steroids). Two major groups of steroid hormones—estrogens and Progestogens—are secreted by the ovaries.

Estrogens promote the growth and maturation of internal and external sex organs and are responsible for the female sex characteristics that develop at puberty. Estrogens also act on mammary glands to promote breast development by stimulating ductal and stromal growth and accumulation of adipose tissue.

Progestogens prepare the internal sex organs, mainly the uterus, for pregnancy by promoting secretory changes in the endometrium (discussed in the section on cyclic changes in the endometrium). Progestogens also prepare the mammary gland for lactation by promoting lobular proliferation. Both hormones play an important role in the menstrual cycle by preparing the uterus for implantation of a fertilized ovum[10].

2.1.1.4.physiology:- During embryonic development, primordial germ cells migrate from the yolk sac and colonize the embryonic gonadal ridges. Here, the germ cells differentiate into oogonia through the process of mitosis and then enter the first phase of meiotic division without completing it. The germ cells become arrested in this state of development and are now called primary oocytes . Primordial follicles are also formed during fetal life and consist of a

primary oocyte surrounded by a single layer of squamous follicular cells. Beginning at puberty and under the influence of pituitary hormones, some selected primordial follicles grow and enlarge to become primary, secondary, and large mature follicles, which can span the cortex and extend deep into the medulla of the ovary. The cortex of a mature ovary is normally filled with numerous ovarian follicles in various stages of development. In addition, the ovary may contain a large corpus luteum of a previously ovulated follicle and a corpus albicans of a degenerated corpus luteum. Also, most ovarian follicles in various stages of development (primordial, primary, secondary, and maturation) may undergo a process of degeneration called atresia, which are then phagocytosed by macrophages. Follicular atresia is common in an ovary. It occurs before birth and continues throughout the reproductive period of the individual. [8]

2.1.1.4.1. Menstrual cycle:- In the course of a normal menstrual cycle, the ovary will go through three phases: Follicular, Ovulation, and Luteal phase.

Follicular phase:- The initial stages of follicular development are independent of hormone stimulation. However, follicular development will fail at the preantral stage and follicular atresia will ensue if pituitary hormones LH and FSH are absent. FSH levels rise in the first days of the menstrual cycle, when oestrogen, progesterone and inhibin levels are low. This stimulates a cohort of small antral follicles on the ovaries to grow. As the follicles grow and oestrogen secretion increases, there is negative feedback on the pituitary.

Ovulation phase:- By the end of the follicular phase, which lasts an average of 14 days, the dominant follicle has grown to approximately 20 mm in diameter. As the follicle matures, FSH induces LH receptors on the granulose cells to compensate for lower FSH levels and prepare for the signal for ovulation. Production of oestrogen increases until they reach the necessary threshold to exert a positive feedback effort on the hypothalamus and pituitary to cause the LH surge. This occurs over 24–36 hours, during which time the LH-induced luteinization of granulosa cells in the dominant follicle causes progesterone to

be produced, adding further to the positive feedback for LH secretion and causing a small periovulatory rise in FSH. Androgens, also rise around the time of ovulation and this is thought to have an important role in stimulating libido, ensuring that sexual activity is likely to occur at the time of greatest fertility.

Luteal phase:- After the release of the oocyte, the remaining granulose and theca cells on the ovary form the corpus luteum. The granulosa cells have a vacuolated appearance with accumulated yellow pigment, (corpus luteum) which undergoes extensive vascularization in order to supply granulosa cells with a rich blood supply for continued steroidogenesis. Ongoing pituitary LH secretion and granulosa cell activity ensures a supply of progesterone which stabilizes the endometrium in preparation for pregnancy. Progesterone levels are at their highest in the cycle during the luteal phase. This also has the effect of suppressing FSH and LH secretion to a level that will not produce further follicular growth in the ovary during that cycle. The luteal phase lasts 14 days in most women, without great variation. In the absence of beta human chorionic gonadotrophin (ß- HCG) being produced from an implanting embryo, the corpus luteum will regress in a process known as luteolysis. If implantation of ovum to the endometrium does not occur, the endometrium of the uterus degenerates and menstruation follows. [11] New preantral follicles begin to be stimulated and the cycle begins anew. [10]

2.1.1.5. Pathology:- Ovarian disorders include the following:

2.1.1.5.1. premature ovarian failure (POF). is defined as cessation of periods before 40 years of age. It is usually unexplained, but may be due to chemotherapy, radiotherapy, autoimmune disease or chromosomal disorders (e.g. Turner's 45XO/46XX).

2.1.1.5.2. anovulation (Polycystic ovarian syndrome):-

PCOS is a syndrome of ovarian dysfunction along with the cardinal features of hyper androgenism and polycystic ovary morphology Its clinical manifestations include menstrual irregularities, signs of androgen excess (e.g. hirsutism) and obesity. Elevated serum LH levels and insulin resistance and are

also common features. It affects around 5–10 per cent of women of reproductive age. The prevalence of polycystic ovaries seen on ultrasound is much higher at around 25% .

2.1.1.5.3. Benign ovarian tumours:- The causes of benign ovarian tumours will vary with age. Functional cysts are common in young girls, adolescents and women in their reproductive years. Germ cell tumours occur more commonly in young women whereas benign epithelial tumours occur more commonly in older women.

Functional ovarian cysts: This group includes follicular, corpus luteal and theca luteal cysts.

Inflammatory ovarian cysts: Tubo-ovarian abscess .

Germ cell tumours(Benign teratoma): These are the most common ovarian tumours in young women, peak incidence is in the early 20s accounting for more than 50 per cent of ovarian tumours in this age group. The most common form of benign germ cell tumour is the mature dermoid cyst (cystic teratoma),

Epithelial tumours :- Benign epithelial tumours increase with age and are most common in peri-menopausal women. The most common epithelial tumours are :Serous cystadenoma , Mucinous cystadenoma , and Brenner tumour.

Sex cord stromal :- Ovarian fibromas are the most common sex cord stromal tumours. They are solid ovarian tumours composed of stromal cells. They present in older women often with torsion due to the heaviness of the ovary. Thecomas are benign oestrogen-secreting tumours. They often present post-menopause.

Other ovarian cysts:- Other cysts occasionally presenting as ovarian tumours include fimbrial cysts, paratubal cysts and other uncommon embryologically derived cysts such as cysts of Morgani.

2.1.2. Ovarian cancer

2.1.2.1. epidemiology:- Ovarian cancer is the second most common gynaecological malignancy and the major cause of death from a gynaecological

cancer. Ovarian cancer is more prevalent in developed nations, there are variations in incidence with ethnicity, Caucasian women have the highest incidence at approximately 14 per 100 000, whereas Asian women have a lower incidence at 10per 100 000. Eighty per cent of ovarian cancers are derived from the ovarian epithelium, other cell types include germ cell and sex cord-stroma. [11]

2.1.2.2. Risk Factors:- While there is currently no effective early detection test available, there are some known risk factors that may increase a woman's chance of developing ovarian cancer. They include: Older age (age 63 and older), Obesity, Use of estrogen replacement therapy after menopause, Having a blood relative who had ovarian, breast, or colorectal cancer Inherited genetic factors (but note that most cases of ovarian cancer occur in people with no family history of the disease and no genetic risk). Having a personal history of breast cancer. [12]

Family history and genetics: Heredity is a strong risk factor for ovarian cancer. Women who have a mother, daughter or sister with ovarian cancer have an increased risk of the disease. BRCA1 and BRCA2 genetic mutations, those associated with Lynch Syndrome and other hereditary genetic mutations have been linked to an increased risk of developing ovarian cancer.

age: The average age of diagnosis for ovarian cancer is 63, but ovarian cancer can also occur in older and younger women.

pregnancies: Women who have never been pregnant have an increased risk of ovarian cancer.

menstruation: More menstrual cycles/early menarche (first menstrual period) is associated with an increased risk of ovarian cancer.

Hormonal replacement therapy: Women who use hormone therapy may be at an increased risk for ovarian cancer. [13]

2.1.2.3. Symptoms of ovarian cancer:- The frequency and/or number of symptoms are key factors in the diagnosis of ovarian cancer. Symptoms

include: Bloating, Pelvic or abdominal pain, Difficulty eating or feeling full quickly, Urinary symptoms (urgency or frequency). [13]

2.1.2.4. Classification of ovarian cancer:- Ovarian cancer has several different tumor types including: epithelial tumors, germ cell carcinoma tumors, stromal carcinoma tumors and small cell carcinoma of the ovary **.** [13]

2.1.2.4.1. Epithelial ovarian cancer:- Is due to malignant transformation of the ovarian epithelium, this is the same as peritoneal mesothelium. Epithelial tumours of the ovary can be benign, malignant or borderline. Approximately 10 per cent of epithelial tumours are classified as borderline tumours. These tumours are well differentiated with some features of malignancy but are characterized by not invading the basement membrane, borderline tumours can spread to other structures (peritoneum, omentum) and rarely recur following initial surgery. The majority of borderline tumours are serous, mucinous borderline tumours may arise from the large bowel (appendix) and can be associated with pseudomxyoma peritonei.

2.1.2.4.2. Sex cord stromal tumours: These tumors account for approximately 10 per cent of ovarian tumors, The peak incidence is around the menopause, the exception is juvenile granulosa cell tumour, commonly presenting in girls under ten years of age and causing precocious puberty. Overall, granulosa cell tumors are the most common sex cord stromal cell tumors accounting for over 70 per cent of sex cord stromal tumors.

2.1.2.4.3. Germ cell tumours: Malignant germ cell tumours occur mainly in young women and account for approximately 10 per cent of ovarian tumours. They are derived from primordial germ cells within the ovary and because of this may contain any cell type. [11]

2.1.2.5. Diagnosing Ovarian Cancer:- Diagnosis of ovarian cancer starts with a physical examination (including a pelvic examination), a blood test (for CA-125 and sometimes other markers), and transvaginal ultrasound . The diagnosis must be confirmed with surgery to inspect the abdominal cavity, take biopsies (tissue samples for microscopic analysis), and look for cancer cells in

the abdominal fluid. This helps to determine if an ovarian mass is benign or malignant. [14] Tests will be used to confirm the diagnosis are : Ultrasound or x-ray tests which can reveal if there is a tumor on one or both ovaries, though they cannot tell if the tumor is cancer. Blood tests can show if you have the right number of the different kinds of blood cells. The tests may also look for tumor marker proteins such as CA-125, which may be higher than normal women with ovarian cancer. doctor may obtain a small tissue sample from a tumor to determine if the tumor is cancer. [12] In patients in whom pregnancy is a possibility, BHCG level can be measured during the diagnosis process. Serum alpha-fetoprotein, neuron-specific enolase, and lactate dehydrogenase can be measured in young girls and adolescents with suspected ovarian tumors. [14] Other tumor markers for ovarian cancer include CA19-9, CA72-4, CA15-3, immunosuppressive acidic protein, haptoglobin-alpha, OVX1, mesothelin, lysophosphatidic acid, osteopontin, and fibroblast growth factor 23. CT scanning is preferred to assess the extent of the tumor in the abdomino-pelvic cavity, though magnetic resonance imaging can also be used. Fluid from the abdominal cavity can also be analyzed for cancerous cells. If cancer is found, this procedure can also be used to determine the extent of its spread (which is a form of tumor staging).[15]

2.1.2.6. Staging and grading of ovarian cancer:- Stages of ovarian cancer are classified as Roman numerals I-IV, the higher the number, the more the disease has spread: **Stage I**: The cancer is confined to one or both ovaries. **Stage II**: Ovarian cancer has spread to another area within the pelvis without spreading elsewhere in the abdomen. **Stage III**: Cancer has spread from one or both ovaries to the lining of the abdomen or lymph nodes. **Stage IV**: cancer has spread beyond the abdomen to distant organs, such as the lung or liver.

There are three grades of ovarian cancer: Grade 1, Grade 2 or Grade 3. The lower the grade, the slower the cancer cells grow. [13]

2.1.2.7. Treatment:- Treatment options vary depending on the stage of the cancer, and are assessed taking into account the following variables: Tumour

size, Tumour position, Degree of spread, Patient's physical condition, and genetic mutations, such as BRCA. Treatment includes a combination of options, often starting with surgery, then chemotherapy and possibly, in rare cases, radiation therapy. Other potential treatments being evaluated in clinical trials include immunotherapy, gene therapy and hormone therapy. [13]

Surgery:- Surgery is used to remove as much of the tumour as possible. This is known as debulking surgery or cytoreduction. Patients most commonly have both ovaries removed (bilateral oophorectomy) and a hysterectomy (removal of the uterus). surgery followed by chemotherapy is recommended. [16]

Chemotherapy:- Chemotherapy after surgery is referred to as 'front-line' or 'first-line' treatment and involves a combination of a platinum and taxane-based chemotherapy (usually carboplatin and paclitaxel). [17]

2.1.3. Tumour Markers:-

Tumour markers are substances that can be found in the body when cancer is present. They are usually found in the blood or urine. They can be products of cancer cells or of the body in response to cancer. Most tumour markers are proteins. For many reasons, tumour marker itself is usually not enough to diagnose or rule out cancer. Most tumour markers can also be made by normal cells as well as by cancer cells. Sometimes, non-cancerous conditions can also cause elevation of some tumour markers to be higher than normal. Besides, not every cancer patient may have raised level of a tumour marker. For these reasons, only a handful of tumour markers are commonly used by most doctors. [18]

2.1.4. Role of Tumour Markers in Cancer:-

For Screening and Early Detection of Cancer: Screening refers to looking for cancer in people who have no symptoms of the disease, while early detection is finding cancer at an early stage. Although tumour markers were first developed to test for cancer in people without symptoms, very few tumour markers have been found to be helpful in this way because most tumour markers have not been shown to detect cancer much earlier than they would

have been found otherwise. **Diagnosing Cancer** In most cases, cancer can only be diagnosed by a biopsy and tumour markers are usually not used to diagnose cancer. However tumour markers can help determine if a cancer is likely in some patients. It can also help diagnose the origin of the cancer in patients presenting with advanced widespread disease. **Determining the Prognosis** (Outlook) for Certain Cancers: Some newer tumour markers help to assess how aggressive a cancer is likely to be or even how well it might respond to certain drugs. **Determining the Effectiveness of Cancer Treatment**: One of the most important uses for tumour markers is to monitor patients being treated for cancer. If the initially raised tumour marker level goes down with treatment, it indicates that the treatment is working and is having a beneficial effect. On the other hand, if the marker level goes up, then the treatment is probably not working and change of treatment should be considered. **Detecting Recurrent Cancer Markers**: are also used to detect cancers that recur after initial treatment. Some tumour markers can be useful once treatment has been completed and with no evidence of residual cancer left. These include PSA (for prostate cancer), HCG (for gestational trophoblastic tumours & germ cell tumours of ovaries & testicles), and CA 125 (for epithelial ovarian cancer). [18]

2.1.5. Role of Tumour Markers in the Management of Ovarian Cancer:-

Currently the only tumour marker to have a well-defined and validated role in the management of ovarian cancer is CA125. Changes in the level of CA125 can be used as a reliable indication of response or progression according to various criteria, but it does not yet have a clear place in diagnosis or prognosis. Its value as part of a screening tool and during routine follow-up remains the subject of ongoing trials. Other markers remain experimental and do not have a well-defined contribution to make at present The best available marker for epithelial ovarian cancer is the mucin, CA125. The reference interval most frequently quoted for CA125 is 0-35 kU/L, although 99% of apparently healthy post-menopausal women have levels below 20 kU/L. In apparently healthy pre-menopausal women, levels of 100 kU/L or higher can

occur during menses.[5] Patients with ovarian germ cell tumours often have raised levels of HCG and /or AFP, which are useful in diagnosis and follow-up.[18]

2.1.6. Survivin:-

Survivin, the protein that is also called baculoviral inhibitor of apoptosis repeat-containing 5 or BIRC5, is the smallest and last member of the Inhibitor of apoptosis (IAP) gene family. [19] Its function as an IAP has been conserved across evolution and its autologs have been found in other organisms such as yeast, worms and flies. [20] Alternative splicing of survivin gene results in different functional transcripts including survivin, survivin-2B, Survivin-delta-Ex-3, survivin-3B and survivin 2α . [21] Interestingly, Functional assays have shown that survivin 2α can attenuate the anti-apoptotic activity of survivin. [22] the expression of survivin 2α is decreased in tumoral tissues. [23] Survivin is undetectable in normal adult tissues, but highly expressed in several types of cancer.2,3 Survivin expression in the primary tumor has been associated with a worse prognosis, resistance to anticancer agents, and metastasis.[24] Furthermore, attenuation of survivin expression can induce apoptosis and sensitize cancer cells to conventional chemotherapeutics. The inhibition of apoptosis by survivin is associated with the mitotic spindle assembly checkpoint. Therefore, survivin may be a potential target for OC therapy. However, the prognostic significance of survivin in ovarian cancer is still unknown; more data are needed to confirm its value.[25] Survivin expression in normal tissues is developmentally regulated and the protein was found to be absent or low in most terminally differentiated tissues . However, recent studies tend to attribute a role to survivin in regulating the function of normal adult cells including vascular endothelial cells, polymorph nuclear cells, T cells, erythroid cells and hematopoietic progenitor cells. Moreover, survivin expression was reported in adult liver cells, gastrointestinal tract mucosa and ovarian granulosa cells. However, although survivin is expressed in normal tissues characterized by self-renewal and proliferation, its expression is

significantly lower than in transformed cells. In fact, several studies have demonstrated strong survivin expression in most human solid tumor types and hematologic malignancies. Expression of survivin has also been detected in a variety of benign and preneoplastic lesions including melanocytic nevi[26] polyps of the colon, breast adenomas, Bowen's disease and hypertrophic actinic keratosis [27], suggesting that re-expression of survivin may occur early during malignant transformation or following a disturbance in the balance between cell proliferation and cell death. [28]

2.1.7. Survivin in ovary tumors:-

Survivin is expressed in human carcinomas, but its expression levels in tissues are different, that is associated with the poor outcome of patients. Many studies have demonstrated that rate of expression and sub-cellular localization of survivin correlated with the progression and prognosis of ovarian carcinoma [29].several studies have shown that positive survivin expression in ovarian cancer was associated with disease aggressiveness, unfavorable clinical outcomes, and chemotherapy resistance[25] Furthermore, recent research showed that serum survivin was positively correlated with advanced stage and poor disease free survival in ovarian cancer[30] . Survivin has been proposed as a chemoresistance and radioresistance factor, and survivin inhibitors are intensively investigated for cancer therapy[31] Survivin status had no relationship with age, family tumor history, blood type, and preoperative CA-125[25]. patients with positive survivin expression are more likely to be chemoresistant and have higher mortality rates than those of negative expression. Therefore, it is feasible that survivin inhibitors can be used for at least a subgroup of ovarian cancer patients [32]

3. Materials and Methods

3.1 Materials:

Archived tissue block obtained from ovarian samples previously diagnosed as ovarian carcinoma were selected for this study, Tissue Microarray slides were prepared and stained immunohistilogically for survivin expression**.**

3.2Methods:

3.2.1 Study design:

This is descriptive retrospective laboratory based study aimed to detect the immune expression survivin in ovarian cancer among Sudanese women using immunohistochemistry.

3.2.2 Study samples:

36 paraffin blocks previously diagnosed as ovarian cancer were selected from El-rahama medical center. Patient identification (age, grad and diagnosis) were obtained from patients records.

3.2.3 Study area:

ELrhama medical center in Khartoum from march to august 2018.

3.2.4 Inclusion criteria:-

- ❖ no patients were treated with preoperative chemotherapy.
- ❖ the first diagnosis were performed in this medical center.
- ❖ the pathological results were diagnosed and documented as ovarian cancer
- ❖ patient data were available.

3.2.5 Immunohistochemistry staining:

From the previously diagnosed ovarian cancer blocks, representative samples from the defined areas, Tissue cores with a diameter of 0.6 µm were punched from each specimen and arrayed on a recipient paraffin block. The immunohistochemical procedure was done as follows:

The tissue microarray section from formalin-fixed, paraffin-embedded block were cut and mounted onto salinized slides (Thermo). Following deparaffinization in xylene, slides were rehydrated through a graded series of alcohol and were placed in distilled water. Samples were steamed for antigen

retrieval for survivin using high PH (9) by water bath at 95C for 40 min. After washing with PBS for 3 min Endogenous peroxides activity were blocked with 3% hydrogen peroxide and methanol for 10 min, and After washing with PBS for 3 min then Slides were incubated with (100 µ L) of (Rabbit monoclonal antibody to survivin quartett), against LMP1 for 30 min at room temperature in a moisture chamber. After washing with PBS for 3 min, binding of antibodies will be detected by incubating for 20 min with dextrin labeled polymer (Dako). Finally, the sections washed in three changes of PBS, followed by adding 3, 3 di amino benzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After washing with distal water for 3 min Slides were counterstained with haematoxylin (MAYER'S) for one min were washed in running tap water for several minutes 7-10 (bluing), then dehydrate and, cleaned, mount in DBX, then slides investigated microscopically for survivin expression.

3.2.5. Result interpretation:-

All positive samples show cytoplasmic expression of survivin tumor marker. Detected by the brown color of (DAB) in the cytoplasm of ovarian cancerous cells.

3.2.6. Positive control:-

Colon cancer samples : show nuclear expression of survivin marker.

3.2.7. Statistical analysis:-

The collected data were analyzed statistically and the frequency of age of patients, frequency of survivin expression, and it relation with different types of tumors was calculated ,they were considered significant at P-value < 0.05. the (IBM SPSS Statistics 22 License Authorization Wizard) program was used to perform statistical analyses.

3.2.8. Ethical consideration:-

Specimens and data was taken According to the laboratory guidelines and regulation , ethically after taken ethical clearance.

4. Results:-

To investigate the status of survivin in ovarian cancer, we examined the expression of survivin in 36 samples of malignant ovarian tumors tissues by immunohistochemistry .The age of study population ranged between 32 and 65 years with mean of 50 years, the patient with less than 50 represents 16 (44.4%) and 20 patients(55.6%) are 50 years or more **(table: 4.1)** .

the histopathological diagnosis of study samples includes:- stromal origin16.7% (6), germ cell 5.6% (1), and epithelial cell origin 80.5% (29). The differentiated ovarian neoplasms72.2% (26), un differentiated ovarian neoplasms 27.8% (10). specific survivin immuno staining was observed in the cytoplasm compartment of tumour cells **(Photomicrograph 3.1)** in 94.4% (34/36) of cases of malignant tumors**(table: 4.2).** The expression rate was 93% (27/ 29) in epithelial origin neoplasms, and was 100% for the stromal and germ cell origin neoplasms . (**Table 4.3)**. According to the type of epithelial neoplasm, the expression rates was (90.5%%)for adenocarcinoma(19/ 21), (100%) for serous and mucinous epithelial neoplasms(**Table 4.4)**. according to the grade of tumor :- the expression rate in differentiated ovarian neoplasms was 92.3% (24/ 26),compared to un differentiated ovarian neoplasms (100%)(**Table 4.5)**, these results show a strong relation between cytoplasmic survivin expression and ovarian cancer, but not with the origin, grade, and type of cancer (*p-value* = 0.774, 0.869 , 0.369) respectively.

Table (4.1): Shows the distribution of age among study population.

Age group	Frequency	Percent
Less than 50	16	44.4
50 or more	20	55.6
Total	36	100.0

Table (4-2): Shows the distribution of Survivin expression.

Survivin expression	Frequency	Percent
Negative	2	5.6
Positive	34	94.4
Total	36	100.0

Table (4-3): Shows the relation between the origin of ovarian cancer and survivin expression.

Lesion	Survivin expression		Total
	Negative	Positive	
Epithelial	2	27	29
Stromal	0	6	6
germ cell	0	1	1
Total	2	34	36

p-value = 0.774

Table (4.4): Shows the relation between the type of epithelial and survivin expression.

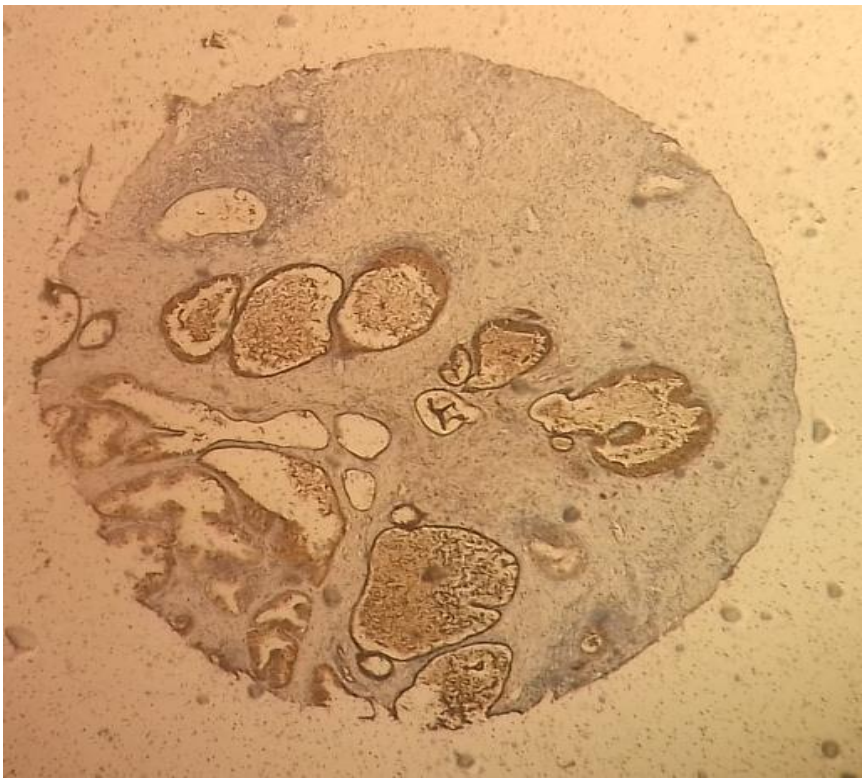
Type of epithelial	survivin expression		Total
	Negative	Positive	
Tcc	0	1	1
Serous	0	5	5
mucinous	0	2	2
adenocarcinoma	2	19	21
Total	2	26	29

p-value = 0.869

Table (4.5): Shows the relation between the grade of tumor and survivin expression.

Grade	Survivin expression		Total
	Negative	Positive	
Differentiated	2	24	26
Undifferentiated	0	10	10
Total	2	34	36

p-value = 0.367



Photomicrograph (4.1): epithelial ovarian carcinoma showed cytoplasmic positive expression of survivin.

5.1. Discussion

The present study include 36 samples of ovarian neoplasms for expression by immunohistochemical staining on fixed paraffin impeded tissue sections using mouse monoclonal Ab (survivin quartett). In our data set, the positive rate of survivin expression in female ovarian cancer tissue was 94.4%, as shown in **table (4.2)** this result agree with ***Danuta Plewka et al*** [33]. who study survivin expression in serous and mucinous ovarian cancer in Polandian women and found that the expression rate was 85.5%(41/ 47) in ovarian cancer . and agree with ***Ferrandina et al*** [29] in the patients admitted to the Division of Gynecologic Oncology, Catholic University of Rome and found that the Cytoplasmic survivin immuno reaction was observed in 84.5% cases and did not show any relationship between survivin positivity rate and ovarian cancer. And also agree with ***N. Zaffaroni et al*** [34] who examine the Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer and found the immunohistochemistry expression of survivin in 90/124 (73%) advanced ovarian carcinomas, and ***Cynthia Cohen et al*** [7] who demonstrate the survivin in 74% of ovarian cancers samples.

According to the origin of tumor The expression in epithelial tumors was 80.5% which seems to be agree with ***Lifeng Chen et al*** [25], who found the positive rate of survivin in epithelial tumors was 60%,the expression in serous and mucinous epithelial tumors was 100% which is near to ***Danuta Plewka et al*** [33] results which is 91% for serous and 80% for mucinous tumors.

The statistical analysis failed to demonstrate any significant relation between the survivin and the origin, grade and type of ovarian cancer and the differentiation, in spite of its relation to clinic-pathological data And this may be due to the small size of sample and lack of study to the control group of normal ovarian tissue samples. But ***Danuta Plewka et al*** [33] Reported that the survivin is strongly related to the degree of malignancy and tumor differentiation. ***Also Z. Liguang et al*** [35] found statistical significance of (P = 0.008) for survivin expression and histological type of tumor (serous,

mucinous), A significant positive correlation (P = 0.026)was observed between survivin mRNA expression rate and histological grade.

In this study there is no relation between survivin expression and the age of patient, this finding agree with ***Lifeng Chen et al*** [25] who reported that Survivin status had no relationship with age, family tumor history, blood type .

6.1. Conclusion

This study include 36 sample of tissues from patient with ovarian cancer ,Survivin expression was studied among them by immunohistochemistry ,majority of samples express survivin in their cytoplasm with no statistical significant relation to the origin, grade, and the subtype of ovarian tumor.

6.2. Recommendations

-We recommend to implement other studies to detect the survivin expression on the ovarian tumors, with larger sample size, with addition of control group.

-We recommend to implement other studies to detect the survivin expression on the ovarian tumors of borderline and benign types .

- We recommend to implement other studies that detect the survivin expression before and after treatment to assess drug resistance and the effect of cancer therapy on the survivin expression

- It is suggested that Patients diagnosed as ovarian malignancies either as clinical or histological basis are strongly recommended for serum ovarian tumor markers(survivin detection), for diagnosis of bulk of ovarian tumors. This tumor marker(survivin) may help oncologist / clinicians for diagnosis, prognosis and treatment of patients.

-We recommend the clinicians to add the survivin tumor marker to the panel of ovarian diagnostic tumor markers

- finally It is suggested that blocking survivin production genetically may have therapeutic potential for the treatment of ovarian cancers, especially those with drug-resistant characteristics.

6.3. References

1. Shaikh NA, M.F., Samo RP, *TUMOR MARKERS; Efficacy of CA-125, CEA, AFP, & Beta HCG An institutional based descriptive & prospective study in diagnosis of ovarian malignancy* Professional Med J, 2014. **21**(4): p. 621-627.
2. Ferlay J, e.a., *Cancer Incidence and Mortality Worldwide: IARC CancerBase No.* GLOBOCAN 2012 v1.0, 2013. **11**.
3. Vergote I, V.J., Heus P, Hoogendam J.P, Damen J.A.A.G, van de Wetering F t, van der Baan F.H, Bourgain, D.G.J. C, Debruyne D, Fastrez M, Goffin F, Huizing M, Kerger J, Kridelka F, Stroobants S, Tjalma W, Van, and V.d.C.V. Dam P, Villeirs G, Vuylsteke P, Fairon N, Zweemer R.P, Hooft L, Scholten R.J.P.M, Verleye L, *Ovarian cancer: diagnosis, treatment and follow-up.* Good Clinical Practice (GCP) Brussels: Belgian Health Care Knowledge Centre (KCE), 2016. **KCE Reports 268. D/2016/10.273/49**.
4. Wisal Adam, R.A.G., Moawia Alsadig Humida and F.G. Abdelaziz, *Ovarian Cancer in Sudan.* Journal of Medical and Biological Science Research September 2017. **Vol. 3**(4): p. 37-41.
5. DM, D.R.M., *Role of Tumour Markers in the Management of Ovarian Cancer.* NDIAN JOURNAL OF MEDICAL & PAEDIATRIC ONCOLOGY, 2007. **27**(1): p. 8-11.
6. S. Kim Suvarna , C.L., John D. Bancroft, Bancroft's, *Theory and Practice of Histological Techniques.* British Library Cataloguing in Publication Data, 2013. **7th ed**(616′.07583 – dc23).
7. Cynthia Cohen, M.D., Christina M. Lohmann, M.D., George Cotsonis, Ph.D., Diane Lawson, M.T.,Robert Santoianni, *Survivin Expression in Ovarian Carcinoma: Correlation with Apoptotic Markers and Prognosis* United States and Canadian Academy ofbPathology, Inc., 2003. **vol.16**(6): p. 574-583.
8. Victor P. Eroschenko, P., *diFIORE'S ATLAS OF HISTOLOGY WITH FUNCTIONAL CORRELATIONS.* Library of Congress, 2013. **12th ed**.

9. Barbara Young, G.O.D., Phillip Woodford, *Wheater's Functional Histology A Text and Colour Atlas.* Library of Congress, 2014. **sixth ed**.
10. Wolfgang Kuehnel, M.D., *Color Atlas of Cytology, Histology, and Microscopic Anatomy* Thieme Stuttgart · New York, 2003. **4th edition**.
11. Ash Monga BMed (Sci) BM BS MRCOG, S.D.M.F., *GYNAECOLOGY by Ten Teachers.* by Taylor & Francis Group, LLC, 2011 **19th Edition**.
12. Survivor, S.M.O.G.o.M.a.C., *UNDERSTANDING OVARIAN CANCER.* society of gynecologic oncology SGO, 2015.
13. frankly, *Ovarian Cancer*. Cancer Support Community, June 2015
14. Longo DL, K.D., Jameson JL, Fauci AS, Hauser SL, Loscalzo J. Harrison's, *Gynecologic Malignancies.* Principles of Internal Medicine, 2012. **18th ed**.
15. Grossman, D.C.C., Susan J.; Owens, Douglas K.; Barry, Michael J.; Davidson, Karina W.; Doubeni, Chyke A.; Epling, John W.; Kemper, Alex R.; Krist, Alex H.; Kurth, Ann E.; Landefeld, C. Seth; Mangione, Carol M.; Phipps, Maureen G.; Silverstein, Michael; Simon, Melissa A.; Tseng, Chien, *Screening for Ovarian Cancer.* JAMA, 13 February 2018. **319**(6).
16. al., G.J.e., *ovarian cancer.* Journal of Ovarian Research 2010. **3**(8).
17. al, H.B.e., *Ovarian cancer* Lancet 2009. **9698**: p. 1371-1382.
18. YING, D.A.C.H., *Tumour Markers.* HKACS.org/ profficional practice, april 2013. **30 april**: p. 45-54.
19. DC, A., *Molecular cloning of effector cell protease receptor-1, a novel cell surface receptor for the protease factor Xa.* J Biol Chem, 1994. **269**(31): p. 39-42.
20. Tamm I, W.Y., Sausville E, et al, *IAP-family proteinsurvivin inhibits caspase activity and apoptosis induced by Fas (CD95), Bax, caspases, and anticancer drugs.* Cancer Res, 1998. **58**(53): p. 15-20.
21. Caldas H, F.J., Boué DR, Holloway MP, Altura RA, *Dissecting the role of endothelial survivin ΔEx3 in angiogenesis.* Blood, 2007. **109**(14): p. 79-89.

22. Cheng Z, H.L., Fu W, Zhang Q, Liao X, *Expression of survivin and its splice variants in gastric cancer.* J Huazhong Univ Sci Technolog Med Sci, 2007. **27**(39): p. 3-8.

23. Elaheh Soleimanpour, E.B., *Survivin as a Potential Target for Cancer Therapy.* Asian Pac J Cancer Prev, October 2015. **16**(15): p. 6187-6191.

24. 6. Zhang M, C.J., Suzuki Y, et al, *Survivin is a potential mediator of prostate cancer metastasis.* Int J Radiat Oncol Biol Physics, 2010. **78**: p. 1095-1103.

25. Lifeng Chen, M., * Lizhi Liang, MD,Þ Xiaojian Yan, MD,* Naihua Liu, PhD,þ Lihua Gong, MD,*Shishi Pan, MD,* Feng Lin, MD,* Qian Zhang, MD,* Hongqin Zhao, MD,* and Feiyun Zheng, MD*, *Survivin Status Affects Prognosis and Chemosensitivity in Epithelial Ovarian Cancer.* International Journal of Gynecological &Cancer 2013. **23**(2): p. 256-263.

26. Marzia Pennati, M.F.a.N.Z., *Targeting survivin in cancer therapy: fulfilled promises and open questions.* Carcinogenesis, 2007. **.28**(6): p. 1133–1139.

27. Altieri, D.C., *Survivin, versatile modulation of cell division and apoptosis in cancer.* Oncogene, 2003. **22**: p. 8581–8589.

28. Velculescu, V.E.e.a., *Analysis of human transcriptomes.* Nat.Gen, 1999. **23**: p. 387–388.

29. Ferrandina, G., et al., *Survivin expression in ovarian cancer and its correlation with clinico-pathological, surgical and apoptosis-related parameters.* British journal of cancer, 2005. **92**(2): p. 271.

30. No JH, J.Y., Kim YB, et al, *Quantitative detection of serum survivin and its relationship with prognostic factors in ovarian cancer.* Gynecol Obstet Invest, 2011. **71**: p. 136-140.

31. Ma X, W.S., Zhou J, et al, *Induction of apoptosis in human ovarian epithelial cancer cells by antisurvivin oligonucleotides.* Oncol Rep, 2005. **14**: p. 275-279.

32. Zaffaroni N, P.M., Colella G, et al, *Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer.* Cell Mol Life Sci, 2002. **59**: p. 1406-1412.
33. Danuta Plewka, B.-B., Michal Morek,Edyta Bogunia , Marek Bienioszek, Hubert Wolski, Daniel kotrych , Karolina Dziekan, Agnieszka Seremak-Mrokiewicz ,Andrzej Plewka, *survivin in ovary tumors.* gine kol pol, 2015. **86**: p. 525-530.
34. N. Zaffaronia, M.P., G. Colellaa, P. Peregoa, R. Supinoa, L. Gattia, S. Pilotti b, F. Zuninoa and M. G. Daidonea, *the Expression of the anti-apoptotic gene survivin correlates with taxol resistance in human ovarian cancer* CMLS, Cell. Mol. Life Sci, 2002. **59**: p. 1406–1412.
35. Z. Liguang1, L.P., M. Hongluan, J. Hong2, W. Rong, M.S. Wachtel, E.E. Frezza, *Survivin expression in ovarian cancer* Exp Oncol, 2007. **29**(2): p. 121-125.

Appendices

Appendix 1: Materials and instrument used for processing and staining of the specimens include:

- Disposable gloves
- Microtome knife
- Positively charged slide
- Cover glass
- Dry oven
- Water bath
- Embedding center
- Coplin jar
- Humidity chamber
- Ethanol (100%, 90%, 70%, 50%)
- Mayer s haematoxylin (haematoxylin , DW,K or ammonium alum ,sodium iodated ,citric acid ,chororal hydrate)
- Reaction buffer
- Primary antibody (EBV)
- Tris EDTA buffer (PH9)
- Phosphate buffer saline (PH7.4)
- Peroxides blocker(3% hydrogen peroxide in methanol)
- Secondary anti body (dextran polymer conjugated secondary – HRP)
- DAB (3,3 di amino benzidin tetra hydrochloride)substrate solution
- Bluing Reagent (0.1MLi2 CO3 , 0.5 M Na2CO3)
- Xylene
- DPX mounting media

Appendix II:- Survivin monoclonal Ab (quartett) sheet:-

quartett

Cat. No.	2-SU044-10
Lot No.	351430
Quantity	3 ml

Antibody to Survivin

Host:	**Rabbit**
Immunogen:	Synthetic peptide corresponding to residues residues on the N terminus of human Survivin.
Subclass:	IgG
Presentation:	Prediluted antibody in TRIS, pH 7.4, containing < 0.1 % sodium azide.
Titre:	Used in a corresponding system, vial is sufficient for 30 slides using 100 µl per slide. Incubate primary antibody for at least 60 min.
Assay system:	IHC(p,f)
Fixation:	1) NOTOXhisto 2)Formalin
Treatments:	Staining of formalin/paraffin tissues is enhanced by deparaffinazation, rehydration and antigen retrieval. The recommended method is HIER (Heat Induced Epitope retrieval), including boiling of tissue sections in ProTaqs Antigen Enhancer I, Cat# 401602092, for 20 min, followed by cooling at RT for 20 min. The optimal method of antigen retrieval must be determined in user's own system.
Reactivity:	Human, others not tested.
Cell. Local.:	Nuclear/Cytoplasmic
Control:	Colon and Colon cancer
Storage:	Aliquot and store at -20 °C. Do not use after expiration date indicated on the vial.

Errors excepted. This data sheet is a general information. The product attribute can diversify with changing Lot No as well as variations in tissue selection, tissue processing, antigen retrieval and detection systems. We do not take responsibility for any possible damage including personal injury, time or effort on economic loss caused by this product. Our warranty is limited to the price paid for the product. The product may only be used by authorized and skilled personnel. Non-application as prescribed in this data sheet leads to loss of all liability.

FOR IN VITRO USE, NOT FOR DIAGNOSTIC USE

Manufacturer:
BIOCYC GmbH & Co. KG, Am Mühlenberg 11, 14476 Potsdam; certified by
ISO 13485:2012 and AC: 2012, ISO 9001:2008; Registry number: 60018916, 60018917

Distributed by:
quartett Immunodiagnostika + Biotechnologie GmbH
Schichauweg 16, 12307 Berlin, Germany
Tel.: ++49 (030) 765 925-0, Fax.: ++49 (030) 765 925-55
E-Mail: **info@quartett.com** Internet: www.quartett.com

Appenaix III:- ovarian cancer Data sheet.

Shendi University

Faculty of Graduate Studies and Scientific Research

Data sheet for ovarian cancer

1. Name……

2. Age of patient ☐

3. Type of ovarian cancer…….

4.Tumor origin:-

Epithelium tumor ☐ **Stromal tumors** ☐ **Germ cell tumors** ☐

5.Grades of ovarian cancer

Grade I ☐ **Grade II** ☐

Grade III ☐ **Grade IV** ☐

6. survivin expression result:-

Positive ☐ **Negative** ☐

Nuclear ☐ **Cytoplasmic** ☐