بسم الله الرحمن الرحيم

Republic of Sudan

Ministry of Higher Education and Scientific Research

Shendi University

Faculty of Graduate Studies and Scientific Research

Assessment of P53 Expression in Ovarian Cancer among Sudanese Women by Immunohistochemical Technique

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BSc. in Haematology & Histopathology & Cytology

Shendi University 2014

A Thesis submitted for the Fulfilment of the Master Degree in Medical Laboratory Sciences (Histopathology & Cytology)

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August – 2018

بسم الله الرحمن الرحيم

قال تعالى :

﴿ وَإِذَا مَرِضْتُ فَهُوَ يَشْفِينِ ﴾

صدق الله العظيم

الآية (80) من سوره الشعراء

DEDICATION

To that gentle man, I love and respect

''MY FATHER''

To the beating heart of my life

''MY MOTHER''

To my lovely family

Special dedication,

To who share with me all moments of happiness and sadness

'' my grandma, my lovely sister and my brothers

And my sweet friends''

To all whom not mentioned

ACKNOWLEDGEMENT

All thanks to Allah from the start to the end…..

And pray for Prophet Mohammed peace be upon him

I would like to acknowledge the contribution of my supervisor

Dr: Asma El-Ameer Mohammed

Who guide me, helped me to make this research as accurate and useful as possible.

My thanks also extend to my colleges and my teachers.

Abstract

This is descriptive retrospective study which was conducted at ELrhama medical center during the period from April 2018 to August 2018.

The aim of this study was to assess the express of p53 protein in ovarian cancer among sudanese women .

40 tissue microarray samples were collected from patients previously diagnosed as ovarian carcinoma, analyzed using immunohistochemical techniques. The data obtained was analyzed using SPSS program version 22. The age of study population ranged between 32 and 65 years. Most patients were less than 50 years represent 19 (47.5%) and the remaining 21(52.5%) were more than 50 years.

A total of 17 cases showed positive nuclear p53 staining, Types of malignant tumour showed no correlation between tumour protein p53 and types of malignant tumour (p = **0.186**), types of epithelial origin show no correlation between tumour protein p53 and types of epithelial origin (p=**0.532**) and grades of malignant tumour showed no correlation between tumour p53 and grades of malignant tumour (p=**0.196**).

The study concluded that the expression of tumor protein p53 was associated with malignancy tumours of ovaries with no correlation with both types or grades of ovarian cancer.

ملخص البحث

أجريت هذه الدراسة الوصفية المعملية الاسترجاعية، في مركز الرحمة الطبي، في الفترة من أبريل إلى أغسطس 2018م، هدفت الدراسة لتقييم تواتر كيمياء الأنسجة المناعية لتحديد بروتين الورم ب 53 لدي النساء السودانيات المصابات بسرطان المبيض.

جمع أربعون قالب شمعي باستخدام الطريقة المصفوفة للأنسجة من سيدات تم تشخيصهن مسبقا بسرطان المبيض و استخدمت تقنية كيمياء الأنسجة المناعية. كذلك استخدام برنامج الحزم الإحصائية للعلوم الاجتماعية النسخة 22 لتحليل البيانات.

تراوحت أعمار المريضات بين 32–65 عام. أظهرت الدراسة ان نسبة المريضات اللاتي تقل أعمارهن عن 50 سنة، كان عددهن 19 مريضة بنسبة (47.5%) و نسبة المريضات اللائي تزيد اعمارهن عن 50 سنة كان عددهن 21 مريضة بنسبة (52.5%).

أظهرت الدراسة أن ب 53 النووية كان موجب الظهور في 40/17 عينة من أورام المبيض.

فيما يتعلق بأنواع الأنسجة الطلائية المنشأ ، أظهرت عدم وجود علاقة ذات دلالة إحصائية بين ظهور بروتين الورم ب 53 و أنواع الأنسجة الطلائية المنشأ (القيمة الاحتمالية =0.532).

فيما يتعلق مراحل تمايز الورم الخبيث، أظهرت عدم وجود علاقة ذات دلالة إحصائية بين ظهور بروتين الورم ب 53 و مراحل تمايز الورم الخبيث (القيمة الاحتمالية =0.196). .

خلصت الدراسة أن ظهور بروتين الورم ب53 له ارتباط مع سرطان المبيض مع عدم وجود علاقة مع النوع النسيجي للأورام الخبيثة.

ايجابية ب 53 تحدد وجود ورم خبيث بالمبيض بينما سلبية ب 53 لا يستبعد وجود ورم خبيث بالمبيض.

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Chapter One

Introduction

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1.1 Introduction

Ovarian cancer is the seventh most common cancer, and it is the most common cause of mortality from gynecological cancers worldwide. In developing countries it is ranked the second most common gynecological cancer, and constitutes the fourth most common of all cancers in women. [4] Ovarian cancer (OC) accounts for an estimated 239,000 new cases and 152,000 deaths worldwide annually. [10]

The incidence rate of ovarian cancer in the entire Sudan has yet to be identified; however, in a hospital-based data set from the National Cancer Institute, Gezira University, Central Sudan and Radiation Isotopes Center in Khartoum, collected between 2000 and 2006, ovarian cancer accounted for 6.8% (949) of all recorded cancers (n=226,652). Additionally, in a more recent data set (2009–2010) from the National Cancer Registry for Khartoum State alone, ovarian cancer was the fourth most common cancer in women, with an estimated incidence rate of 188 per 100,000 populations, a gender-specific rate of 8.0 per 100,000 populations, and an age-standardized rate (ASR) of 7.0 per 100,000 populations. [4]

Immunohistochemistry (IHC) is a method used to determine the expression of biomarkers in tissue. It is used in the pathology laboratory as an aid in the differential diagnosis and classification of cancer, and for certain other diseases, including infections. [32] IHC allows for precise tissue localization of stem cells, dying neurons, and metastasizing cancer cells, as well as subtle intracellular changes in protein activation and trafficking within the cell. Immunohistochemical analysis can be used to demonstrate accumulation of mutant p53 protein in tumor cells. [33]

P53 (Tp53, tumor protein p53) is one of the most relevant human oncosuppressor genes. Accordingly, inactivation of p53 by direct mutation of the gene is one of the most frequent genetic lesions in human tumors, indicating that the *TP53* gene plays a crucial role in preventing cancer formation. [11, 7]

1.2 Rationale

Ovarian tumors is the second most common gynecological neoplasm , the majority of cases present at advanced stages ,at which the disease is raely curable by existing treatment schemes . The most frequent alterations contribute to ovarian carcinogenes is p53 pathway. The high mortality rate of ovarian cancer is due to the lack of a screening strategy to detect early-stage disease, in this study well find the value of introduce this marker in routine panel of markers used for diagnosis and prognosis of ovarian cancer.

1.3 Objectives

1.3.1 General objective:

To assesses p53 expression in ovarian cancer among Sudanese women by histochemical technique.

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1.3.2 Specific objectives:

1. To evaluate P53 tumour protein activity using p53 immunohistochemical staining in ovarian cancer.
2. To correlate P53 expression with types of ovarian cancer and grades.

Chapter Tow

Literature review

2. Literature Review

The female reproductive system consists of the internal reproductive organs (the paired ovaries and oviducts, the uterus, and the vagina), and the external genitalia (the clitoris, the labia majora, and the labia minora). [15]

2.1: Ovary

The ovaries are considered the female gonads. The paired ovaries, located within the pelvis, are almond-shaped bodies 3 cm long, 1.5 to 2 cm wide, and 1 cm thick, each weighing approximately 14g. [15]

Each ovary is whitish in color and located along side the lateral wall of the uterus in a region called the ovarian fossaThe ovarian fossa is the region that is bounded by the external iliac artery and in front of the ureter and the internal iliac artery. This area is about 4 cm x 3 cm x 2 cm in size. The ovaries are surrounded by a capsule, and have an outer cortex and an inner medulla. [29, 3]

The ovaries are suspended in the broad ligament of the uterus by an attachment called the mesovarium, a special fold of the peritoneum that conveys blood vessels to the ovaries. [15]

- paired glands:
  - Exocrine function: Maturation and release of oocytes, developing female germ cells.
  - Endocrine function: Secretion of estrogen and progesterone. [25]

2.1.1: Function

At puberty, the ovary begins to secrete increasing levels of hormones. Secondary sex characteristics begin to develop in response to the hormones. The ability to produce eggs and reproduce develops. The ovary changes structure and function beginning at puberty.

- Gamete production, the ovaries are the site of production and periodical release of egg cells, the female gametes.

- Hormone secretions, at maturity, ovaries secrete estrogen, testosterone, inhibin, and progesterone.
- Ovarian aging, as women age, they experience a decline in reproductive performance leading to menopause. [1]

2.1.2: Development of the Ovary

Around the end of the first month of embryonic life, a small population of primordial germ cells migrates from the yolk sac to the gonadal primordia. In the gonads these cells divide and transform into oogonia. Division is so intense that in the second month of intrauterine life there are around 600,000 oogonia, and around the fifth month more than 7 million. Beginning in the third month, oogonia begin to enter the prophase of the first meiotic division but stop at the diplotene stage and do not progress to other stages of meiosis. These cells are the primary oocytes (Gr. oon, egg, + kytos, cell), and they become surrounded by flattened cells called follicular cells. By the seventh month of pregnancy, most oogonia have been transformed into primary oocytes. Many primary oocytes, however, are lost through a degenerative process called atresia. As a result, around puberty the ovaries contain about 300,000 oocytes. Atresia continues over the entire span of the woman's reproductive life so that by 40–45 years of age about 8000 oocytes are left. Because generally only one oocyte is liberated by the ovaries in each menstrual cycle (average duration, 28 days) and the reproductive life of a woman lasts about 30–40 years, only about 450 oocytes are liberated. All others degenerate through atresia. [6]

2.1.3: Ovarian Follicles

The primary role of the follicle is oocyte support. From birth, the ovaries of the human female contain a number of immature, primordial follicles. These follicles each contain a similarly immature primary oocyte. At puberty clutches of follicles begin folliculogenesis, entering a growth pattern that ends in death (apoptosis) or in ovulation (the process where the oocyte leaves the follicle). During follicular

development, primordial follicles undergo a series of critical changes in character, both histologically and hormonally. First they change into primary follicles and later into secondary follicles. The follicles then transition to tertiary, or antral, follicles. At this stage in development, they become dependent on hormones, particularly follicular stimulating hormone (FSH) which causes a substantial increase in their growth rate. The late tertiary or preovulatory follicle ruptures and discharges the oocyte (that has become a secondary oocyte), ending folliculogenesis. [21]

2.2: Pathology of the ovary

2.2.1: Inflammation

Acute inflammation of the ovary occasionally complicates acute salpingitis and this combination may progress to atubo ovarian abscess. Oophoritis is, however rare. [5]

2.2.2: Oophoritis

Inflammation of the ovaries is always secondary to disease of the fallopian tubes or peritoneum. In the case of the tube, the inflamed fimbrial end becomes adherent to the ovary and direct spread of infection occurs. Tubo-ovarian inflammation is also associated with the presence of an intra uterine contraceptive device. [12]

2.2.3: Non neoplastic cysts

Cystic change occurs with some frequency in graafian follicles and corpora lutea.[5]

Ovarian changes of functional origin:

The control mechanisms of ovarian function frequently develop faults resulting in abnormalities in structure. [12]

2.2.3.1: Follicular cysts

Are so common as to be virtually physiologic variants, are found in the cortex. They originated in unruptured graafian follicles or in follicles that have ruptured and immediately sealed. These may be single or multiple; the maximum diameter of normal graafian follicle is 1.5-2 cm.Single follicular cysts may be several centimeters in diameter. Their smooth lining is formed of flattened granulose cells

which may secrete sufficient estrogen to inhibit pituitary FSH secretion and lead to anovulatory cycles with consequent endometrial hyperplasia. [5, 12, 13]

2.2.3.2: Corpus luteum cysts

Are usually solitary, contain either altered blood or clear amber fluid, are lined by luteinised granulosa and theca cells and although usually asymptomatic, can rupture and bleed into peritoneal cavity. [5]

2.2.3.3: Polycystic ovaries

In 1935 Stein and Leventhal described a syndrome characterized by secondary amenorrhea, obesity, hirsutism, infertility, and bilaterally enlarged polycystic ovaries. The exact pathophysiology of this condition is obscure but it appears that this particular type of polycystic ovary secretes, possibly because of an enzyme defect, an excess of androstenedione. [13,5]

2.3: Neoplastic changes

Tumors of the ovary are common forms of neoplasia in women. Among cancers of the female genital tract, the incidence of ovarian cancer ranks below only carcinoma of the cervix and endometrium.It accounts for 6% of all cancers in the female and is the fifth most common form of cancer in women in the United State America U.S.A (excluding skin cancer). Ovarian cancer is the seventh most common cancer in women worldwide (18^{th} most common cancer overall), with 239,000 new cases diagnosed in 2012. In addition, because many of these ovarian neoplasms are highly aggressive and lethal, they account for a disproportionate number of fatal cancers, being responsible for almost half the deaths from cancer of the female genital tract. The age specific incidence rate for ovarian cancer revealed that the disease increases from 35 years of age and reaches a peak between the ages 55 and 64. There are numerous types of ovarian tumors, both benign and malignant. About 80% are benign, and these occur mostly in younger women between the age of 20 and 45 years. The malignant tumors are more common in older age groups, between 40 and 65. [13, 16, 2]

2.3.1: Classification of ovarian tumours

The many different ovarian tumours are classified on the basis of their cell or tissue origin, it being believed that these neoplasms arise from undifferentiated cells in mature tissues which retain the same potentiality for differentiation as is possessed by the embryonic cells which are the precursor of that tissue.

A simplified form of the complex classification of ovarian tumours defines four main groups:

I. Tumours derived from the surface epithelium.
II. Tumours of sex cord and stromal origin.
III. Tumours derived from germ cells.
IV. Metastatic tumours. [5]

2.3.2: Genetic

Germ line mutations in *BRCA1* and *BRCA2* are the most common genetic aberrations in hereditary ovarian carcinomas, by far the most frequent alterations in sporadic Epithelial Ovarian Carcinoma EOC are in the p53 and Retinoblastoma RB pathways. Defects in these two tumor suppressor pathways are present in over eighty percent of human cancers and have been associated with poor prognosis in ovarian carcinomas. [24]

2.3.3: Staging

Ovarian cancer is staged using the International Federation of Gynecology and Obstetrics FIGO staging system and uses information obtained after surgery. The American Joint Committee on Cancer AJCC stage is the same as the FIGO stage. The AJCC staging system describes the extent of the primary tumor (T), the absence or presence of metastasis to nearby lymph nodes (N), and the absence or presence of distant metastasis (M). [23]

Stage	Description
I	Cancer is completely limited to the ovary.
II	Pelvic extension of the tumor (must be confined to the pelvis) or primary peritoneal tumor, involves one or both ovaries.
III	Cancer found outside the pelvis or in the retroperitoneal lymph nodes, involves one or both ovaries.
IV	Distant metastasis (i.e. outside of the peritoneum).

AJCC/TNM stages of ovarian cancer

Stage	Description
T	Primary tumor
N	Regional lymph node metastasis
M	Distant metastasis

2.3.4: Grading

Grade 1 tumors have well differentiated cells (look very similar to the normal tissue) and are the ones with the best prognosis. Grade 2 tumors are also called moderately well differentiated and they are made up of cells that resemble the normal tissue. Grade 3 tumors have the worst prognosis and their cells are abnormal, referred to as poorly differentiated. [9]

Metastasis in ovarian cancer is very common in the abdomen, and occurs via exfoliation and can also travel through the lymphatic system. [3]

2.3.5: Method of diagnosis of ovarian tumour

Diagnosis of ovarian cancer starts with a physical examination, a blood test (for Cancer Antigen-125 CA-125 and sometimes other markers), and transvaginal ultrasound. Sometimes a rectovaginal examination is used to help plan a surgery. The diagnosis must be confirmed with surgery to inspect the abdominal cavity, take

biopsies, and look for cancer cells in the abdominal fluid. This helps to determine if an ovarian mass is benign or malignant. [3]

2.3.6: Prevention

People with strong genetic risk for ovarian cancer may consider the surgical removal of their ovaries as a preventive measure. This is often done after completion of childbearing years. This reduces the chances of developing both breast cancer (by around 50%) and ovarian cancer (by about 96%) in people at high risk. Women with *BRCA* gene mutations usually also have their Fallopian tubes removed at the same time (salpingooophorectomy), since they also have an increased risk of Fallopian tube cancer. However, these statistics may overestimate the risk reduction because of how they have been studied. [22, 19]

2.3.7: Management

Once it is determined that ovarian, fallopian tube, or primary peritoneal cancer is present, treatment is scheduled by a gynecologic oncologist, can perform surgery on and give chemotherapy to women with ovarian cancer. A treatment plan is developed. [20]

Treatment usually involves surgery and chemotherapy, sometimes radiotherapy, regardless of the subtype of ovarian cancer, Hormonal therapy and Immunotherapy. [27, 28]

2.4: Tumours derived from the surface epithelium

Approximately 60% of all ovarian tumours and 90% of those which are malignant, and are found in adult life, very rarely in children, Derived from the surface epithelium: they are collectively known as the common epithelial tumours. [5, 12]

The great majority of primary neoplasms in the ovary fall within this category. There are three major types of such tumors, depending on whether the epithelium is serous, mimicking the fallopian tube epithelium, mucinous, mimicking the endocervical epithelium, or endometrioid, recapitulating the endometrial lining. [13]

2.4.1: Serous tumours

As with all ovarian tumors, these serous lesions occur at any age, but are most common between 20 and 50 years, the malignant form being seen later in life. They are quite uncommon prior to puberty. These common cystic neoplasms are lined by tall, columnar, ciliated epithelial cells and are filled with serous fluid, the two distinctive features of these tumours. Together the benign, borderline and malignant types account for about 30% of all ovarian tumours. In overall spectrum of serous tumours, about 10% are benign, 30% borderline, and 60% malignant .Thus, the ratio of benign to malignant serous tumors is 1 to 9. [13]

2.4.2: Mucinous tumours

These tumours closely resemble their serous counterpart. They are somewhat less common than the serous form and account for about 20% of all ovarian neoplasms. Mucinous cystadenocarcinomas are rare and account 5–10% of epithelial ovarian cancers, histologically, they are similar to intestinal or cervical adenocarcinomas, and are often actually metastases of appendiceal or colon cancers. Advanced mucinous adenocarcinomas have a poor prognosis, generally worse than serous tumors, and are often resistant to platinum chemotherapy, though they are rare. [24]

They occur principally in middle adult life and rare before puberty and after menopause. The benign variants are much more common than the malignant in the ratio of approximately 7 to 1.It compose of nonciliated, tall, columnar, mucous secreting cells. [13]

2.4.2.1: Pseudomyxoma peritonei

Pseudomyxoma peritonei refers to a collection of encapsulated mucous or gelatinous material in the abdominopelvic cavity, which is very rarely caused by a primary mucinous ovarian tumor. More commonly, it is associated with ovarian metastases of intestinal cancer. [23]

2.4.3: Small cell carcinoma

Small cell ovarian carcinoma is rare and aggressive, with two main subtypes: hypercalcemic and pulmonary. It is typically fatal within 2 years of diagnosis. Hypercalcemic small cell ovarian carcinoma overwhelmingly affects those in their 20s, causes high blood calcium levels, and affects one ovary. Pulmonary small cell ovarian cancer usually affects both ovaries of older women and looks like oat-cell carcinoma of the lung. [23]

2.4.3.1: Primary peritoneal carcinoma

Primary peritoneal carcinomas develop from the peritoneum, a membrane that covers the abdominal cavity that has the same embryonic origin as the ovary. They are often discussed and classified with ovarian cancers when they affect the ovary. They can develop even after the ovaries have been removed and may appear similar to mesothelioma. [23]

2.4.4: Clear cell carcinoma

Clear cell ovarian carcinomas do not typically respond well to chemotherapy and may be related to endometriosis. They represent approximately 5% of all endometrial cancers. Japanese women develop clear-cell ovarian cancer more frequently than other groups of women. [23]

2.4.5: Clear cell adenocarcinoma

Clear cell adenocarcinomas are histopathologically similar to other clear cell carcinomas, with clear cells and hobnail cells. They represent approximately 5–10% of epithelial ovarian cancers and are associated with endometriosis in the pelvic cavity. They are typically early-stage and therefore curable by surgery, but advanced clear-cell adenocarcinomas (approximately 20%) have a poor prognosis and are often resistant to platinum chemotherapy. [23]

2.4.6: Endometrioid tumours

These cystadenomas and carcinomas are composed of epithelial cells that are cytologically the same as those of the endometrium. The problem is to separate the benign neoplasms from cystic endometriosis, and the differences have not as yet been clearly delineated. The epithelial cells of benign lesions are columnar, with pale flocculent cytoplasm and small oval nuclei, somewhat variable in position but usually placed near the centres of the cells. There may be vacuolation of the cytoplasm, reminiscent of the secretory change that occurs in the endometrium, and the adjacent stroma sometimes has a cytogenic appearance. The carcinomas are well differentiated neoplasms sometimes acanthoid, but so may be the carcinomas that arise in implantation endometriosis. The justification for separating the endometrioid carcinomas from other celomic epithelial neoplasms is that the evidence to date suggests that the prognosis for patients with them is considerably better. [30]

2.4.6.1: Malignant mixed müllerian tumour (carcinosarcoma)

Mixed müllerian tumors make up less than 1% of ovarian cancer. They have epithelial and mesenchymal cells visible and tend to have a poor prognosis. [23]

2.4.7: Undifferentiated epithelial

Undifferentiated cancers - those where the cell type cannot be determined make up about 10% of epithelial ovarian cancers and have a comparatively poor prognosis. When examined under the microscope, these tumors have very abnormal cells that are arranged in clumps or sheets. Usually there are recognizable clumps of serous cells inside the tumor. [23]

2.4.7.1: Brenner Neoplasms (fibroepithelioma, Urothelioma)

These fibroepithelial neoplasms are considerably less common than the other types of celomic cell tumours. They are usually solid neoplasms related to adenofibromas but there may be a cystic component. Generally they are benign and hormonally inactive, but there are exceptions to both of these generalizations. A variety of

theories concerning the pathogenesis of these neoplasms have been suggested over the years, but the evidence supports origins from surface germinal epithelium and rete ovarii. The usual neoplasm consists of branching cords and tunnels forming a root-like network of epithelial cells with knobby and cystic projections surrounded by dense fibrous tissue. The epithelial cells are stratified and resemble those of the urinary system and the cell nests of von Brunn that arise from them. Often there is a small central lumen or cystic space about which the cells assume a low columnar configuration. Malignant Brenner tumours may retain the Basie structural features of cell nests in a fibrous stroma but may also assume an adenomatous or squamoid appearance. [30]

2.4.7.2: Transitional cell carcinoma

Transitional cell carcinomas represent less than 5% of ovarian cancers. Histologically, they appear similar to bladder carcinoma. The prognosis is intermediate better than most epithelial cancers but worse than malignant Brenner tumors. [23]

2.5: Tumours of sex cord and stromal origin

Included within this category are all ovarian neoplasms originating either from the sex cords of the embryonic gonad (which precede the differentiation of gonadal mesenchyme into male or female) or from the stroma of the ovary. [30]

These may be divided into two broad groups:

- Those tending to produce excess oestrogen: granulose cell tumours and thecomas.
- Those produce androgens and virilisation: Sertoli-Leydig cell tumours, hilus cell tumours and lipid cell tumours. [12]

2.5.1: Granulosa-Theca Cell Group of Neoplasms

This is the largest group of gonadal stromal tumours and many exert a feminizing effect owing to the production of estrogens; however, a few may cause a masculinization of the host. There is an association between these neoplasms and

endometrial carcinoma that cannot be explained by chance, but whether there is truly a cause and effect relationship has not been clearly established. Neoplasms of this group have been produced experimentally in small animals by x-irradiation, by the administration of chemical carcinogens, and by ovarian transplants into spleens in gonadectomized animals. Three basic types of neoplasms are recognized in this group, granulosa cell, theca cell, and thecogranulosa tumours. [30]

2.5.1.1: Granulosa cell tumour (folliculoma)

This neoplasm has a distinctive epithelial appearance and arises either from granulosa cells of follicles o from precursor cells in the ovarian mesenchyme. It is the most common tumour in the granulosa theca cell group. Before puberty this tumour causes precocious development and after the menopause enlargement of the uterus and breasts with endometrial hyperplasia and bleeding. During the reproductive period symptoms and signs due to it hormonal activity may not be apparent. The tumours are either solid or cystic and usually have a distinct yellow hue. Microscopically the epithelial cells are uniform in appearance, cuboidal polygonal, and form a variety of patterns even the same neoplasm. The more common patterns of growth are folliculoid, trabecular, cylindroid and pseudoadenomatous. There may be stromal hyperplasia in these tumours but not the extent that confusion with thecogranulosa tumours is likely to occur. Roughly 25% of these neoplasms behave as carcinomas. [30]

2.5.1.1.1: Adult granulosa cell tumour

Adult granulosa cell tumors are characterized by later onset (30+ years, 50 on average). These tumors produce high levels of estrogen, which causes its characteristic symptoms: menometrorrhagia; endometrial hyperplasia; tender, enlarged breasts; postmenopausal bleeding; and secondary amenorrhea. The mass of the tumor can cause other symptoms, including abdominal pain and distension, or symptoms similar to an ectopic pregnancy if the tumor bleeds and ruptures. [23]

2.5.1.1.2: Juvenile granulosa cell tumour

2.5.2: Theca cell tumour (thecoma)

In most series these tumours occur less frequently than granulosa cell tumours and later in life. They are generally feminizing but if there is associated luteinization (luteoma) they may cause masculinization and produce progesterone effect with the formation of decidua in the endometrium. Most thecomas are preceded by nodular cortical hyperplasia in the ovary or hyperplasia of the Stein-Leventhal syndrome. Grossly these neoplasms are solid fibrous tumours with a distinctly yellow appearance, and microscopically they consist of large, plump, pale, spindled and polygonal cells that contain stainable cytoplasmic lipids. Areas of hyalinization may be prominent, and it is possible that some so called fibromas may represent thecomas that for some reason regressed and became hyalinized. Very few thecomas are malignant. [30]

2.5.3: Thecogranulosa tumour

Approximately 20% of the neoplasms in the granulosa-theca cell group is a mixture of the two elements, both being neoplastic. It is suggested that they arise from gonadal mesenchyme which possesses the potentiality to differentiate into both granulosa and theca cells. These tumours consistently exert action, but whether they have any greater malignant potential than the other members of the group has not been established. [30]

2.5.3.1: Sertoli-Leydig cell group of neoplasms

These neoplasms, although rare, have attracted considerable attention because of the dramatic systemic changes that they often produce. They are generally thought to cause defeminization and masculinization but a few actually cause feminization with signs of excessive estrogenic activity. They arise from the sex cord elements and mesenchyme in the hilar region. Structurally they resemble and are often identical to the similarly named tumours that occur in the testis. Three histologically different neoplasms comprise the group:

- pure Leydig cell tumour.
- pure Sertoli cell tumour.
- And Sertoli-Leydig cell tumour or arrhenoblastoma, in which both types of cells are present often with a nondescript mesenchyme. [30]

2.5.3.1.1: Leydig cell tumour (hilus, interstitial cell or Bergers tumour)

These small neoplasms arise in the ovarian hilum and cause masculinization due in part to the production of testosterone. They occur in women near menopausal age and for a time thereafter. Histologically they consist of clusters, sheets and/or cords of eosinophilic polygonal cells that contain some lipid and lipochrome pigment. The cells sometimes become spindled and in the larger neoplasms there is often considerable central hyaline change. The hallmark of these lesions is the presence of intracytoplasmic eosinophilic crystalloids of Reinke, but these cannot always be identified. With one exception, the examples reported thus far have been benign. A few of these tumours have occurred in dysgenetic ovaries. [30]

2.5.3.1.2: Sertoli cell tumour (folliculoma lipidique, androblastoma)

These are feminizing neoplasms that rise from the sex cord remnants in the ovarian hilum. In children they cause precocious development of secondary sex characteristics. They consist of tubules and cords of eosinophilic cuboidal cells that in some instances contain a good deal of lipid. Most of these tumours are histologically benign and behave so, but we have recently seen a poorly differentiated Sertoli cell tumour in a young girl which was histologically malignant and which metastasized. There is a tendency for Sertoli cell tumours to develop in dysgenetic ovaries. [30]

2.5.3.1.3: Sertoli-Leydig cell tumour (arrhenoblastoma)

These neoplasms are mixtures of Sertoli cells, Leydig cells, and undifferentiated mesenchyme in various proportions. The majority of them cause masculinization but a few may actually be feminizing and occasionally both effects are noted. They occur mainly in the early reproductive years. Testosterone is produced by some of

these neoplasms and is presumed to come from the Leydig cells; the estrogens are thought to be produced by the Sertoli cells. Approximately 20% of these neoplasms are malignant. [30]

2.6: Tumours derived from germ cells

Germ cells tumours represent 15% to 20% of all ovarian tumours. The vast majority (95%) is the benign cystic teratomas, but the reminder, which are found principally in children and young adults, have a higher incidence of malignant behavior and pose problems in histologic diagnosis and in therapy. [13]

Tumour may show no evidence of either embryonic or extra embryonic differentiation such un differentiated germ cell neoplasms known as **dysgerminomas** and microscopically identical in all respect to the seminomas of the testis, sharing with this neoplasm also the attributes of early dissemination to the para-aortic lymph nodes and a marked degree of radiosensitivity. A germ cell tumor may however, differentiate along extra-embryonic pathways into either placental tissue, resulting in the rare and highly malignant ovarian choriocarcinoma, or into yolk sac tissue to produce the equally un common malignant yolk sac carcinoma also known as an endodermal sinus tumour, occur in young girls and like yolk sac tumour of the testis has a complex histological structure with elements resembling primitive yolk sac, similarity further accentuated by the ability of these neoplasms to secrete alpha-fetoprotein. [5]

2.6.1: Teratomas

Teratomas develop from totipotential germ cells, and consequently contain all three germ cell layers: ectoderm, mesoderm and endoderm. Teratomas are classified into immature (malignant), mature (dermoid cyst) and monodermal (struma ovarii, carcinoid).

Dermoid cysts contain mature tissue, and upon gross examination skin, teeth, bone, hair, sebaceous glands and neural tissue predominate; whilst cartilage, respiratory and intestinal epithelium are also common .They are cystic tumours

with a firm capsule. Monodermal teratoma comprise mainly one tissue element. For example the most common type of monodermal teratoma, Struma ovarii, is comprised of at least 50% mature thyroid tissue (of any type). Argentaffin cells in dermoid cysts are usually the site of origin for ovarian carcinois, although this is rare. Immature teratomas account for approximately 20% of all malignant GCT. They are classified as Grade I, II or III if they have 0 or1, 3 or less, or 4 or more lowpower fields (x-40) containing immature neuroepithelium per section, respectively. Immature teratomas are solid tumours containing immature or embryonal tissues. Immature neuroepithelium is the predominant immature tissue found. [30]

2.6.2: Dysgerminoma

Dysgerminomas have a solid, lobulated, tan, flesh-like gross appearance with a smooth surface. Microscopically dysgerminoma cells are round and ovoid, contain abundant cytoplasm, irregularly shaped nuclei, >1 prominent nucleolus. These cells have a propensity to aggregate forming cords and sheets. Lymphocytic and granulocytic infiltration of the fibrous septa is often evident. [30]

2.6.3: Endodermal Sinus Tumour (EST)

Gross examination of EST, also known as yolk sac tumour, demonstrates smooth, glistening, hemorrhagic and necrotic surfaces. Histology reveals a wide range of patterns (microcystic, endodermal sinus, solid, alveolar-glandular, papillary, macro-cystic, hepatoid, primitive endodermal). The classic pattern contains Schiller-Duval bodies (central capillary surrounded by simple papillae) and eosinophilic globules containing AFP. Intracellular and extracellular hyaline droplets (periodic acid-Schiff positive) are also seen in EST. [30]

2.6.4: Embryonal Carcinoma

Gross examination of embryonal carcinoma reveals a solid, haemorrhagic, necrotic tumour, resembling a larger form of EST. Embryonal glands, glandlike clefts (embryoid bodies), and syntrophoblastic giant cells are present microscopically. [31]

2.6.5: Choriocarcinoma

Choriocarcinoma is a very rare solid, haemorrhagic tumour, composed of malignant cytotropohoblast and syncytiotrophoblast. Nongestational and gestational choriocarcinoma have identical histologies. [31]

2.6.6: Mixed Germ Cell Tumour

As the name suggests, mixed germ cell tumours contain >1 histological type. Dysgerminoma with EST and immature teratomas with EST are frequent combinations. [31]

2.6.7: Polyembryoma

Histological analysis of polyembryoma demonstrates erythroid bodies in different stages of presomite development. [31]

2.6.8: Squamous cell carcinoma

Primary ovarian squamous cell carcinomas are rare and have a poor prognosis when advanced. More typically, ovarian squamous cell carcinomas are cervical metastases, areas of differentiation in an endometrioid tumor, or derived from a mature teratoma. [24]

2.6.9: Secondary ovarian cancer

Ovarian cancer can also be a secondary cancer, the result of metastasis from a primary cancer elsewhere in the body. Common primary cancers are breast cancer, colon cancer, appendiceal cancer, and stomach cancer (primary gastric cancers that metastasize to the ovary are called Krukenberg tumors). Krukenberg tumors have signet ring cells and mucinous cells. Endometrial cancer and lymphomas can also metastasize to the ovary. [23]

2.6.10: Low malignant potential tumours

Low malignant potential ovarian tumors, also called borderline tumors, have some benign and some malignant features. LMP tumors make up approximately 10%-15% of all ovarian tumors. They develop earlier than epithelial ovarian cancer, around the age of 40–49. They typically do not have extensive invasion; 10% of

LMP tumors have areas of stromal microinvasion (<3mm, <5% of tumor). LMP tumors have other abnormal features, including increased mitosis, changes in cell size or nucleus size, abnormal nuclei, cell stratification, and small projections on cells (papillary projections). Serous and/or mucinous characteristics can be seen on histological examination, and serous histology makes up the overwhelming majority of advanced LMP tumors. More than 80% of LMP tumors are Stage I; 15% are stage II and III and less than 5% are stage IV. Implants of LMP tumors are often non-invasive. [23]

2.7: Metastatic tumours

The ovary is more often involved by metastatic processes than any of the other pelvic genital organs. Two groups of malignancies contribute to this incidence: carcinomas arising within the other pelvic organs and carcinomas arising within the upper gastrointestinal tract, stomach, biliary tract, and pancreas. The term **krukenberg tumour** is sometimes applied to bilateral metastatic ovarian tumours causing massive enlargement of the ovaries. And is characterized by the presence of mucin-containing "signet ring" cells scattered in a fibrous stroma which is extremely cellular and resembles a sarcoma. This florid stromal reaction is seen before the menopause. Metastases in post menopausal ovaries usually have the same appearances as elsewhere. [5, 13]

2.8: P53

Tumor protein p53, also known as p53, cellular tumor antigen p53, is any isoform of a protein encoded by homologous genes in various organisms, such as *TP53*.

The TP53 gene is the most frequently mutated gene (>50%) in human cancer, indicating that the *TP53* gene plays a crucial role in preventing cancer formation. The name p53 was given in 1979 describing the apparent molecular mass; Sodium Dodecyl Sulfate Polyacrylamid Gel Electrophoresis SDS-PAGE analysis indicates that it is a 53-kilodalton (kDa) protein. However, the actual mass of the full-length p53 protein (p53α) based on the sum of masses of the amino acid residues is only

43.7 kDa. This difference is due to the high number of proline residues in the protein, which slow its migration on SDS-PAGE, thus making it appear heavier than it actually is. [11]

P53 has many mechanisms of anticancer function and plays a role in apoptosis, genomic stability, and inhibition of angiogenesis. [11]

2.8.1: In its anticancer role, p53 works through several mechanisms

It can activate Deoxyribonucleic DNA repair proteins when DNA has sustained damage. Thus, it may be an important factor in aging.

It can arrest growth by holding the cell cycle at the Gap1/Synthesis phase G1/S regulation point on DNA damage recognition (if it holds the cell here for long enough, the DNA repair proteins will have time to fix the damage and the cell will be allowed to continue the cell cycle). It can initiate apoptosis (i.e., programmed cell death) if DNA damage proves to be irreparable. It is essential for the senescence response to short telomeres. [11]

2.9: P53 role in ovarian cancer

In the event of injury, human cells are dependent on a functional p53 for DNA repair, or if the damage is irreparable, for apoptosis. The p53 protein, acting as a transcription factor, activates or alternatively represses the transcription of genes leading to the expression of specific elements necessary for the inhibition of cell growth and the induction of apoptosis. The absence of functional p53 thus can lead to deregulated cellular proliferation. Missense mutations of the p53 gene result in proteins that have longer half life than their wild type counterparts and are resistant to degradation. As a result, these mutations appear to give rise to p53 over expression by immunohistochemical (IHC) techniques with a variety of different antibodies. Null mutations (insertion, deletion, splice site aberrations, and nonsense) result in a truncated protein product that cannot bind DNA or induce apoptosis. Such mutations generally do not result in increased p53 protein stability, and the truncated proteins often go undetected by conventional IHC techniques.

Ovarian carcinoma, appear to display variation in the occurrence and range of p53 abnormalities according to certain disease characteristics such as histologic subtype and stage. In Ovarian Carcinoma OC, p53 is altered in 30–80% of cases. [7, 8]

2.10: Analysis of p53 mutations

Four analytical methods

(1) Molecular analysis: can be used to identify the type of mutation in the p53 gene.

(2) Immunohistochemical analysis: can be used to demonstrate accumulation of mutant p53 protein in tumour cells.

(3) Serological analysis can be used to detect anti-p53 antibodies in the serum of patients.

(4) Functional analysis can be used to measure the transactivating activity of p53. [26]

2.10.1: Immunohistochemical analysis

An important feature of mutant p53 proteins is their longer half-life. This makes it possible to perform an immunocytochemical diagnosis (coupled with histological analysis) on tumor tissue to directly visualize the accumulation of inactive mutant p53 protein. This approach has been used in many types of cancer, generally with good correlation between the molecular results (presence of mutation) and the immunohistochemical findings (accumulation of mutant protein). However, 100% agreement is never attained, because 10–15% of p53 mutations consist of nonsense mutations or microdeletions that do not produce detectable levels of protein. Despite this drawback, this approach has the advantage that it can be used for routine testing in anatomopathology laboratories. Several groups have produced new monoclonal antibodies directed against human p53. These antibodies have been used for immunohistochemical diagnosis under a wide variety of conditions such as p53 detection in paraffin embedded sections after formol fixation or Bouin staining. [26]

2.11: Previous studies

1) A study from patients undergoing laparotomy in hospitals throughout the northwest of England of the 50 cases examined, 28 (56%) were p53 positive and there was no significant correlation between p53 status and differentiation stage, clinical (FIGO) stage. [14]
2) A study at the Mayo Clinic, Rochester, MN, between 1976 and 1990, reported that two hundred eighty-four patients A total of 177 cases (62%) showed immunoreactivity with the p53 antibody. Seven (4% of positives) showed 1+ reactivity; 31 (18% of positives) showed 2+ reactivity; and 139 (78% of positives) showed 3+ reactivity Staining was negative for p53 in 107 cases (38%). [18]
3) A study at Clinical Pathology Associates, Austin, TX, USA, 2011, reported that a total of 57 ovarian carcinomas, Tumors with wild-type TP53 displayed a wide range of immunolabeling patterns, with the most common pattern showing < 10% of positive cells in 6 cases (46%). Mutant TP53 was associated with 60–100% positive cells in 23 cases (64% of cases). This pattern of staining was also seen in three cases with wild type TP53. Tumors that were completely negative (0% cells staining) had a mutation of TP53 in 65% of cases and wild-type TP53 in 11%. [19]
4) A study at the University of Iowa Hospitals and Clinics between January 1, 1990 and December 31, 1996, reported that tumor specimens from 171 consecutive epithelial ovarian carcinomas were examined for overexpression of p53 protein with DO7 antibody. Overall, 48.5% of the samples showed p53 over expression. [8]

5) Study at the Department of Gynecology and Obstetrics, Virchow-Klinikum of the Humboldt-University in Berlin between 1981 and 1995, reported that Tumor tissues from 179 patients with epithelial ovarian carcinoma were used for immunohistochemical analysis with monoclonal antibody DO1 and BP 53-12-1 on formalin-fixed, paraffin-embedded tissue, a total of 78 cases (44%) showed positive nuclear p53 staining. [34]

Chapter Three

Materials and Methods

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 Array. Tumour tissue were embedded in paraffin and 3µm sections stained with HαE were obtained to identify viable, representative areas of the specimen, 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, 5µm sections of these tissue array blocks were cut and placed onto salinized slides, These sections were used for immunohistochemical analysis.

3.2 Methods:

3.2.1 Study design:

This is descriptive retrospective study aimed to assess the expression p53 in ovarian cancer among Sudanese women patients using immunohistochemistry technique.

3.2.2 Sample size:

40 tissue microarray previously diagnosed as ovarian cancer were collected in El rahama medical center. Patient identification (age, grad and diagnosis) were obtained from patients records.

3.2.3 Study area:

This study was conducted at EL rhama medical center.

3.2.4 Immunohistochemistry staining:

The immunohistochemical procedure was done as follows:

Tissue array block from formalin-fixed, paraffin-embedded tumours mounted onto salinized slides. Following deparaffinization in xylene, slides was rehydrated through a graded series of alcohol and was placed in distilled water. Samples were steamed for antigen retrieval for p53 using high PH (9) by water bath at 95C for 40

min. After washing with PBS for 3 min Endogenous peroxides activity was blocked with 3% hydrogen peroxide and methanol for 10 min, and After washing with PBS for 3 min then Slides was incubated with (100 µ L) of (Monoclonal Mouse Anti-Human P53Clone DO-7, p53 Dako) against nuclear p53 protein, 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.

Each slide was evaluated with investigator then the results were confirmed by consultant histopathologist.

3.2.5 Result interpretation:

All quality control measure was adopted positive and negative control slides were used during immunohistochemical staining. Detection cells with brown nuclei considered as positive result.

3.2.6 Statistical analysis:

The data were processed using SPSS 22 and the frequencies were determined. Correlation between p53 and histology was tested by using Chi Squire Test.

3.2.7 Ethical consideration:

The samples were collected after permission from EL rhama medical center, According to the laboratory guidelines and regulation.

Chapter Four

Results

4. Results

The age of study population ranged between 32 and 65 years. Most patients were less than 50 years represent 19/40 (47.5%) and the remaining 21/40(52.5%) were more than 50 years as indicated in table (4.1).

Tumour protein p53 positive expression was found in 17/40 (42.5%) samples while 23/40 (57.5%) samples show negative expression as indicated in table (4.2).

Types of malignant tumour show positive expression for tumour protein p53 in 13(32.5%) samples of epithelial origin, 3(7.5%) samples of sex-stromal, 1(2.5%) sample of germ cell, this result show no correlation between tumour protein p53 and types of malignant tumour (p = **0.186**) as showed in table (4.3).

Types of epithelial origin show positive expression for tumour protein p53 in 1(2.5%) TCC, 1(2.5%) Serous, 1(2.5%) Mucinous and 9(22.5%) adenocarcinoma, no detected samples of invasive papillary cyst. This result show no correlation between tumour protein p53 and types of epithelial origin (p=**0.532**) as showed in table (4.4).

Grades of malignant tumour show positive expression for tumour protein p53 in 11(27.5%) Differentiated types, 6(12.5%) Un differentiated types. This result shows no correlation between tumour p53 and Grades of malignant tumour (p=**0.196**) as showed in table (4.5).

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

Age group	Frequency
Less than 50	19
50 or more	21
Total	40

Table (4.2): Shows the frequency of p53 expression among study population.

P53 expression	Frequency
Negative	23
Positive	17
Total	40

Table (4.3): Shows the relation between tumor protein p53 expression and types of ovarian tumor.

Types	P53 expression		Total	
	Negative	Positive	No	%
Epithelial	18	13	31	77.5%
Stromal	5	3	8	20%
Germ	0	1	1	2.5%
Total	23	17	40	100%

P=0.186

Table (4.4): Shows the relation between p53 expression and types of epithelial.

Types of epithelial	P53 Expression		Total	
	Negative	Positive	No	%
TCC	0	1	1	2.5%
Serous	4	1	5	12.5%
Mucinous	1	1	2	5%
Invasive papillary cyst	1	0	1	2.5%
Adenocarcinoma	12	9	21	52.5%
Total	18	12	30	75%

P=0 .532

Table (4.5) Show the relation between p53 expression and grades of ovarian tumour.

Grade	P53 expression		Total	
	Negative	Positive	No	%
Undifferentiated	4	6	10	25%
Differentiated	19	11	30	75%
Total	23	17	40	100%

P =0.196

Figure (4.1): Shows the description of age groups among the study population.

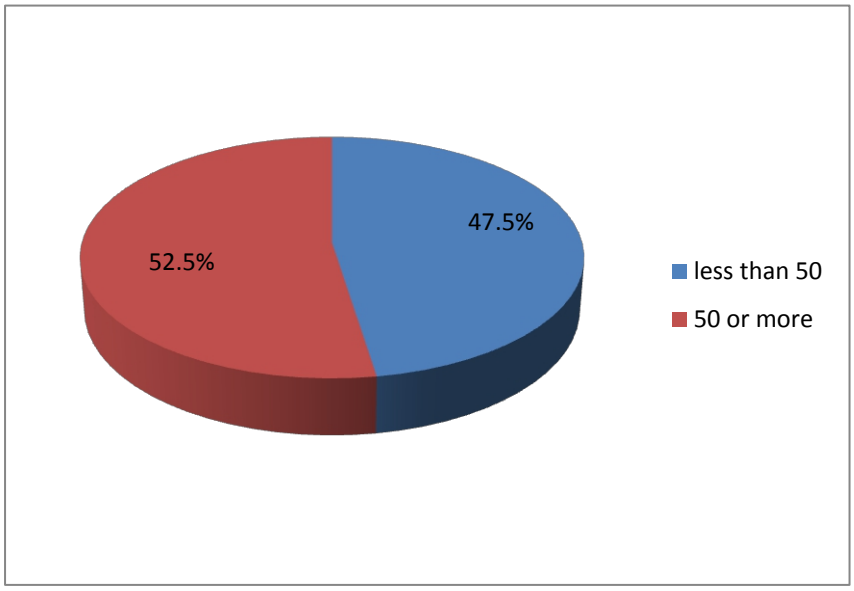
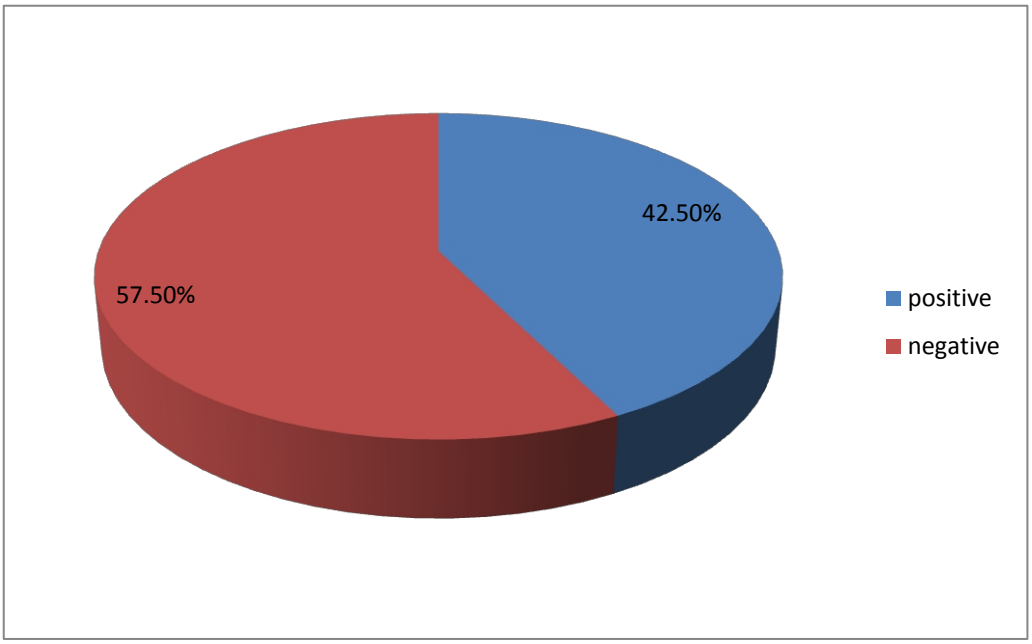


Figure (4.2): Shows the description of p53 expression among study population.



Chapter Five

Discussion

Conclusion

Recommendations

5.1 Discussion

The present study included 40 samples of ovarian carcinoma for expression of p53 nuclear protein by immunohistological staining on fixed paraffin-embedded tissue sections using Monoclonal Mouse Anti-Human P53Clone DO-7 (p53 Dako) Concerning the age group of the study population, the study revealed that the most patients were more than 50 years indicating that patients more than 50 years are more affected with ovarian cancer, this result agree with Surendra Kumar Saini, et al(2007-2009) who reported that the age specific incidence rate for ovarian cancer revealed that the disease increases from 35 years of age and reaches a peak between the ages 55 and 64.

Tumour protein p53 show positive expression in (42.5%) samples, this result close to Angela Reles, et al who reported that Tumor tissues from 179 patients with epithelial ovarian carcinoma who were treated at the Department of Gynecology and Obstetrics, Virchow-Klinikum of the Humboldt-University in Berlin between 1981 and 1995, were used for immunohistochemical analysis with monoclonal antibody DO1 and BP 53-12-1 on formalin-fixed, paraffin-embedded tissue, a total of 78 cases (44%) showed positive nuclear p53 staining. Types of epithelial origin show positive expression for tumour protein p53 revealed there was no association between tumour protein p53 and types of epithelial origin. Also differentiation stages of malignant tumour show positive expression for tumour protein p53 revealed there was no association between tumour protein p53 and differentiation stages of malignant tumour. This result compatible with J. Renninson, et al (1991), who reported that a series of 50 cases of epithelial ovarian adenocarcinoma for expression of p53 by immunohistological staining on fixed, paraffin embedded tissue sections using the polyclonal antibody 28 (56%) were p53 positive and there was no significant correlation between p53 status and differentiation stage, clinical (FIGO) stage.

5.2 Conclusion

On the basis of this study we concluded that:

- Expression of tumor protein p53 was associated with malignancy tumours of ovaries among Sudanese women with no correlation with types or grades of ovarian cancer, and tends to occur in age group more than 50 years old.

5.3 Recommendations

On the basis of this study we recommended that:

- Further studies should be done with large sample size.
- And p53 tumour protein combine with other tumor markers using advancing techniques.

Chapter Six

References

Appendix

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6.2 Appendices:

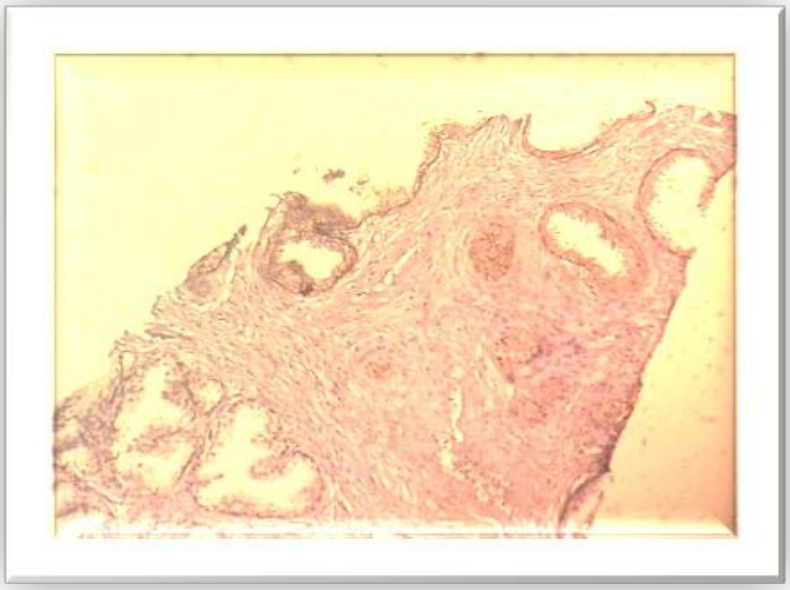
Appendix 1:

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
- 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 (P53)
- Tris EDTA buffer (PH9)
- Phosphate buffer saline (PH7.4)
- Peroxides blocker(3% hydrogen peroxide in methanol)
- Secondary anti body (dextran polymer conjugated secondary – P53)
- DAB (3,3 di amino benzidin tetra hydrochloride)substrate solution
- Bluing Reagent (0.1MLi2 CO3 , 0.5 M Na2CO3)
- Xylene
- DPX mounting media

Appendix 2:

Microphotograph: Shows positive reaction of p53 in ovarian cancer



Shendi University

College of Higher Studies and research

Data sheet for ovarian cancer

1. Name

……..……

2. Age of patient

……

3. Type of ovarian cancer

……

4. Tumor origin

1. Epithelium tumors ☐ 2. Stromal tumors ☐ 3.Germ cell tumors ☐

5. Grades of ovarian cancer

Grade I ☐ Grade II ☐ Grade III ☐ Grade IV ☐

6. P63 expression result

A. positive ☐ B. Negative ☐