University of Shandi

College of Graduate Studies

Immunohistochemical Expression of Vimentin in Ovarian Tumarous among Sudanese Patients

By

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Thesis submitted in partial fulfillment for the requirements of MSc Degree in Histopathology and Cytology

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August 2018

بسم الله الرحمن الرحيم

صدق الله العظيم

DEDICATON

To my parents …

Who encouraged me at all stages of life

To my brother and sisters …

For their unlimited support …

.

ACKNOWLEDGEMENT

I would like to express my sincere gratitude and thankfulness to my supervisor Dr. Ashwag Mohammed Mukhtar for her guidance, meticulous supervision, revising and discussing all aspects of this study, her valuable advices and comments are highly appreciated.

My great thanks also extend to the colleges and staff in Shendi University, and for everyone who contributed in a way or another for the success of this study. For AlRahma lab and RIC (Radio Isotope center) histopathology lab.

ABBREVIATIONS

IHC	Immunohistochemistry
AFP	Alpha-Fetoprotein
AT	Alpha-1-Antitrypsin
BHCG	Beta Human Chorionic gonadotropin
Ca Ovary	Cancer Of The Ovary
CA125	Cancer antigen125
DAB	DiaminobenzidineTetrahydrochloride
DAKO	Dako Denmark A\s
EGFR	Epidermal Growth Factor Receptor
EMA	Epithelial Membrane Antigen
EMT	Epithelial–Mesenchymal Transition
FIGO	Federation of gynecology and obstetrics
LMP	Tumor of low malignant potential
SCCO	Small cell carcinoma
StABC/HRP	Streptomyces Avidin Biotin Complex-Horse Radish Peroxidase
TBS	Hydroxymethylamine Buffered Saline
TMA	Tissue microarray
TVS	Transvaginal ultrasound graphy

ABSTRACT

Background: Ovarian cancer is the most lethal gynecologic malignancy, in Sudan although many researchers worked on ovarian cancer but they do not describe the Immionohistochemical Expression of vimintin to within the benign and malignant ovarian Cancer.

Objective: to evaluate the Immunohistochemical Expression of Vimentin among Sudanese Patients with different ovarian lesions.

Methodology: case control laboratory based study. The Immunohistochemical stain was applied for 80 paraffin blocks of ovarian tissue including 40 malignant lesions as case and 40 benign lesions as control. Data were collected, and analyzed using SPSS .

Results: the study revealed that the expression of Vimentin in all group was 61 positive results (76.3%). While 11 were negative(13.8%). The histopathological diagnosis showed that (55%) of the benign group were diagnosed as Serous cyst (22.5%) Musinous Cyst (20%) Teratoma and Fibroma (2.5%) . while in case group the study revealed that more than half them were diagnosed with adenocarcinoma (75%), papillary tumor (5%), Granuloma (17.5%).The immunohistochemical stain showed (90.0%) was positive for vimentin expression in case group. And (62.5%) of the benign group was positive. while the cross tabulation test revealed that there was no significant relation in the expression of vimentin in comparing the bengnin and malignant group.(p value 0.05).

Conclusion: The study found the majority of cases group (90.0%) had positive expression for vimentin and (62.5%) of the benign group was positive although there was no difference in the expression of vimentin according to the statistical tests.

ملخص الأطروحه

الخلفية: سرطان المبيض هو السبب الأكثر شيوعا للوفيات الناجمة عن السرطانات النسائية في جميع أنحاء العالم ، في السودان على الرغم من أن العديد من الباحثين عملوا على سرطان المبيض ، ولكن لم يتم وصف ظهور الفيمنتين في سرطانات المبيض الحميدة والخبيثة بين المرضى السودانيين.

الهدف: تقييم التعبير المناعى الكيميائى للفيمنتين بين المرضى السودانيين المصابين بآفات مبيضية مختلفة.

المنهجية: دراسة مستندة إلى مقارنة الحالة ؛ قمنا بتطبيق تقنية الصبغ المناعي الكيميائي على 80 كتلة بارافينية من الأنسجة المبيضية بما في ذلك 40 سرطان خبيث و 40 سرطان حميد. تم جمع البيانات وتحليلها باستخدام .SPSS

النتائج: أظهرت الدراسة أن وجود الفيمنتين كان في 61 نتيجة إيجابية (76.3٪). بينما كانت 11 سلبية (13.8 ٪) .

أظهر التشخيص النسيجي أن (55٪) من المجموعة الحميدة تم تشخيصها على أنها كيس مصلي (22.5٪) كيس مخاطي (20٪) مسخي ورأب ليفي (2.5٪). بينما كشفت الدراسة في مجموعة الحالات أن أكثر من نصفهم تم تشخيصهم بالورم الغدي (75٪) ، الورم الحليمي (5٪) ، الورم الحبيبي (17.5%)

أظهرت نتائج صبغة الاميون هستوكمستري (90.0 ٪) إيجابية لتعبير فيمنتين في مجموعة الورم الخبيث بينما (62.5٪) من المجموعة الحميدة كانت إيجابية.

ووجدت الدراسة أن النتائج الإيجابية لفيمنتين قورنت بشكل كبير مع معدل الإيجابية بين مجموعة التحكم (قيمة ب =0.05)

الاستنتاج: وجدت الدراسه ان غالبيه مجموعه الحالات(90.0%)كان لها تعبير ايجابي للفيمنتين و (62.5%)من المجموعه كانت ايجابيه علي الرغم من عدم وجود اختلاف في التعبير عن الفايمنتين وفقا للاختبارات الاحصائيه .

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Chapter One

Introduction and literature review

Cancer is a disease in which cells in the body grow out of control. Cancer is always named for the part of the body where it starts, even if it spreads to other body parts later. When cancer starts in the ovaries, it is called ovarian cancer. Women have two ovaries that are located in the pelvis, one on each side of the uterus. When ovarian cancer is found in its early stages, treatment is most effective (CDC, 2009). When this process begins, there may be no or only vague symptoms.(Cho KR., 2009) Symptoms become more noticeable as the cancer progresses. These symptoms may include bloating, pelvic pain, abdominal swelling, and loss of appetite, among others. Common areas to which the cancer may spread include the lining of the abdomen, lymph nodes, lungs, and liver. (Cho KR, 2009)

1.1 EPIDEMIOLOGY

Ovarian cancer is the seventh most common cancer, and it is the most common cause of mortality from gynecological cancers worldwide, with 238,619 incident cases in 2012. In developing countries, it is ranked the second most common gynecological cancer, and constitutes the fourth most common of all cancers in women, with 17,755 incident cases in 2012. Essentially, the highest incidence rates of ovarian cancer are found in the developed countries. Northern Europe has the highest incidence rate (13.3 per 100,000 person-years), followed by Western Europe (11.3 per 100,000 person-years) and Northern America (10.7 per 100,000 person-years),

whereas North Africa has the lowest incidence rate (2.6 per 100,000 person-years). (Abuidris, 2016).

Internationally, the incidence ranges from 3.1 cases per 100,000 women in Japan to 21 cases per 100,000 women in Sweden. Around the world, more than 200,000 women are estimated to develop ovarian cancer every year and about 100,000 die from the disease. Epithelial ovarian cancer occurs most commonly in white women in the industrialized countries of northern and Western Europe and North America and least commonly in India and Asia. Asian women have low risk unless they relocate to North America or Europe. Scandinavian and Norwegian women have the highest risk.(Abuidris, 2016).

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), and it was ranked the sixth most common cancer for both genders. (Abuidris, 2016).

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 population, a gender-specific rate of 8.0 per 100,000 population, and an age-standardized rate (ASR) of 7.0 per 100,000 population. Furthermore, neither the morality rate for ovarian cancer nor the survival rate in Sudan has previously been described due to a lack of the availability of death certificates, the majority of patients presenting with advanced stage

disease were not thoroughly investigated or treated symptomatically. (Abuidris, 2016).

1.2 Histological appearance of ovarian tissue

From prepubertal to postmenopausal periods, the ovary exhibits a wide spectrum of appearance and functional activities. The adult ovary is composed of three zones: outer cortex, inner medulla and the hilus. Follicular structures such as follicle and corpus luteum are located in the cortex. After ovulation, the corpus luteum is gradually converted to a scar, *i.e*., the corpus albicans. Aged corpus albicans is either replaced by stroma or embedded in dense collagen fibers in the medulla. The surface of the ovary consists of a single layer of peritoneal cells. These surface cells are fragile and often invisible in resected ovaries. These mesodermally derived lining cells are not pure "epithelial" cells. They are immunoreactive for epithelial markers such as cytokeratin, Ber-EP4 and Wilms tumor gene (WT1) (Shimizu *et al.,* 2000). In addition, they are positively stained for mesothelial markers such as calretinin and vimentin. Therefore, these cells may be called "modified mesothelium/peritoneal cells". Epithelial inclusion cysts are often observed in normal ovaries, and they are embedded in the cortex and infrequently, in the medullary stroma. The lining cells of inclusion cysts sometimes connect with ovarian surface cells, and are also stained for WT1 and other epithelial and mesothelial markers, supporting the notion that they are originated from surface lining cells. It is accepted that ovarian surface cells can undergo epithelial-mesenchymal transition (EMT) both *in vitro* and *in vivo*, especially in the process of post-ovulatory remodeling (Auersperg *et al.,* 2009). Although most of inclusion cysts remain inactive, the notion is widely accepted that inclusion cyst is potential

precursor lesion of ovarian cancer. Biological dynamics of the cyst is great research interest among gynecologic oncologists. Several studies demonstrate differential molecular expression patterns between inclusion cyst-lining cells and quiescent surface cells (Cai *et al.*, 2007, Gendronneau *et al.*, 2012).

1.3 Pathology

The etiology of sporadic ovarian cancers is not fully understood, but a series of investigations suggest that certain conditions such as genetic predisposition and benign inflammatory diseases are involved in the molecular mechanism of carcinogenesis. The pathologic studies have revealed differential background of ovarian cancers depending on histologic types (Bell DA, 2005, Kurman RJ, 2008). There are four representative histologic types; serous, mucinous, endometrioid and clear cell types. The other types such as transitional cell and squamous cell cancers are reported, if any, at low frequency.

The ovarian surface is exposed to the peritoneal cavity. In most cases, cancer cells proliferate inward, and when thin cyst walls rupture, cancer cells spread into peritoneal cavity. Cancer cells may grow outward in papillary form, then directly expose themselves to the peritoneal cavity. Therefore, ovarian cancers preferentially metastasize to multiple surface sites of intraperitoneal organs including the omentum. Hematogenous and lymphatic metastases are also associated with distant metastasis of this malignancy. Serous cancer is divided into low- and high-grade subtypes (Smith S *et al*., 2003, Singer G, 2005). Currently, it is widely accepted that low-grade and high-grade serous cancers develop through different carcinogenic pathways, respectively (Bell DA, 2005) The former is characterized by mutations of *KRAS*, *BRAF* and

ERBB2 (Singer G, 2005, 2003). The latter is characterized by *TP53* mutations and not accompanied by mutations of *KRAS*, *BRAF* and *ERBB2* (Singer G, 2005, Dehari *et al.*, 2007)

1.4 Types of Ovarian Cancer

1.4.1 Malignant germ cell tumors

Malignant germ cell tumors (GCTs), which include dysgerminoma, endodermal sinus tumor, malignant teratoma, embryonal carcinoma, and choriocarcinoma, are thought to derive from primitive germ cells in the embryonic gonad. GCT of the ovary is much rarer than GCT of the testis in males, and much of the development of the management approach has been based on experience with male GCT.(Bell , 2005).

Common characteristics of these tumors include rapid growth, a predilection for lymphatic spread, frequent mixtures of tumor types, and a predominantly unilateral pattern of ovarian involvement (except for dysgerminoma). GCT is much more common in young women but occasionally occurs in infants and older women. (Bell, 2005).

Many GCTs produce tumor markers that can be measured in the blood and then used to monitor response to treatment and for follow-up care. Endodermal sinus tumors secrete alpha-fetoprotein and choriocarcinoma, and dysgerminomas occasionally secrete beta human chorionic gonadotropin (bHCG). (Bell , 2005)

Dysgerminoma may secrete lactate dehydrogenase and placental alkaline phosphatase.No factors have been established related to etiology, apart from an increased incidence associated with dysgenetic gonads.Although these

tumors may be asymptomatic and present as a palpable mass, many patients present with abdominal pain. (Mackenzie, 2015).

The mass may lead to acute pain due to torsion, rupture, or hemorrhage, or, patients may have abdominal distension, vaginal bleeding, or fever.Most are stage I and confined to the ovary at the time of diagnosis. (Mackenzie, 2015).

1.4.2 Dysgerminoma

This is the most common malignant GCT and represents 3-5% of all ovarian malignancies. Ninety percent occur in people younger than 30 years, and 75% occur in the second and third decades, with a median age of 22 years.Dysgerminomas are bilateral in 10-35% of cases. Five percent occur in phenotypic females with abnormal gonads. (Mackenzie, 2015).

They may have a 46XY karyotype with pure gonadal dysgenesis or androgen insensitivity syndrome, or, they may have a 45X, 46XY karyotype with mixed gonadal dysgenesis. Dysgerminomas may be large and usually are solid, with a smooth external surface and a fleshy pink-tan color inside. The majority are confined to the ovary at diagnosis, but approximately 25% of otherwise stage I dysgerminomas have lymph node metastasis. (Mackenzie, 2015).

Teratomas are germ cell tumors commonly composed of multiple cell types derived from one or more of the 3 germ layers. Inconsistent nomenclature often confuses discussions of various subtypes of teratomas. The word is derived from the Greek *teras,* meaning monster, which Virchow coined in the first edition of his book on tumors.(Mackenzie, 2015).

Teratomas range from benign, well-differentiated (mature) cystic lesions to those that are solid and malignant (immature). Additionally, teratomas may

be monodermal and highly specialized. Rarely, within some mature teratomas certain elements (most commonly squamous components) undergo malignant transformation. (Mackenzie, 2015).

For those who continue to make a distinction, dermoids are tumors that maintain rather orderly arrangements, with well-differentiated ectodermal and mesodermal tissues surrounding endodermal components. Teratomas, specifically solid teratomas, are essentially devoid of organization; thus, the presence of some degree of organization, a high degree of cellular differentiation, and cystic structure differentiates dermoids from teratomas (Mackenzie, 2015).

1.4.3 Immature teratoma

This is the second most common GCT. It occurs mostly in females aged 10-20 years but may occur after menopause. The tumor spreads most commonly to peritoneal surfaces. (NSSO, 2016).

1.4.4 Other germ cell tumors

Endodermal sinus tumor occurs at a mean age of 18 years, and one third occur before puberty. Embryonal carcinoma and choriocarcinoma are extremely rare. (NSSO, 2016).

1.4.5 Sex-cord stromal tumors

These include tumors arising from the sex cords; granulosa cells; Sertoli cells; and the specialized stroma of the genital ridge, theca, and Leydig cells. They comprise fewer than 5% of all ovarian tumors.Although granulosa cell tumors are malignant and Sertoli-Leydig cell tumors less so, they behave in a much less malignant fashion than epithelial ovarian cancers. (Kurman, 2008).

Benign tumors in the group include thecoma and fibroma. Granulosa cell tumors and pure Sertoli cell tumors commonly secrete estrogen, while Leydig cell tumors and combined Sertoli-Leydig tumors often secrete androgens. (Kurman, 2008)

1.4.6 Granulosa cell tumor

This is the most common malignant sex-cord stromal tumor. Ninety percent of granulosa cell tumors are stage I at the time of diagnosis. This tumor account for approximately 2% of all ovarian tumors and can be divided into adult (95%) and juvenile (5%) types based on histologic findings. Juvenile granulosa cell tumor is a variant of granulosa cell tumor that is rarely malignant. It most often presents in young girls with isosexual precocious puberty. The tumor is usually unilateral and confined to the ovary and can be managed with surgery alone. (Kurman, 2008).

Granulosa cell tumor can occur at any age, with a mean age of the early 50s. Because of the secretion of estrogen, the presenting features depend on the patient's age. Prepubertal girls typically present with precocious sexual development, women of reproductive age have heavy or irregular periods, and postmenopausal women may have postmenopausal bleeding.(McCluggage, 2011).

At all ages, the tumor may present with acute abdominal pain due to rupture or hemorrhage.The tumors vary in size and may be solid or partially cystic.The cut surface may be gray-white or yellow, depending on lipid content. Necrosis and hemorrhage often are present, with cystic compartments filled with fluid or clotted blood. The microscopic features are granulosa cells in a wide variety of patterns, and characteristic Call-Exner bodies may be present. (McCluggage, 2011).

1.4.7 Sertoli-Leydig cell tumor

These tumors are rare. They are a form of low-grade malignancy that typically produces androgens and rarely estrogens. (McCluggage, 2011).

1.4.8 Tumors of low malignant potential

Tumors of low malignant potential (LMP), or borderline tumors, are a distinct variety of epithelial ovarian cancer that behave in a much less aggressive fashion and have a very favorable prognosis. (Cho, 2007).

These tumors cause great anxiety to patients, and the concept of LMP sometimes is difficult to explain. They comprise approximately 20% of malignant ovarian tumors. The mean age of diagnosis is younger than for invasive epithelial ovarian cancer, at approximately 48 years, and no large peak of incidence is observed.In contrast to epithelial ovarian cancer, however, most LMP tumors are stage I at presentation, with a distribution as Stage IA: 51%, Stage IB: 6% Stage IC: 18%, Stages II-III: 15% and Stage IV: 2% (Cho, 2007).

LMP tumors can cause a range of symptoms similar to epithelial ovarian cancer, including increasing abdominal girth, an abdominal mass, abdominal pain, abnormal uterine bleeding, urinary symptoms, and gastrointestinal symptoms. They may be asymptomatic and found on routine physical examination or ultrasound scan. (Cho, 2007).

1.4.9 Stromal carcinoma tumors

Ovarian stromal carcinoma accounts for about five percent of ovarian cancer cases. It develops in the connective tissue cells that hold the ovary together and those that produce the female hormones estrogen and progesterone. The two most common types are granulosa cell tumors and sertoli-leydig cell tumors. Unlike epithelial ovarian carcinoma, 70 percent of stromal carcinoma cases are diagnosed in Stage I. (Matz, 2017).

1.4.10 Small cell carcinoma of the ovary

Small cell carcinoma of the ovary (SCCO) is a rare, highly malignant tumor that affects mainly young women, with a median age at diagnosis of 24 years old. The subtypes of SCCO include pulmonary, neuro-endocrine and hypercalcemic. SCCO accounts for 0.1 percent of ovarian cancer cases. Approximately two-thirds of patients with SCCO have hypercalcemia. The symptoms are the same as other types of ovarian cancer. (Matz, 2017).

Small-cell carcinoma is a rare type of carcinoma that occurs in females aged 2-46 years. It often is associated with hypercalcemia.The most common form of sarcoma in the ovary is the mixed mesodermal sarcoma or carcinosarcoma.Metastatic tumors of the ovary arise from direct extension and spread within the bloodstream or lymphatic system or within the peritoneal cavity. (Doubeni, 2016).

Sites of origin include the endometrium; cervix; and nongynecologic sites such as breast, colon, and stomach. The classic Krukenberg tumor refers to bilateral enlargement of the ovaries from metastases from a signet-ring carcinoma of the stomach. (Doubeni, 2016).

1.5 Histopathological diagnosis

Current diagnosis of ovarian cancer relies on pelvic exam, transvaginal ultrasonography (TVS), abdominal ultrasonography, and exploratory or diagnostic laparoscopy when evaluating a pelvic mass. One tumor biomarker, the cancer antigen 125 (CA125) is commonly used preoperatively to help predict potential for malignancy (Sundar, 2015).

Disease stage at diagnosis is a powerful prognostic variable for predicting patient outcome in ovarian cancer. Patients with International Federation of Gynecology and Obstetrics (FIGO) stage III ovarian cancer, indicating tumor dissemination and seeding of the peritoneal lining outside of the pelvis, have a 5-year survival rate of approximately 35%. This survival rate drops to less than 10% in patients diagnosed with stage IV ovarian cancer, where disease has spread to distant metastasis. (Sundar, 2015).

For patients with advanced stage III and IV ovarian tumors, the most important prognostic factor for predicting favorable outcome is success of complete cytoreductive surgery and minimal residual tumor volume. An ovarian cyst is a fluid-filled sac in an ovary. Ovarian cysts can develop from the neonatal period to postmenopause but most occur during infancy and adolescence, which are hormonally active periods of development. With the more frequent use of ultrasonography in recent years, the diagnosis of ovarian cysts has become more common (Sundar, 2015).

The normally functioning ovary produces a follicular cyst six to seven times each year. In most cases, these functional masses are self-limiting and resolve within the duration of a normal menstrual cycle. In rare situations, they persist longer or become enlarged. At this point, they represent a pathological condition.Adnexal masses present a diagnostic dilemma; the differential diagnosis is extensive, with most masses representing benign processes. However, without histopathologic tissue diagnosis, a definitive diagnosis is generally precluded. (Sundar, 2015).

Physicians must evaluate the likelihood of a pathologic process using clinical and radiologic information and balance the risk of surgical intervention for a benign versus malignant process. Since ovaries produce

physiologic cysts in menstruating women, the likelihood of a benign process is higher. In contrast, the presence of an adnexal mass in prepubertal girls and postmenopausal women heightens the risk of a pathologic etiology.A review by Suh-Burgmann and Kinney suggests that surgical evaluation of adnexal masses is appropriate as Symptomatic masses, Masses associated with other signs of malignancy (eg, elevated cancer antigen 125 [CA125] levels in a postmenopausal patient, ascites), Women at high genetic risk for ovarian cancer, Large masses (>10 cm), which are less likely to regress, have a higher risk of symptoms, and are often more difficult to characterize on ultrasound

Ultrasound features associated with malignancy include Irregular solid tumor, Ascites, At least four papillary projections, Irregular multilocular solid tumor ≥10 cm and Very strong intratumoral blood flow (Sundar, 2015).

1.6 Expression of vimentin

Vimentin protein is a type III intermediate filament protein that is widely expressed in mesenchymal tissue. Collectively with other microtubules and actin microfilaments, vimentin plays a significant role in making up the cytoskeleton to maintain the cell shape, integrity, and stabilizing epithelial mesenchymal cellular interactions. (Czernobilsky, 2009).

Additionally, vimentin works as an organizer of a number of other proteins involved in attachment, migration, and cell signaling. Vimentin expression by immunohistochemistry is widely applied and is a well-known marker reported to be, in combination with other markers, a helpful marker in differentiating endometrial and endocervical adenocarcinomas. (Wud, 2016).

Studies found that Vimentin mRNA expression is higher in solid metastases compared to primary carcinomas and effusions from OC patients, further attesting to the dynamic nature of EMT in OC as function of anatomic site, and suggesting a role in tumor progression. (Wud,2016).

Interestingly, exposure of clinical specimens from patients diagnosed with advanced-stage ovarian carcinoma to cisplatin, as well as the OVCA433 cell line, results in reduced E-cadherin and increased Snail, Slug, Twist, and Vimentin mRNA levels, as well as the increased cell surface expression of CSC markers, which correlated with activation of the MAPK member ERK2, presenting a mechanism for development of resistance in ovarian carcinoma. (Köbel, 2016).

Vimentin is encoded by a single-copy gene located on chromosome 10p13. Initially, vimentin promoter was shown to be composed of three different elements that regulate its expression Later, several cis-elements and associated factors were identified within the human vimentin promoter, suggesting that vimentin gene is subjected to complex control. These include a TATA box, eight putative GC-boxes NF-κB binding site, AP-1 binding site , PEA3 binding site, Sp/XKLF binding site and ZBP-89 binding site. Furthermore, vimentin expression was shown to be transactivated by β-catenin/ TCF, binding to the putative site 468 bp upstream of the transcription initiation site of vimentin promoter and thus increasing the tumor cell invasive potential. It has been shown that NF-κB, a key protein regulating the immune and inflammatory process, also plays an important role in regulating EMT process and its inhibition in the mesenchymal cells reversed the EMT process, suggesting the importance of NF-κB in both

activation and maintenance of EMT. Since vimentin is over expressed during EMT (Rittling SR and Baserga R,1987).

1.7 Literature review

In a Study conducted by(Abd El hafez *et al.,* 2014) they found that ovarian cancer is rare in women younger than 40. Same study done by (Kriplani, 2013) revealed that ovarian cancers develop after menopause. Half of all ovarian cancers are found in women with 63 years of age or older. other study showed that two-thirds of women diagnosed with ovarian cancer are 55 or older. (Scully, 2011).

(Reid *et al.,* 2017) showed that in serous tumors, the expression of vimentin was related to the degree of tumor differentiation. But, it was consistently identifiable in the better differentiated tumors; against the other study where it was defined in borderline and malignant serous tumors. Also he revealed that more than 90% of malignant ovarian tumors are epithelial in origin, 5%–6% of tumors constitute sex cord-stromal tumors (e.g., granulosa cell tumors, thecomas, etc.), and 2%–3% are germ cell tumors.

Other study done by (Cho, 2009). explained Grade 2 (moderately differentiated) cancers and Grade 3 (poorly

differentiated) ovarian cancers that show increasing abnormality of appearance compared to normal cells. Other studies stated the results demonstrated that the low expression of vimentin positively correlates with the low proliferation rates of ovarian cancer cells. (Scully, 2011). Others found that Malignant ovarian cancer, are comprised of five main histotypes: high-grade serous endometrioid, clear cell (10%), mucinous (3%), and low-grade serous (<5%).(Cho, 2009).

(Prat *et al.*, 2012) they stated that Vimentin was coexpressed with high intensity in 62.5% of serous carcinomas, mild intensity in 25% of mucinous adenocarcinoma, and moderate intensity in single case of endometrioid adenocarcinoma. Moreover they claimed that there was a significantly increased expression of vimentin in serous cystadenoma and serous carcinoma, compared with their mucinous counterparts.

Chapter two

Rationale and objective

2.1 Rationale

Ovarian cancer is the most lethal gynecologic malignancy, with the majority of patients dying within 5 years of diagnosis(Hippisley-CoxJ and Coupl , 2011) . in Sudan although many researchers worked on ovarian cancer but the prevalence of disease is not completely identified furthermore they do not describe the Immionohistochemical Expression of vimintin among Sudanese Patients to distinguish between benign and malignant ovarian Cancer.

2.2- Objectives

2.2.1 General Objective

●**To investigate** the Immunohistochemical Expression of Vimentin among Sudanese Patients with overian lessions.

2.2.2- Specific Objectives

●To detect the frequency of Immunohistochemical Expression of Vimintin among patients with ovarian cancer.

●To compare the expression of Vimentin Immunohistochemical Expression in bengin and malignant ovarian tumors.

●To correlate the vimentin expression with the grade of tumors, patient's age and different types of lesions.

Chapter three

Material and method

3-1 Study design

It is analytical case control study.

3.2- Study area

Alrahma histopathology lab.

3.3- Study population

Paraffin embedded block of ovarian tissue which previously diagnosed with ovarian lesion

3.4- Study variable

Patients' age, Tumor grades and Histopathological diagnosis .

3-5- Sample size

A total of 80 samples were collected, 40 of them as case and 40 served as control.

3-6- Ethical considerations

The study was approved by the department of histopathology at Shandi university and permission was taken from Alrahma laboratory to collect the samples.

3-7 Sample preparation

3-7-1Tissue microarray

The targeting area was identified according to the previous stained section with H and E then the origin block was subjected to 3 mm, sections were brought in recipient paraffin bock, the surface of TMA block were then pressed by preheated clean glass slide until the surface became smooth, then the block were placed in refrigerator until cooling, glass slide was then detached and the block was ready for cutting.

3-7-2 Sectioning

TMA block was sectioned by using rotary microtome (leica RM 2125) and tremining in 4 micron as sickness by using disposable knife then section floated in water bath 45 degree then section picked up by slide and left for 2 hrs at 60 degree.

3-8 Immunohistochemistry procedure

Two slides de-waxed in xylene and dehydrate through graded alcohol to DW, slide placed in preheated buffer Dako (Dako Denmark A\S) at ph 9.0 at 96 degree in water bath for 40 mins after completion of retrieval the coplinjar were removed from water bath and allowed to cool in RT.

Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 20 mins then slide was incubated with 100µl of vimintin primary Ab for 20 mins at RT in moisture chamber and then was washed with PBS for 3 mins binding Abs will be detected by incubating for 2o mins with dextrane polymerase (thermo) finally the section was washed in three change in PBS followed by adding 3,3

diaminobinzidine tetra hydrochloride (DAB) as chromogen to produce the characteristic brown stain for the visualization of the Ab – enzyme complex for up to 3 mins, was counter stain with hematoxylin.

3-9-1Interpretation of IHC result

The positive expression of vimentin was expressed as a brown color, cytoplasmic in origin the absence of this expression indicate the negative result.

Chapter four

Results

4.1 Results

This study was conducted to detect the frequency of vimentin expression in 80 paraffin block sample who divided into two study groups, 40 as cases (diagnosed with malignant ovarian tumors) and 40 as controls (diagnosed with begin ovarian disease). The study showed that more than half of the study participants (61.3%) were within (41 – 60) years old while only (10%) of them were above 60 years. (Table: 1).

Comparison of age group in benign and malignant showed that those with age 21-40 years represent 10% in malignant and 25% in benign, while those from 41-60% represent 70% in malignant and 52.5% in benign, furthermore those with age 61-80% involved in malignant only and represent 20%, the comparison appeared significantly (p value 0.005), (figure 8).

Regarding the expression of Vimentin 61 (76.3%) showed positive results while (13.8%) were negative (10%) were missed duo to detached of sections in immunohistochemical stain.

Table (1) Distribution of age among the study groups

Age - years	Frequency	Percent
21-40	14	17.5
41-60	49	61.3
61-80	8	10.0
Missing	9	11.3
Total	80	100.0

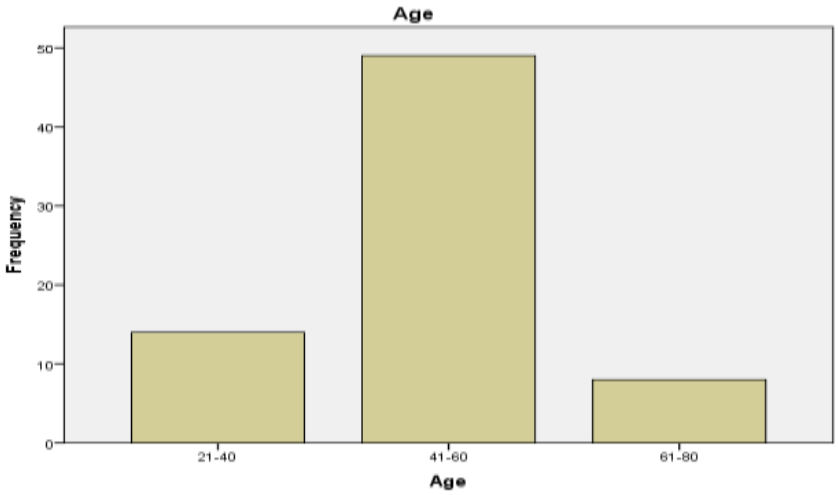

Figure (1): Distribution of age among the study group.

Table (2) Distribution of Vimentin expression among the study group

Vimentin result	Frequency	Percent
Positive	61	76.3
Negative	11	13.8
Missing	8	10.0
Total	80	100.0

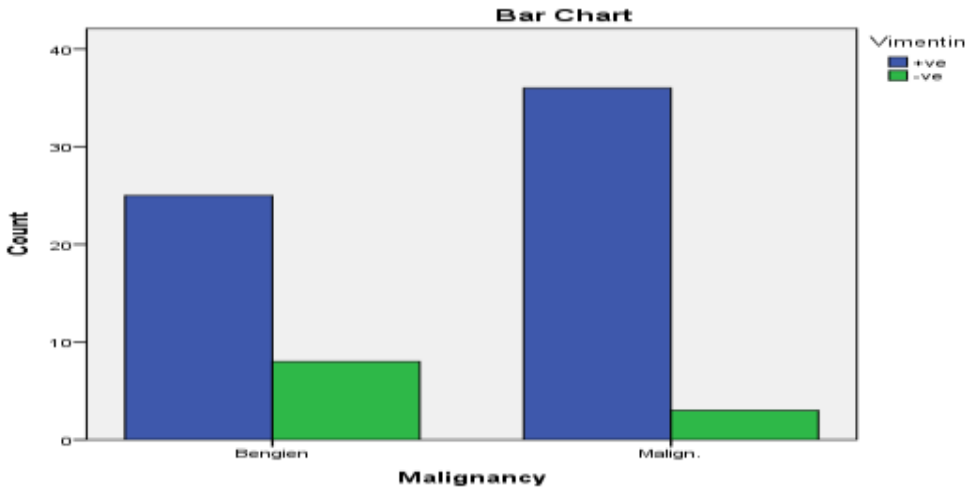

Figure (2): Distribution of vimentin expression among benign and malignant.

Control group (Benign Ovarian Disease)

The histopathological diagnosis showed that 22 (55%) of the benign group were diagnosed as Serous cyst (22.5%) Musinous Cyst (20%) Teratoma and (2.5%) Fibroma .(table 4)

the age distribution showed that more than half of Benign group (52.5%) were within (41 – 60) years old , while (25%) of them were less than 40 years .

The immunohistochemical staining showed that (62.5%) of the benign group was positive for Vimentin expression, while (20%) were negative (table 3).

Statistical tests revealed that there was no significant relationship between the histopathological diagnoses and the expression of Vimentin (p value = 0.145), or between the diagnosis and the age (p value = 0.665) (tables 6 and 7)

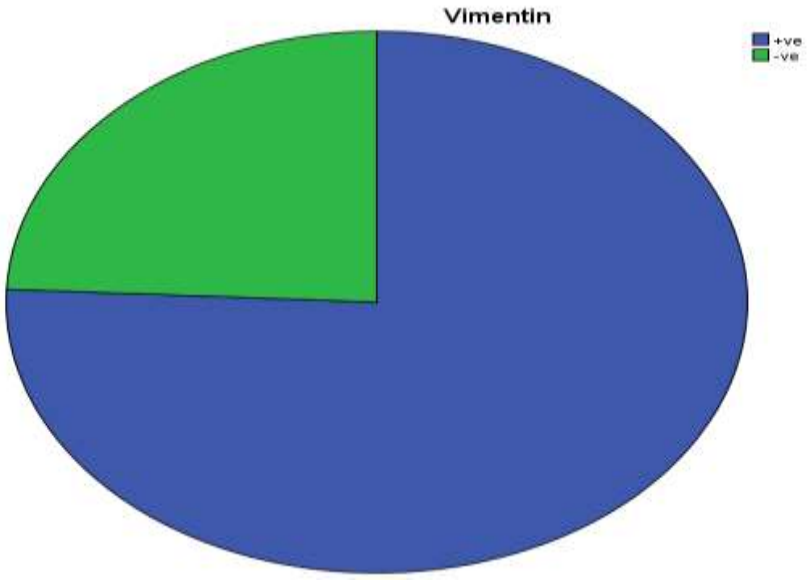

Figure (3) distribution of Vimentin result among the benign group

Table (3) the distribution of Histopathology Diagnosis among the benign group

Diagnosis	**Frequency**	**Percent**
Fibroma	1	2.5
Musinous. Cyst	9	22.5
Serous cyst	22	55.0
Teratoma	8	20.0
Total	40	100.0

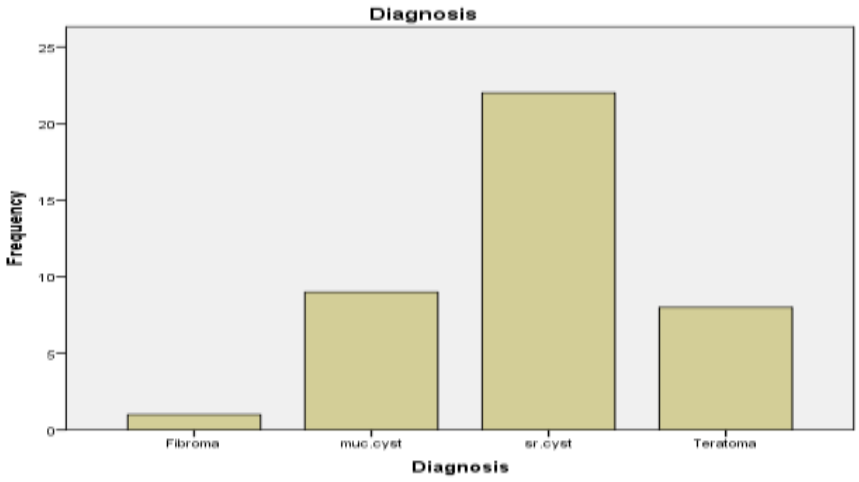

Figure (4) the distribution of Diagnosis among the benign group

Table (4) the distribution of age among the benign group

Age - years	Frequency	Percent
21-40	10	25.0
41-60	21	52.5
61-80	0	0.0
Missing	9	22.5
Total	40	100.0

Table (5) the relationship between the histopathological diagnosis and the Vementin expression among benign group.

		Vimentin		Total	P value
		+ve	-ve		
Diagnosis	Fibroma	0	1	1	0.145
	Mucinous. cyst	5	3	8	
	Serous. Cyst	15	2	17	
	Teratoma	5	2	7	
Total		25	8	33	

Cases group (Malignant Ovarian tumors)

Regarding the age distribution among the Malignant group, it is revealed that (70%) of them were 41 - 60 years of age while (20%) of them were 61 - 80 years old and only (10%) were from 21 to 40 years of age (table 8).
The histopathological diagnosis revealed that more than half of the study participants were diagnosed with adenocarcinoma 30 (75%), papillary tumor (5%), Granuloma (17.5%) of Malignant group(table 9)

Table (6) the distribution of age among the malignant group

Age - years	Frequency	Percent
21-40	4	10.0
41-60	28	70.0
61-80	8	20.0
Total	40	100.0

Table (7) the distribution of diagnosis among the malignant group

Diagnosis	Frequency	Percent
Adenocarcinoma	30	75.0
Papillary tumore	2	5.0
Granulosa.	7	17.5
Total	40	100.0

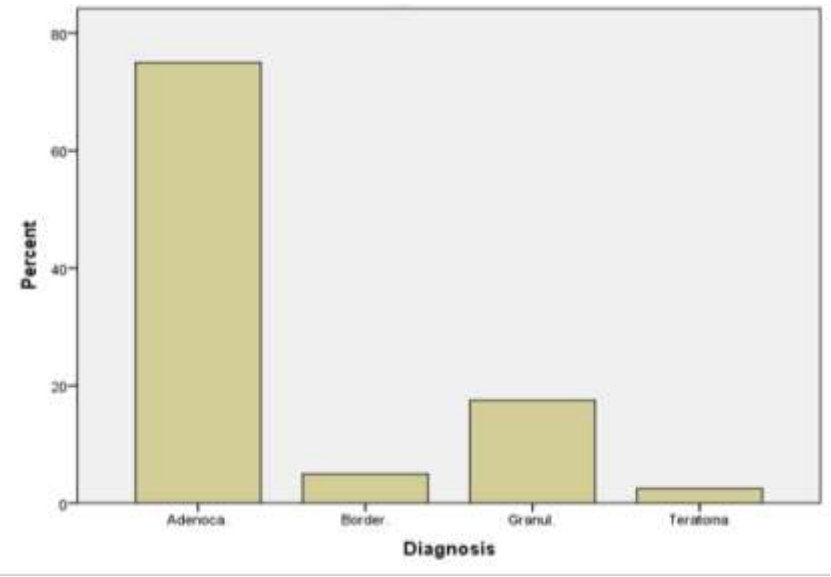

Figure (5) the distribution of histopathological diagnosis among the malignant group

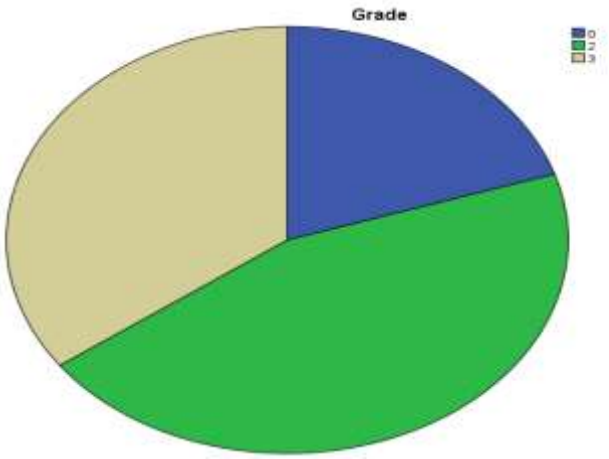

Figure (6) the distribution of tumor grades among the malignant group

The immunohistochemical stain showed (90.0%) was positive for vimentin expression while(7.5%) were negative (table 8) ,Cross tabulation tests revealed that there is no significant relationship between the histopathological diagnoses and the expression of Vimentin (p value = 0.772), or between the grade with the Vimentin expression (p value = 0.586) in cases group (tables 6 and 7)

The study revealed that there was no significant relation in the expression of vimentin in comparing the bengnin and malignant group.(p value 0.05).

Table (8) the distribution of Vimentin results among the malignant group

Vimentin results	**Frequency**	**Percent**
Positive	36	90.0
Negative	3	7.5
Missing	1	2.5
Total	40	100.0

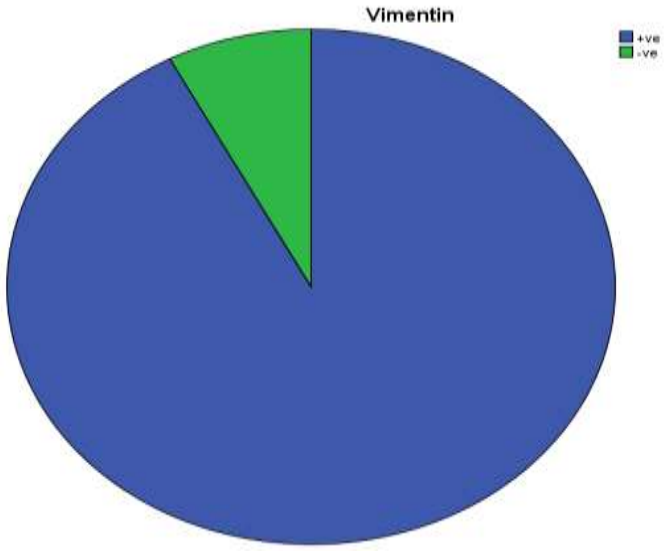

Figure (7) the distribution of Vimentin results among the malignant group

Table (9) the relationship between the histopathological diagnosis and the Vementin expression among malignant group.

		Vimentin		Total	P value
		+ve	-ve		
Diagnosis	Fibroma	26	3	29	0.772
	Mucinous cyst	2	0	2	
	Serous Cyst	7	0	7	
	Teratoma	1	0	1	
Total		36	3	39	

Table (10) the relationship between the grade and the Vementin results among malignant group(n=40)

		Vimentin		Total	P value
		+ve	-ve		
Grade	1	8	0	8	**0.586**
	2	15	2	17	
	3	13	1	14	
Total		36	3	39	

Table (11) the difference in **Vimentin** results between two study groups.

		Vimentin			Total
		+ve	-ve	Missing	
Study group	Case	36	3	1	40
	Control	25	8	7	40
Total		61	11	8	80

Chi square = 8.756 ***P value = 0.05***

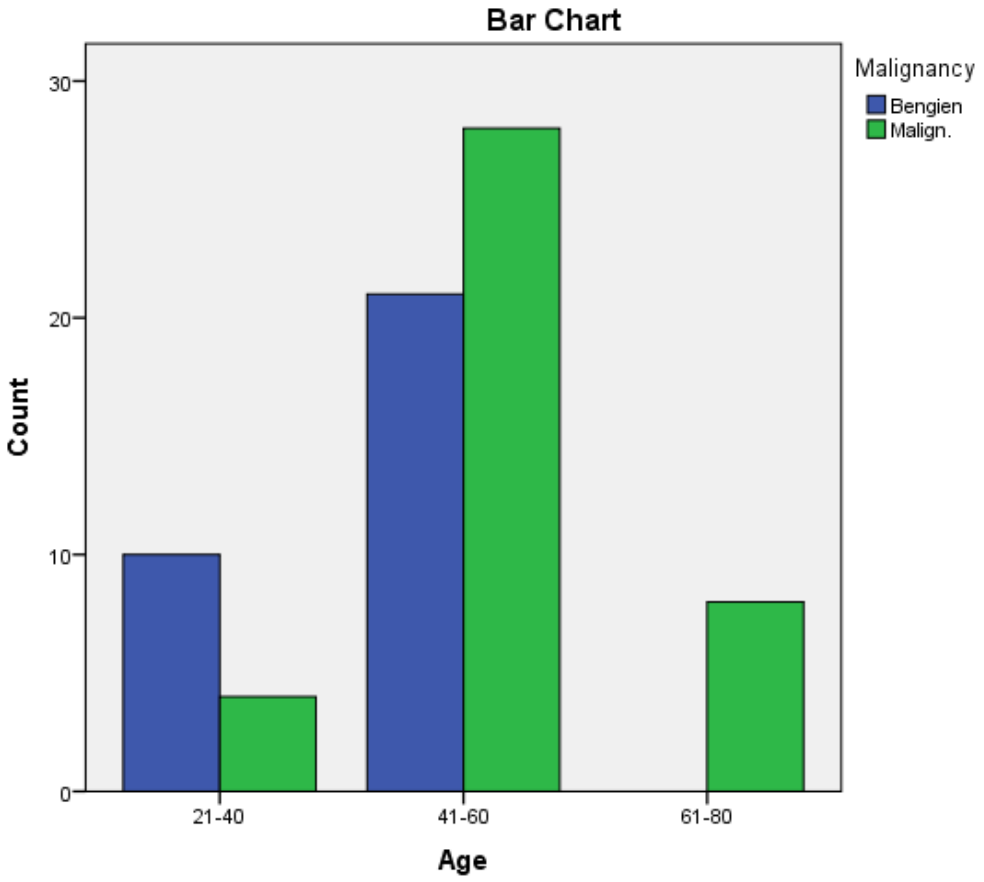

Figure (8): comparison of age in benign and malignant

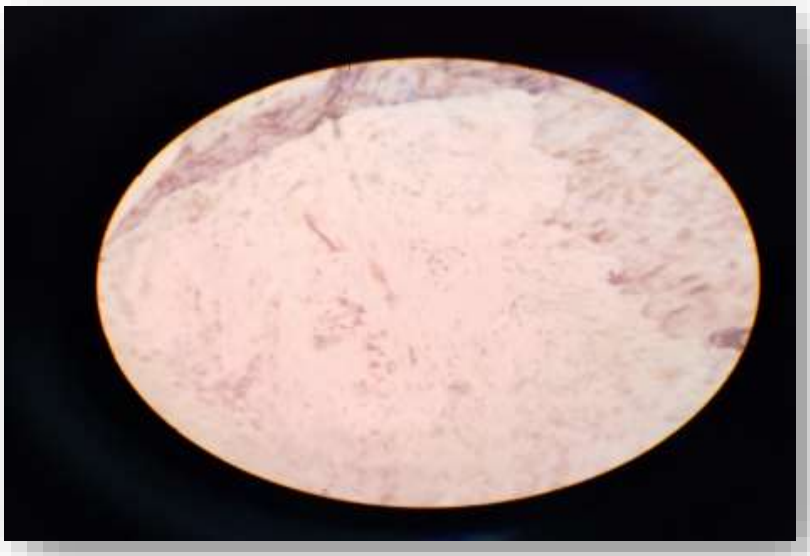

Figure (9) benign ovarian tissue diagnosis positive IHC expression of vimentin

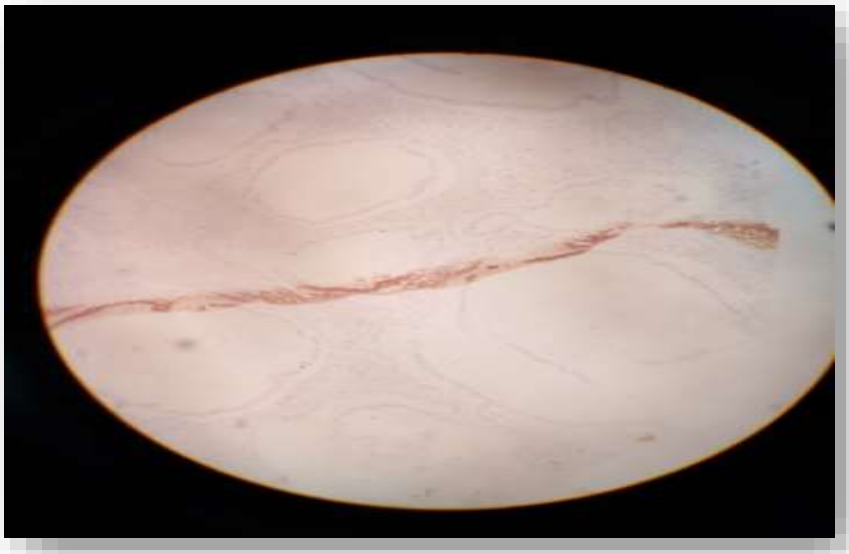

Figure (10) benign ovarian tissue diagnosis negative IHC expression of vimentin

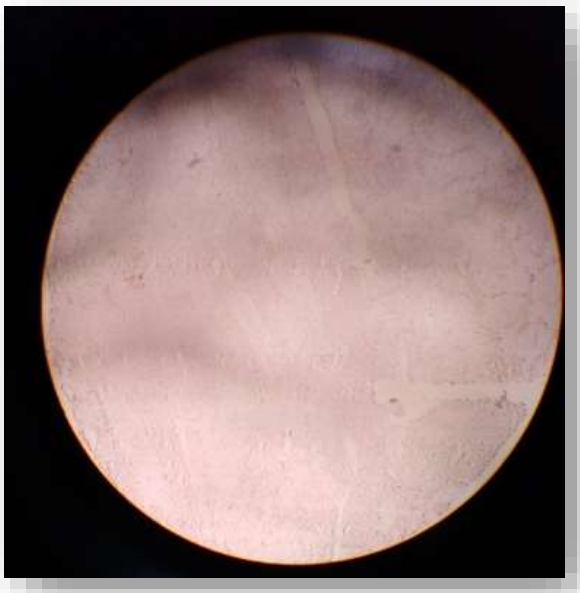

Figure (11) malignant ovarian tissue diagnosis positive IHC expression of vimentin

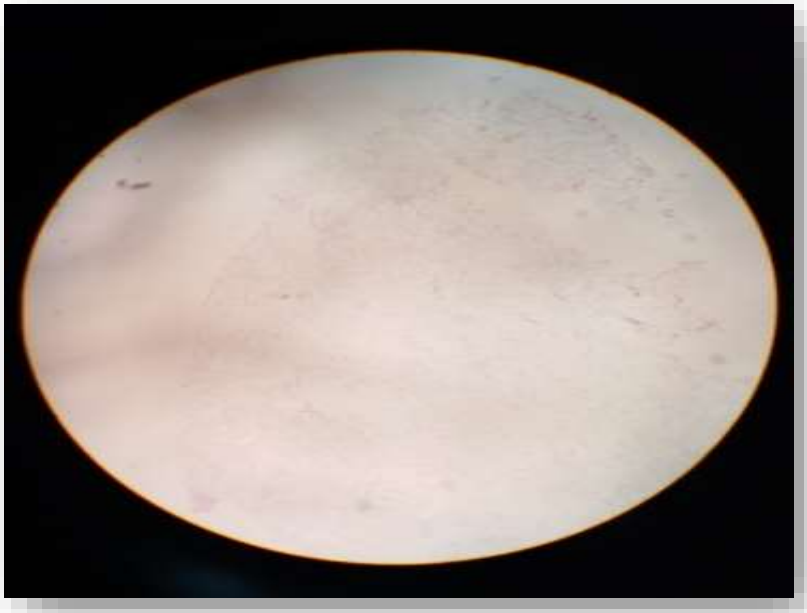

Figure (12) malignant ovarian tissue diagnosis negative IHC expression of vimentin

Figure (13) tissue microarray sample for benign and malignant group

Chapter Five

Discussion, Conclusion and Recommendations

5.1 Discussion

This study aimed to investigate the Immunohistochemical Expression of Vimentin in 80 study type of paraffin block sample divided into two study groups, forty cases (diagnosed with malignant ovarian tumor) and 40 controls (diagnosed with benine ovarian tumor). The study showed that more than half of the study participants (61.3%) were 41 - 60 years in age while only (10%) of them were above 60 years. Other studies agreed with these findings when stated that the risk of developing ovarian cancer gets higher with age.(Abd El hafez, 2014). Moreover, they found that ovarian cancer is rare in women younger than 40. Most ovarian cancers develop after menopause. Half of all ovarian cancers are found in women in 63 years of age or older. (Kriplani, 2013) other showed that two-thirds of women diagnosed with ovarian cancer are 55 or older. (Scully, 2011).

Our study found that (55%), of participants were diagnosed with sereous cyst and (22.5%) with Mucinous Cyst ,Teratoma were found in (20%) and Fibroma in only (2.5%).

A study conducted by Reid et al., showed that in serous tumors the expression of vimentin was related to the degree of tumor differentiation But it was consistently identifiable in the better differentiated tumors(Reid, 2017).

Our study showed that (75%) of participants were diagnosed with adenocarcinoma, (5%) papillary tumor,(17.5%) Granuloma and (2.5%) Teratoma. And it had been showed that in developed countries, more than 90% of malignant ovarian tumors are epithelial in origin, 5%–6% of tumors constitute sex cord-stromal tumors and 2%–3% are germ cell tumors (Reid, 2017).

The study found that less than half of the cases group (45%) classified as grade 2, (35%) as grade 3 and only (20%) as grade 4. Other study explained Grade 2 (moderately differentiated) cancers and Grade 3 (poorly differentiated) ovarian cancers that show increasing abnormality of appearance compared to normal cells. They are also increasingly more likely to spread and recur. (Cho, 2009).

Our study revealed that (90.0%) of the cases group had positive results for Vimentin while (7.5%) were negative , Other studies stated that this results demonstrated that the low expression of vimentin positively correlates with the low proliferation rates of ovarian cancer cells. (Scully, 2011) So, vimentin plays a

significant role in making up the cytoskeleton to maintain the cell shape, integrity, and stabilizing epithelial mesenchymal cellular interactions. (Czernobilsky, 2009).

Cross tabulation was done to assess the possible relationships between the result of Vimentin among the study participants. The analysis showed that there is no significant relationship between the diagnoses with the result of Vimentin (p value = 0.772) , or between the grade with the Vimentin results in cases group (p value = 0.586).

The pattern of epithelial cells staining with vimentin was peripheral. The intermediate filaments coexpression was associated with the type of tumor and unrelated to the degree of differentiation. It was similar to the research conducted by Ried et al. they found no strong vimentin positivity was identified in benign, borderline, and malignant mucinous tumors, which was correlated with the current study (Ried, 2017).

5.2 Conclusion

- The study found that (90.0%) of the cases group had positive results for Vimentin while (7.5%) were negative compared with control (62.5% positive and 20% negative).

- There is no significant relationship in the expression of Vimentin with the histopathological diagnoses and with the tumor grade in the case group.

5.3 Recommendation

1. Further studies are highly recommended with larger sample size to cover wider scope of ethnic/nationalities variation to account for genetic diversity and it role in the expression of vimentin.

2. Health education should be given to population to increase their awareness toward ovarian cancer to reduce the risk of disease incidence and the detection of the disease within the late stages.

Chapter Six

References and appendix

6.1 References

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6.2 Appendices

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)
- DAB (3,3 di amino benzidin tetra hydrochloride)substrate solution
- Bluing Reagent (0.1MLi2 CO3 , 0.5 M Na2CO3)
- Xylene
- DPX mounting media