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Immunohistochemical Expression of Ki-67 among Sudanese Females with Ovarian Tumors in Khartoum State

A thesis submitted for partial fulfillment of the requirements of M.Sc. degree in Medical Laboratory Sciences (Histopathology and Cytology)

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الاية

بسم الله الرحمن الرحيم

قال تعالى:

{وَ قُلْ رَبِّ زِدْنِي عِلْماً}

صدق الله العظيم

(طه -114)

Dedication

To my mother and my father

To my family… always…

To my daughter

To all my friends,,

Sahar

Acknowledgment

All the thanks to Allah for the gift of life, health and faith and for the strength and desire that he gave me to continue this way, and look always for the best.

I am grateful to my supervisor Dr. Mohammed Abdelgader Elsheikh Mohammed for his help, patience, care, invaluable support and for providing me with the materials that I need to carry out this study.

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DAB……..diaminobenzidine tetra hydrochlorid

DPX…….....Distyrene plasticizer xylene

EOC……..Epithelial Ovarian Cancer

FFPE……..Formalin Fixed Paraffin Embedded

FIGO……....International Federation of Gynecology and Obstetrics

HGSOC……High-grade serous ovarian carcinoma

IHC…….……...Immunohistochemistry

ISH .....…..In situ hybridization

MLS……. Medical Laboratory Sciences

MIB-1…… Mind bomb E3 ubiquitin protein ligase 1

NACT……..Neo Adjuvant Chemotherapy

NCI…….....National Cancer Institute

PBS……. phosphate buffer saline

PCR……..….Polymerase chine reaction

PI……..…….Proliferation index

SEOC……..Surface Epithelial Ovarian Carcinoma

SPSS…….Statistical Package for the Social Sciences

TMA…….....tissue microarray

Abstract

Background; Ovarian tumors include several different types of tumors that all arise from cells of the ovary. Most commonly, tumors arise from the epithelium, or lining cells, of the ovary. Ki-67 tumor maker immunostaining to detect proliferation of tumor cells is one such recommended prognostic marker and is a nearly new method for investigating the proliferative index (PI) of a tumor lesion. Over expression of Ki-67 antigens is associated with tumor aggression, vascular invasion, tumor spread, reserved prognosis and poor response to chemotherapy.

Materials and methods; This was a descriptive cross sectional study conducted in Khartoum state-Sudan, during the period from September 2017 to May 2018. Forty paraffin-embedded tissue blocks with ovarian tumors were included in this study; samples were subjected to detect Ki67 antigen using immunohistochemistry technique.

Results; Ki67 antigen was detected in 30 cases (75%) with no statistically significant different, the p value was 0.664. There was no statristical correlation of tumor grade with mraker expression, as the p.value was 0.579. Also there was no statistical correlation between age and marker expression, as the p.value was0.330.

Conclusions; There was no relationship between tumors type and ki67 expression. There was no relationship between grade of tumors and ki67 expression.

المستخلص

الخلفية؛ تشتمل أورام المبيض على عدة أنواع مختلفة من الأورام تنشأ من خلايا المبيض. وتعبتر الأورام التي تنشأ من الخلايا المبطنة للمبيض هي الأكثر شيوعا. الكشف عن الأنتجين (كي آي 67) عن طريق تقنية الصبغ المناعي إحدى الوسائل الجديدة للكشف عن تكاثر الخلايا وهو إحدى العلامات التنبؤية الموصى بها للكشف عن عامل تكاثر الخلايا. ظهور الأنتجين (كي آي 67) بكثرة يترافق مع أنتشار الورم وأنتقاله عن طريق الأوعية الدموية أيضا له دلالة على أستجابة الورم للعلاج.

الطرق والمواد؛ أجريت هذة الدراسة المتعلقة بالمشاهدة والتي شملت مجموعة الحالة بولاية الخرطوم في الفترة من سبتمبر 2017 الي مايو 2018. أشتملت الدراسة على 40 عينية نسيجية من المبيض في شمع البرافين جميع العينات شخصت على انها أورام مبيضية. أخضعت هذه العينات للكشف عن انتجين (كي اي 67) عن طريق تقنية الصبغ المناعي.

النتائج؛ ظهر انتجين (كي اي 67) في ثلاثون عينة والتي تمثل نسبة 75% مع عدم وجود دلالة ذات قيمة أحصايئة وكانت القيمة الأحتمالية 0.664. أيضا أتضح أنه ليست هناك علاقة بين تدرج الورم وظهور الأنتجين (كي اي 67) وكانت القيمة الاحتمالية 0.579. أيضا أتضح انه ليست هناك علاقة بين العمر وظهور الانتجين(كي اي 67) وكانت القيمة الأحتمالية 0.330.

الأستنتاج؛ لا توجد علاقة بين ظهور الأنتجين (كي اي 67) وتدرج الورم او العمر.

CHAPTER ONE
INTRODUCTION

1.1. Background

Ovarian tumors include several different types of tumors that all arise from cells of the ovary. Most commonly, tumors arise from the epithelium, or lining cells, of the ovary. These include epithelial ovarian, fallopian tube, and primary peritoneal cancer. These are all considered to be one disease process. There is also an entity called ovarian low malignant potential tumor; these tumors have some of the microscopic features of a cancer, but tend not to spread like typical cancers. (Green *et al*., 2013) There are also less common forms of ovarian tumor that come from within the ovary itself, including germ cell tumors and sex cord-stromal tumors. (Green *et al*., 2013) High-grade serous ovarian carcinoma (HGSOC) accounts for 67% of all ovarian tumors and is the most aggressive subtype. These cancers arise due to DNA changes in cells that lead to the development of cancer. (Chen, 2014) Ovarian cancer is the fifth most frequent female cancer type. (Jemal *et al*., 2004) Ovarian tumors account 30% of all female genital tract cancers. More than 90% of the tumors cases of the ovary are SEOS and is the common cause of death of gynecologic neoplasms. (Prat, 2012; Kurman*et al*., 2014; Khandakar *et al*., 2014) Ovary tumors are ten times less common than breast cancer; it is associated with late stage where the tumors are metastasized beyond the pelvis in 70-75% of cases. The presence of recurrence after surgical removal and chemotherapy is high with high number of deaths in due course of cancer. (Stewart and Kleihues, 2003; Khandakar *et al*., 2014) Early detection plans and new therapeutic approaches to minimize death have been mainly ineffective due to absence of specific tumorigenesis and diagnostic aids for screening. (Kurman and Shih, 2010; Liu *et al*., 2012, Heeran *et al*., 2013, Ezzati *et al*., 2014) There are no proven methods of prevention. If diagnosed and treated while the

disease is confined to the ovary, the 5-year survival rate is 95%; unfortunately, 70% of cases are diagnosed at late stages and prognosis is dismal. (Niloff *et al*., 1984) Most patients initially respond to chemotherapy, the majority succumb to recurrent chemoresistant tumors. Efforts to overcome chemoresistance have been largely unsuccessful. The mortality rate remains high. (Jemal *et al*., 2004) Cell proliferation exhibits an important role in the clinical behavior and aggressiveness of tumor of the ovary. Detection of proliferation activity has been demonstrated to be of a diagnostic with prognostic value and many methods are used to investigate the number of proliferating cells. (Mita *et al*., 2004; Gursan *et al*., 2009; Heeran *et al*., 2013; Ezzati *et al*., 2014)

Ki-67 tumor maker immunostaining to detect proliferation of tumor cells is one such recommended prognostic marker and is a nearly new method for investigating the proliferative index (PI) of a tumor lesion by the Mind bomb E3 ubiquitin protein ligase 1 (MIB-1 antibody) immunohistochemistry (IHC). Ki-67 tumor marker over expressed in ovarian tumor compared to benign or borderline tumors of surface epithelial origin. Over expression of Ki-67 antigens is associated with tumor aggression, vascular invasion, tumor spread, reserved prognosis and poor response to chemotherapy. Its expression can be used to direct the clinical management of ovary cancer both as diagnostic and prognostic tool. (Scholzen *et al*., 2000; Mita *et al*., 2004; Kritpracha *et al*., 2005; Aune *et al*., 2011, Prat, 2012; Khandakar *et al*., 2014) This study aimed to correlate the Ki-67 antigen expression with histological subtypes, grades and investigating the clinical value of Ki-67 antigen as a proliferative marker for diagnostic purpose.

1.2. Rationale

Ovarian cancer is the fourth common cancer in Sudan. Usually women present late in disease due to lack of efficient education, efficient health care system and low socioeconomic status. No published data in Sudan that correlate Ki-67 proliferating gene with ovarian cancer development, progression and prognosis. Detection of KI 67 in ovarian cancer tissues may play important role in reliable diagnosis and further detection of tumor stage, progression, prognosis and thus my lead to sufficient treatment and follow up.

1.3. Objectives

1.3.1. General Objective

1- To detect Ki- 67 in tissues taken from Sudanese females with ovarian tumors using immunohistochemical monoclonal MIB-1antibody.

1.3.2. Specific Objectives

1- To correlate ki-67 immunoexpression with histological subtypes.

2- To correlate Ki- 67 expression score with tumor grade.

3- To correlate Ki-67 immune stain with patient's age.

CHAPTER TWO
LITERATURE REVIEW

2.1. Ovarian tumors

Epithelial ovarian tumor accounts for a majority of all ovarian tumors. It is generally thought of as one of three types of tumors that include ovarian, fallopian tube, and primary peritoneal tumor. All three tumors behave, and are treated the same way, depending on the type of cell that causes the tumor. The four most common cell types of epithelial ovarian tumors are serous, mucinous, clear cell, and endometrioid. These tumors arise due to DNA changes in cells that lead to the development of tumor. The serous cell type is the most common variety. It is now thought that many of these tumors actually come from the lining in the fallopian tube, and fewer of them from the cells on the surface of the ovary, or the peritoneum. However, it is often hard to identify the sources of these tumors when they are found at advanced stages, which is very common. (Chen, 2014)The type of cell where the cancer begins determines the type of ovarian tumors you have. Ovarian tumors types include; epithelial tumors, which begin in the thin layer of tissue that covers the outside of the ovaries. About 90 percent of ovarian tumors are epithelial tumors. The second type is stromal tumors, which begin in the ovarian tissue that contains hormone-producing cells. These tumors are usually diagnosed at an earlier stage than other ovarian tumors. About 7 percent of ovarian tumors are stromal. The last type is germ cell tumors, which begin in the egg-producing cells. These rare ovarian cancers tend to occur in younger women. (Desai *et al*., 2014)

Ovarian cancer may not cause any specific symptoms, particularly in its early stages. When it does cause symptoms, these may be nonspecific and vague. Symptoms can include; abdominal enlargement or swelling, abdominal fullness, early satiety (feeling full early), changes in bowel or bladder habits or clothes not fitting well. Other signs and symptoms can

include shortness of breath, leg swelling, and pain in the abdomen or pelvis. Fatigue may be present, but it is considered another nonspecific symptom. (Rochester, 2013)

2.2. Risk factors of ovarian tumors

Risk factors associate with the development of ovarian cancer are; age, obesity, reproductive history, birth control, gynecologic surgery, fertility drugs, androgens, estrogen therapy and hormone therapy, family history of ovarian cancer, breast cancer, or colorectal cancer, family cancer syndromes, diet analgesics, smoking and alcohol use. (Havrilesky *et al*., 2013) Although the etiology of ovarian tumors is not clear, certain factors are implicated in the etiology of this disease, such as ovulation, gonadotropic and steroid hormones, germ cell depletion, oncogenes and tumor suppressor genes, growth factors, cytokines, and environmental agents. Family history of breast or ovarian cancer is a prominent risk factor for ovarian cancer, with 5–10% of ovarian tumors due to heritable risk. (Fariba *et al*., 2008)

2.3. Ki-67 and ovarian cancers

Estimation of proliferative activity of the neoplasm has been reported to be a diagnostic and prognostic value with known clinicopathologic features in many tumors such as those of lymphatic system, lung, brain, breast, cervix, uterus, ovary, prostate, and in soft tissue sarcoma. There are many methods which can consider detecting the number of proliferating cells by proliferation index (PI). Recently Ki-67 tumor marker immunostaining is a promising objective for PI which required further methodological fine tuning. (Brown and Gatter, 2002; Mita *et al*., 2004; Gursan *et al*., 2009; Aune *et al*., 2011; Choudhury *et al*., 2011; Liu *et al*., 2012) Gerdes*et al*., demonstrated Ki-67 as nuclear non-histone protein. (Gerdes *et al*., 1991) The

Ki-67 immunoexpression was of great value to identify as a tumor marker of cell proliferation because of its complete expression in proliferating tissues and the absence in quiescent cells. The Ki-67 gene is present on the long arm of human chromosome 10 (10q25). Ki-67 is also frequently measured both as a fixed tumor marker for proliferation activity and with many measurements during treatment, as a potential active intermediate or alternative tumor marker of treatment efficacy. (Gerdes *et al*., 1991; Scholzen and Gerdes, 2000) MIB-1 antibody has now been considered as a reference monoclonal mouse antibody for the detection of PI in formalin-fixed, paraffin embedded tissues for Ki-67 antigen. It reacts with the Ki-67 tumor marker nuclear protein which is present in two isoforms of 345 and 395 KDa. The Ki-67 antigen/ MIB-1 immunostaining are exist during all active stages of each cell's cycle (G1, S, G2 and M-phase) but absent in resting cells (G0 phase, quiescent state of the cell. (Scholzen and Gerdes, 2000; Mita *et al*., 2004; Kritpracha *et al*., 2005; Giurgea *et al*., 2012) Ki-67 is an easily available, inexpensive, rapid, applied on formalin-fixed paraffin embedded sections and a more reproducible biomarker available in developing countries, compared with other biomarkers such as proliferating cell nuclear antigen and bromodeoxy uridine. Ki-67 immunostaining needs only small tissue samples, allowing it to be used even in case of patients who are candidates for Neo Adjuvant Chemotherapy (NACT). (Scholzen and Gerdes, 2000; Brown and Gatter, 2002; Kritpracha *et al*., 2005; Giurgea *et al*., 2012) Ki-67 immunostaining is evaluated in most positively stained areas and all identifiable nuclear staining is interpreted as positive immunoreactivity regardless of intensity. Immunostaining is usually confined to the nucleus and cytoplasmic positivity is observed only during mitosis. Many studies have correlated this tumor marker in SEOC with other

prognostic markers such as histologic subtype, tumor grade, FIGO stage, chemotherapy response and also with survival rates. (Gerdes *et al*., 1991; Mita *et al*., 2004; Munstedt *et al*., 2004; Kobel *et al*., 2008; Liu *et al*., 2012; Marinas *et al*., 2012; Giurgea*et al*., 2012; Heeran *et al*., 2013; Khandakar *et al*., 2014) The SEOC expresses high Ki- 67 L1 than benign and borderline tumors. (Min and Park, 2007; Khouja *et al*., 2007; Choudhury *et al*., 2011) Sylvia et al., studied 60 respective cases of epithelial ovarian neoplasms and found that; Ki- 67 L1 was highest in cancer cases (Mean PI-48.6 ± 26.76) followed by borderline and lowest in benign. (Sylvia *et al*., 2012) In malignant group, serous had high index followed by endometroid and lowest in mucinous. They also documented that; CA-125 levels did not have a significant correlation with PI. Similar results were observed by Asha *et al*., they concluded that; the level of Ki-67 L1 was high in serous group and no correlation was found between CA-125 levels with L1. (Asha *et al*., 2017) Several studies have reported difference in Ki-67 expression in different histological subtype and have shown various distribution of immunostaining in serous with its subtype, mucinous, endometroid and clear cell. (Mita *et al*., 2004; Aune *et al*., 2011; Sylvia *et al*., 2012; Giurgea *et al*., 2012; Heeran *et al*., 2013)

In a retrospective study of 500 ovarian tumors by Kobel et al., Ki-67 immunohistochemical expression along with other biomarkers was assessed and it was concluded that; there is marked significance variation in PI between different subtypes but is not of prognostic significance within any subtype. (Kobel *et al*., 2008) Asha *et al*., study also concluded that; no significant association was found between the subtypes and also serous versus nonserous histology. (Asha *et al*., 2017)

Heenan*et al*., observed that; Ki-67 expression elevated with histological grade (p > 0001), (Heeran*et al*., 2013) and many authors have documented the same findings. (Jemal *et al*., 2007, Min and Park, 2007, Gursan *et al*., 2009, Aune *et al*., 2011; Patil *et al*., 2011; Asha *et al*., 2017) It has been documented in the serous carcinoma subtypes; the Ki-67 L1 was higher in the HGSC than the LGSC. (Henriksen *et al*., 1994; Rohke *et al*., 1997; Kobel *et al*., 2008; Prat, 2012) The study by Giurgea *et al*., noted the expression of both Ki-67 and p53 in 125 diagnosed cases of epithelial ovarian neoplasms and found serous to be the most common histological subtype (92.3%) and demonstrated high level of Ki-67 L1 in HGSC. (Giurgea *et al*., 2012) Similar results were obtained by Asha *et al*., they concluded that; there was a significant difference (p=0.001) in Ki-67 L1 expression between HGSC (65.34%) as compared to the LGSC. (37.96%) (Asha *et al*., 2017)

The Ki-67 immunostaining pattern has been detected as focal and heterogeneous in more number of low grade neoplasms as compared to diffuse pattern in higher grade. (Mita *et al*., 2004; Aune *et al*., 2011; Giurgea *et al*., 2012; Asha *et al*., 2017)

The FIGO system has been identified as independent prognostic factor with higher stage reflecting more aggressive state due to change in tumor biology. (Aune *et al*., 2011; Ezzati *et al*., 2014) Khouja *et al*., found a correlation between high Ki-67expression with higher grade, poor differentiation, ascitis, the presence of residual disease after primary surgery and advanced FIGO stage. (Khouja *et al*., 2007) In Asha *et al*., study, high Ki-67 L1 was noted with advanced FIGO stage (stage III-70.6%) which was statistically significant (p> 0.001). (Asha *et al*., 2017)

Munstedt *et al*., found a significant correlation between Ki-67 determined tumor growth fraction and incidence of tumor recurrence ($p > 0.001$) in early stage ovarian carcinoma. (Munstedt *et al*., 2004) Several studies which have examined the relation between Ki-67 antigen expression and long term survival have reported that; the PI is a good predictor of patient outcome in epithelial ovarian cancer. The median survival of patients whose carcinoma had a high Ki-67 expression was lower compared to patients whose tumors demonstrated low Ki-67 expression. (Garzetti *et al*., 1995, Kritpracha *et al*., 2005, Liu *et al*., 2012, Heeran *et al*., 2013)

The assessment of the PI by Ki-67 L1 determines the proliferative potential of SEOC in diagnosing the high grade, HGSC and advanced stage along with routine histopathological report as aggressive tumors. This helps in prognosis and therapy, need of tailoring chemotherapy and longer survival rate. Even patient with early stage ovarian carcinomas with high Ki-67 L1 are likely to benefit from adjuvant chemotherapy despite of the tumor grade and type. (Kaern *et al*., 2005; Khandakar *et al*., 2014) The exclusive study on clear cell adenocarcinomas indicates the survival rate of the patients with high Ki-67 antigen expression was significantly greater than for low Ki-67 antigen expression and suggested low proliferation activity may contribute to chemoresistance. (Itamochi *et al*., 2002)

Conventional treatment for SEOC consists of surgical removal of tumor, followed by Platinum/Taxane based chemotherapy. (Sassen *et al*., 2007)

Currently, sandwich therapy is preferred for advanced stage disease of the FIGO staging system that is, NACT with interval debulking surgery and post-surgery chemotherapy. (Sassen *et al*., 2007; Miller *et al*., 2008) Studies by Miller *et al*., and Khandakar *et al*., found a decrease in Ki-67 L1 subsequent to NACT and significant differences in the tumor

histomorphology as compared to the indigenous neoplasms with better survival outcome. (Miller *et al*., 2008; Khandakar *et al*., 2014)

Recent studies suggest that; Ki-67 is potentially an attractive therapeutic target in cancer due to the ubiquitous expression in all proliferating cells. Inactivation of the proliferation marker Ki-67 will lead to cell death specifically in proliferating cells and thus could be a potential strategy for the treatment not only of ovarian cancer but also of numerous other malignancies. (Rahmaanzadeh *et al*., 2010; Li *et al*., 2015)

2.4. Epidemiology

According to the National Cancer Institute (NCI), in 2015 there were an estimated 21,290 new cases of ovarian tumor and 14,180 deaths from the disease. The vast majorities of the cases are EOC and are found at stage 3 or later, meaning the cancer has spread beyond the pelvis or to the lymph nodes. This is mostly due to the lack of definite symptoms at the early stages of cancer growth. Around 1.3% of women will be diagnosed with cancer of the ovary at some point in life, thus it is relatively rare. The median age of diagnosis is 63. However, approximately 25% of cases are diagnosed between ages 35 and 54. Caucasian women have the highest rate of diagnosis. (National Cancer Institute, 2015) In 2016, an estimated 1,685,210 new cases of cancer will be diagnosed in the United States and 595,690 people will die from the disease. The most common cancers in 2016 are projected to be breast cancer, lung and bronchus cancer, prostate cancer, colon and rectum cancer, bladder cancer, melanoma of the skin, non-Hodgkin lymphoma, thyroid cancer, kidney and renal pelvis cancer, leukemia, endometrial cancer, and pancreatic cancer. (National Cancer Institute, 2016)

Like many other cancers, when ovarian cancer is found at an early stage (for example, localized to the ovary or fallopian tube) the survival at 5 years is very good (about 92%); most women at stage 1 will still be alive at 5 years. However, the 5-year survival for all women diagnosed with ovarian cancer is only 45%. This is because it is often found at an advanced stage in which the disease has already spread within the abdomen. Survival is also dependent on the type of care the patient receives. Women suspected of having ovarian cancer should be referred to a gynecologic oncologist. These are physicians with special training in gynecologic (ovarian, uterine, cervical, vulvar, and vaginal) cancers. If a woman does not involve a doctor with this specialized training in her care, then studies show that her survival is significantly worse, often by many years. For this reason, every woman with this disease ideally will obtain a referral to a gynecologic oncologist before she starts any treatment or has any surgery. (American Cancer Society, 2015)

A total of 224,747 new cases of ovarian cancer were reported worldwide in 2008, with 99,521 cases being diagnosed in more developed regions, and 125,226 being diagnosed in less developed regions. Ovarian cancer was the seventh most common cancer diagnosis among women in the world overall, and fifth most common cancer diagnosis among women in more developed regions. The world rate is estimated to be 6.3 per 100,000, and is higher in developed countries and regions (9.3) compared to others. (Ferlay *et al*., 2010) Incidence rates for selected region. Rates range from 3.8 in the Southern and Western African regions to 11.8 in the region of Northern Europe. Continental rates are highest in Europe (10.1), followed by North America (8.7), Australia (including New Zealand, 7.8), South America (6.2), Asia (5.1), and Africa (4.2). (Ferlay *et al*., 2010)

In the United States, 20,749 ovarian cases were diagnosed in 2007 (the most recent year for which data are available), for an incidence rate of 12.2 per 100,000 women. (National Cancer Institute, 2105) Koper *et al*. reported a rate of 14.9 in the Netherlands, similar to that found in the United States. (Koper *et al*., 1996) In Alexandria, Egypt, the rate was 3.16. (Mahdy *et al*., 1999) An Italian network of cancer registries reported 7,690 cases of ovarian cancer from 1986 through 1997. (Zambon *et al*., 2004) Few countries publish trends in ovarian cancer incidence over time. This may be due to differing methods of data collection and data quality issues, especially for countries that do not have a national registry. In the United States, a recent report estimates that ovarian cancer incidence has been decreasing since 1998, with a significant decline of 2.3% per year from 2003-2007. (Kohler *et al*., 2011) The reasons for this decrease are unclear, but are likely not artefactual due to the long-standing high-quality data available for the United States. A Japanese analysis based on data from several regional cancer registries reported a 1.5 fold increase in ovarian cancer rates from 1975 to 1993. (Tamakoshi *et al*., 2001) The Chinese Shanghai Cancer Registry also reported an increase in ovarian cancer incidence from 1979-1989. Some of these increases may be due to increases in population coverage or completeness of data within the registry; however, the Chinese increase is thought to be a birth cohort effect in women born between1925-1935. (Jin *et al*., 1993) studies in Egypt, (Dey *et al*., 2010) and Italy, (Minelli *et al*., 2007) have found ovarian cancer rates to be higher in urban compared to rural areas. Globally, a lack of reliable screening modalities has restricted the opportunities for early diagnosis and cancer detection, leading to a significant proportion of women worldwide presenting at an advanced stage of the disease. Due to this late presentation, available treatments are

ineffective, and the majority of patients relapse following treatment-induced regression. (Holschneider *et al*., 2000) Furthermore, there is substantial geographic variation in the incidence of ovarian cancer and mortality, with higher incidence observed in developed countries (9.4 per 100,000 women) compared with women living in the developing world (5.0 per 100,000 women). (Jemal A *et al*., 2011)

2.5. Ovarian tumors in Sudan

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 (Mohammed et al., 2000–2006). 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. (Saeed et al., 2009–2010) ...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. (Saeed *et al*., 2014)

2.6. Previous studies

Several studies which have examined the relation between Ki-67 antigen expression and long term survival have reported that; the PI is a good predictor of patient outcome in epithelial ovarian cancer. The median survival of patients whose carcinoma had a high Ki-67 expression was lower compared to patients whose tumors demonstrated low Ki-67 expression. (Garzetti *et al*., 1995; Kritpracha *et al*., 2005; Liu *et al*., 2012; Heeran *et al*., 2013) The assessment of the PI by Ki-67 L1 determines the proliferative potential of Squamous Epithelial Ovarian Cance in diagnosing the high grade, HGSC and advanced stage along with routine histopathological report as aggressive tumors. This helps in prognosis and therapy, need of tailoring chemotherapy and longer survival rate. Even patient with early stage ovarian carcinoms with high Ki-67 L1 are likely to benefit from aduvant chemotherapy despite of the tumor grade and type. (Kaern*et al*., 2005; Khandakar *et al*., 2014)

The exclusive study on clear cell adenocarcinomas indicates the survival rate of the patients with high Ki-67 antigen expression was significantly greater than for low Ki-67 antigen expression and suggested low proliferation activity may contribute to chemoresistance. (Itamochi *et al*., 2002)

Recent studies suggest that; Ki-67 is potentially an attractive therapeutic target in cancer due to the ubiquitous expression in all proliferating cells. Inactivation of the proliferation marker Ki-67 will lead to cell death specifically in proliferating cells and thus could be a potential strategy for the treatment not only of ovarian cancer but also of numerous other malignancies. (Rahmaanzadeh *et al*., 2010; Li *et al*., 2015)

2.7. Immunohistochemistry

Immunohistochemistry (IHC) is a technique for identifying cellular or tissue constituents (antigens) by means of antigen-antibody interactions, the site of antibody binding being identified either by direct labeling of the antibody or by use a secondary labeling method. The recent introduction of prognostic and predictive markers in IHC has made a tremendous impact on patient treatment and management. Immunohistochemistry is widely employed in establishing diagnosis, predicting prognosis and response to therapy and in the study of disease pathogenesis. (Bancroft and Gamble, 2008)

CHAPTER THREE

MATERIALS

&

METHODS

3.1. Study design

This was descriptive cross-sectional conducted to study the immunohistochemical expression of Ki-67 in ovarian tumors among Sudanese patients.

3.2. Study duration

This study was carried out during the period from September 2017 to May 2018.

3.3. Study area

The current study was conducted at Elrahma Medical Centre- Khartoum north- Sudan.

3.4. Study populations, samples and samples size

Populations subjected in this study were Formalin Fixed Paraffin Embedded (FFPE) tissue blocks previously diagnosed with ovarian tumors as case group. Sample size recruited in this study was forty samples with ovarian tumors.

3.5. Sampling technique

Convenient sampling technique was used to collect samples in this study.

3.6. Data collection tools and variables

Master sheets were used to record all patients and samples data. Master sheets were also used to record all IHC results. Ki-67 was detected using IHC method.

3.7. Quality control

Positive and negative control sections were used to evaluate the working solutions and to evaluate the testing slides. All precautions and quality approaches were issued as manufacture instructions.

3.8. Sample processing

One section from each block measured four microns was cut using Leica microtome (Leica Micro systems, RM 2125 RT, serial NO. 8843/04-2005-China) and then stained in Haematoxylin & Eosin (H & E) to confirm diagnosis of each block and to select the tumor area for preceding tissue microarray (TMA). All FFPE tissues were assembled in one TMA block using conventional mechanical pencil tips; a hollow needle was used to remove tissue cores as small as 1mm in diameter from regions of interest in FFPE tissue samples. These tissue cores were then inserted in a recipient paraffin block in a precisely spaced array pattern (1 mm), then the array block was covered by microscopic glass slide, after that introduced into dry oven at 37 ^{0}C until the block was warmed, then the slide was rotated over the cores, then let to cool at room temperature (RT), then incubated in the refrigerator freezer for complete solidification, then the TMA block was ready for sectioning.

One section from TMA block measured four microns was cut using the same type of microtome and then floated consecutively in 70% ethanol and water bath (Electrothermal ser NO.18861434-China) at 40c^{0}. The floated section was mounted on positive charge immune slide (Thermo Scientific- Italy) to detect immune expression of Ki-67. Then the slide contained section was dried in dry oven (WTC binder 7200 TUTTLINGEN, B28, NO.88485-USA) at 60c^{0} for 30 minutes.

3.9. Methods of detection

3.9.1. H & E staining method

Section of 4 micron thickness was stained using haematoxylin and eosin (Mayer's technique). Each section was dewaxed in hot plate oven and cleared in two changes of xylene for two minutes, then hydrated through ethanol (100%, 90%, 70%, 50%) and water two minutes for each, then stained in Mayer's haematoxylin for 7 minutes, then washed and blued in running tap water for ten minutes, then stained in eosin for three minutes, then washed in distilled water (D.W)... and hydrated through ascending grades of ethanol, cleared in xylene and mounted in Disterene, a plasticizer (polystyrene) and xylene (DPX).

3.9.2 Method of immunohistochemistry (IHC) technique

The immunohistochemical procedure was done as followed; following deparaffinization in xylene, slide was rehydrated through a graded series of alcohol and placed in running water. Then slide was steamed for antigen retrieval for Ki-67 using water bath in coplin jar containing sodium citrate buffer (pH 9.0). Endogenous peroxidase activity was blocked with 3% hydrogen peroxidase and methanol for 10 minutes, and then slide was incubated with 100-200 µl of primary monoclonal antibody for Ki-67 for 20 min at RT, then rinse in phosphate buffer saline (PBS) for 3 min. Then binding of antibody site was detected by incubating slide for 20 minutes with dextran labeled polymer. Finally, the sections was washed in three changes of PBS, followed by applying (DAB) as a chromogen to produce the characteristic brown stain for up to 5 min. Slide was then counter stained with haematoxylin for 5 minutes.

3.10 Interpretation of immunostaining slides

Negative staining achieved when no crisp brown staining were observed in the nucleus and/or cytoplasm of the target malignant cells (score is 0 and over expression assessment is negative). Observed nucleus and/or cytoplasm less than 10% of tumor cells regarded also as negative (score is 0 and over expression assessment is negative). A faint/barely perceptible nucleus and/or cytoplasm staining is detected in more than10% of tumor cells and incomplete staining regarded also as negative (score is 1+ and over expression assessment is negative). A weak to moderate complete nucleus and/or cytoplasm staining is observed in more than 10 % of tumor cells regarded as weakly positive (score is 2+ and over expression is weakly positive). A strong complete nucleus and/or cytoplasm staining is observed in more than 10% of tumor cells regarded as positive (score is 3+ and over expression assessment is positive). (Rajeev *et al*., 2011)

3.11 Data analysis and presentation

All obtained results were analyzed by Statistical Package for the Social Sciences (SPSS) version 22.0, with Pearson's chi-square test used to assess intergroup significance. Other variables, frequencies, mean values were calculated and presented in form of figures and tables. The p value equal or <0.05 was considered statically significant.

3.12 Ethical consideration

This study was approved by the board of Medical Laboratory Sciences (MLS) at Shendi University. A written agreement was a signed prior to sample collection with each hospital and laboratory administration. Also permission for this study was obtained from the local authorities in the area

of the study. The aims and the benefits of this study were explained well with assurance on confidentiality.

CHAPTER FOUR

RESULTS

4. 1Results

A total of 40 cases (patients with histopathologically confirmed ovarian tumors) were included in this study. The age of patients was ranged from 32-65 years with average mean of 49.9 years. The ages were divided into two age groups the first category of less than or equal to 45 years old (younger adult), this age group was represented 13 cases (32.5%) the second age group was older than 45 years old (menopausal), and this age group was represented 27 cases (67.5%) as indicated in figure 4.1.

Figure 4.2 summarizes the frequency of types of tumors among patients with ovarian tumors; adenocarcinoma was most frequent type was found in 29/40 cases (72.5%), followed by granulosa cell tumor 8/40 (20%), followed by immature teratoma, transitional cell carcinoma, mesenchymal cell tumor 1/40 (2.5%) respectively.

Figure 4.3 demonstrates the frequency of tumor grade. Of the 40 cases, grade II was observed in 21/40 (52.5%) followed by grade 1 and grade III constituting 14/40 (35%) 5/40 (12.5%) consecutively.

Figure 4.4 demonstrates the frequency of marker expression. Of the 40 cases 30 cases (75%) were positive and 10 cases (25%) were negative.

Figure 4.5 demonstrates the frequency of marker score expression. Of 40 cases, score I (mild expression) was recorded 24/40 (60%), followed by score zero (negative expression) 10/40 (25%), followed by score II (moderate expression) 4/40 (10%) and score III (high expression) 2/40 (5 %) respectively.

Table 4.1 illustrates the association of tumor types with marker positive expression. Of the 30 positive samples for Ki-67, adenocarinoma was the most commn type that showed positive expression 23/30 cases (76.7%), followed by granulosa cell tumors 5/30 (16.7%), followed by imaturte

teratoma 1/30 (3.3%), transitional cell cancer 1/30 (3.3%) and mesenchymal cell tumor 0.00/40 (0.00%), the p value was 0.327.

Table 4.2 illustrates the comparison between tumor grade and Ki-67 positive expression. Grade II was most positive grade expression 17/30 (56.7%) followed by grade I 10/30 (33.3%) and grade III 3/30 (10%), the p value was 0.579.

Table 4.3 illustrates the correlation of patients age with Ki-67 expression. Out of 40 cases, the age group up to 45 years (13 cases), 11/40 (27.5%) samples were showed positive expression, the remainder 2 samples (5%) were negative. Regarding the other age group of above than 45 years old (27 cases), 19/40 (47.5%), the remainder 8 samples (20%) were negative, the p value was 0.330.

Table 4.4 shows the association between Ki-67 and tumor grade. Out of 40 samples, score I was present in 24 (60%) samples as followed;

Eight samples out of 14 samples of grade I [8/14=57.1%], 13 samples out of 21 samples grade II [13/21=61.9%], 3 samples out of 5 samples of grade III [3/5=60%].

Score II was present in 4 (10%) out of 40 samples as followed;

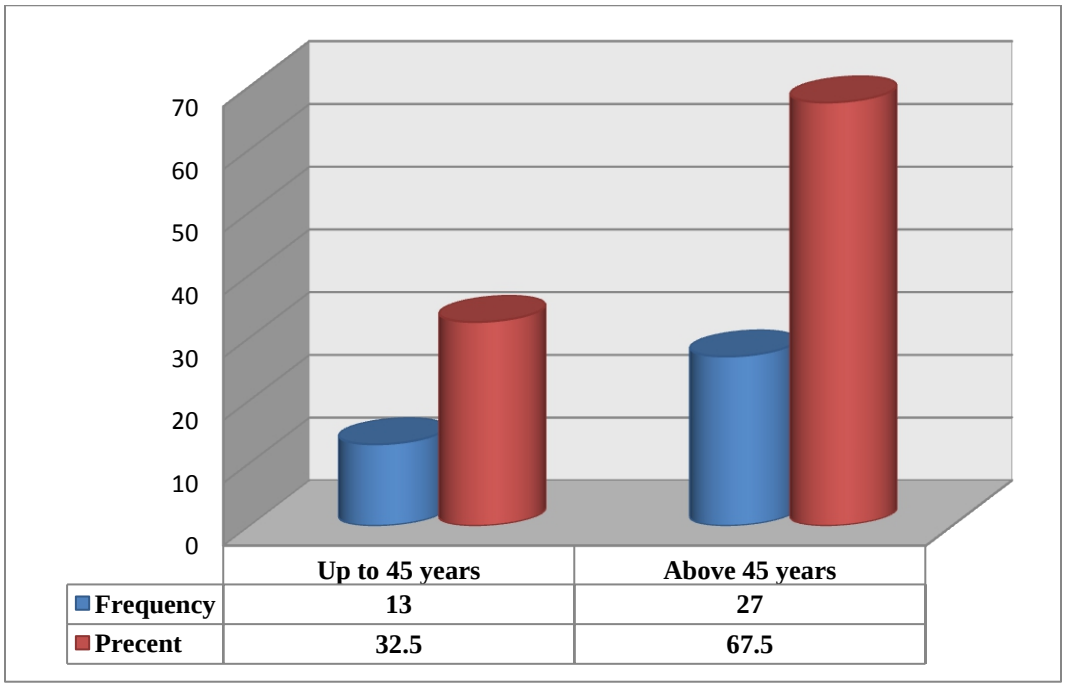
Only one sample out of 14 samples of grade 1 [1/14=7.1%], 4 samples out of 21 samples grade II [4/21= 19%], zero- sample out of 5 samples of grade III [0.000/5=0.00%).

Score III was present in 2 (5%) out of 40 samples as followed;

Only one sample out of 14 samples of grade I [1/14=7.1%], 1 sample out of 21samples of grade II [1/21=4.8%], zero sample out of 5 samples of grade III [0.00/5=0.00%].

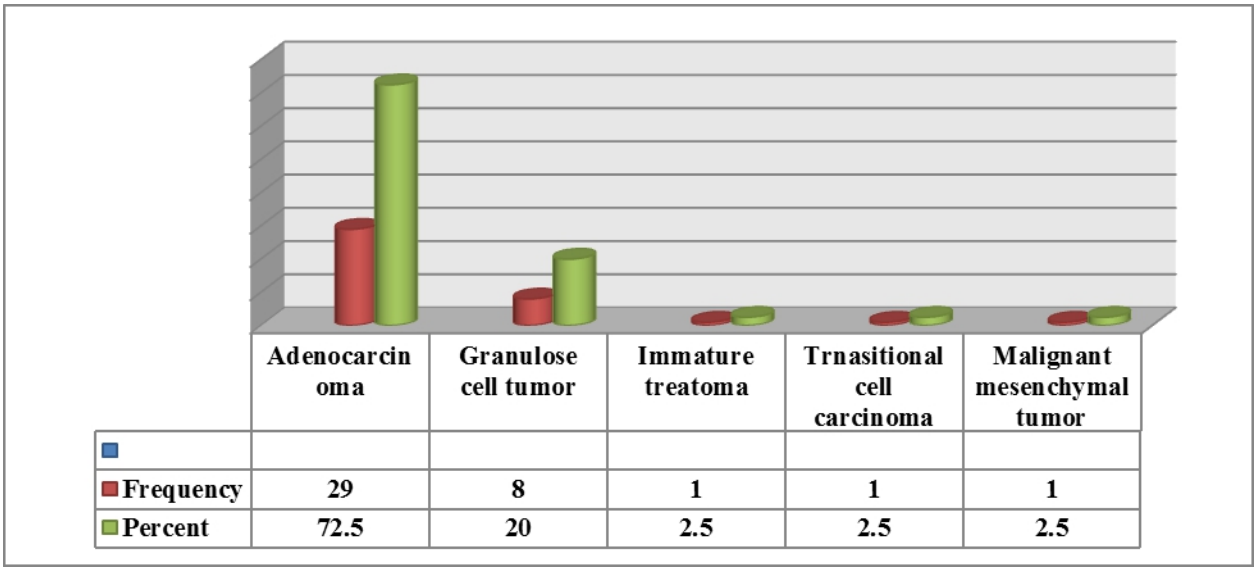
Score zero was present in 10 samples (25%) out of 40 samples as followed;

Four samples out of 14 samples of grade I [4/14=28.6%], 3 samples out of 21samples of grade II [3/21=14.3%], 2 samples out of 5 samples of grade III [2/5=40%], the p value was 0.728.



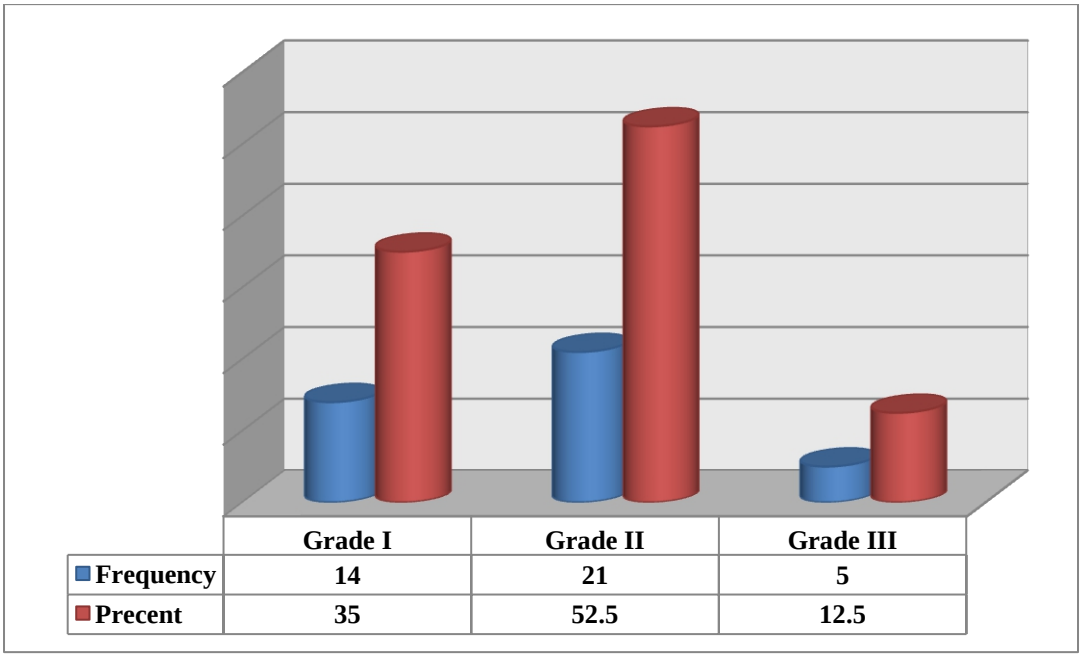
	Up to 45 years	Above 45 years
Frequency	13	27
Precent	32.5	67.5

Figure (4.1): Shows frequency of age group among study populations.



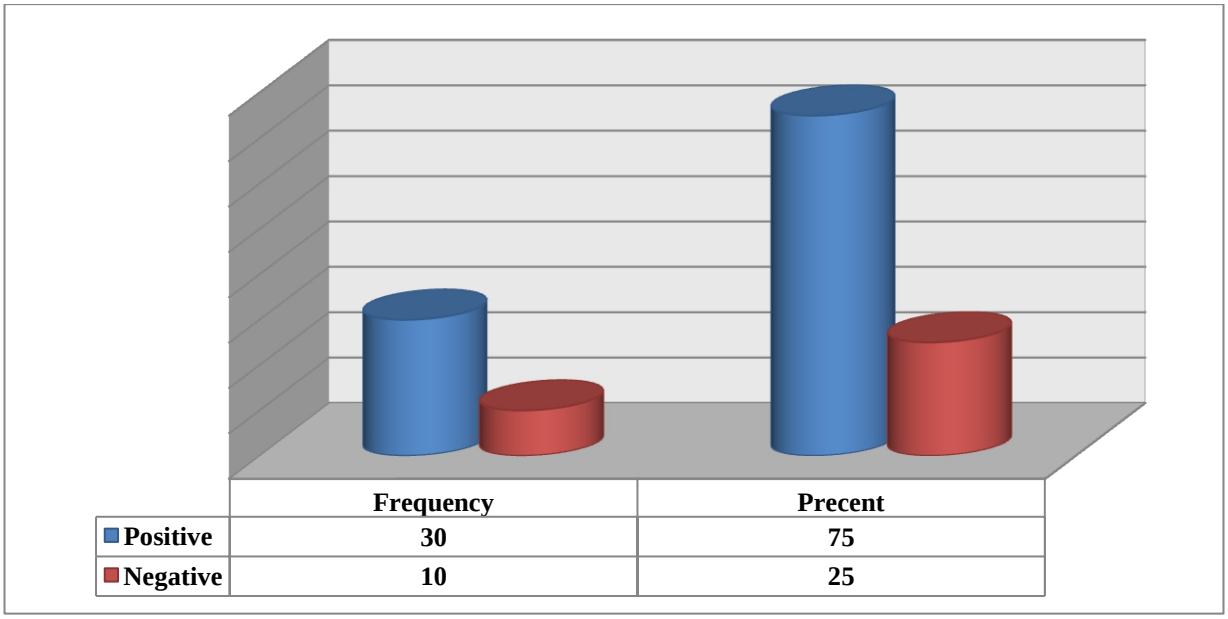
	Adenocarcinoma	Granulose cell tumor	Immature treatoma	Trnasitional cell carcinoma	Malignant mesenchymal tumor
Frequency	29	8	1	1	1
Percent	72.5	20	2.5	2.5	2.5

Figure (4.2): Shows frequency of tumor types among study samples.



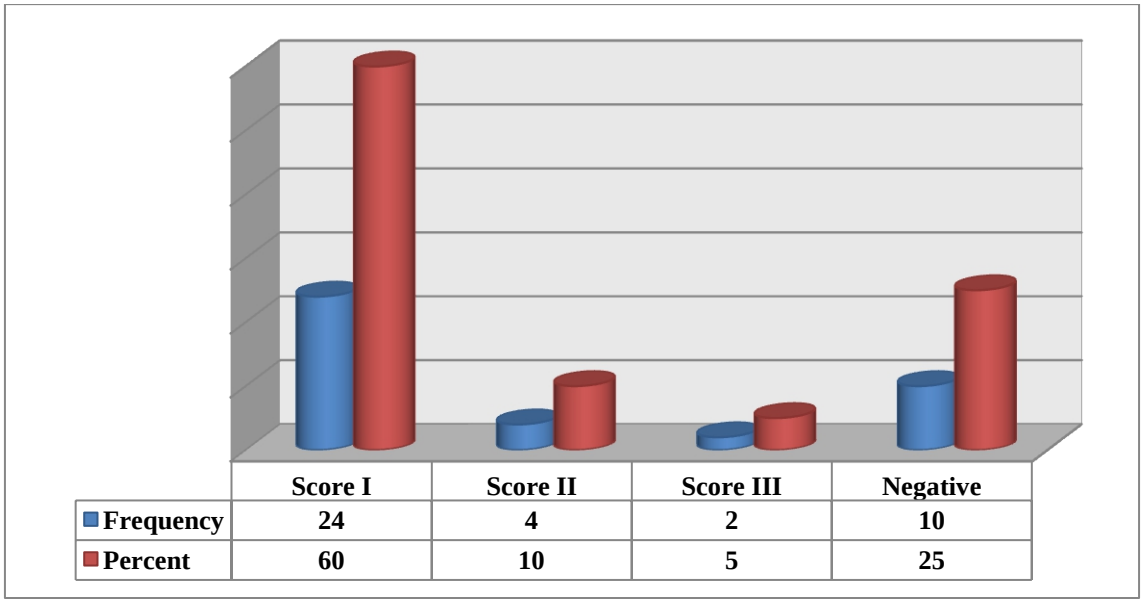
	Grade I	Grade II	Grade III
Frequency	14	21	5
Precent	35	52.5	12.5

Figure 3: Shows frequency of tumor grades among study samples.



	Frequency	Precent
Positive	30	75
Negative	10	25

Figure 4: Shows frequency of Ki-67 expression among study samples.



	Score I	Score II	Score III	Negative
Frequency	24	4	2	10
Percent	60	10	5	25

Figure 5: Shows frequency of Ki-67 expression per scores among study samples.

Table 4.1: Correlation of Ki-67 expression with tumor types.

	Ki-67 expression			p. value
Tumor types	**Positive**	**Negative**	**Total**	
Adenocarcinoma	23	6	29	
Granulose cell tumor	5	3	8	
Immature teratoma	1	0	1	
Transitional cell carcinoma	1	0	1	
Malignant mesenchymal tumor	0	1	1	
Total	30	10	40	0.327.
N=40				

Table 4.2: Correlation of Ki-67 expression with tumor grades.

	Ki-67 expression			p. value
Tumor grade	**Positive**	**Negative**	**Total**	
Grade I	10	4	14	
Grade II	17	4	21	
Grade III	3	2	4	
Total	30	10	40	0.579
N=40				

Table 4.3: Correlation of Ki-67 expression with patient's age.

	Marker expression			p. value
Patient age	**Positive**	**Negative**	**Total**	
Up to 45 years	11	2	13	
Above 45 years	19	8	27	
Total	30	`10	40	0.330.
N=40				

Table 4.4: Correlation of Ki-67 expression score with tumor grades.

	Tumors grade				p. value
Ki-67 expression score	**Grade I**	**Grade II**	**Grade III**	**Total**	
Score I	8	13	3	24	
Score II	1	3	0	4	
Score III	1	1	0	2	
Score zero	4	4	2	10	
Total	14	21	5	40	
N=40					0.728

CHAPTER FIVE

DISCUSSION, CONCLUSION & RECOMMENDATIONS

5.1. Discussion

Ovarian cancer is the third most common malignancy among women accounting for 5.5% of all cancers (Gupta *et al*., 2003). About 80% of all ovarian tumors are benign and occur mostly in young women between 25 and 40 years of age (Crum, 2004). In the present study, the age of patients with ovarian cancers was between 32-65 years old; this finding was similar to that study of the Romanian authors (Luminta *et al*., 2012). 40 cases of ovarian tumors were distributed into 2 groups; the first age group was included samples from patients with years old up to 45 years old, the another age group contained samples from patients above 45 years old (this age division was done depending on the WHO menopausal ages (pre and post-menopausal respectively).

Regarding association between incidence of ovarian cancer and the age of patients at presentation; our study summarized that; the ovarian tumors occurs more frequently in females above than 45 years old, this result is consistent with the outcome of the study conducted by (Pooja, 2015), which concluded that; the peak incidence for benign tumors was in the third decade of life while for malignant tumors it was the fifth and sixth decades.

In this study, the Ki-67 positive immunoexpression was predominant over negative expression, this result is near to the outcome of that study conducted by (Luminta *et al*., 2015), which concluded that; the Ki-67 was positive expression in 61.5% in ovarian tumors.

In the present study there was no statistically significant different between ki-67 expression and subtypes of ovarian tumor, as the p value was above 0.05, there was no published data that correlated Ki-67 with the subtypes of ovarian tumor, the outcome of the Indian study conducted by (Kumudini, 2018), concluded that; the present of significant different of ki-67 positivity

was observed between malignant, borderline and benign ovarian tumors, the difference between our study and Indian study in that; our study concentrated only on malignant ovarian tissues.

In the present study there was no statistically significant different between ki-67 positivity with tumor grade, as the p value was above 0.05, this result is inconsistent with the outcome of the study conducted by (Asha, 2017), which concluded that; the present of significant different of ki67 positivity and tumor grade (p. value 0.001), this difference in the results may be due to variability in the grade of samples as the commonest grade in the current study samples was grade II, followed by grade I and III respectively, also may be due to variability in the sample number of each grade, also small sample size in our study may play a role in this variability.

In the present study we found there was no relation between score of Ki-67 expression and tumor grade, this results do not agree with the results in Indian study obtained by Monisha *et al*., in 2011, which found that; the Ki-67 index was higher in advanced stage tumors; and also concluded that; a higher Ki-67 index points toward the aggressive clinical behavior. This difference in the results may be due to variability in the grade of samples, also may be due to variability in the sample number of each grade, also small sample size in our study may play a role in this variability.

5.2. Conclusion

On the base of the obtained results we concluded that;

- The incidence rate of ovarian tumors was more present in the post-menopausal women.
- Adenocarcinoma was the most frequent type of ovarian tumors.
- Ki-67 expression invalid to differentiate between different types of ovarian tumors.
- Ki-67 not reliable marker in grading ovarian tumors.
- Ki-67 expression score improper way to grade ovarian tumors.

References

- American Cancer Society, (2015). https:// www. ncbi.nlm.nih.gov/pubmed/ 2032235.
- Aune D.; Navarro Rosenblatt D.A.; Chan D.S.; Abar L.; Vingeliene S.; Vieira A.R.; Greenwood D.C.; Norat T.; et al., (2015). Anthropometric factors and ovarian cancer risk: a systematic review and nonlinear dose-response meta-analysis of prospective studies. Int J Cancer. 136(8):1888-98.
- Asha M.; Shruthi K.; and Manjunath V., (2017). Diagnostic and Prognostic Significance of Ki-67 Immunohistochemical Expression in Surface Epithelial Ovarian Carcinoma. J Clin Diagn. 11(2): EC08–EC12.
- Bancroft J.D.; Gamble M.,(2008). Theory and Practice of histological Techniques. Churkhil Livinstone London UK. 6:433-469.
- Bast R.C.; Klug T.L.; Schaetzl E.; Lavin P.; Niloff J.M.; Greber T.F.; Zurawski V.R .; Knapp R.C.,(1984). Monitoring human ovarian carcinoma with a combination of CA 125, CA 19-9, and carcinoembryonic antigen. Am J Obstet Gynecol. 149(5):553-9.
- Battista M.J.; Mantai N.1.; Sicking I.; Cotarelo C.; Weyer V.; Lebrecht A.; Solbach C.; Schmidt M.,(2014). Ki-67 as an independent prognostic factor in an unselected cohort of patients with ovarian cancer: results of an explorative, retrospective study. Oncol. 31(5):2213-9.
- Binny Kh.; Sandeep R.; Mathur Lalit Kumar; Sunesh Kumar; Siddhartha Datta Gupt; Venkateswaran K.; Iyer and M. Kalaivani.,(2014).Tissue Biomarkers in Prognostication of Serous Ovarian Cancer following Neoadjuvant Chemotherapy. BioMed Research International. Article ID 401245: 9.

5.3. Recommendations

On the base of the obtained results we recommended that;

- ❖ Further study should be conducted in ovarian tumors using larger sample size.
- ❖ For future study, advance techniques like polymerase chain reaction (PCR) and In situ hybridization (ISH) should be conducted to detect the exact role of Ki-67 in ovarian cancer.
- ❖ Further study should include normal, benign lesions and malignant lesions of ovary.

- Brown D.C.; Gatter K.C.,(2002). Ki67 protein: the immaculate deception. Histopathology. 40(1):2-11.
- B.W. Stewart; P. Kleihues.,(2003). World Cancer Report. IARC Publications.
- Chen L.,(2014). Epithelial carcinoma of the ovar., fallopian tube, and peritoneum: Clinical features and diagnosis. http://www.uptodate.com/home. Accessed Feb. 18.
- Choudhury M.; Goyal S.; Pujani M., (2011). A cytohistological study of Ki-67 expression in ovarian tumors. Indian J Pathol Microbiol. 54:21-4.
- Desai A.; Xu J.; Aysola K.; Qin Y.; Okoli C.; Hariprasad R; Chinemerem U.; Gates C.; Reddy A.; Danner O.; Franklin G.; Ngozi A.; Cantuaria G.; Singh K.; Grizzle W.; Landen C.; Partridge E.E.; Rice V.M.; Reddy E.S.; Rao V.N., (2014). Epithelial ovarian cancer: An overview. World J Transl Med. 3(1):1-8.
- Dey A.; Hablas I.A.; Seifeldic K.; Ismail M.; Ramadan H.; El-Hamzawyc M.L.; Wilsona M.; Banerjee P.; Boffetta J.; Harford S.D.; Merajver; and A.S. Solimana.,(2010). Urban–rural differences of gynaecological malignancies in Egypt (1999–2002). BJOG. 117(3): 348–355.
- Ezatti M.; Abdulah A.; Shariffabrizi A.; Hou J.; Kopf M.; Stedman JK., (2014). Recent Advancements in Prognostic Factors of Epithelial Ovarian Carcinoma. International Scholalrly Research Notices. 2014:953509, 10 pages.
- Fariba Salehi ; Lesley Dunfield ; Karen P. Phillips ; Daniel Krewski & Barbara C. Vanderhyden ., (2008).Pages 301-321 | Published online.
- Feng L.P.; Chen HL.; Shen M.Y., (2014). Breastfeeding and the risk of ovarian cancer: a meta-analysis. J Midwifery Women's Health. 59(4):428-37. doi: 10.1111/jmwh.12085.

- Ferlay J.; Shin H.R.; Bray F.; Forman D.; Mathers C.;Parkin D.M., (2010). Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer. 127(12):2893-917.
- Garzetti G.G.; Ciavattini A.; Goteri G.; De Nictolis M.; Stramazzotti D.; Lucarini G.; et al., (1995). Ki-67 antigen immunostaining (MIB-1 monoclonal antibody) in serous ovarian tumours: index of proliferative activity with prognostic signif-icance. Gynecol Oncol. 56:169–74.
- Gerdes J.1.; L.i.;Schlueter C.; Duchrow M.; Wohlenberg C.; Gerlach C.; Stahmer I.; Kloth S.; Brandt E., Flad H.D.,(1991). Immunobiochemical and molecular biologic characterization of the cell proliferation-associated nuclear antigen that is defined by monoclonal antibody Ki-67. Am J Pathol. 138(4):867-73.
- Giurge L.N.; Ungureanu C.; Mihailovici M.S., (2012). The immunohistochemical expression of p53 and Ki67 in ovarian epithelial borderline tumors. Correlation with clinicopathological factors. Rom J MorpholEmbryol. 53(4):967-73.
- Green, Andrew, *et al*., (2013). Ovarian cancer. Medscape.
- Gupta M.C; Mahajan B.K., (2003). Epidemiology of non-communicable diseases. New Delhi: Jaypee Brothers. 3:306-28.
- Gursan N.; Sipal S.; Calik M.; Gundogdu C., (2009). p53, Bcl2, Ki-67 LI (Labeling index) Status in Benign, Proliferative, And Malignant Ovarian Surface Epithelial Neoplasms. The Eurasian Journal of Medicine. 41(41):10-14.
- Havrilesky L.J.; Moorman P.G.; Lowery W.J.; Gierisch J.M.; Coeytaux R.R.; Urrutia R.P.; Dinan M.; McBroom A.J.; Hasselblad V.; Sanders

G.D.; Myers E.R., (2013). Oral contraceptive pills as primary prevention for ovarian cancer: a systematic review and meta-analysis. Obstet Gynecol.

- Heeran M.C.; Hogdal C.K.; Kjaer S.K.; Christensen L.; Jensen A.; Blaaker J.; et al., (2013). Prognostic Value of Tissue Protein Expression Levels of MIB-1(Ki-67) in Danish Ovarian Cancer Patients. From the MALOVA Ovarian Cancer Study. APMIS. 121(12):1177-86.
- Henriksen R.; Strang P.; Backstrom T.; Wilander E.; Tribukait B.; Oberg K., (1994). Ki-67 immunostaining and DNA flow cytometry as prognostic factors in epithelial ovarian cancers. Anticancer Res. 14(2B):603–08.
- Hiroaki Itamochi; Junzo Kigawa; Naoki Terakawa,(2008). Mechanisms of chemoresistance and poor prognosis in ovarian clear cell carcinoma.
- Holschneider C.H.; Berek J.S., (2000). Ovarian cancer: epidemiology, biology, and prognostic factors. Semin Surg Oncol. 19(1):3-10.
- Intisar E.; Saeed Hsin-Yi.; Weng; Kamal H.; Mohamed and Sulma I.; Mohammed.,(2010), Cancer incidence in Khartoum, Sudan: first results from the Cancer Registry, 2009–2010. Cancer Med. 2014 Aug; 3(4): 1075–1084.
- Itamochi H.; Kigawa J.; Sugiyama T.; Kikuchi Y.; Suzuki M.; Terakawa N.,(2002). Low proliferation activity may be associated with chemoresistance in clear cell carcinoma of the ovary. Obset Gynecol. 100:281-87.
- Jin F.; Shu X.O.; Devesa S.S.; Zheng W.; Blot, W. J. &Gao Y.T., (1993). Incidence trends for cancers of the breast, ovary, and corpus uteri in urban Shanghai, 1972-89. Cancer Causes Control., Vol 4, No. 4(July 1993), pp. (355-360).

- Jemal A.; Tiwari R.C.; Murray T.; Ghafoor A.; Samuels A.; Ward E.; Feuer EJ.; Thun MJ.; Cancer statistics., (2004). CA Cancer J Clin. 2004 Jan-Feb;54(1):8-29.
- Jemal A.; Siegel R.; Ward E.; Murray T.; Xu J.; Thun MJ., (2007). Cancer statistics. CA: A Cancer Journal for Clinicians. 57(1):43–66.
- Jemal A.; Bray F.; Center, MM.; Ferlay J.; Ward E.; Forman D., (2011). Global cancer statistics. CA Cancer J Clin.; 61:69–90. doi: 10.3322/caac.
- Jin F.; Shu X.O.; Devesa S.S.; Zheng W.; Blot W. J. & Gao Y.T., (1993). Incidence trends for cancers of the breast, ovary, and corpus uteri in urban Shanghai, 1972-89. Cancer Causes Control., Vol 4, No. 4, pp. (355-360)
- Kaern M.; Aghmesheh M., Nesland JM.; Danielsen H.E.; Sandstad B.; Friedlander M., et al., (2005). Prognostic factors in ovarian carcinoma stage III patients. Can biomarkers improve the prediction of short- and long-term survivors? International Journal of Gynecological Cancer. 15(6):1014–22.
- Keam B.; Im S-A, Lee K-H, et al., (2011). Ki-67 can be used forfurther classification of triple negative breast cancer intotwo subtypes with different response and prognosis. BreastCancer Res, 13, 22.
- Khandakar B.; Sandeep R.; Mathur, Kumar L.; Kumar S.; Gupta SD., et al., (2014). Tissue Biomarkers in Prognostication of Serous Ovarian Cancer Following Neoadjuvant Chemotherapy. BioMed Research International. 2014: 401245, 9 pages.
- Khouja M.H; Baekelandt M; Nesland J.M.; Holm R., (2007).The clinical importance of Ki-67, p16, p14, and p57 expression in patients with advanced ovarian carcinoma. Int J Gynecol Pathol. 26(4):418-25.

- Köbel M.; Kalloger S.E.; Boyd N.; McKinney S.; Mehl E. ; Palmer C.; Leung S.; Bowen N.J.; Ionescu DN.; Rajput A.; Prentice L.M.; Miller D.; Santos J.; Swenerton K.; Gilks CB.; Huntsman D.,(2008). Ovarian carcinoma subtypes are different diseases: implications for biomarker studies. PLoS Med. 5(12):e232.
- Kohler B.A.; Ward E.; McCarthy B.J; Schymura M.J.; Ries L.A.; Eheman C.; Jemal A.; Anderson R.N.; Ajani U.A.; Edwards B.K.,(2011). Annual Report to the Nation on the Status of Cancer 1975–2007 Featuring Tumors of the Brain and Other Nervous System. J Natl Cancer Inst. 103 (9):714–736.
- Koper N.P.; Kiemeney L.A.; Massuger L.F.; Thomas C.M.; Schijf C.P.; & Verbeek A.L., (1996). Ovarian cancer incidence (1989-1991) and mortality (1954-1993) in The Netherlands. Obstet. Gynecol. 88(3): 387-393.
- Krittaya Kritpracha, Jitti Hanprasertpong, Alan Geater, Verapol Chandeying, (2005). Survival analysis in advanced epithelial ovarian carcinoma in relation to proliferative index of MIB-1 immunostaining. Journal of Obstetrics and Gynaecology Research. 31(3):268- 76.
- Kumudini Sardar; DrJitendra Singh; Sarita Tirkey.,(2018). Ki67 immunoexpression in ovarian tumours. I OSR Journal of Dental and Medical Sciences. 17(2):34-37.
- Kurman R.J.; Shih IeM., (2010).The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. Am J Surg Pathol.
- Kurman R.J.; Carcangiu ML.; Herrington C.S.; Young R.H., (2014). WHO Classification of Tumors of Female Reproductive Organs. Lyon: IARC press.
- Leon F. A. G.; massugrr, chris M. G.; thomas, charle P. T.; schijf, and andrl L.M.; vrebeek,(1996). Ovarian Cancer Incidence (1989-1991) and Mortality

(1954-1993) in the Netherlands. 0029-7844/ 96/ $15.00 387 PII 50029-7844(96)00198-6. 88(3).

- Li L.T.; Jiang G.; Chen Q.; Zheng J.N., (2015). Ki67 is a promising molecular target in the diagnosis of cancer (Review). Molecular Medicine Reports. 11:1566-72.
- Liu J.; Cristea M.C.; Frankel P.; Neuhausen S.L.; Steele L.; Engelstaedter V.; Matulonis U.; Sand S.; Tung N.; Garber JE.; Weitzel JN., (2012).Clinical characteristics and outcomes of BRCA-associated ovarian cancer: genotype and survival. Cancer Genet. 205(1-2):34-41.
- Longacre T.A.; Gilks C.B., (2009).Surface epithelial stromal tumors ofovary. In: Nucci M.R, Oliva E.; (eds), Gynecologic pathology, Elsevier Churchill Livingstone, 390–450.
- lutnit A.G. ; carmenu maria M.,(2012).The immunohistochemical expression of p53 and Ki67 in ovarian epithelial borderline tumors. Correlation with clinicopathological factors. Rom J Morphol Embryol 53(4):967–973.
- Ma Y.; Chapman J.; Levine M.; Polireddy K.; Drisko J.; Chen Q.,(2014). High-dose parenteral ascorbate enhanced chemosensitivity of ovarian cancer and reduced toxicity of chemotherapy. Sci Transl Med. 6(222):222ra18.
- Marinas, M. C.; Mogos G.; Ciurea R.; Mogos D.G.,(2012). EGFR, HER2/neu and Ki67 immunoexpression in serous ovarian tumours. Romanian Journal of Morphology and Embryology. 53(3):563-67.
- Miller K.; Price, J.H.; Dobbs S.P.; McClelland R.H.; Kennedy K.; Mc Cluggage W.G.,(2008). An immunohistochemical and morphological analysis of post chemotherapy ovarian carcinoma. Journal of Clinical Pathology. 61(5):652–57.

- Min K.W.; Park M.H., (2007). The expression of c-erbB-2, EGFR, p53 and Ki-67 in ovarian borderline tumours and carcinomas of the ovary. Korean J Pathol. 41:296-306.
- Minelli L.; Stracci F.; Cassetti T.; Canosa A.; Scheibel M.; Sapia I.E; *et al*.,(2007). Urban-rural differences in gynaecological cancer occurrence in acentral region of Italy: 1978–1982 and 1998–2002. Eur J Gynaecol Oncol. 28: 468–472.
- Mita, Akihito Nakai; Shotaro Maeda, and Toshiyuki Takeshita, (2004). Prognostic Significance of Ki-67 Antigen Immunostaining（MIB-1 Monoclonal Antibodyin Ovarian Cancer. J Nippon Med Sch. 71(6).
- Mohammad Ezzati; Amer Abdullah; Ahmad Shariftabrizi; June Hou; Michael Kopf; Jennifer K.; Stedman Robert Samuelson, and Shohreh Shahabi,(2014). Recent Advancements in Prognostic Factors of Epithelial Ovarian Carcinoma. Int Sch Res Notices. 953509.
- Mohammed Elimam Mohammed; Ammar Mohamed Hassan; Hala Ahmed Abdelhadi; Mohamed Gamaleldin Elsadig; Dalal Mohamed Adam; Khalid Elmamoun; Rania Hamid; Hiba Elias; Mohamed Abdallah; Zaki Abdelkarim; Nasr Eldin Elwali; and Sulma Ibrahim Mohammed, (2006). Burden and Pattern of Cancer in the Sudan, 2000-2006. British Journal of Medicine and Medical Research. 4(5): 2231-0614.
- Munstedt K.; von Georgi R.; Franke F.E., (2004). Correlation between MIB1 determined tumour growth fraction and incidence of tumour recurrence in early ovarian carcinomas. Cancer Invest. 22:185–94.
- Nakaji S.; Umeda T.; Takahashi I.; Matsuzaka M.; Iwane K. Epidemiological features of ovarian cancer in Japan and the world. PMID: 23156290.

- National Cancer Institute,(2105). Cancer Stat Facts: Ovarian Cancer. Annual Report.
- National Cancer Institute,(2016). Cancer Statistics Cancer Stat Facts: Ovarian Cancer. Annual Report.
- National Cancer Institute, (2018). Understanding Cancer. Cancer Statistics.
- Nesrin Gursan; Sare Sipal; Muhammed Calik.; and Cemal Gundogdu1.,(2009). p53, Bcl-2, Ki-67 Li (Labeling Index) Status in Benign, Proliferative and Malignant Ovarian Surface Epithelial Neoplasms. Eurasian J Med. 41(1): 10–14.
- N.H Mahdy; Moataz Abdel-Fattah,(1999). Ovarian cancer in Alexandria from 1988 to 1997: Trends and survival. East Mediterr Health J. 5(4):727-39.
- Niloff J.M.; Knapp R.C.; Schaetzl E.; *et al*., (1984). CA125 antigen levels in obstetric and gynecologic patients. Obstet Gynecol. 64:703-707.
- Prat J., (2012). Ovarian carcinomas: five distinct diseases with different origins, genetic alterations, and clinicopathological features. Virchow's Arch. 460:237–49.
- Prat J., (2012). New Insights into Ovarian Cancer Pathology. Annals of Oncology. 23(10): 111-17.
- Pooja S.; Naik; Sanjay Deshmukh; Siddhi Gaurish Sinai Khandeparkar; Avinash Joshi; Shridhar Babanagare; Jyostna Potdar; and Neelesh Sharad Risbud,(2015). Epithelial ovarian tumors: Clinicopathological correlation and immunohistochemical study. J Midlife Health. 6(4): 178–183.
- Salehi F.; Dunfield L.; Phillips K.P.; Krewski D.; Vanderhyden B.C., (2008). Risk factors for ovarian cancer: an overview with emphasis on hormonal factors. 11(3-4):301-21.

- Rahmanzadeh R.; Rai P.; Celli J.P.; Rizvi I.; Baron-Luhr; B.; Gereds J.; *et al*., (2010). Ki-67 as a molecular target for therapy in an in vitro three-dimensional model for ovarian cancer. Cancer Res. 70(22):9234-42.
- Rajeev Singhai, Vinayak W. Patil, Sanjog R. Jaiswal, Shital D. Patil, Mukund B. Tayade, and Amit V. Patil, Mukund B. Tayade and Amit V. Patil, (2011). E-cadherin as a Diagnostic Biomarker in Breast Cancer. North American Journal of medical sciences. 3(5):227-233.
- Rochester; Ask Mayo Expert, (2013). What are the symptoms of ovarian cancer? Minn. Mayo Foundation for Medical Education and Research.
- Röhlke P.; Milde-Langosch K.; Weyland C.; Pichlmeier U.; Jonat W.; Löning T., (1997). p53 is a persistent and predictive marker in advanced ovarian carcinomas: multivariate analysis including comparison with Ki67 immunoreactivity. J Cancer Res Clin Oncol. 123(9):496–501.
- Saeed I.E.; Weng H.Y.; Mohamed K.H.; Mohammed S.I., (2014). Cancer incidence in Khartoum, Sudan: First results from the cancer registry. Cancer Med. 3:1075–1084.
- Sassen S.; Schmalfeldt B.; Avril N.; Kuhn W.; Busch R.; Hofler H.; *et al*., (2007). Histopathologic assessment of tumour regression after neo-adjuvant chemotherapy in advanced-stage ovarian cancer. Human Pathology. 38(6):926–34.
- Scholzen T.; Gerdes J., (2000).The Ki-67 protein: from the known and the unknown. J Cell Physiol. 182(3):311-22.
- Stewart B.W.; Kleihues P., (2003). World Cancer Report. Lyon: IARC Press.
- Sylvia MT.; Kumar S.; Dasari P., (2012). The expression of immunohistochemical markers estrogen receptor, progesterone receptor, Her-

2-neu, p53 and Ki-67 in epithelial ovarian tumours and its correlation with clinicopathologic variables. Indian J Pathol Microbiol. 55:33-37.

- Tamakoshi K.; Kondo T.; Yatsuya H.; Hori Y.; Kikkawa F.; & Toyoshima H., (2001). Trends in the mortality (1950-1997) and incidence (1975-1993) of malignant ovarian neoplasm among Japanese women: analyses by age, time, and birth cohort. Gynecol. Oncol. 83(1):64-71.
- www.merckmanuals.com/professional/gynecology-and-obstetrics/benign-gynecologic-lesions/benign-ovarian-masses
- Zambon P.; & La Rosa F., (2004). Gynecological cancers: cervix, corpus uteri, ovary. Epidemiol Prev. 28(2):68-74.

Appendix

Preparation of Mayer's haematoxylin

Haematoxylin ……1 g

Distilled water……1000 ml

Sodium iodate……..0.2 g

Potassium alum…….....50 g

Citric acid……...1 g

Chloral hydrate…….50 g

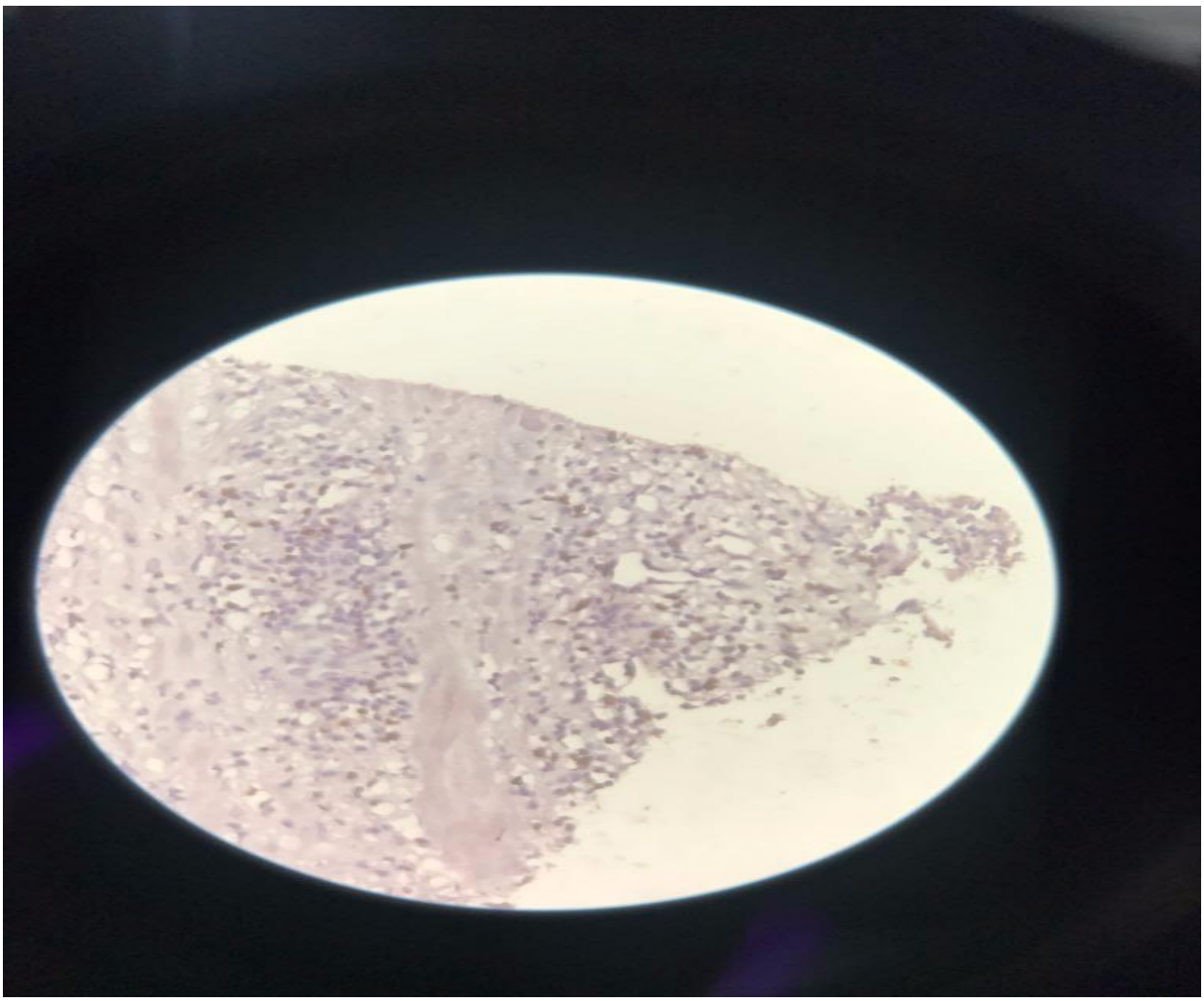
Preparation of eosin Y

Eosin Y…….1 g

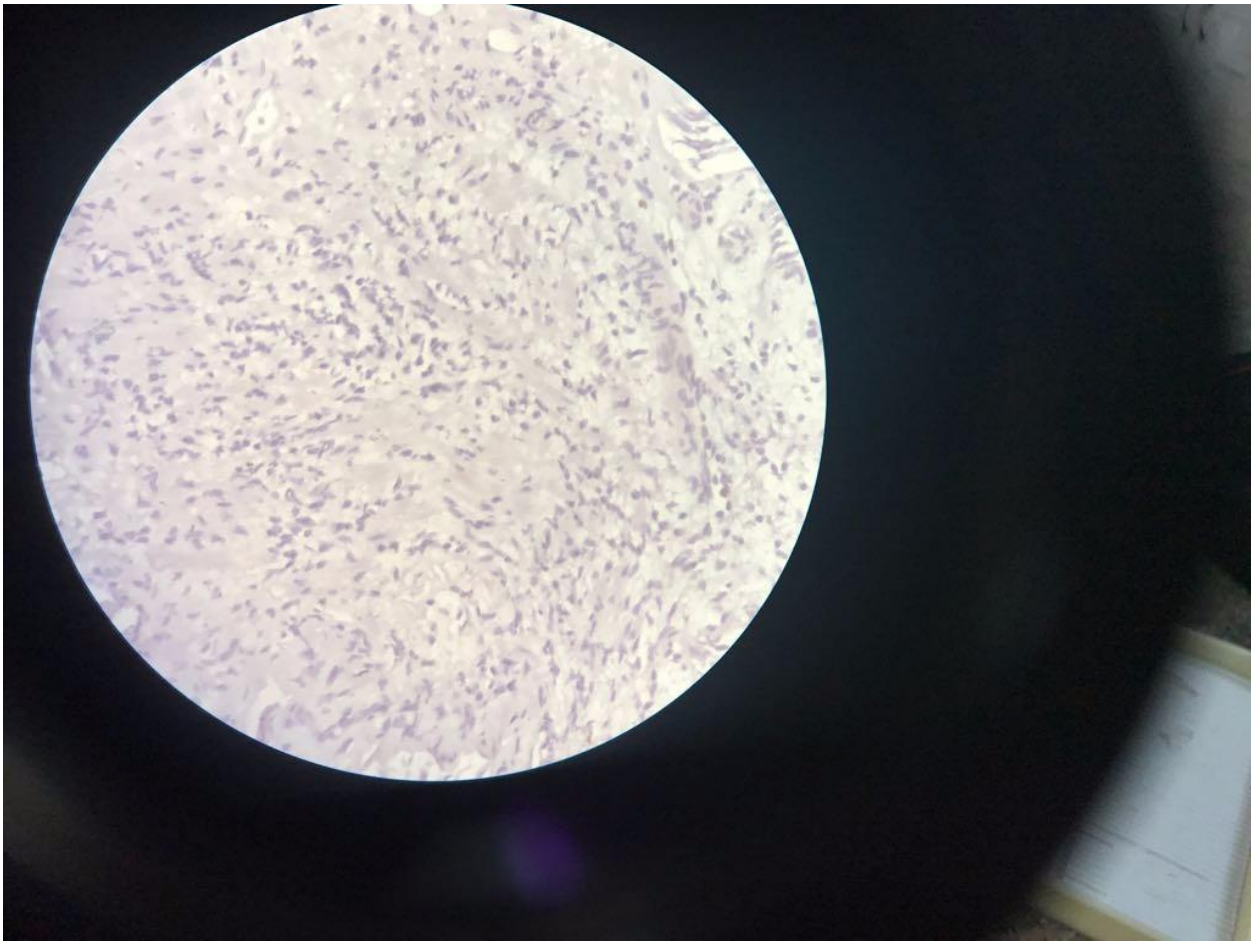
Distilled water…….....100 ml

Glacial acetic acid……...0.05 ml

Crystal thymol…….....small amount



Picture 1: Shows positive Ki-67 immunohistochemical expression.



Picture 2: Shows negative Ki-67 immunohistochemical expression.

Shendi University

Faculty of Graduate Studies and Scientific Research

Questionnaire sheet

Immunohistochemical Expression of Ki-67 among Sudanese Females with Ovarian Tumours in Khartoum State

NO.	Age	Cancer type	Grade	Ki-67 result	Expression score