**Sudan University of Sciences and Technology**

**College of Graduate Studies**

# Immunohistochemical Detection of Ki67 Expression in Endometrial Cancer among Sudanese women

**الكشف الكيميائي النسيجي المناعي لإفراز (ki67) في سرطان بطانة الرحم لدى النساء السودانيات**

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

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# الاية

## بسم الله الرحمن الرحيم

قال الله تعالي:

( يَا أَيُّهَا الَّذِينَ آَمَنُوا إِذَا قِيلَ لَكُمْ تَفَسَّحُوا فِي الْمَجَالِسِ فَافْسَحُوا يَفْسَحِ اللَّهُ لَكُمْ وَإِذَا قِيلَ انْشُزُوا فَانْشُزُوا يَرْفَعِ اللَّهُ الَّذِينَ آَمَنُوا مِنْكُمْ وَالَّذِينَ أُوتُوا الْعِلْمَ دَرَجَاتٍ وَاللَّهُ بِمَا تَعْمَلُونَ خَبِيرٌ (11))

صدق الله العظيم

سورة المجادلة الاية (١١)

# Dedication

***This work is dedicated to my family and my friends they mean the world to me. Thank you for your support all along the way***

***Thank you***

## Acknowledgement

*First of all I have to thank Allah, the most gracious who gave me an amazing opportunity to life and who helped me in bringing this work to light.*

*I would like to express my gratitude and greatest indebtedness to.*

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*Who devoted great deal of his valuable time, untiring effort, continuous guidance, providing scientific comments and encouragement to complete this work.*

*Thank you all*

# Abstract

Endometrial carcinoma is one of the commonest gynecological malignancies.Ki67is a typical immunohistochemical marker for cell proliferation. Ki67monoclonal antibody is a promising tool for determining cell proliferation on routine histologic materialit is increasingly popular due to its minimal tissue requirements and suitability to routinely fixed tissues.

Thisdescriptive, retrospective hospital based study aimed to detect the expression of Ki67 in endometrial cancer,it is was conducted in Omdurman Maternity Hospital during the period from January 2021 to May 2021

Fiftyformalin fixed paraffin embedded tissue blocks were selected, then applied to immunohistochemistry technique

The Fifty women age ranged between 21 years to 76years, the mean age was 48.5 years old, result of immunohistochemistry showed that;Ki67 expression as follow 29 (58%) female were less than 38% considered as negative result and 21 (42%) were more than 38% considered as positive result,expression of more than 38% were distributed not detected in grade I, 3(6%)cases seen in grade II and 18(36%) cases seen in grade III, with significant relation between the expression of ki67 and cancer grade ( p.value :0.000)

The study concludethat there is a significant correlation between Ki67 expression and cancergrade.

## المستخلص

سرطان بطانة الرحم من أهم وأكثر الأورام النسائية انتشارا. بروتين ki67هو علامة كيميائية مناعية نموذجية لتكاثر الخلايا. فهي تعد ذات تطبيق واسع من حيث استخدامها لجزء بسيط من الأنسجة وملائمتها للانسجة الثابتة بشكل روتيني .

اجريت هذه الدراسة الوصفية التراجعية المستشفوية في مستشفى الولادة امدرمان خلال الفترة من يناير الى مايو2021,هدفت هذه الدراسة لتحديد افراز ki67 في سرطان بطانه الرحم.

تضمنت الدراسة50 قالب شمعي محتوي علي نسيج رحمي وبعد ذلك تم فحصه بواسطة طريقة المناعة النسيجية الكيميائية.

تراوحت اعمار المرضى من 21 سنة الى 76 سنة بمتوسط عمر 48.5.أظهرتنتيجة الكيمياء المناعية أن: تعبير Ki67 على النحو التالي 29 (58 %) من العينات أقل من 38 % واعتبرت نتيجة سلبية بينما 21 (42 %) من العينات كان أكثر من 38 % وتعتبر نتيجة إيجابيةوكان توزيع نتيجة العينات الايجابية وفق درجة الورم على النحو التالى لم تسجل اى حالة في الدرجة الاوليو3(6%) حالات وجدت في الدرجة الثانية و18(36%) حالة وجدت في الدرجةالثالثة، مع وجود علاقة ذات دلالةاحصائية بين افراز ki67 ودرجة الورم( القيمة الإحتمالية0.000).

خلصت الدراسة لوجود علاقة ذات دلالة إحصائية بين إفراز ki67ودرجة الورم لسرطان بطانة الرحم.

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# List of abbreviations

CAH: Complex Atypical Hyperplasia

CH: Complex Hyperplasia

D&C:Dilatation and curettage

DNA: Deoxyribo Nucleic Acid

E2: Estrogen 2

EC: Endometrial Carcinoma

EEC: Endometrialendomtrioid carcinoma

ESC: Endometrial Serous Carcinoma

FIGO:International Federation of Gynecology and Obstetrics

IHC: Immunohistochemistry

MIB-1:methylation-inhibited binding protein 1

NCI: National Cancer Institute

P53: Protein 53

SAH: Simple Atypical Hyperplasia

TMA: Tissue Microarray

WHO: World Health Organization

# List of photos

# Chapter One

# Introduction

## 1.1 Introduction

Endometrial cancer is the most common gynaecologicaltumor in developed countries, and its incidence is increasing. The most frequently occurring histological subtype is endometrioid adenocarcinoma. Patients are often diagnosed when the disease is still confined to the uterus(Morice,*etal*, 2016).

Endometrial cancer has increased 21% in incidence since 2008, and the death rate has increased more than 100% over the past two decades. Precursor lesions of complex hyperplasia with atypia are associated with an endometrial carcinoma in more than 40% of cases(Sorosky, 2011).The incidence of endometrial cancer varies widely throughout the world. The highest rates occur in North America and Europe, whereas rates in developing countries and Japan are four to five times lower. The incidence is also about twice as high in whites compared to blacks. However, the proportion of endometrial cancer related deaths is higher in blacks due to a relative increase in the incidence of high-risk endometrial carcinoma in the black population. The reason for this is not well understood but access to and quality of health care as well as genetics is considered possible factors (Ellenson, *etal*, 2011).

In the majority of cases, the neoplasm is histologically diagnosed as an endometrial carcinoma of endometrioid type (type I) and its stage at the time of diagnosis as type I according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). The second majorGroup of endometrial cancer is histologically classified as serous carcinoma (type II) (Amant, *etal*, 2009).clinically relevant

biomarkers for cancer diagnosis (Njoku, *etal, 2019*). Ki67 proliferative activity was significantly increased with a decrease of the histological grading and with the myometrial invasion of human endometrial cancer (Semczuk, *etal, 2001*).Ki67 is useful in distinguishing STUMP from cellular leiomyomas (Mittal, and Demopoulos, 2001). Ki67monoclonal antibody is a promising tool for determining cell proliferation on routine histologic material; MIB-1 assays are increasingly popular because of their minimal tissue requirements and suitability to routinely fixed tissues (Spyratos, *etal, 2002*).

## 1.2 Rationale

Increase number of endometrialcancerare noted in last few years among population, but there is a few studies concerning the behavior and expression pattern of tumor markers, such as diagnostic markers that used for differentiation between benign and malignant. So our study will concentrate in the detection of Ki67 expression on endometrial tissue, which may help in distinguishing between different histopathological grading of endometrial tumor.

## 1.3 Objectives

### 1.3.1 General objective:

To detect expression ofKi67 in endometrial cancer among Sudanese women

### 1.3.2 Specific objectives:

1. To detectKi67 in endometrial carcinoma using immunohistochemistry
2. To correlate between the Ki67expression and histopathological grading of cancer

# Chapter Two

# Literature Review

## 2.1 Anatomy of endometrium

The endometrium is a complex and dynamic tissue, even in the absence of pregnancy. It is composed of multiple cell types, including glandular and luminal epithelium, stroma, endothelium, and multiple immunocytes. Endometrium is unique among tissues in that it undergoes proliferation, neovascularization, differentiation, large changes in resident immunocyte number and type, apoptosis, tissue shedding, bleeding, and subsequent healing and regrowth as a normal physiological process. (Young, 2014).

## 2.2 Endometrial cancer epidemiology

Endometrial cancer is the commonest gynaecological cancer mostly affecting women in the post-menopausal age group. Rates vary worldwide and are highest in white women in Western populations (Purdie,. and Green,., 2001Endometrial carcinoma (EC) is one of the major gynecological malignancies. It is a significant health problem in Sudan (Mohager, 2013)

## 2.3 Frequency of endometrium

The lifetime risk for developing this disease is approximately 2.8%. The peak ages of diagnosis are between ages 55 and 64 years (median 62 years). Though the risk for endometrial cancer is slightly lower in American black women than white women (24.8 versus 26.3 new cases/100,000 per year), the lethality of endometrial cancer in black women significantly exceeds the lethality of this disease in white women (8.1 versus 4.2 deaths/100,000 per year)(Casey, *etal*,2018).

### 2.3.1 Obesity

.There are several mechanisms whereby obesity is hypothesized to increase endometrial cancer risk, including increased endogenous sex steroid hormones, insulin resistance, chronic inflammation and adipokines. (Manih&Azzo , 2021)

### 2.3.2 Estrogens and progestins

During the follicular phase of the menstrual cycle, when the ovaries produce E2but virtually no progesterone, epithelial tissue and stromal fibroblasts in the upper two-thirds of the endometrium(“functional “layer) proliferate (this is referred to Asthe “proliferativephase “of the endometrium). High proliferationrates continue until ovulation, when plasma E2 levels reach a nadir, and then decline rapidly during the luteal phaseof the menstrual cycle, because of the increase in levels of progesterone, which antagonizes the proliferative actions of E2. (Kaaks, *etal, 2002*)

### 2.3.3 Family history in endometrial carcinoma

Women with a first-degree family history of endometrial cancer or colorectal cancer have a higher risk of developing endometrial cancer than those without a family history (Win,*etal,* 2015).

## 2.4 Classification of endometrial carcinoma

The classification of endometrial carcinomas is based on pathological assessment of tumor cell type;

- Endometrioid
- Serous
- Carcinosarcoma

- Mixed
- Undifferentiated
- clear cell

theyare associated with distinct molecular alterations. This current classification system for high-grade subtypes, in particular the distinction between high-grade endometrioid (EEC-3) and serous carcinomas (ESC), is limited in its reproducibility and prognostic abilities. (Murali, *etal*, 2014).

## 2.5 Diagnosis of endometrial carcinoma

### 2.5.1 Tissue microarray (TMA):

Tissue microarray has been developed as a method to evaluate numerous samplesof tissue in a short period. Battifora (1986) first introduced the concept of puttingtogether multiple pieces of tissue in a single block called a sausage block.(Kononen*et al*1998) used this mechanism for examining several histological sections at onetime by arraying them in paraffin block. Today's tissue microarrays use multipletissues in a single paraffin block using a precise size and shape to prepare therecipient block(Bancroft& Gamble, 2008)

Tissue microarray are proven tool in clinical laboratory for histologic applicationfor Immunohistochemistry standardization and optimization of novel antibodies byperforming simultaneous assays on hundreds of clinical samples in sequentialtissue microarray sections. TMA design includes multitissue/ multitumor screeningarrays, tumor specific arrays and tumor progression arrays. TMAs offer a muchneeded validation tool and statistical power by arraying hundreds of clinicalspecimen that provide critical prognostic significance of newly identifiedbiomarkers.(Hongbao& Young, 2014)

### 2.5.2 Ki67

The ihcDirect Ki67 Ab is a polyHRP conjugated antibody. Ki67 is a nuclear proteinexpressed in all proliferating cells and is a widely used biomarker to estimate the proportion of dividing cells to help grade human tumor cells. More specifically, Ki67 has often been used as a marker of proliferation for tumor cells of the breast and prostate. During the interphase of a cell cycle including the G1, S, G2, and M phases, but not the (G0) phase, Ki67 appears to help control heterochromatin organization.

It is increasingly popular due to its minimal tissue requirements and suitability to routinely fixed tissues (Liu, *etal*, 2020)

### 2.9 Management of endometrial carcinoma

Management of endometrial cancer consists of surgical staging with adjuvant therapy guided by risk factors, though some women cannot undergo surgery due to comorbidities (Gebhardt,*etal*, 2019).

### 2.10Previous studies

In study done on Medical University, Chongqing, People's Republic of China The Ki67 was demonstrated to be a useful prognostic factor in patients with stages I–II endometrial cancer, and the Ki67 labeling index 38.0% was optimal cut-off value for predicting recurrenceThe multivariate Cox regression analysis demonstrated that the histotypes (*P*=0.012), myometrial invasion (*P*=0.014), cervical stromal invasion (*P*=0.001), Ki67 (*P*=0.002), estrogen receptor (ER) (*P*=0.045) and P53 (*P*=0.032) were significant prognostic predictors for recurrence of endometrial cancer. **(**Peng Jiang.*et al*, (2020)**)**

Study done on Europe (2001) showed that  Ki67 was positive in 19 out of 29 cases (65.5%) showed a positive correlation with the grading.(Cherchi,*etal*, 2001).

Study done on Poland (2001) showed that the mean Ki67Proliferation Index was 43.8%, with a median of 36.0%. A significant relationship was noted between Ki67expression and histological grading ($p$=0.0004) and myometrial invasion of cancer ($p$=0.01). Ki67Proliferation Index that was nearly twice as high as in those neoplasias that stained positively for retinoblastoma (70.33% and 42.14%, respectively; $p$=0.09; Mann-Whitney-$U$ test).

# Chapter Three

# Materials and Methods

## 3.1 Study design:

This study was descriptive, retrospective hospital based study.

## 3.2 Study area

Tissue Samples were collected from female block inKhartoum state; the collected samples were processed and examined in Omdurman Maternity Hospital.

## 3.3 Study population:

Paraffin blocks prepared from patients with endometrial cancer of all age groups who undergo hysterectomy during the period between January- may in 2021, were enrolled in this study.

## 3.4 Sample size:

Fifty blocks were taken from 50 patients samples confirmed with endometrial cancer. Was carried out in Khartoum state.

## 3.5 Data collection tool:

The available demographic, data according to the laboratory guidelines, were obtained from patients records.

## 3.6 Methods:

### 3.6.1Microtomy:

Two slides each of one contain thirty tissue sections, were prepared using tissue microtome technology. One for Heamatoxylin and Eosin stain, one is to immunohistochemistry techniques. Other twenty slide section of 5μm in thickness hadbeen obtained from each formalin fixed paraffin wax embedded tissue using rotary microtome for H&E and IH.

### 3.6.2Immunohisochemical techniques (Ki67):

The immunohistochemical procedure was done as follows: One section (3μm) from formalin-fixed, paraffin-embedded tumors was cut and mounted onto salinized slides (Thermo). Following deparaffinization in xylene, slides was rehydrated through a graded series of alcohol and was placed in distilled water. Samples were heated for antigen retrieval for Ki67 using high PH (9) by water bath at 95C for 40 min. After washing with PBS for 3 min Endogenous peroxides activity was blocked with 3% hydrogen peroxide and methanol for 10 min, and after washing with PBS for 3 min then slides were incubated with (100 μL) of (mouse monoclonal antibody (Clone and Ki67) against antigen for 30 min at room temperature in a moisture chamber. After washing with PBS for 3 min, binding of antibodies were detected by incubating for 20 min with secondary Abpolymer (HRP). Finally, the sections washed in three changes of PBS, followed by adding 3, 3 diaminobenzidine tetra hydrochloride (DAB) as a chromogen to produce the characteristic brown stain for the visualization of the antibody/enzyme complex for up to 5 min. After washing with distaled water for 3 min slides was counterstained with haematoxylin (MAYER'S) for one minthen was washing in running tap water for several minutes 7-10 (bluing), then dehydrate and, cleaned, mount in DBX. Then examined under microscope .

### 3.7 Data analysis:

The data was analyzed using version 20.0 SPSS computer program frequencies, means and chi square test values were calculated.

The significant value ( p =0.05).

### 3.8 Ethical considerations:

Blocks were taken with permission from Omdurman Maternity Hospital administration

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# Chapter four

## Results

Fifty block tissue samples included in this study were diagnosed with endometrial carcinoma. The total of Ki67 estimation according to grade was found as follow; grade I seen in 15 (30%) female, grade II seen in 9 (18%) female and grade III seen in 26 (52%) female (Table “4-1”).

Result of Ki67 expression as follow 29 (58%) female were less 38% and 21 (42%) were more than 38% (Table “4-2”).

The result of Ki67 expression more than 38% (cutoff point) as follow zero(0%) cases seen in grade I, 3(6%) cases seen in grade II and 18(36%) cases seen in grade III. P.value was 0.00 which is statistically significant, that mean there is relation between endometrial carcinoma grading and Ki67 expression (Table “4-3”

Table (4-1)Distribution of endometrial cancer grade among study population

| Cancer grade | Frequency(%) |
|---|---|
| Grade I | 15(30%) |
| Grade II | 9(18%) |
| Grade III | 26(52%) |
| Total | 50(100%) |

Tale (4-2) The expression of Ki67 results among population

| Ki67 result | Frequency(%) |
|---|---|
| less than 38% | 29(58%) |
| 38% or more | 21(42%) |
| Total | 50(100%) |

38% cutoff point

Table (4-3): Relation between Ki67 results and cancer grade

| Ki67 result | Cancer grade | | | P.value |
|---|---|---|---|---|
| | Grade I | Grade II | Grade III | |
| less than 38% | 15(30%) | 6(12%) | 8(16%) | 0.00 |
| 38% or more | 0(0%) | 3(6%) | 18(36%) | |
| Total | 15(30%) | 9(18%) | 26(52%) | |
| | 50(100%) | | | |

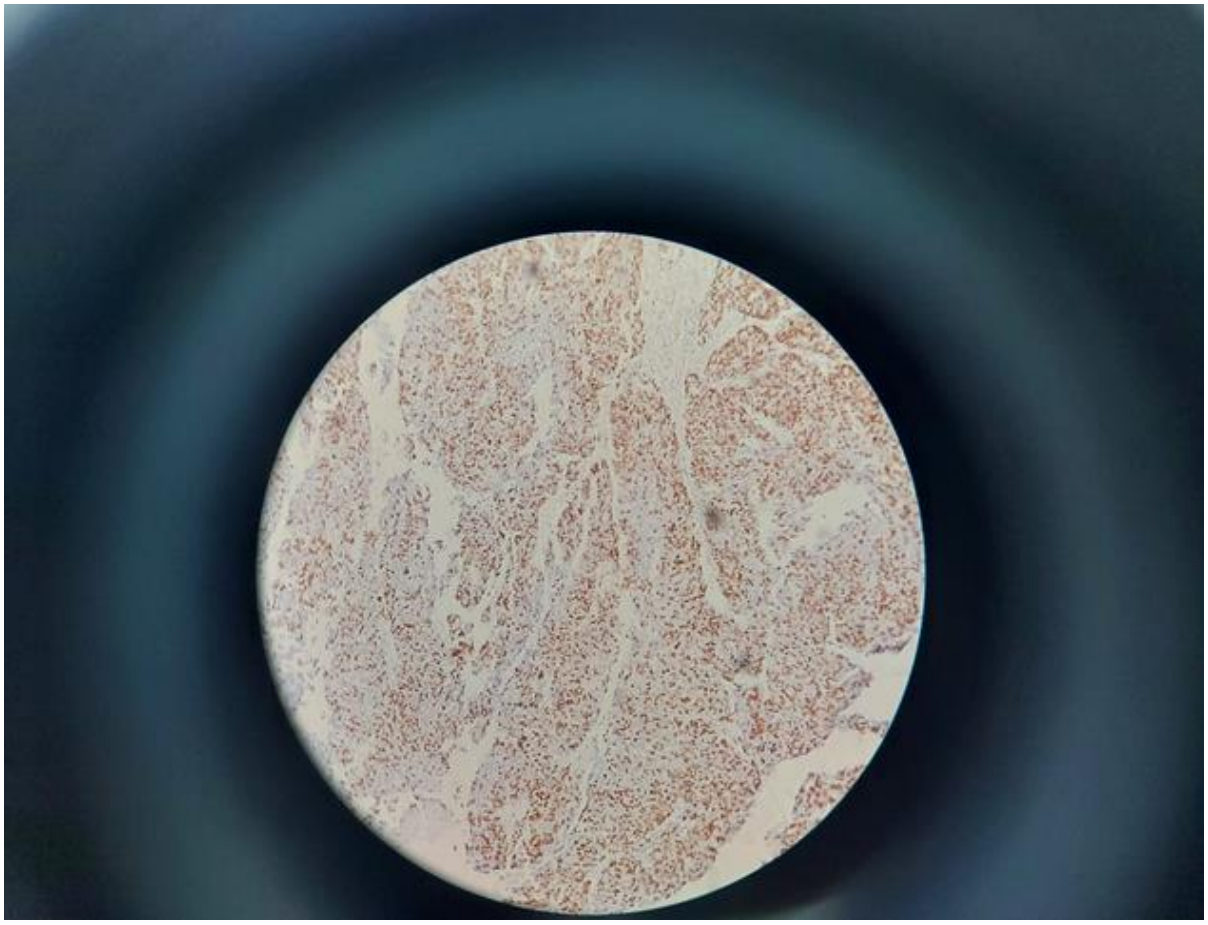

Photo 4-1: High expression of Ki67 in endometrial cancer x 10

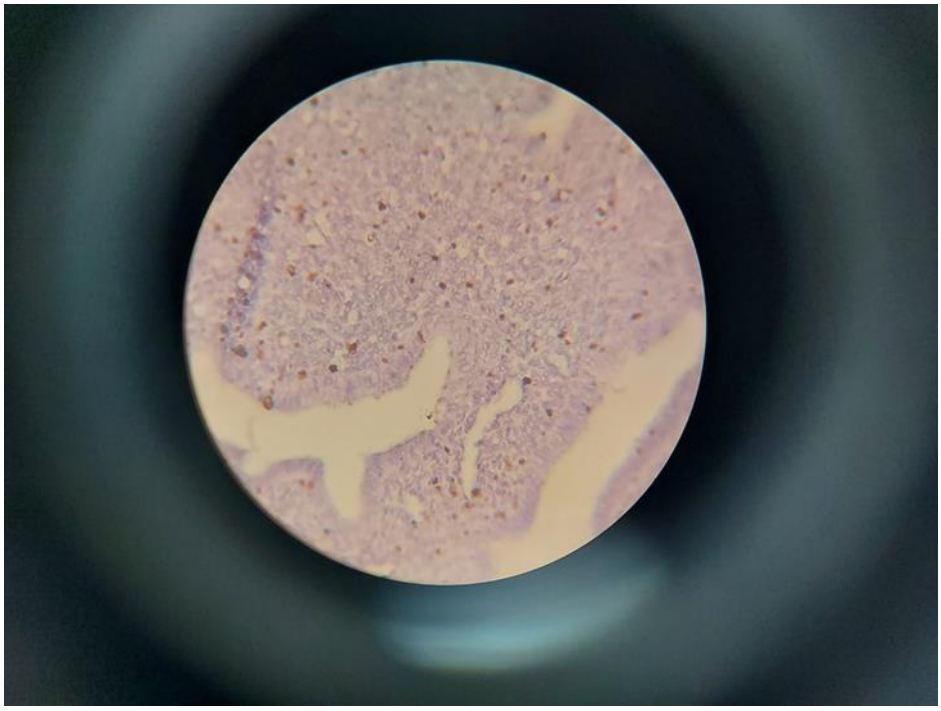

Photo 4-2: Low expression of Ki67 in endometrial cancer x 40

# Chapter five

# DISCUSSION, CONCLUSION & RECOMMENDATION

## 5.1 Discussion

The present study identify the ki67expression and endometrial cancer grade as follow; grade I seen in 15 (30%) female, grade II seen in 9 (18%) female and grade III seen in 26 (52%) female thiswas agree with study done at Bangkok (2008) which showed that Ki-67 27 cases with (41.5%) having > 35% positive nuclearstainingand 38 cases (58.5%) had < 35% nuclear staining(Negative). (Suthipintawong, *etal*, 2008).

Also agree with study done on India (2017) which showed that Ki67 labeling index increased as the severity of lesion increased from EH to endometrial carcinoma (Masjeed,*etal* , 2017).

This study agree with study done in Egypt on (2020) which conclude that Ki-67 are significantly associated with poor tumor characteristics (Gharib,*etal*,2020)

This study agree with study done at Craiova university which conclude that a significant correlation with the degree of differentiation marker ($p<0.005$) and stage of lesion ($p<0.005$) (Stoian,*et al*, (2011)).

This study disagree with study done in Sudan by Ali at (2018) which showed that Ki67 antigen was detected in 30 cases (75%) which was not significant different was found. Also there was no statristical correlation of tumor grade with mraker expression (Ali, 2018). the difference was due to sample size included.

## 5.2 Conclusions

There is a significant relation between Ki67expression and histological grade of endometrial carcinoma.

## 5.3 Recommendations

On the base of above result we recommended that :

- Ki67should be used as marker for grading of endometerial tumors.
- Further study with large sample size is needed to give more accurate result.
- Other markers should be used to investigate the pathogenesis of endometrial carcinoma such as (PRB-CD10-K-ras).

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# Appendix

## 1. Instrument and materials:

### 1.1Instrument:

-Rotary microtome

-Oven

-Coplinjare

-Staining racks

-Stainless microtome blade

-Dako coated slides

-PT link

-Cover glass

-Water bath

-Dako pen

-Moisture chamber

-Work station

-Pipettors

### 1.2 Materials:

-Xylene

-Ethyle alcohol

s haematoxylene‘-Mayer

-Distilled water

-Citrate buffer

-Peroxidase blocker

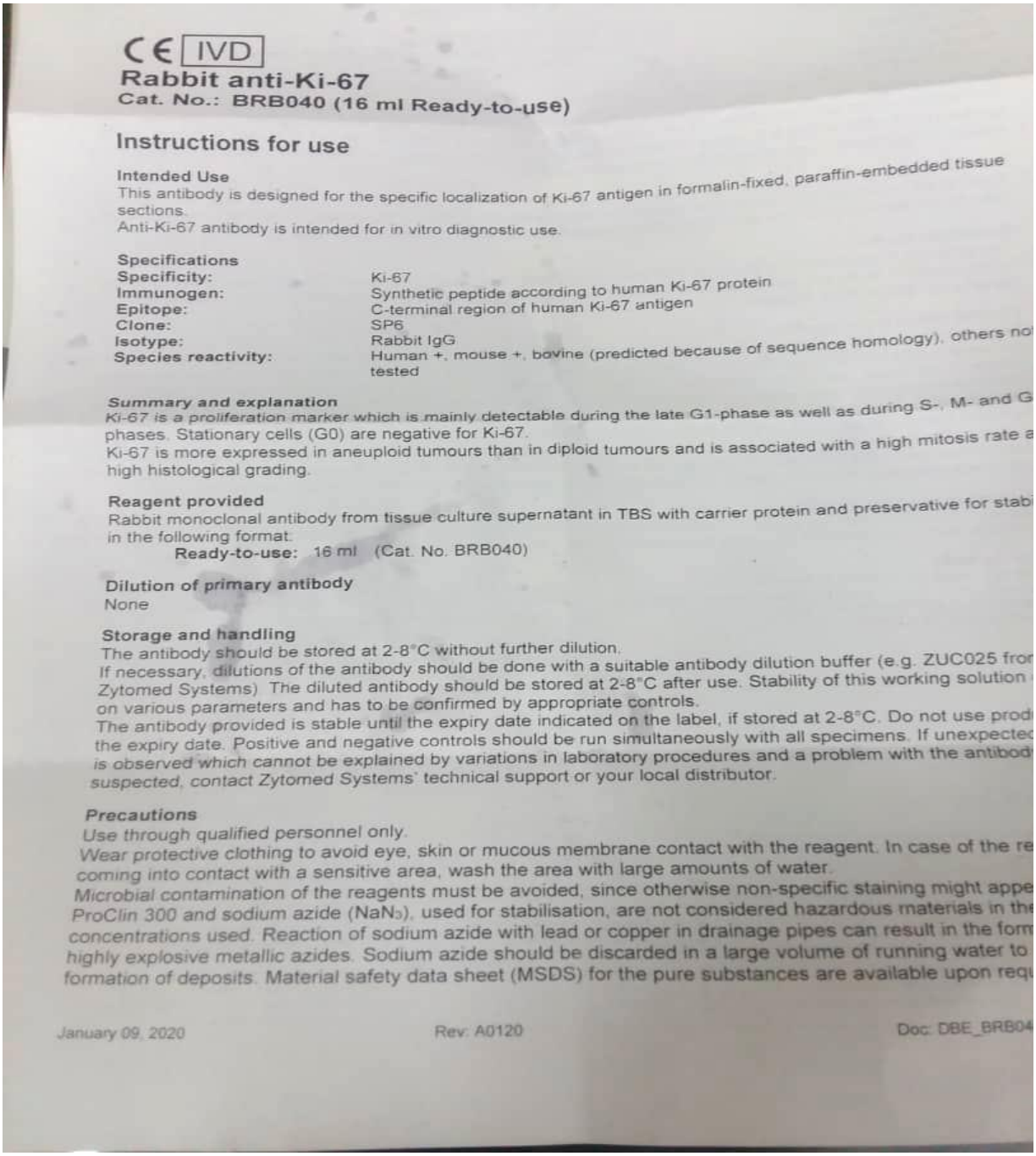
Appendix ii

-Anti E cadherin antibodies (primary antibody)

-Dextran polymer conjugated secondary antibodies and HRP

-3,3diaminobenzidinetetrahydrochloride in substrate buffer

-DPX mounting media



CE IVD

**Rabbit anti-Ki-67**

**Cat. No.: BRB040 (16 ml Ready-to-use)**

## Instructions for use

**Intended Use**

This antibody is designed for the specific localization of Ki-67 antigen in formalin-fixed, paraffin-embedded tissue sections.

Anti-Ki-67 antibody is intended for in vitro diagnostic use.

**Specifications**

| | |
|---|---|
| **Specificity:** | Ki-67 |
| **Immunogen:** | Synthetic peptide according to human Ki-67 protein |
| **Epitope:** | C-terminal region of human Ki-67 antigen |
| **Clone:** | SP6 |
| **Isotype:** | Rabbit IgG |
| **Species reactivity:** | Human +, mouse +, bovine (predicted because of sequence homology), others no tested |

***Summary and explanation***

*Ki-67 is a proliferation marker* which is mainly detectable during the late G1-phase as well as during S-, M- and G phases. Stationary cells (G0) are negative for Ki-67.

Ki-67 is more expressed in aneuploid tumours than in diploid tumours and is associated with a high mitosis rate a high histological grading.

**Reagent provided**

Rabbit monoclonal antibody from tissue culture supernatant in TBS with carrier protein and preservative for stabi in the following format:

**Ready-to-use:** 16 ml (Cat. No. BRB040)

**Dilution of primary antibody**

None

**Storage and handling**

The antibody should be stored at 2-8°C without further dilution.

If necessary, dilutions of the antibody should be done with a suitable antibody dilution buffer (e.g. ZUC025 fro Zytomed Systems). The diluted antibody should be stored at 2-8°C after use. Stability of this working solution on various parameters and has to be confirmed by appropriate controls.

The antibody provided is stable until the expiry date indicated on the label, if stored at 2-8°C. Do not use prod the expiry date. Positive and negative controls should be run simultaneously with all specimens. If unexpected is observed which cannot be explained by variations in laboratory procedures and a problem with the antibod suspected, contact Zytomed Systems' technical support or your local distributor.

***Precautions***

Use through qualified personnel only.

Wear protective clothing to avoid eye, skin or mucous membrane contact with the reagent. In case of the re coming into contact with a sensitive area, wash the area with large amounts of water.

Microbial contamination of the reagents must be avoided, since otherwise non-specific staining might appe ProClin 300 and sodium azide ($NaN_3$), used for stabilisation, are not considered hazardous materials in the concentrations used. Reaction of sodium azide with lead or copper in drainage pipes can result in the form highly explosive metallic azides. Sodium azide should be discarded in a large volume of running water to formation of deposits. Material safety data sheet (MSDS) for the pure substances are available upon requ

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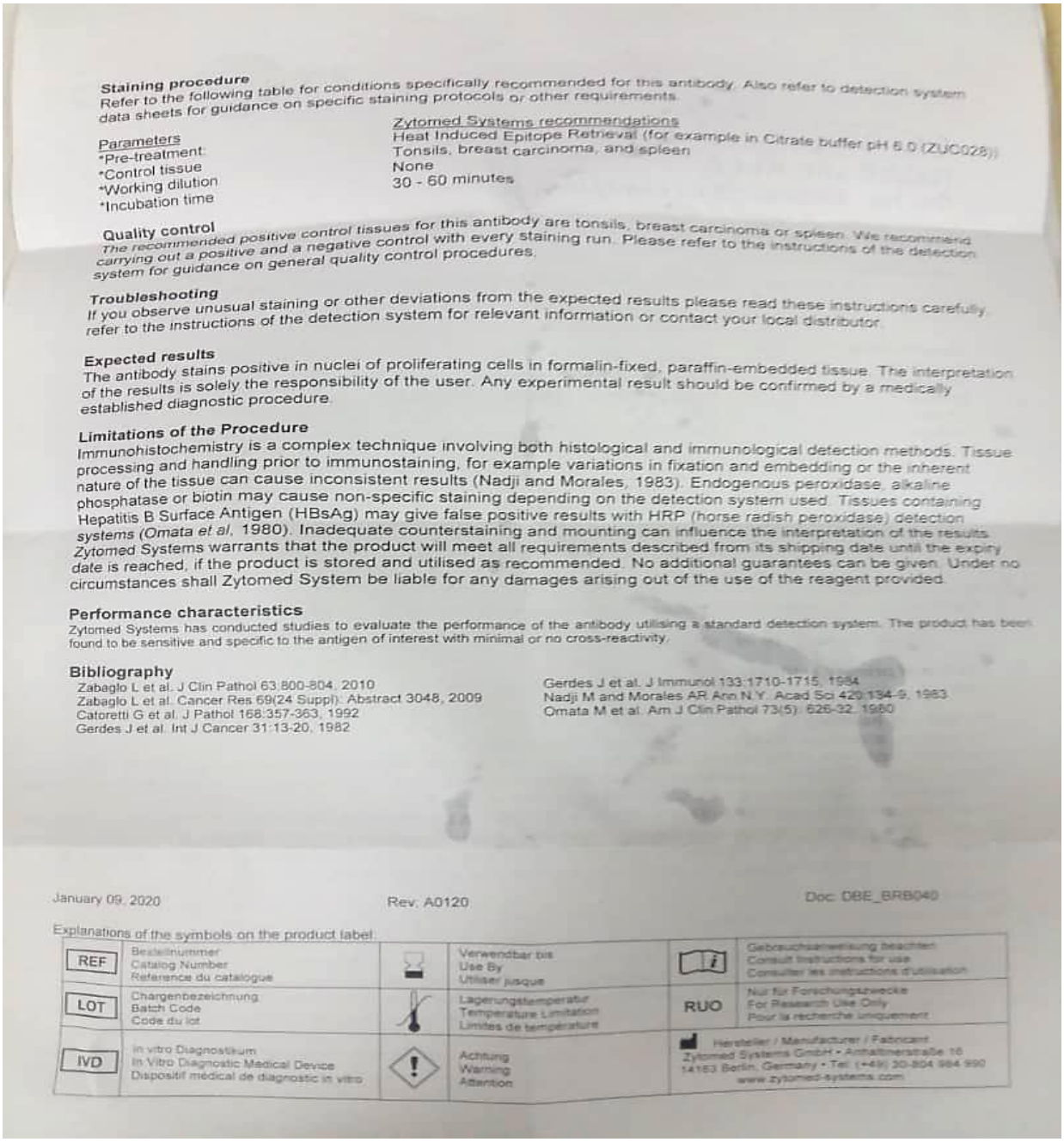
Leaflet of kits



**Staining procedure**
Refer to the following table for conditions specifically recommended for this antibody. Also refer to detection system data sheets for guidance on specific staining protocols or other requirements.

| Parameters | Zytomed Systems recommendations |
|---|---|
| *Pre-treatment: | Heat Induced Epitope Retrieval (for example in Citrate buffer pH 6.0 (ZUC028)) |
| *Control tissue | Tonsils, breast carcinoma, and spleen |
| *Working dilution | None |
| *Incubation time | 30 - 60 minutes |

**Quality control**
*The recommended positive control tissues for this antibody are tonsils, breast carcinoma or spleen. We recommend carrying out a positive and a negative control with every staining run. Please refer to the instructions of the detection system for guidance on general quality control procedures.*

***Troubleshooting***
*If you observe unusual staining or other deviations from the expected results please read these instructions carefully, refer to the instructions of the detection system for relevant information or contact your local distributor.*

**Expected results**
The antibody stains positive in nuclei of proliferating cells in formalin-fixed, paraffin-embedded tissue. The interpretation of the results is solely the responsibility of the user. Any experimental result should be confirmed by a medically established diagnostic procedure.

**Limitations of the Procedure**
Immunohistochemistry is a complex technique involving both histological and immunological detection methods. Tissue processing and handling prior to immunostaining, for example variations in fixation and embedding or the inherent nature of the tissue can cause inconsistent results (Nadji and Morales, 1983). Endogenous peroxidase, alkaline phosphatase or biotin may cause non-specific staining depending on the detection system used. Tissues containing Hepatitis B Surface Antigen (HBsAg) may give false positive results with HRP (horse radish peroxidase) detection *systems (Omata et al*, 1980). Inadequate counterstaining and mounting can influence the interpretation of the results. *Zytomed* Systems warrants that the product will meet all requirements described from its shipping date until the expiry date is reached, if the product is stored and utilised as recommended. No additional guarantees can be given. Under no circumstances shall Zytomed System be liable for any damages arising out of the use of the reagent provided.

**Performance characteristics**
Zytomed Systems has conducted studies to evaluate the performance of the antibody utilising a standard detection system. The product has been found to be sensitive and specific to the antigen of interest with minimal or no cross-reactivity.

**Bibliography**

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Nadji M and Morales AR Ann N.Y. Acad Sci 420:134-9, 1983
Omata M et al. Am J Clin Pathol 73(5): 626-32, 1980


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Explanations of the symbols on the product label:

| | | | | | |
|---|---|---|---|---|---|
| REF | Bestellnummer<br>Catalog Number<br>Référence du catalogue | | Verwendbar bis<br>Use By<br>Utiliser jusque | | Gebrauchsanweisung beachten<br>Consult Instructions for use<br>Consulter les instructions d'utilisation |
| LOT | Chargenbezeichnung<br>Batch Code<br>Code du lot | | Lagerungstemperatur<br>Temperature Limitation<br>Limites de température | RUO | Nur für Forschungszwecke<br>For Research Use Only<br>Pour la recherche uniquement |
| IVD | In vitro Diagnostikum<br>In Vitro Diagnostic Medical Device<br>Dispositif médical de diagnostic in vitro | | Achtung<br>Warning<br>Attention | | Hersteller / Manufacturer / Fabricant<br>Zytomed Systems GmbH • Anhaltinerstraße 16<br>14163 Berlin, Germany • Tel: (+49) 30-804 994 990<br>www.zytomed-systems.com |

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