Evaluation of Serum Human Epididymis Secretory Protein 4 (HE4) in Benign Endometrial Disease and Endometrial Cancer

Thesis

Submitted for Partial Fulfillment of Master Degree in Obstetrics and Gynaecology

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2018

Acknowledgment

First and foremost, I feel always indebted to **ALLAH**, *the Most Kind and Most Merciful.*

I'd like to express my respectful thanks and profound gratitude to **Professor/ Sabry Sayed Mohamed,** *Professor of Obstetrics and Gynecology Faculty of Medicine - Ain Shams University for his keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

I am also delighted to express my deepest gratitude and thanks to **Dr/ Mohamed Abd Elhameed Abd Elhafeez,** *Assistant Professor of Obstetrics and Gynecology Faculty of Medicine - Ain Shams University, for his kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

I am deeply thankful to **Dr/ Hayam Fathy Mohammad,** *Assistant Professor of Obstetrics and Gynecology Faculty of Medicine - Ain Shams University, for her great help, active participation and guidance.*

I wish to introduce my deep respect and thanks to **Dr/ Nashwa Nagy El-Khazragy,** *Lecturer of Clinical Pathology Ain Shams University, for her kindness, supervision and cooperation in this work.*

Fatma Mohammed Abukra

List of Contents

List of Tables

List of Figures

List of Abbreviations

Abb.	Full term
ACS	American caner society
BMI	Body mass index
CA125	Cancer antigen 125
CDC	Center for disease control and prevention
EAC	Endometrial adenocarcenoma
EC	Endometrial cancer
ELISA	Enzyme linked immunosorbent assay
FIGO	International Federation of Gynecologists and Obstetricians
GOG	Gynecologic oncology group study
HE4	Human epididymis protein 4
HNPCC	Hereditary nonpolyposis colorectal cancer
IUD	Intrauterine device
MPA	Medroxyprogesterone acetate
MRI	Magnetic resonant image
mTOR	Mammalian target of rapamycin
NCCN	National comprehensive cancer network
NHANES	National Health and Nutrition Examination Survey
OSHR	Office of state human resources
PCOS	Polycystic ovarian syndrome
PI3K	Phosphatidylinositol/3 kinase
PORTEC-1	Post operative radiation therapy in endometrial carcinoma
PS3	Phosphoprotein gene –S3
PTEN	Polycystic ovarian syndrom
P-value	Probability value

List of Abbreviations Cont...

Abb.	Full term
ROC	Receiver operator characteristic
TVUS	Transvaginal ultrasound
VEGF	Vascular endothelial growth factor
WAP	Whey acidic protein
$WFDC_2$	WAP four-disulfide care domain protein 2 gene

Abstract

Objective: The incidence of endometrial cancer is constantly growing. More aggressive types of endometrial cancer as well as the incidence in younger women is being observed. More than 80% of cases is diagnosed in early stages due to early symptoms like abnormal bleeding. Aim of our study to evaluate the ability of serum HE4 concentration to differentiate between benign endometrial disease and endometrial cancer and asses correlation it with prognosis of EC.

Material and Methods: Serum HE4 level was measured in 90 patients with abnormal uterine bleeding. Based on histology after curettage the study group was divided into the benign and malignant endometrial pathology groups. Statistical analysis was performed using Mann-Whitney test

Results: The difference of serum HE4 level between benign endometrial pathology and cancer was significant (p=0.000) and the cut-off for identification of patients with endometrial cancer was 62 pmol/L yield. There was a significant difference between Stage (I – II) endometrial cancer, and Stage (III – IV) p=0.01, Patients who needed lymphadenectomy had significantly higher HE4 level than those who had no indications for this procedure (p=0,001).

Conclusion: HE4 is a useful biomarker in diagnosing endometrial cancer. HE4 is associated with high grade endometrial cancer. It can also serve as an useful preoperative counseling tool to identify patients, who may require pelvic lymphadenectomy.

Key words: endometrial cancer / HE4

Protocol
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INTRODUCTION

Endometrial cancer represents the most common gynecologic cancer, and it is expected to become an even greater public health concern as the prevalence of obesity, one of the most common risk factors for endometrial cancer, increases worldwide, Approximately 42,160 cases are diagnosed annually, 7,780 deaths occur ***(Renehan et al., 2008).***

The diagnosis is usually done at an early stage, and approximately 70% of endometrial cancers are diagnosed as stage I; this results in better prognosis, with a 5-year overall survival rate of 90% to 95% ***(Jemal et al., 2009).***

Almost 20% of patients with endometrial cancer are in the premenopausal state and 10% are asymptomatic. In such a case, it is much harder to make an early diagnosis and usually they are probably diagnosed at advanced stages ***(Li et al., 2009).***

An earlier diagnosis represents an imperative goal to improve survival and prognosis of patients of endometrial cancer. Actually, there are no certified screening tools for endometrial cancer. Pelvic ultrasound as screening for endometrial cancer reaches 80.5% of sensitivity, when endometrial echo is > 5 mm, but it dramatically decrease to 20% in asymptomatic women; moreover, specificity is low (61%) ***(Jacobs et al., 2011).***

HE4, a putative protease inhibition containing two (Whey Acid Protein) WAP domains, is significantly increased in the endometrioid subtype of EC ***(Drapkin et al., 2005).***

Tissue microarray and PCR studies confirmed a high level of HE4 expression in both endometrioid and serous types

of EC ***(Jiang et al., 2013)***, this results are consistent with those from other laboratories showing increased HE4 mRNA and protein expression in endometrial cancer tissues ***(Moore el al., 2008; Bignotti et al., 2011).***

Subsequent investigation demonstrated that HE4 are detectable in various normal tissues with varying expression levels, yet their levels are significantly increased in EC compared to normal endometrium ***(Jiang et al., 2013).***

Human epididymis protein 4 (HE4) is a member of the whey-acidic protein (WAP) family and it is encoded by the WAP four-disulfide core domain protein 2 (WFDC2) gene in humans. HE4 was first isolated from the human epididymis ***(Kirchhoff et al., 1991).***

This protein is also known as Epididymal secretory protein E4, Major epididymis specific protein E4 and putative protease inhibitor WAP5. WFDC-2 gene product was originally thought to be a protein specifically expressed in the epididymis and was dubbed as a tissue marker for the same ***(Kirchhoff et al., 1991).***

Angioli et al. (2013), found HE4 cutoff of 70pmol/l yields the best sensitivity and specificity for detecting endometrial cancer (59.4%) sensitivity and 100% specificity) with a positive predictive value 100% and negative predictive value equal to 71.52% for the 70 pmol/l cutoff, also found that HE4 marker was never increased in patients with benign disease.

Kalogera et al. (2012) found that HE4 is elevated in high proportion of endometrial cancer patients and it is correlated with myometrial invasion (> 50% P< 0.00) also found that

lymph node, statue correlates with the HE4 values there is a statistical significant difference comparing stage I versus stage III ($P < 0.0010$.

This findings suggest that HE4 could be useful as a preoperative indicator to identify patients suitable for pelvic and para aortic lymphadenectomy.

AIM

- To evaluate the ability of serum HE4 concentration to differentiate between benign endometrial lesions and endometrial cancer.
- To asses correlation it with prognosis of EC.

Patients and Methods

- Type of study: case control study

- **Study setting:** The study will be conducted in the Ain Shams University Maternity Hospital.

- Study Period: Starting from December 2017

- Study population:

This study will be conducted on women with abnormal uterine bleeding they will be divided into two equal groups:

1- Control group: 45 Patients with abnormal uterine bleeding and diagnosed benign endometrial pathology by endometrial biopsy.

2- Case group: 45 Patients with abnormal uterine bleeding and diagnosed endometrial cancer.

Inclusion criteria for control group:

Age (40 – 70 yr old).

Exclusion criteria for control group:

1. Age more than 70 yr and less than 40 yr.
2. Abnormal cardiac hematological renal hepatic functions.
3. Breast cancer or other malignancies.
4. Concomitant benign and for malignant adnexal pathologies.
5. Hormonal medication.
6. Patient taking or having chemo-radiotherapy.
7. Patients unfit for surgical intervention.
8. Smoker.

Study procedures

- History: will be taken from each patient apart of personal history (age, age of menarch, duration of uterine bleeding, parity) and history of hormonal therapy, family history of endometrial or breast cancer, previous surgical intervention.
- Medical history (DM or HTN).
- General examination will be do for all patients: measurement of weight to calculate body mass index (BMI=(kg/m^2) and blood pressure measurement.
- Pelvic ultrasound (trans-vaginal-abdominal) will be do for all patients to assess of endometrial thickness and to exclude adnexal mass.
- Endometrial biopsy will be obtained form all patients and will be reviewed by the pathologists.
- Computed axial tomography (CT scan) will be do for patient with endometrial carcinoma (case study) for detect tumor metastasis.
- Pre-operative complete investigation will be do for patient underwent staging lapratomy (CBC, RFT, LFT, FBS and coagulation profile to rule out systemic causes of bleeding.
- Blood sample will be taken from all patients to assess serum level of HE4:

Five milliliters of venous blood will withdrawn from all participants. The samples will left to clot. The separated sera will stored at -20° until all samples will obtained. Frozen samples will allowed to reach room temperature (20-25°) prior to use. Samples