A STUDY OF TUMOR MARKERS AND HORMONE RECEPTORS IN NORMAL AND ABNORMAL ENDOMETRIUM

Thesis
Submitted in partial fulfillment of M.D. degree in
Obstetrics and Gynecology

By
Cherifa Mahmoud Sharaf
Specialist in Obstetrics and Gynecology,
Preventive Centre, M.B., B.Ch., M.Sc.

Supervised By

Prof. Dr. Hossam Badrawi
Professor of Obstetrics and Gynecology
Cairo University

Prof. Dr. Mahmoud Emad El-Din Salem
Professor of Obstetrics and Gynecology
Cairo University

Prof. Dr. Bahaa Ihab Mounir
Professor of Pathology
Cairo University

Dr. Ahmed Nehad Ahmed Hatem Askalany
Lecturer of Obstetrics and Gynecology
Cairo University

Faculty of Medicine

Cairo University

2009

ACKNOWLEDGEMENT

I would like to express my deep and sincere gratitude to my supervisor, ***Professor Hossam Badrawi.*** *His wide knowledge and logical way of thinking have been of great value for me. His comprehension, encouragement and personal guidance provided a good basis for the present thesis. His ideals and concepts have had a remarkable influence on my entire career in the field of Ob/Gyn.*

I wish to express my warm and sincere thanks to ***Prof. Dr Mahmoud Salem*** *for the honor of working under his kind supervision,his detailed and constructive comments, and for his important support throughout this work.*

My sincere thanks and appreciation are presented to ***Prof.Dr.Bahaa Ihab Mounir*** *for his valuable advice and creative remarks, without which, this work could not have been accomplished.*

I am grateful and thankful to ***Dr. Ahmed Nehad Ahmed Hatem Askalany*** *for his kind, helpful supervision and careful review of this work.*

My special gratitude is due

To my family

And son Youssef

For their loving support

ABSTRACT

This study aims at determination of Cathepsin-D (Cath-D) and Estrogen recoptor (ER) expression in normal, hyperplastic and malignant endometrial tissue through immunohistochemical analysis. The study included 85 patients suffering form abnormal uterine bleeding in addition to 15 women under infertility investigations as a control group. High Cath-D expression (> 10% of cells) was seen in 50% 70.6% 37.9% and 27.3% of proliferative, secretory, hyperplastic and malignant cells, respectively, cyclin D1 was overexpresed more than 60% of cells in atypical hyperplasia 42.85% & obviously cells group 69.44%, The mean percentagfe of Erialpha positive cells was 90.3% , 45.1% 77.3% and 33.6% in the proliferative, secretory, hyperplastic and malignant cells, respectively.

Key ward :-

- Cathepsin –D
- Cyclin – D 1
- Estrogen receptor
- Endometrial hyperplastic
- Malignant cells

CONTENTS

	Page
Introduction	1
Aim of the Work	4
Chapter 1: Anatomy and physiology of the endometrium	5
Chapter 2: Endometrial Hyperplasia	12
Chapter 3: Endometrial Cancer	26
Chapter 4: Tumor Markers In Abnormal Endometrium	43
Chapter 5: Hormone Receptors And Endometrial Cancer	53
Subjects and Methods	62
Results	71
Discussion	96
Conclusion	112
Summary	113
References	117
Arabic Summary	

LIST OF TABLES

		Page
Table I:	Results of 4 Prospective Follow-Up Studies of Endometrial Hyperplasia	24
Table II:	Tumor classification of the international Federation of Obstetrics and Gynecology (FIGO), Clinical Staging	39
Table III:	Tumor classification of the international Federation of Obstetrics and Gynecology (FIGO), Surgical Staging	39
Table IV:	Molecular events in sporadic endometrial cancer	44
Table 1:	The Clinical and Histopathological Diagnosis of Patients involved in the study	72
Table 2:	Clinical characteristics and the 4 studied groups	73
Table 3:	Clinicopathological features of endometrial carcinoma cases	73
Table 4:	High CathD expression in different types of endometrial hyperplasia	76
Table 5:	Correlation of CathD expression with clinicopathologic features in endometrial carcinoma cases	77
Table 6:	CathD expression according to histopathological diagnosis	77
Table 7:	Expression of cyclin D1 in different endometrial patterns among study among the study sample	79
Table 8:	Nuclear intensity of cyclin D1 in different endometrial patterns among study among the study sample	80
Table 9:	A comparison of cyclin D1 immunoreactivity in non cancer cases versus cancer cases	80
Table 10:	A comparison of cyclin-D1 immunoreactivity in low risk cases (proliferative, secretory, simple hyperplasia & complex hyperplasia) versus high risk cases (atypical hyperplasia and cancer)	81
Table 11:	Comparison of the percentages of ER-alpha positive cells between groups	82

LIST OF FIGURES

		Page
Figure 1:	Distribution of the four Studied Groups	71
Figure 2:	Histopathological diagnosis of endometrium	74
Figure 3:	Moderately +ve CathD expression in proliferative Endometrium (x 400).	84
Figure 4:	Moderately +ve CathD expression in proliferative Endometrium (x 100).	84
Figure 5:	Moderately +ve CathD expression in hyperplastic Endometrium (x 400).	85
Figure 6:	Strong +ve CathD expression in hyperplastic Endometrium (x 400).	85
Figure 7:	+ve CathD expression in secretory Endometrium (x 100).	86
Figure 8:	+ve CathD expression in secretory Endometrium (x 400).	86
Figure 9:	Weak +ve CathD expression in hyperplastic Endometrium (x 400).	87
Figure 10:	Negative CathD expression (x 100).	87
Figure 11:	Negative CathD expression (x 400).	88
Figure 12:	Negative CathD expression in hyperplastic Endometrium (x 100).	88
Figure 13:	Negative CathD expression in hyperplastic Endometrium (x 400).	89
Figure 14:	Strong +ve ER expression in proliferative Endometrium (x 100).	89
Figure 15:	Strong +ve ER expression in proliferative Endometrium (x 400).	90
Figure 16:	Strong +ve ER expression in hyperplastic Endometrium (x 400).	90
Figure 17:	Moderate +ve ER expression in hyperplastic Endometrium (x 100).	91

Figure 18:	Moderate +ve ER expression in hyperplastic Endometrium (x 400).	91
Figure 19	Patchy +ve ER expression in hyperplastic Endometrium (x 100).	92
Figure 20:	Patchy +ve ER expression in hyperplastic Endometrium (x 400).	92
Figure 21:	Cyclin-D1 immune expression in simple endometrial hyperplasia	93
Figure 22:	Cyclin-D1 immune expression in simple endometrial hyperplasia	93
Figure 23:	Cyclin-D1 immune expression in atypical endometrial hyperplasia	94
Figure 24:	Cyclin-D1 immune expression in atypical endometrial hyperplasia	94
Figure 25:	Cyclin-D1 immune expression in well differentiated endometrial carcinoma	95
Figure 26:	Cyclin-D1 immune expression in well differentiated endometrial carcinoma	95

LIST OF ABBREVIATIONS

AGUS: Atypical glandular cells of undetermined significance
AH: Adenomatous hyperplasia
CAH: Complex hyperplasia with atypia
CH: Complex hyperplasia
CYP: Cytochrome P
DAB H2O2: Diamino Benzadine H2O2
DNA: Deoxyribonucleic acid
EC: Endometrial Cancer
EIN: Endometrial intraepithelial Neoplasia
ER : Estrogen receptor
ERE: Estrogen responsive elements
GPR30: G protein coupled receptor
H&E: Hematoxylin& Eosin
hMLH1: Human-mut-L homologue1
hMSH2: Human-mut-S homologue2
HNPCC: Hereditary non polyposis colorectal cancer
IRS: Immunihistochemical reaction score
MCF-7: Breast Cancer cell line
MIB1 : Molecular immunology Bortel
MMAC1: Mouse monoclonal antibody C1
MSI: Microsatellite Instability
MSS: Microsatellite Stable
MT : Metallothionein
PBS: Phosphate buffered saline
PCNA: Proliferating cell nuclear antigen
PCOS: Polycystic ovarian syndrome
PEPI: Post menopausal estrogen/progestin interventions
PR: Progesterone receptor
SAH: Simple hyperplasia with atypia
SH: Simple hyperplasia
VEGF: Vascular endothelial growth factor
VPS: Volume percentage stroma
WHI: Women health initiative

INTRODUCTION

The normal human endometrium is characterized by hormone-dependent variations during the menstrual cycle. This tightly controlled system is disturbed in endometrial hyperplasia and carcinomas and a series of changes initiate and promote progression towards the malignant phenotype ***(Ioachin, 2005)***.

Estrogen and progesterone are known modulators of endometrial proliferation and differentiation via their receptors. The association between unopposed estrogen - either endogenous or exogenous - and endometrial hyper stimulation is well recognized ***(Disaia, 1997)*.**

Endometrial hyperplasia and endometrial carcinoma are often viewed as points on a continuum, which includes simple and atypical hyperplasia and adenocarcinoma.

Although endometrial hyperplasia is considered to be a precancerous lesion of endometrial adenocarcinoma, and the latter is the most common malignant neoplasm of the female genital tract, the pathogenic relationship has not been analyzed to the same extent as that of cervical carcinoma.

It is now widely accepted that most human cancers arise from a series of genetic alterations in oncogenes and tumor suppressor genes that are associated with progression from normal cells to cancer ***(Pohlod et***

***al., 2002)*.** The molecular events that contribute to the development and progression of the lesion remain poorly understood.

These changes can be subdivided into discrete steps, involving activation of oncogenes, inactivation of tumor suppressor genes deregulation of cell cycle regulators or other proteins involved in tumor invasion and progression. Immunohistochemical expression of different biomarkers such as hormone receptor status (ER, PR), proliferation associated indices (PCNA, MIB1), oncogenes (c-erbB-2), tumor suppressor gene products (pRb, p53 protein), cell cycle related proteins (Cyclin D1, Cyclin E, p21/WAF1) anti-apoptotic protein (bcl-2), adhesion molecule (CD44s), proteolytic enzyme (Cathepsin D), heat shock protein (hsp27) and metallothionein (MT) has shown the contribution of these molecules to endometrial carcinogenesis in a hormone-dependent or independent manner as an early or late event. In addition, these biomarkers seem to be correlated with tumor differentiation or myometrial invasion, and therefore could be considered as indicators of the biological behavior of endometrial carcinoma.

Furthermore, the interrelationships of these molecular markers show that these genetic deregulations could be implicated in the control of cell proliferation and differentiation, and thereby in the multi step process of endometrial carcinogenesis ***(Ioachin, 2005)*.**

Cyclin is a 36 KD nuclear protein, it is necessary with other D type cyclins and cyclin dependent kinases for transition from G1 to S phase

(Peters et al., 1996). Amplification of the cyclin D1 gene locus occurs in many solid tumors.

Leland H. Hartewell , R. Timothy Hunt, and Paul M. Nurse won the 2001 Nobel Prize in physiology for their complete description of cyclin and cyclin-dependant kinase mechanisms, central molecules in the regulation of the cell cycle.**(Journal of the National Cancer Institute;2001).**

The lysosomal acidic protease Cathepsin D, a recognized independent predictor of prognosis in human breast cancer, has not been studied widely in patients with endometrial adenocarcinoma ***(Nazeer et al., 1992).***

AIM OF THE WORK

The aim of the current work is to examine and compare the expression of the markers cyclin D1 and Cathepsin D as well as estrogen receptors as contributing factors to the development of cancer, independent of estrogen effect, in proliferative, secretory endometrium, endometrial hyperplasia and endometrial carcinoma.

ANATOMY AND PHYSIOLOGY OF THE ENDOMETRIUM

The human endometrium is a dynamic tissue that, in response to the prevailing steroid environment of sequential ovarian estrogen and progesterone exposure, undergoes well-characterized cycles of proliferation, differentiation, and tissue breakdown on a monthly basis. If pregnancy fails to be established, then the endometrium is shed and regenerates ***(Jabbour et al., 2006)***.

Menstruation is the reproductive process whereby the upper two thirds of the endometrium (functional layer) is shed and regenerated on a repetitive basis. The endometrium is consequently a site of recurrent physiological injury and repair ***(Critchley et al., 2001)***.

The role of ovarian steroids, estradiol, and progesterone in regulating the changes in endometrial conformation across the menstrual cycle is well-established. Progesterone is essential for the establishment and maintenance of pregnancy consequent upon the transformation of an estrogen-primed endometrium. Sex steroids, acting via their cognate receptors initiate a cascade of gene expression and events crucial for successful implantation and early stages of pregnancy ***(Jabbour et al., 2006)***.

Application of knowledge from the human genome, utilizing microarray technologies, has allowed several groups to contribute to a rapidly expanding literature on gene profiles during the "putative window" of implantation ***(Carson et al., 2002; Kao et al., 2002; Riesewijk et al., 2003)***.

Menstruation is the response of the endometrium to the withdrawal of progesterone (and estrogen) that occurs with the demise of the corpus luteum in the absence of pregnancy. The molecular mechanisms by which sex steroids induce these events within the endometrium at the time of menstruation, involves complex interactions between the endocrine and immune system. Crucial structural components in the endometrium during the menstrual process are the component blood vessels and the dynamic population of leukocytes that influx at this time ***(Critchley et al., 2001)***.

ENDOMETRIAL STRUCTURE

There are three classic phases of the menstrual cycle: an estrogen-dominated preovulatory phase, a postovulatory and progesterone-dominated secretory phase, and a menstrual phase following progesterone withdrawal that accompanies demise of the corpus luteum ***(Buckley & Fox, 1989)***.

The endometrium is composed of two layers and is a target tissue for steroid hormones. The upper functional layer is shed at menstruation. The endometrium regenerates after menstrual shedding from an

underlying basal layer. Estrogen is the steroid responsible for proliferative changes during the follicular phase of the ovarian cycle, and exposure of the endometrium to ovarian progesterone results in differentiation during the secretory phase ***(Jabbour et al., 2006)***.

The progesterone-dominated latter half of the menstrual cycle is constituted by an early, mid, and late secretory phase. The pattern of sex steroid receptor expression in the endometrium across the secretory phase reflects the fact that the early secretory phase is regulated by both estrogen and progesterone; the mid secretory phase is regulated by progesterone alone as estrogen receptor (ER) is down-regulated in the glands and stroma at this time; and the late secretory phase is associated with progesterone withdrawal and, consequently, menstruation ***(Snijders et al., 1992)***.

Evidence that endometrial genes are regulated has been demonstrated by the observed changes in gene expression from late proliferative to mid secretory ***(Kao et al., 2002)*** and early to mid secretory phase ***(Carson et al., 2002)*** in microarray studies. Interestingly, the failure to make a transition in gene expression has been demonstrated in endometriosis with dysregulation of specific genes during the mid secretory phase in this condition ***(Kao et al., 2003)***.

It is notable that the exogenous administration of sex steroids produces a marked modulation of the classic histological features such as the glandular structure, mitotic status of glandular cells, and secretions in