Ain Shams University
Faculty of Science
Biochemistry Department

Human epididymis protein 4 as a novel diagnostic ovarian cancer biomarker: comparison with cancer antigen 125 in Egyptian ovarian cancer patients

A thesis submitted in partial fulfillment of the requirements for M.Sc. degree in Biochemistry

By

Mohamed Hassan Abd El-Magied
B.Sc. in Biochemistry (2008)

Under the supervision of

Prof. Dr. Mohamed Ragaa Mohamed
Professor of Biochemistry and Molecular Biology
Faculty of Science
Ain Shams University

Dr. Amal Fawzy Said
Assistant Professor of Clinical Pathology
National Cancer Institute
Faculty of Medicine - Cairo University

Dr. Mohamed A. M. Ali
Assistant Professor of Biochemistry
Faculty of Science
Ain Shams University

Faculty of Science
Ain Shams University
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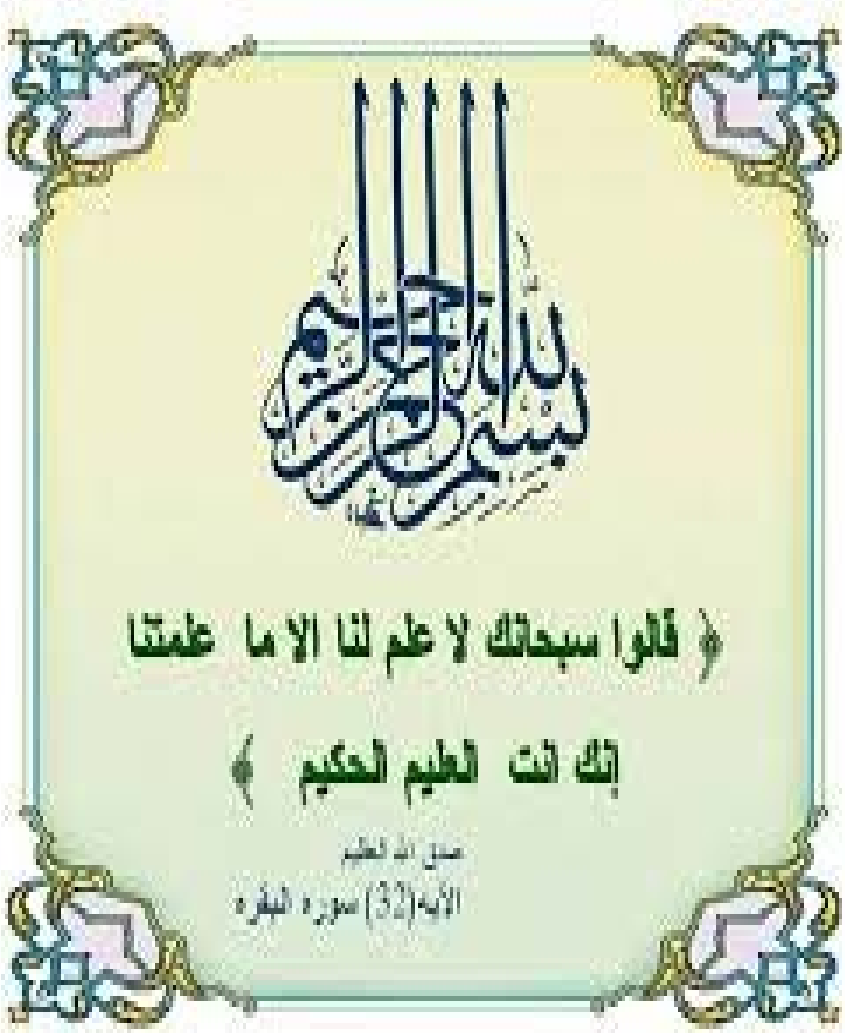
بسم الله الرحمن الرحيم
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This thesis has not been submitted
to this or any other university

Mohamed Hassan Abd El-Magied

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Mohamed Hassan Abd El-Magied

DEDICATION

This thesis is proudly dedicated to my beloved family
(my parents and my wife)

Thanks for your endless love, prayers, sacrifices and support

Mohamed Hassan Abd El-Magied

CONTENTS

LIST of ABBREVIATIONS

Abbreviation		Full name
ARID1A	:	AT-rich interactive domain 1A
AUC	:	area under the curve
β-ME	:	β-mercaptoethanol
BRAF	:	v-Raf murine sarcoma viral oncogene homolog B
BRCA1	:	breast cancer type 1 susceptibility protein
BRCA2	:	breast cancer type 2 susceptibility protein
CA125		cancer antigen 125
CA72-4	:	carbohydrate antigen 72-4
CCC	:	clear cell carcinoma
CMIA	:	chemiluminescent microparticle immunoassay
CT	:	computed tomography
Ct	:	cycle threshold
CTNNB1	:	catenin [cadherin-associated protein] beta 1
DUSP4	:	dual specificity phosphatase, 4
EC	:	endometrioid carcinoma
EGFR	:	epidermal growth factor receptor
EIA	:	enzyme immunoassay
EOC	:	epithelial ovarian cancer
FIGO	:	international federation of gynecology and obstetrics
FISH	:	fluorescence in situ hybridization
GAPDH	:	glyceraldehyde 3-phosphate dehydrogenase
GCIG	:	gynecologic cancer inter-group
HBOC	:	hemoglobin-based oxygen carrier
HE4	:	human epididymis protein 4
HER2/ ERBB2	:	epidermal growth factor receptor 2

HGEC	:	high-grade endometrioid carcinoma
HGSC	:	high-grade serous carcinoma
hMLH1	:	human mutL homolog 1
hMSH2	:	human mutS homolog 2
HNF1	:	hepatocyte nuclear factor 1
HNPCC	:	hereditary non-polyposis colorectal carcinoma
HRD	:	homologous repair deficiency
HRP	:	horseradish peroxidase
HRR	:	homologous recombination repair
IQR	:	interquartile range
KRAS	:	Kirsten rat sarcoma viral oncogene homolog
LGEC	:	low-grade endometrioid carcinoma
LGSC	:	low-grade serous carcinoma
LMP	:	low malignant potential
MAb	:	monoclonal antibody
MAP	:	mitogen-activated protein
mBRCA	:	breast cancer susceptibility gene mutation
MC	:	mucinous carcinoma
MEK	:	mitogen extracellular signal-regulated kinase
MMMT	:	malignant mixed mesodermal tumors
MMR	:	mismatch repair
MPF	:	megakaryocyte potentiation factor
MR	:	magnetic resonance
mTOR	:	mammalian target of rapamycin
MUC16	:	mucin 16
NPV	:	negative predictive value
NRAS	:	neuroblastoma rat sarcoma viral oncogene homolog
OPN	:	osteopontin
p53	:	tumor protein 53
PARP	:	poly (ADP-ribose) polymerase
PAX2	:	paired box gene 2

PCR	:	polymerase chain reaction
PFI	:	platinum-free interval
PI		predictive index
PIK3CA	:	phosphatidylinositol-4,5-bisphosphate 3-kinase catalytic subunit alpha
PP	:	predicted probability
PPP2R1A	:	protein phosphatase 2, regulatory subunit A, alpha
PPV	:	positive predictive value
PTEN	:	phosphatase and tensin homolog
qRT-PCR	:	quantitative real time polymerase chain reaction
RLUs	:	relative light units
RMI	:	risk of malignancy index
ROC	:	receiver operating characteristic
ROMA	:	risk of ovarian malignancy algorithm
RT-PCR	:	reverse transcriptase polymerase chain reaction
RV	:	reaction vessel
SERPINA5	:	serpin peptidase inhibitor, clade A (alpha-1 antiproteinase, antitrypsin), member 5
TGF-β	:	transforming growth factor-beta
TKR	:	tyrosine kinase receptor
TMB	:	3, 3′, 5, 5′-tetramethylbenzidine
UC	:	undifferentiated carcinomas
US	:	ultrasound
VEGF	:	vascular endothelial growth factor
WAP		whey acidic protein
WFDC2	:	whey acidic protein four-disulfide core domain 2
WHO	:	world health organization
ZNF217	:	zinc finger protein 217

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ABSTRACT

Ovarian cancer remains a major worldwide health care issue due to the lack of satisfactory diagnostic methods for early detection of the disease. Prior studies on the role of serum cancer antigen 125 (CA125) and human epididymis protein 4 (HE4) in detecting ovarian cancer present conflicting results. New tools sought to improve the accuracy of identifying malignancy are urgently needed. We aimed to evaluate the diagnostic utility of tissue CA125 and HE4 gene expression in comparison to serum CA125 and HE4 in discriminating benign from malignant pelvic masses. One-hundred Egyptian women were enrolled in this study, including 60 epithelial ovarian cancer (EOC) patients, 20 benign ovarian tumor patients as well as 20 apparently healthy women. Preoperative serum levels of CA125 and HE4 were measured by immunoassays. Tissue expression levels of genes encoding CA125 and HE4 were determined by quantitative real time polymerase chain reaction (qRT-PCR). The diagnostic performance of CA125 and HE4, measured either as mRNA or protein levels, was evaluated by receiver operating characteristic (ROC) curve. Serum CA125+serum HE4 combination and serum HE4 with area under the curve (AUC) values of 0.935 and 0.932, respectively, performed significantly better than serum CA125 alone (AUC=0.592; $P<0.001$). Tissue CA125 and HE4 (AUC=1) performed significantly better than serum CA125 ($P<0.001$), serum HE4 ($P=0.016$) and serum CA125+serum HE4 combination ($P=0.018$). Measurement of tissue CA125 and HE4 gene expression not only improves the discriminatory performance, but also broadens the range of differential diagnostic possibilities in distinguishing EOC from benign ovarian tumors.