

Clinical Utility of Third Trimester's Endocan-1 Levels in Pregnant Women with Diabetes Mellitus

Thesis

*Submitted for Partial Fulfillment of Master Degree
in Clinical Pathology*

By

Mai Mohamed Mohamed Farid

M.B.B.Ch

Faculty of Medicine, Ain Shams University

Supervised by

Professor/ Mona Mohamed Zaki

Professor of Clinical Pathology

Faculty of Medicine, Ain Shams University

Professor / Adel Ahmed Elazab Elged

Professor of Clinical Pathology

Faculty of Medicine, Ain Shams University

Doctor/ Doaa Mostafa Awad

Lecturer of Clinical Pathology

Faculty of Medicine, Ain Shams University

Faculty of Medicine

Ain Shams University

2017



Acknowledgement

*First of all, all gratitude is due to **Allah** for blessing this work, until it has reached its end, as a part of His generous help, throughout my life.*

*Really I can hardly find the words to express my gratitude to **Professor. Mona Mohamed Zaki**, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for her supervision, continuous help, encouragement throughout this work and tremendous effort she has done in the meticulous revision of the whole work. It is a great honor to work under her guidance and supervision.*

*I would like also to express my sincere appreciation and gratitude to **Professor. Adel Ahmed Elazab Elged**, Professor of Clinical Pathology, Faculty of Medicine, Ain Shams University, for his continuous directions and support throughout the whole work.*

*I owe much to **Doctor. Doaa Mostafa Awad**, Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her continues help, valuable suggestions and final revision of the manuscript.*

*Last but not least, I dedicate this work to **my family**, and specially **my dear mother, father and my husband** whom without their sincere emotional support, pushing me forward this work would not have ever been completed.*

 **Mai Farid**

Contents

Subject	Page No.
List of Abbreviations	I
List of Figures	VI
List of Tables	VIII
Introduction.....	1
Aim of the Study	4
Review of Literature.....	
I. Diabetes in pregnancy.....	5
A. Definition.....	5
B. Types	5
C. Prevalence	6
D. Risk Factors of GDM.....	6
E. Dynamics of Glucose and Insulin In Normal Pregnancy	8
F. Pathogenesis of Pregnancy Associated with Diabetes.....	9
G. Complications	17
H. Diagnosis of Diabetes in Pregnancy	29

Contents (Cont.....)

Subject	Page No.
II. Endocan-1	35
A. Introduction.....	35
B. Origin and Chemical Structure of Endocan-1	37
C. Sources and Distribution.....	39
D. Regulation of Endocan-1.....	39
E. Physiological Functions and Binding Properties of Endocan-1.....	43
F. Clinical Utility of Endocan-1	44
G. Assessment of Endocan-1	52
Subjects and Methods	61
Results	77
Discussion	93
Summary	100
Conclusion	103
Recommendations	104
References	105
Arabic Summary	-

List of Abbreviations

Abb.	Full term
2hPP BG	2hour post prandial blood glucose
ACOG	American college of obstetrics and gynecology
ACS	Acute coronary syndrome
ADA	American Diabetic association
Alb/creat ratio	Albumin/creatinine ratio
ALT	Alanine transaminase
AST	Aspartate transaminase
AUC	Area under the curve
BMI	Body mass index
CAD	Coronary artery disease
CBC	Complete blood count
CDKAL1	Cyclin-dependent kinase 5 regulatory subunit-associated protein 1-like 1
CKD	Chronic Kidney disease
CNS	Central nervous system
CS	Chondroitin sulfate
CVD	Coronary vascular disease
DKA	Diabetic ketoacidosis
DM	Diabetes mellitus
DS	Dermatan sulfate
ECAM	Epithelial Cell adhesion molecule

Abb.	Full term
ECM	Extracellular matrix
EGF	Endothelial growth factor
ELISA	Enzyme linked immunosorbent assay
ESM-1	Endothelial cell specific molecule-1
FBG	Fasting blood glucose
FFA	Free fatty acids
FGF-2	Fibroblast growth factor-2
FN	False negative
FP	False positive
FPG	Fasting plasma glucose
GAG	Glycosaminoglycan
GCT	Glucose challenge test
GDM	Gestational diabetes mellitus
GFR	Glomerular filtration rate
GLUT-4	Glucose transporter type 4
HCC	Hepatocellular carcinoma
HDL-C	High density lipoproteins Cholesterol
HELP	Hemolysis, elevated liver enzymes, low platelet count
HGF/SF	Hepatocyte growth factor/scatter factor
HPLC	High performance liquid chromatography
HRP	Horseradish. Peroxidase

Abb.	Full term
HS	Heparan sulfate
HsCRP	Highly sensitive C reactive protein
HSPGs	Heparan sulfate proteoglycans
HTN	Hypertension
HUVEC	Human umbilical vein endothelial cell
ICAM-1	Intracellular adhesion molecule-1
IFG	Impaired fasting glucose
IGT	Impaired glucose tolerance
Il-6	Interleukin -6
IQR	Interquartile range
IRS-1	Insulin receptor substrate-1
IUFD	Intra uterine fetal death
KCNQ1	Potassium channel voltage-gate KQT-like subfamily member 1
KS	Keratan sulfate
LDH	Lactate dehydrogenase
LDL	Low density lipoproteins
LFA-1	Lymphocyte function-associated antigen-1
LGA	Large for gestational age
MDH	Malate dehydrogenase
mRNA	Messenger RNA
MVD	Micro vessel density

Abb.	Full term
NAD	Nicotinamide adenine dinucleotide
NPV	Negative predictive value
OGTT	Oral glucose tolerance test
PBS	Phosphate buffer saline
PCO	Polycystic ovary
PCR	Polymerase chain reaction
PE	Pre-eclampsia
PG	Proteoglycan
PI3K	Phosphatidylinositol 3-kinase
PKB	Protein kinase B
PKC/NF-κB	Protein kinase C/ nuclear factor kappaB
PLT	Platelet
PPH	Postpartum hemorrhage
PPV	Positive predictive value
PTE	Pulmonary thromboembolism
PVDF	Polyvinylidene fluoride
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
RBCs	Red blood cells
ROC	Receiver-operating characteristic
RT-PCR	Reverse transcriptase polymerase chain reaction
SD	Standard deviation

Abb.	Full term
SDS-PAGE	SDS-polyacrylamide gel electrophoresis
SGA	Small for gestational age
SH	Severe hypoglycemia
TCF7L2	Transcription factor 7-like 2
TG	Triglycerides
TGA	Transposition of great arteries
TMB	Tetramethylbenzidine
TN	True negative
TNFα	Tumor necrosis factor - α
TP	True positive
UAE	Urinary albumin excretion
VCAM-1	Vascular cell adhesion molecule-1
VEGF	Vascular endothelial growth factor
VSD	Ventricular septal defect
WBC	White blood cell

List of Figures

Figure No.	Title	Page No.
Fig. (1)	The pathogenesis of GDM	13
Fig. (2)	The role of adiponectin in gestational diabetes	15
Fig. (3)	The role of leptin in gestational diabetes	16
Fig. (4)	Structural determinants and composition of endocan-1	38
Fig. (5)	The regulation mechanism of endocan-1 expression	41
Fig. (6)	Western blot technique for assessment of endocan-1	56
Fig. (7)	ROC curve analysis showing the diagnostic performance of endocan-1 for discriminating group Ia from group Ib	87
Fig. (8)	ROC curve analysis showing the diagnostic performance of Alb/Creat Ratio for discriminating groups Ia from group Ib	88
Fig. (9)	ROC curve analysis showing the diagnostic performance of HBA1c and their combination for discriminating groups Ia from group Ib	89
Fig. (10)	Multi ROC curve analysis showing the diagnostic performance of endocan-1 and its combination with Alb/Creat Ratio for discriminating between groups Ia and group Ib	90

Figure No.	Title	Page No.
Fig. (11)	ROC curve analysis showing the diagnostic performance of Endocan; HBA1c and their combination for discriminating group Ia from group Ib	91
Fig. (12)	ROC curve analysis showing the diagnostic performance of HBA1c and albumin/creatinine ratio for discriminating groups Ia from group Ib	92

List of Tables

Table No.	Title	Page No.
Table (1)	Congenital malformations	26
Table (2)	Criteria for the Diagnosis of Diabetes	31
Table (3)	ACOG and ADA Recommendations for Diagnosing GDM	33
Table (4)	Descriptive and Comparative Statistics of Demographic Data in the Studied Groups using Student's t test for Parametric Data	81
Table (5)	Descriptive and Comparative Statistics of Routine Laboratory tests in the Studied Groups using Student's t test for Parametric Data and Wilcoxon's Rank Sum test for Non Parametric Data	82
Table (6)	Descriptive Statistics of Various Studied Parameters in the Different Studied Groups	83
Table (7)	Descriptive Statistics and Statistical Comparison Between Group Ia and Group II as regards the Various Studied Parameters Using Student's t test for Parametric Data and Wilcoxon's Rank Sum test for Non Parametric Data	83
Table (8)	Descriptive Statistics and Statistical Comparison Between Group Ib and Group II as regards the Various Studied Parameters using Student's t Test for Parametric Data and Wilcoxon's Rank Sum Test for Non Parametric Data	84

Table No.	Title	Page No.
Table (9)	Descriptive Statistics and Statistical Comparison Between Group Ia and Ib as regards the Various Studied Parameters Using Student's t Test For Parametric Data and Wilcoxon's Rank Sum Test for Non-parametric Data	84
Table (10)	Statistical Correlation Between Serum Endocan-1 Level and Different Studied Parameters among Group Ia using Ranked Spearman's Correlation Coefficient Test for Non Parametric data	85
Table (11)	Statistical Correlation Between Serum Endocan-1 Level and Different Studied Parameters among Group Ib using Ranked Spearman's Correlation Coefficient Test for Non Parametric data	85
Table (12)	Statistical Correlation Between Serum Endocan-1 Level and Different Studied Parameters among Group II using Ranked Spearman's Correlation Coefficient Test for Non Parametric data	86
Table (13)	The Diagnostic Performance of Endocan-1 in Discriminating between Group Ia (Pre-existing DM) and Group Ib (GDM)	87

Table No.	Title	Page No.
Table (14)	The Diagnostic Performance of albumin/creatinine ratio in Discriminating Group Ia (Pre-existing DM) from Group Ib (GDM)	88
Table (15)	The Diagnostic Performance of HbA1c in Discriminating Group Ia (Pre-existing DM) from Group Ib (GDM)	89
Table (16)	The Diagnostic Performance of Endocan-1 and albumin/creatinine ratio in Discriminating Group Ia (Pre-existing DM) from Group Ib (GDM)	90
Table (17)	The Diagnostic Performance of Endocan-1 and HbA1c in Discriminating Group Ia (Pre-existing DM) from Group Ib (GDM)	91
Table (18)	The Diagnostic Performance of albumin/creatinine ratio and HbA1c in Discriminating Group Ia (Pre-existing DM) from Group Ib (GDM)	92

Introduction

Diabetes mellitus (DM) is considered one of the most common metabolic complications occurring in pregnancy (*Ben-Haroush et al., 2004*). Both, type 1 and type 2 may be diagnosed before pregnancy and thus called pregestational diabetes. On the other hand, if diabetes is diagnosed for the first time during pregnancy, it is called gestational diabetes (*Rosenstein et al., 2012*). In 2012, Schneider and his co-workers stated that gestational diabetes mellitus (GDM) accounts for about 90% of cases of DM in pregnancy worldwide. They added that pre-existing diabetes accounts for 10 % of such cases. *Kim et al. (2012)* added that first degree relatives with diabetes, increased weight and decreased physical activity were well established risk factors for developing GDM.

Diabetes during pregnancy carries the risk for further complications to both, the mother and the fetus (*Kim et al., 2002*). Maternal complications include pre-eclampsia, hyperglycemic crisis and recurrent urinary tract infection that may progress to pyelonephritis (*Ben-Haroush et al., 2004*). In addition, women with GDM are more likely to develop type 2 DM later in life and have 30-69% higher risk to develop GDM again in future pregnancies