Role of vascular endothelial growth factor receptor and gene polymorphism in preeclampsia

Thesis

Submitted for partial Fulfillment of

M.D. degree in

Clinical and Chemical Pathology

By

Dr. Nesreen Hamdy Mohamed Zawilla

M.B, B.Ch, M.Sc. Faculty of Medicine Cairo University

Supervised by

Prof. Dr. Ahmed El-Sayed El-Taweel

Prof. of Clinical and Chemical Pathology

Faculty of Medicine, Cairo University

Prof. Dr. Amal Abdelwhab Mohamed

Prof. of Clinical and Chemical Pathology

Faculty of Medicine, Cairo University

Prof. Dr. Adel Farouk Ibrahim

Prof. of Gynecology & Obstetrics

Faculty of Medicine, Cairo University

Faculty of Medicine
Cairo University
2011

Abstract

Role of vascular endothelial growth factor receptor and gene polymorphism in preeclampsia

Vascular endothelial growth factor and its receptors may play a pivotal role in the altered function of PE. Many polymorphisms of the VEGF gene have been identified; a few of them have been correlated with variation in VEGF protein production. The aim of this work is to evaluate whether the serum level of soluble VEGF 1 as well as VEGF 936 C/T gene polymorphism are associated with increased risk of preeclampsia. Analysis of serum soluble VEGF-R1 by ELISA and Deoxyribonucleic acid analysis for VEGF gene polymorphism (936C/T) using PCR followed by RFLP was done. a highly statistically significant difference was found between cases and control groups as regards VEGF-R1 serum levels and there was a significant difference in VEGF 936 C/T genotypes between cases and control groups.

Key words : preeclampsia , VEGF gene, VEGF-R1.

ACKNOWLEDGMENT

First and foremost thanks are due to GOD the most beneficial and merciful.

This study was supported and completed with the sincere help of many persons to whom no words of thanks and feelings of gratitude are sufficient.

My first and sincere thanks go to Prof. Dr. Ahmed El-Taweel, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University for granting me privilege of working under his supervision, and for his encouragement, patience and perseverance. His critical evaluations were of great assistance.

I am sincerely indebted to Prof. Dr. Amal Abd El Wahab, Professor of Clinical and Chemical Pathology, Faculty of Medicine, Cairo University, for her great support, unfailing tender advice, and patience in revising the whole study. Major credit of bringing this work to light goes to her.

I am very grateful to Prof. Dr. Adel Farouk, Professor of Gynecology & Obstetrics, Faculty of Medicine, Cairo University, for his help by giving me access to patients in his department.

Eventually I would like to thank all patients who participated in this work wishing them all the best of health.

List of Figures

List of Tables

List of Abbreviations

Abbreviation	Full name
ALT	Alanine transaminase
ANOVA	Analysis of variance
AST	Aspartate transaminase
BP	Blood pressure
CMV	Cytomegalovirus
DBP	Diastolic blood pressure
DDAH	Dimethylarginine dimethylaminohydrolase
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide-triphosphates
EBV	Epstein-Barr virus
EDTA	Ethylenediamine tetra-acetic acid
ELISA	Enzyme linked immune sorbant-assay
eNOS	Endothilial nitric oxide synthase
FIGF	Fos induced growth factor
FLK1	Fetal liver kinase 1
GFR	Glomerular filtration rate
HELLP	Haemolysis, elevated liver enzymes, low platelet count
HSV-2	Herpes simplex virus-2
IgG	Immunoglobulin G
IUGR	Intrauterine growth retardation
KDR	Kinase insert domain receptor
LDH	Lactate dehydrogenase
MgCl2.6H2O	Magnesium chloride hexahydrate
NaCl	Sodium chloride
NaOH	Sodium hydroxide
NK	Natural killer
NO	Nitric oxide
P value	Probability
PCR	Polymerase chain reaction
PDGF	Platelet-derived growth factor
PE	Preeclampsia
PIH	Pregnancy-induced hypertension
PLGF	Placental like growth factor

POEMS	Polyneuropathy, organomegaly, endocrinopathy, monoclonal gammopathy, skin changes
r	Regression coefficient
RFLP	Restriction fragment length polymorphism
ROC	Receiver operating characteristic
SBP	Systolic blood pressure
SDS	Sodium dodecyl sulphate
sEng	Soluble endoglin
sFLt-1	Soluble Fms-like tyrosine kinase-1
SOGC	Society of Obstetricians and Gynecologists of Canada
TBE Buffer	Tris-borate EDTA buffer
TE	Tris-EDTA
TGF	Transformation growth factor
Tris-HCl	Tris – hydrochloric acid
Trisma base	Tris (hydroxy) methyl aminomethane
TXA_2	Thromboxane A_2
VEGF	Vascular endothelial growth factor
VEGF-R1	Vascular endothelial growth factor receptor 1
VEGF-R2	Vascular endothelial growth factor receptor 2
VEGF-R3	Vascular endothelial growth factor receptor 3
VPS	Vascular permeability factor

CONTENTS

Introduction and aim of work

Preeclampsia is a common complication of pregnancy with potentially devasting consequences to both the mother and the baby. It is the leading cause of maternal deaths in developing countries ,in developed countries it is the major cause of iatrogenic premature delivery and contributes significantly to increasing health care cost associated with prematurity ***(Redman et al., 2005).***

There is currently no known treatment for preeclampsia; ultimate treatment involves delivery of the placenta. Although there are several risk factors (such as multiple gestation or chronic hypertension), most patients present with no obvious risk factors ***(Kaufman et al., 2003).***

The molecular pathogenesis of preeclampsia is just being elucidated. It has been proposed that abnormal placentation and an imbalance in angiogenic factors lead to the clinical findings and complications seen in preeclampsia ***(Zhou et al., 2002).***

Vascular endothelial growth factor (VEGF) is a major angiogenic factor and plays an important role as a regulator of endothelial cell proliferation and vascular permeability. Previous studies have shown reduced circulating concentrations of VEGF in preeclamptic patients and increased VEGF expression that is

associated with hypoxia in the placentas of preeclamptic patients. VEGF has also been shown to affect early events in pregnancy, which lead to failed trophoblast invation and placentation ***(Levine et al., 2004).***

The gene encoding VEGF is located on chromosome 6 band p21 and comprises a 14 kb coding region with 8 exons and 7 introns.

Many polymorphisms of the VEGF gene have been identified.

A 936 C/T polymorphism of the VEGF gene was shown to affect VEGF plasma levels, and carriers of a 936 T allele had significantly reduced levels of VEGF ***(Galazios et al., 2009).***

Considering the important role of VEGF in pregnancy, fuctional polymorphisms in the VEGF gene are potentially important as genetic markers of susceptibility of preeclampsia. Based on genetic predisposition, this relationship may be strenghthed by showing an association between genetic polymorphisms of VEGF and an increased risk of developing preeclampsia ***(Geva et al., 2002).***

Also preeclampsia is characterized by high levels of circulating antiangiogenic factors such as soluble vascular

endothelial growth factor receptor 1 (sVEGF-R1) also called fms-like tyrosine kinase-1 (sFLt-1) and soluble endoglin, which induce maternal endothelial dysfunction. These soluble factors are altered not only at the time of clinical disease but also several weeks before the onset of clinical signs and symptoms ***(Maynard et al., 2003).***

Many methods of prediction and surveillance have been proposed to identify women who develop preeclampsia, but studies have been inconclusive. With the recent discovery of the role of angiogenic factors in preeclampsia, novel methods of prediction and diagnosis are being developed to aid obstetricians and midwives in clinical practice ***(Maynard et al., 2003).***

Aim of work

The aim of this work is to evaluate whether the serum level of soluble vascular endothelial growth factor receptor 1 as well as VEGF 936 C/T gene polymorphism are associated with increased risk of preeclampsia.

Chapter 1: Preeclampsia

Hypertension is the most common medical problem encountered during pregnancy, complicating 2-3% of pregnancies. Hypertensive disorders during pregnancy are classified into 4 categories, as reported by the *National High Blood Pressure Education Program Working Group on High Blood Pressure in Pregnancy in 2000:*

1) Chronic hypertension,
2) Preeclampsia,
3) Preeclampsia superimposed on chronic hypertension, and
4) Gestational hypertension (transient hypertension of pregnancy or chronic hypertension identified in the latter half of pregnancy.

The Society of Obstetricians and Gynecologists of Canada (SOGC) released revised guidelines that simplified the classification of hypertension in pregnancy into 2 categories, preexisting or gestational, with the option to add "with preeclampsia" to either category if additional maternal or fetal symptoms, signs, or test results support this ***(Magee et al., 2009).***

Gestational Hypertension

Gestational hypertension is defined as systolic blood pressure ≥140 mmHg and/or a diastolic blood pressure ≥90 mmHg, in the absence of proteinuria, in a previously normotensive pregnant woman at or after 20 weeks of gestation ***(Siabi et al., 2003).***

The blood pressure readings should be documented on at least two occasions at least six hours apart. Gestational hypertension is considered severe when sustained elevations in systolic blood pressure ≥160 mmHg and/or diastolic blood pressure ≥110 mmHg occur for at least six hours ***(Hauth et al., 2000).***

These criteria distinguish gestational hypertension from preeclampsia (proteinuria must be present) and chronic hypertension (hypertension antedates pregnancy or develops before the 20th week of pregnancy) ***(Barton et al., 2001).***

Gestational hypertension is a provisional antenatal term and includes the following mix of patients:

- Women who go on to develop 'preeclampsia'
- Women with 'transient hypertension of pregnancy' (preeclampsia does not develop and blood pressure returns to normal by 12 weeks postpartum).

- Women with previously unrecognized 'chronic hypertension' (blood pressure elevation first detected after the 20th week of pregnancy and persists ≥12 weeks postpartum).

Thus, the diagnosis of gestational hypertension is a temporary one and should only be used during pregnancy in women who do not meet criteria for preeclampsia or chronic hypertension. Reassessment up to 12 weeks postpartum is necessary to establish a final definitive diagnosis ***(Nicholson et al., 2006).***

Gestational hypertension progresses to overt preeclampsia in approximately 10% to 25% of cases. When gestational hypertension is severe, it carries similar risks for adverse outcomes as preeclampsia, even in the absence of proteinuria. A renal biopsy study suggests that a large proportion of women with gestational hypertension have renal glomerular endothelial damage. Hence, gestational hypertension may share the same pathophysiologic criteria as preeclampsia and should be monitored and treated as such ***(Hedderson et al., 2008).***

In a subset of women with gestational hypertension, it may represent a temporary unmasking of an underlying predisposition toward chronic hypertension. Such women often present with a strong family history of chronic hypertension and develop hypertension in the third trimester with a low uric acid and no proteinuria. Although the hypertension often resolves