

Protein Z gene polymorphisms & protein Z plasma levels in preeclamptic patients

Thesis

**Submitted For Partial Fulfillment of
M.D. degree in Clinical and Chemical Pathology**

By: Mona Abdel Rahman Talaat Ibrahim
M.B.B.ch, M.Sc of clinical pathology

Supervisors

Dr. Hanaa Hamed Arnaout

Professor of Clinical Pathology
Faculty of Medicine Cairo University

Dr. Shahira Amin Zayed

Professor of Clinical Pathology
Faculty of Medicine Cairo University

Dr. Iman Maher Mansour

Professor of Clinical Pathology
Faculty of Medicine Cairo University

Dr. Soumaya Mohamed Hassan Abou Elew

Professor of Gynecology and Obstetric
Faculty of Medicine Cairo University

**Faculty of Medicine
Cairo University**

2009

بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ

”سبحانك لا علم لنا الا ما علمتنا

انك انت العظيم الحكيم“

صدق الله العظيم

سورة البقرة آية 31

Abstracts

Protein Z (PZ) is a 62 kDa vitamin K-dependant glycoprotein, synthesized by the liver. PZ has shown to possess procoagulant as well as anticoagulant properties. First PZ enhance the assembly of thrombin with phospholipids surface thus enhancing coagulation. Secondly, it is responsible for binding of PZ dependant protease inhibitor to factor Xa and therefore indirectly act as a natural anticoagulant. High PZ plasma levels may represent a prothrombotic conditions in preeclamptic patients. Thus, estimation of PZ plasma levels is of utmost importance for early diagnosis of preeclampsia before 20th week of gestation. The (A) allele of an intron F polymorphism appears to be a novel protective genetic marker for the risk of preeclampsia being associated with low PZ plasma levels.

Key words:

Protein Z, polymorphisms, preeclampsia

Acknowledgment

I am greatly honored to express my deepest thank and gratitude to **Dr. Hanaa Hamed Arnaout**, Professor of Clinical Pathology, Cairo University, for her constant guidance and unlimited generosity and effort that only a mother could give.

I also, wish to extend my sincere gratitude and appreciation to **Dr. Shahira Amin Zayed**, Professor of Clinical Pathology, Cairo University, for her valuable aid, supervision and encouragement.

I am also deeply indebted and privileged by the supervision of **Dr. Iman Maher Mansour**, Professor of Clinical Pathology, Cairo University.

I wish also to express my deepest thanks to **Dr. Soumaya Mohamed Hassan Abou Elew**, Professor of Gynaecology and Obstetric, Cairo University

I would also like to express my gratefulness and true appreciation to **Dr. Rania Mohamed Fawzy Hammod**, Assistant professor of Clinical Pathology, Cairo University, for her kind help, guidance and patience all through out the preparation of this work.



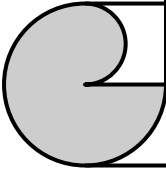
Dedication

First of all, I kneel down
before Allah for
accomplishing this study.

To my mother:

I present every word of this
study which would not have
been accomplished without her
great assistance.

I have every reason to be
grateful. I am and will
always be.



Contents

SUBJECT	pages
▪ INTRODUCTION AND AIM OF THE WORK	1
▪ REVIEW OF THE LITERATURE	4
• Chapter 1 (Haemostasis in normal and complicated pregnancy)	
- Haemostasis in normal pregnancy.	4
- Changes in natural anticoagulant during pregnancy.	17
- Platelet changes during pregnancy.	23
- Placental haemostasis.	25
• Chapter 2 (Protein Z in normal and complicated pregnancy)	
- Protein Z in normal and complicated pregnancy.	31
- Factors affecting PZ expression.	40
- PZ genes polymorphisms and association with its plasma levels.	52
• Chapter 3 (Preeclampsia)	
- Preeclampsia.	57
-Theories of etiological cause of Preeclampsia.	62
- Pathogenesis.	68
- Pathophysiology.	72
- Prediction and prevention of Preeclampsia.	88

▪ SUBJECT AND METHODS	
- Subjects	93
- ELISA	96
- PCR- RFLP	102
▪ RESULTS	113
▪ DISCUSSION	161
▪ SUMMARY AND CONCLUSION	173
▪ REFERENCES	178
▪ ARABIC SUMMARY	

List of tables

Number	Title	Page
1	Haemostatic changes during pregnancy.	29
2	Human anticoagulant proteins and their inhibitors.	33
3	Nucleotide sequence and type of the splice junctions and size of exons.	38
4	Polymorphisms identified in the PZ gene, method of detection, and frequency.	53
5	Mean PZ plasma levels according to gene polymorphisms identified.	56
6	Diagnosis of Hypertensive Disorders Complicating Pregnancy.	58
7	Indications of severity of hypertension disorders during pregnancy.	59
8	Causes of thrombophilia in pregnancy.	79
9	Calibration of PZ.	99
10	Assay procedure of PZ.	100
11	Individual data of control group.	113
12	Individual data of mild preeclamptic group.	115
13	Individual data of severe preeclamptic group.	116
14	Individual data of total preeclamptic group.	117
15	Descriptive statistics of clinical and laboratory data and PZ plasma level of the control group.	122
16	Descriptive statistics of clinical and laboratory data and PZ plasma level of mild preeclamptic patients.	124

17	Descriptive statistics of clinical and laboratory data and PZ plasma level of severe preeclamptic group.	126
18	Descriptive statistics of clinical and laboratory data and PZ plasma level of total preeclamptic group.	128
19	Frequency of primipara and multipara among preeclamptic patients and control group.	129
20	Patients characteristics and laboratory results of Patients and Controls.	133
21	Individual comparisons between mild preeclamptic patients and control with regards to age, clinical and laboratory findings and PZ plasma level.	140
22	Individual comparisons between severe preeclamptic patients and control group as regard the age, clinical and laboratory findings and PZ plasma level.	143
23	Individual comparisons between mild and severe preeclamptic patients as regard the age, clinical and laboratory findings, and PZ plasma levels.	147
24	Level of protein Z as regard min., max., mean, SD, P-value in preeclamptic patients and controls	149
25	Frequency and odd ratio of protein Z intron F G79A and promoter A-13G polymorphism; in preeclamptic patients and controls.	155
26	Association between PZ intron F G79A and promoter A-13G polymorphism genotypes in preeclamptic patients and controls and corresponding PZ plasma level in µg/L.	157
27	Correlations between Protein Z and other variables.	159
28	Mean Protein Z level in different grades of albuminuria.	160

List of figures

Number	Title	page
1	The involvement of blood vessels, platelets and blood coagulation in haemostasis.	6
2	The formation and stabilization of fibrin.	7
3	The classical model of blood coagulation involving a cascade of zymogen activation.	9
4	Activation and action of protein C by thrombin which has bound to thrombomodulin on the endothelial cell surface.	13
5	Activated protein C inactivates factor Va by proteolytic cleavage at three sites in the Va heavy chain. (b) In the factor V Leiden mutation the Arg506Gln polymorphism leads to glutamine at position 506 with less efficient inactivation of factor V and increased risk of thrombosis.	14
6	The action of heparin.	16
7	Fibrinolytic system, tPA: tissues plasminogen activator.	19
8	Mechanism of thrombin-activatable fibrinolysis inhibitor (TAFI) effects toward fibrinolysis.	21
9	Causes of thrombocytopenia during pregnancy.	24
10	Thromboelastography (TEG).	30
11	Two pathways for the inhibitions of factor Xa by protein Z-dependent protease inhibitor (ZPI) with protein Z (PZ).	32
12a	PZ crystalloid structure.	34
12b	PZ chemical structure.	35
13	Master Map: Ideogram of protein Z.	36

14	Organization and SacI restriction map of the gene for human PZ.	36
15	Location of introns in the structure of human protein Z.	37
16	Nucleotide sequence of cyclic DNA coding for PZ and the predicted amino acid sequence.	39
17	Immunofluorescent microscopy of cells stably expressing PZ	43
18	Immunolocalization of PZ in human umbilical vein endothelial cells (HUVEC) and in the endothelium of arterial and venous vessel sections.	47
19	Normal placental implantation shows proliferation of extravillous trophoblasts, forming a cell column beneath the anchoring villous.	63
20	Atherosclerosis is demonstrated in this blood vessel from the placental bed (left, photomicrograph; right schematic diagram of vessel).	64
21	Pathophysiological considerations in the development of hypertensive disorders due to pregnancy.	66
22	(Top) Flt1 on endothelial cells with few sFlt1 in the circulation. Agonists VEGF and PlGF can occupy Flt1. (Bottom) Vessel from preeclamptic patient with abundant sFlt1 that are able to catch VEGF and PlGF so that the growth factors cannot occupy Flt1 on the cell surface.	72
23	Left shows normal placentation and vessel formation. PlGF signals <i>via</i> Flt1.	73
24	Frequency of primipara and multipara among preeclamptic patients and control group.	129

25	Mean systolic and diastolic blood pressures in preeclamptic patients and control.	134
26	Platelet count in preeclamptic patients and control.	134
27	Mean AST in preeclamptic patients and control.	135
28	Mean ALT in preeclamptic patients and control.	135
29	Mean PT in preeclamptic patients and control.	136
30	Mean PC in preeclamptic patients and control.	137
31	Mean PZ level in preeclamptic patients and control.	150
32	Mean PZ level in mild, severe preeclampsia and controls.	150
33	RFLP analysis of PZ gene polymorphism (FG79A) PCR products.	151
34	RFLP analysis of PZ gene polymorphism (A-13G) PCR products.	152
35	RFLP analysis of PZ gene polymorphism (A-13G), (FG79A) PCR products.	153
36	Mean PZ level in relation to FG79A in preeclamptic patients and controls.	158
37	Mean PZ level in relation to A-13G in preeclamptic patients and controls,	158
38	Mean PZ level in relation to different degree of albuminuria in preeclamptic patients.	160

List of abbreviations

ACL	Anticardiolipin antibodies
ACE	Angiotensin-converting enzyme
ALT	Alanine aminotransferase
ADMA	Asymmetric dimethyl arginine
ANP	Atrial natriuretic peptide
APA	Antiphospholipid antibodies
APC	Activated protein C
APC-APTT	Activated protein C – Activated partial thromboplastin time
APCR	Activated protein C resistance
ASE	Age stable element
AST	Aspartate aminotransferase
AT-1 AA	Angiotensin 1 autoantibody
ATP	Adenosine triphosphate
ATIII	Antithrombin III
bp	Base pair
BP	Blood pressure
C4B	Complement 4 binding protein
DIC	Disseminated intravascular coagulopathy.
DOC	Deoxycorticosterone
EGF	Epidermal growth factor
ELISA	immunosorbent assay
ET-1	Endothelin-1
FDP	Fibrin / fibrinogen degradation products
FVL	Factor V Leiden

GCX	gamma- glutamyl caboxylase
Gla	Glutamic acid
HELLP	Haemolysis, elevated liver enzyme and low platelet count.
HMWK	High molecular weight kininogen
HNF4α	Hepatocytes nuclear factor 4
HESC	Human endometrial stromal cells
His	Histidine
IL-1	Interleukin-1
IUGR	Intrauterine growth retardation
KD	Kilodalton
LA	Lupus anticoagulant
LDH	Lactate dehydrogenase
Leu	Leucine
MP	Microparticles.
MT	Mutant type.
MTHR	Methylene tetrahydrofolate reductase
NHBPEP	National High Blood Pressure Education Program
PAP	Plasmin antiplasmin
PAI	Plasminogen activator inhibitor
PC	Protein C
PC	Prothrombin concentration
PGG2	Prostaglandin G2
PGH2	Prostaglandin H2
PGI	Endothelial prostacyclin
PIGF	placental growth factor

PS	Protein S
PSGL-1	P- selectin glycoprotein- 1
PT	Prothrombin time
PZ	Protein Z.
RFLP	Restriction fragments length polymorphism.
ROS	Reactive oxygen radicles
Ser	Serin
sFlt	Serum fms like tyrosine kinase
TAFI	Thrombin activatable fibrinolytic inhbitor
TAT	Thrombin antithrombin
TF	Tissue factor
TFPI	Tissue factor pathway inhibitor
ThSR	Thrombin sensitive region
TM	Thrombomodulin
TNF	Tumor necrosis factor
TPA	Tissue plasminogen activator
Trp-Arg-Arg-Tyr	Tryptophan-Arginine-Arginine-Tyrosine
TTP	Thrombotic thrombocytopenic purpura
TXA2	Thromboxan A2
UPA	Urokinase type plasminogen activator
VEGF	vascular endothelial growth factor
VTE	Venous thromboembolism
UTR	Untranslated region
VWF	vonWillebrand factor
WT	Wild type
ZPI	Protein Z dependent protease inhibitor.