

Dedication

This work is dedicated to:

My father and mother

Thank you for always keeping me in our prayers, believing in me and supporting my decisions.

My wife and daughter

Which were realized by our loss of precious time together, were for me the most painful.

My sisters and brothers

For taking me in and providing the love, support and comfort that can only come from family

Our friends and colleagues

The people who are help me, to perform this work

To all of them I dedicate this work

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Asaad.M.Ahmed

Abstract

The current study is a prospective analytical case control study designed to investigate the relationship between Factor V Leiden G1691A, methyleneterhydrofolate reductase (MTHFR) C677T and to the prothrombin G20210A mutation variant and adverse pregnancy outcomes. The study included hundred Sudanese women who experienced three or more of the adverse pregnancy loss as case group during their reproductive in the Omdurman Maternity Hospital (Sudan) these compared with ninety four control group healthy women with at least more than two normal pregnancies and without any history of adverse pregnancy outcome or recurrent miscarriages during the period from July 2012 to June 2014. The study group data collected using structure questionnaire which was used to collect information about age, parity, medical and obstetric history, smoking, family medical and obstetric history, residency and relative marriage. Blood samples were collected from participants and total genomic DNA was isolated from blood leukocytes and the frequency of these gene mutations in the patients and controls were determined using PCR-restriction fragment length polymorphism then followed by Blood group, PT and PTT.

Results showed: The mutation was detected in 8 out of 100 cases (8.0%) and in 6 out of 94 controls (6.4%) (P- Value > 0.05). In study subjects, the general mutation prevalence of the Prothrombin gene was 3% among cases with (P- Value > 0.05). And there was no any mutant gene detected among control group. The frequency of Heterozygous A/C MTHFR gene was 3.0% in cases with (P- Value > 0.05), there was no mutant gene detected among the controls group. Results showed no significant variations in factor V Leiden, prothrombin G20210A and MTHFR C677T gene mutation distribution among women with RSA and controls.

Prothrombin time (PT) and partial thromboplastin time (PTT) in women RSA in this study were not affected significantly (P > 0.05 and P > 0.05) respectively.

In conclusion the study observed that FV Leiden, FII G20210A mutation and MTHFR C677T do not associated with recurrent spontaneous abortion.

المستخلص

الدراسة الحالية هي دراسة متقدمة أجريت كشاهد لكشف علاقة عن هذه الطفرات الجينية لعامل التخثر الخامس V ليدن ,وجين مختزلة ميثيلين تتروهيدروفولات (C 677T), وجين البروثرومبين (G20210A) مع حدوث نتائج الحمل السلبية (مضاعفات الحمل العكسية), شملت الدراسة مائة امرأة سودانية من اللاتي عانين من ثلاث فاكثر من حالات الاجهاض المتكرر خلال الفترات الانجابية لهن في مستشفى الولادة ام درمان (السودان), وتمت مقارنة النتائج مع اربعة وتسعين امرأة سودانية صحية خضعت كل واحد منهن على الاقل لاكثر من حالتين ولادة طبيعية ولم يكن لهن أي تاريخ لتعرضهن لحالات اجهاض متكرر او نتائج للحمل السليبي واللاتي اعتبرن كشاهد في هذه الدراسة, وقد تمت هذه الدراسة في الفترة من شهر يوليو 2012 الى شهر يونيو 2014, وقد تم جمع البيانات باستخدام الاستبيان الهيكلي لجمع المعلومات حول العمر, عدد مرات الانجاب, والتاريخ الطبي للولادة, ثم تم استخلاص الحمض النووي من كل عينة, وتم الكشف عن هذه الطفرات الجينية باستخدام سلسلة التفاعل المبلمر (PCR), وكذلك اختبار تحديد فصائل الدم لكل عينة للمجموعتين المرضية والسليمة, وكذلك قياس وقت تخثر البروثرومبين, ووقت تخثر الثرومبولاستين. ووفقا لذلك تحصلنا على النتائج الاتية:

فقد تم ايجاد طفرة عامل التخثر الخامس V ليدن G1691A في 8 حالات من اصل 100 حالة من النساء اللتي عانين من حالة الجهاض المتكرر و6 من اصل 94 من السليمات وكانت القيمة الاحصائية هي $P > 0.05$ للنساء اللاتي لم يعانين من أي حالة من الاجهاض المتكرر, وكذلك كان معدل انتشار طفرة جين البروثرومبين (G20210A) بنسبة 3% من الحالات المرضية وكانت القيمة الاحصائية هي $P > 0.05$, ولم تسجل له أي وجود في عينات الحالات السليمة, وايضا اظهرت النتائج معدل انتشار الطفرة في جين مختزلة ميثيلين تتراهيدروفولات (C677T) بنسبة 3% من الحالات المرضية للنساء وكانت القيمة الاحصائية هي $P > 0.05$ ولم يتم الكشف لوجود هذه الطفرة في عينات الحالات السليمة, ووجد ايضا ان النساء اللاتي يعانين من الاجهاض المتكرر ان زمن تخثر المنشط الجزئي الثرومبولاستين وزمن تخثر البروثرومبين قد سجلا قيمتان في المدى الطبيعي لهما وكانت $P > 0.05$, حيث تبين من التحليل الاحصائي لنتيجة الاختبارين أنهما لايتأثران بحالات الاجهاض المتكرر او نتائج الحمل السليبي.

وفي ختام دراستنا وُجد ان الطفرات الجينية لعامل التخثر الخامس V ليدن G1691A, والبروثرومبين (G20210A), وجين مختزلة ميثيلين تتراهيدروفولات (C677T), لا تشكل خطرا مؤثرا يودي لحدوث حالات الاجهاض التلقائي المتكرر لدى النساء خلال الفترات الانجابية.

List of contents

Contents	Page
Dedication	I
Acknowledgements	II
Abstract (English)	III
Abstract (Arabic)	VI
List of contents	VI
List of Tables	X
List of Figures	XI
Abbreviations	XII

Chapter One

	Title	Page
1.1	Introduction	1
1.2	literature review	3
1.2.1	pregnancy	3
1.2.1.1	Physiological and hemostatic change during pregnancy	4
1.2.1.2	Abnormal Pregnancy	5
1.2.1.2.1	Ectopic pregnancy	5
1.2.1.2.2	A Molar Pregnancy	6
1.2.1.2.3	A stillbirth	7
1.2.1.2.4	An an embryonic gestation	7
1.2.2	Recurrent Spontaneous abortion	8
1.2.2.1	Stages and Types of Spontaneous Abortions	9
1.2.2.2	Incidence of recurrent Spontaneous abortion	11

1.2.2.3	Epidemiology of recurrent Spontaneous Abortion	11
1.2.2.4	Etiology of Recurrent spontaneous abortion	14
1.2.2.4 .1	Parental Factors	15
1.2.2.4.1.1	Chromosomal Abnormality	15
1.2.2.4 .2	Maternal Factors	15
1.2.2.4 .2 .1	Age	15
1.2.2.4 .2 .2	Endocrinological Factors	16
1.2.2.4 .2 .3	Anatomic Factors	17
1.2.2.4 .2 .4	Immunological Factors	17
1.2.2.4 .3	Infections	18
1.2.2.4 .2 .1	Toxoplasmosis	19
1.2.2.4 .2 .2	Cytomegalovirus (CMV)	19
1.2.2.4 .2 .3	Rubella	20
1.2.2.4 .4	Thrombophilias	20
1.2.2.4 .4 .1	Inherited thrombophilia	21
1.2.2.4 .4 .1.1	Prothrombin gene mutation (G20210 mutation)	22
1.2.2.4 .4 .1.2	Factor V Leiden mutation	23
1.2.2.4 .4 .1.3	Methylene tetrahydrofolate reductase deficiency	24
1.2.2.4 .4 .1.4	Factor XII	26
1.2.2.4 .4 .1.5	Protein C and Protein S deficiencies	26
1.2.2.4 .4 .1.6	Antithrombin III deficiencies	28
1.2.2.4 .4 .1.7	Plasminogen Activator Inhibitor 1 (PAI1)	29
1.2.2.4 .4 .2	Acquired thrombophilia	29
1.2.2.4 .4 .2.1	Acquired hyperhomocystinemia	30
1.2.2.4 .4 .2.2	Acquired activated protein C resistance	30
1.2.2.4 .4 .2.3	Antiphospholipid syndromes	30
1.2.2.4 .4 .2.4	Disseminated intravascular coagulation (DIC)	31
1.2.2.4 .5	Fetal Factors	32
1.2.2.4 .5.1	Fetal-blocking antibodies	32
1.2.2.4 .5.2	Umbilical cord abnormalities	32

1.2.2.4 .6	Environmental Factors	33
1.2.2.4 .7	Stress factor	33
1.2.2.4 .8	Alcohol and smoking	34
1.2.2.5	Signs and Symptoms of 1.3.4	34
1.2.2.5.1	The followings are considered the main signs	34
1.2.2.5.2	Other possible signs include	35
1.3	Rationale	36
1.4	Hypothesis	37
1.5	Objectives	37
1.5.1	General objective	37
1.5.2	Specific objective	37

Chapter Two		
2.0	MATERIALS AND METHODS	38
2.1	Materials	38
2.1.1	Study area	38
2.1.2	Study Setting	38
2.1.3	Study design	38
2.1.4	Study population	39
2.1.5	Controls	39
2.1.6	Inclusion Criteria	39
2.1.7	Exclusion criteria	39
2.1.8	Sample size	39
2.1.9	Data Collection	39
2.2	Method	40
2.2.1	Sample collection	40
2.2.2	Hemostatic laboratories analysis	40
2.2.2.1	Preparation of platelets poor plasma	40
2.2.2.2	Estimation of Prothrombin Time	40
2.2.2.3	Estimation of Partial Prothrombin Time	40

2.2.3	Molecular analysis	40
2.2.3.1	Blood collection and DNA extraction	41
2.2.3.1.1	GF-1BloodDNA Extraction Kit	41
2.2.3.1.2	Blood Lysis	41
2.2.3.1.3	Removal of RNA	41
2.2.3.1.4	Addition of ethanol	41
2.2.3.1.5	Loading to column	42
2.2.3.1.6	Column washing 1	42
2.2.3.1.7	Column washing 2	42
2.2.3.1.8	DNA elution	42
2.2.3.1.9	DNA Quantification	42
2.2.3.2	Polymerase chain reaction	42
2.2.3.2.1	PCR Components	42
2.2.3.2.2	General PCR Steps	43
2.2.4	Detection of prothrombin gene mutation	43
2.2.4.1	Agarose gel electrophoresis	44
2.2.4.2	Digestion	44
2.2.4.3	Detection of fragments	44
2.2.4.4	Result and interpretation	44
2.2.5	Detection of Factor V Leiden gene mutation	44
2.2.5.1	Agarose gel electrophoresis	44
2.2.5.2	Digestion	45
2.2.5.3	Detection of fragments	45
2.2.5.4	Result interpretation	45
2.2.6	Detection of MTHFR Gene Mutations	45
2.2.6.1	Method of PC	45
2.2.6.2	Agarose gel electrophoresis	45
2.2.6.3	MTHFR digestion	46
2.2.6.4	Result interpretation	46
2.3	Data analysis	46

Chapter Three		
3	RESULTS	47
Chapter Four		
4	Discussion ,Conclusion and Recommendations	57
4.1	Discussion	57
4.2	Conclusion	65
4.3	Recommendations	66
	REFERENCES	67
APPENDICES		
I	Questionnaire	86
II	GF-1 Blood DNA Extraction Kit	88
III	Master Mix preparation	91

List of Tables

Title	page
Table 3.1 Distribution of Study Subjects according to demographic characteristics	49
Table 3.2 Average distribution of PT and PTT in patient and control groups	49
Table 3.3 Frequency of factor V (Leiden) mutation among cases of recurrent pregnancy loss compared to controls	50
Table 3.4 Frequency of Prothrombin mutation among cases of recurrent pregnancy loss compared to controls	50
Table 3.5 Frequency of MTHFR mutation among cases of recurrent pregnancy loss compared to controls	51
Table 3.6 Frequency of factor V (Leiden), Prothrombin and MTHFR related to times of recurrent pregnancy loss	52

List of Figures

Title	page
Figure: 1.2 Showing of Khartoum state map	38
Figure 3.1 Showing of Distribution of recurrent misscreage cases according to age groups	53
Fig 3.2 Showing of PCR amplification of FVL gene mutation	54
Fig 3.3 Showing of PCR amplification of Prothrombin gene mutation	55
Fig 3.4 Showing of PCR amplification of MTHFR gene mutation	56

List of abbreviations

aPC	Activated protein C
APCR	Activated protein C resistance
APL	Antiphospholipid antibodies
APS	Anti phospholipid Syndrome
AVP	Abortion-Very-Preterm-birth
BB	Blocking buffer
bp	Base pair
BV	bacterial vaginosis
Ca Cl ₂	Calcium chloride
CBS	cystathionine beta synthase
CMV	Human cytomegalovirus
CRS	Congenital rubella syndrome
CRH	Corticotropin-releasing hormone
CYP1A2	Cytochrome P450 1A2
DDIA	Dipstick dye immunoassay
DHEA-S	dehydro epiandrosterone sulfate
DIC	Disseminated intravascular coagulation
DNA	Deoxyribonucleic Acid
DNase	deoxyribonuclease
dNTPs	Deoxynucleoside triphosphate
DVT	Deep vein thrombosis
dTMP	Deoxythymidylate
EDTA	Ethylene diamine tetra acetic acid
dUMP	Deoxyuridylate
ELISA	Enzyme linked immunosorbent assay
FAS	Fetal Alcohol Syndrome
FVL	Factor V Leiden mutation
F11	Factor two
dGTP	Deoxy guanine triphosphate

GnRHa	Gonadotropin-releasing hormone analog
hCG	Human chorionic gonadotropin
HHCY	Hyperhomocystinemia
HLA	Human leukocyte antigen
HPA	hypothalamus-pituitary-adrenals
HSV	Herpes simplex virus
hMG	Human menopausal gonadotropin
IgG	Immunoglobulin G
IgM	Immunoglobulin M
IUFD	Intra-uterine fetal deaths
IUGR	Intrauterine growth restriction
IVF	In vitro fertilization
KD	Kilo Dalton
LAT	Latex agglutination test
LH	luteinizing hormone
Mgcl ₂	Magnesium Chloride
MGD	Mean Gestational Diameter
MTHFR	Methylene tetrahydrofolate reductase
LMWH	Low-molecular-weight Heparin
NTD	Neural tube defects
O.D	Optical Density
PAL-1	Plasminogen activator inhibitor
PAR-1	Protease – activated receptor -1
PC	Protein C
PCOS	Polycystic Ovary syndrome
PCR	Polymerase chain reaction
PE	Preeclampsia
PGD	Preimplantation genetic diagnosis
PGS	Preimplantation genetic screening
PS	Protein S

PT	Prothrombin time
PTR	Prothrombin ratio time
PTT	Partial thromboplastin time
PVN	paraventricular nucleus
RCT	Randomized controlled trail
RFLP	Restriction fragment Length Polymorphism
RPL	Repeated pregnancy loss
RNase	Ribonuclease
RPOC	retained products of conception
RSA	Recurrent spontaneous abortion
RM	Recurrent miscarriage
SDS	Sodium Dodecyl Sulfate
SNPs	single-nucleotide polymorphisms
dTT	Deoxy Thymine Triphosphate
TBE	Tris-borate
TE	Thromboembolic
TE buffer	Tris-EDTA buffer
Th-1	T- helper -1
Th-2	T-helper-2
TMB	Tetramethylbenzidine
TNF α	Tumor necrosis factor alpha
t PA	tissue plasminogen activator
UV	Ultraviolet
TPO	thyroid peroxidase
Va	Factor V Activated
VIIIa	Factor VIII Activated
VTE	Venous thrombotic embolism
WHO	World Health Organization