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 مستشفي الولادة ام درمان (السودان),وتمت مقارنة النتائج مع اربعه وتسعين امرأة سودانية صحية خضعت كل واحده منهن على الاقل لاكثر من حالتين ولادة طبيعية ولم يكن لهن أي تاريخ لتعرضهن لحالات اجهاض متكرر او نتائج للحمل السلبي 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 الثرومبوبلاستين . 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 اللائي يعانين من الاجهاض المتكرر ان زمن تخثرالمنشط الجزئي الثرومبوبلاستين وزمن تخثر البروثرومبين قد سجلا قيمتان في المدي الطبيعي لهما وكانت P > 0.05 ,حيث تبين من التحليل الاحصائي لنتيجة الاختبارين أنهما لايتأثران بحالات الاجهاض المتكرر او نتائج الحمل السلبي .

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

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Chapter One

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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 bair
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	Deoxynuclioside 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
TNFα	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