The Frequency of Factor V Leiden Mutation among Sudanese Pregnant Women with Recurrent Miscarriage

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Abstract: The purpose of this study was to determine the prevalence and analysis of factor V Leiden G1691A (Leiden mutation) among Sudanese women with recurrent abortions; further, to identify a subgroup at higher risk of being carriers of these mutations. Design**:** A prospective analytical case controls study between 2012 and 2014.Setting: Omdurman Maternity Hospital (Sudan- Omdurman).Patient(s): Hundred women with 3 or more consecutive miscarriages were reported at 94 controls.Materials and Methods**:** Between July 2012 and June 2014, in a nested case control study, pregnant women with recurrent miscarriages (N=100) as cases and health (N=100) as controls were enrolled in the study. DNA was extracted from 15 CC peripheral bloods and analyzed for the presence of factor V Leiden mutation in these subjects. Result(s): In total, 8(8.6%) of cases and 6(6%) of controls showed the factor V Leiden.

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1. Introduction

Recurrent pregnancy loss (RPL) is defined as two or more consecutive pregnancy losses before twenty weeks of gestation, which affects 1-3% of couples.(1) Thrombophilia is considered still a debated question that may be common in women with unexplained recurrent pregnancy loss, with prevalence as high as 65% in selected populations **(**2,3). The thrombophilias are a number of prothrombinase factors, which can either be inherited or acquired. The inherited thrombophilias include activated protein C resistance [95% due to factor V Leiden (FVL) mutation], protein S deficiency, protein C deficiency, antithrombin III deficiency, FII (prothrombin) mutation and hyper homocysteinaemia (4,5). Pregnancy loss is a common medical problem among reproductive age women. 1 However, relatively few women having one pregnancy loss experience multiple or "recurrent" pregnancy losses (RPLs). Approximately 5% of such women expert-ence of a second pregnancy loss and only 1–2% three or More.1evaluation for RPL often includes ruling out parental chose measure abnormalities, identifying maternal exposures, and testing for underlying maternal conditions.(6,7) Factor V Leiden (FVL) and prothrombin (G20210A) mutations are the 2 most common causes have been implicated as risk factors of hereditary thrombophilias which in turn can result in placenta Ation. Factor V Leiden mutation in the pathogenesis of preeclampsia syndrome among the pregnant population of northern shore of Persian Gulf in Iran and concluded that the pregnant women with factor V Leiden mutation are prone for preeclampsia syndrome during pregnancy, but this Risk factor was not correlated to pregnancy complications in the studied women.(6) The FVL3 allele is linked to pregnancy complications and adverse outcomes and conclude that the FVL mutation is a significant risk factor for pregnancy complications and adverse outcomes.(7)

Factor V Leiden mutation is a result of an amino acid substitution of glutamine for Arginine at amino acid position 506 in the factor V molecule. During normal clotting activated protein C (APC) inactivates factor Va and VIIIa by cleavage at specific sites. In the presence of the mutation in factor V, the cleavage of this factor is deprived, leading to enhanced thrombin generation and hence increased clot formation. The maternal thrombophilia which is due to FVL mutation may result in production of micro thromboses in placental blood vessels and placental infractions, which damage the maternal vessels supplying the placenta (the spiral arteries) leading to low placental perfusion and eventually in fetal death. This mutation is responsible for up to 95% cases of APC resistance in non-pregnant individuals; inheritance of the mutation is autosomal dominant. Prothrombin gene G1691A –A mutation, a single base-pair substitution gives rise to increased prothrombin levels due to unknown mechanisms, predisposing to thrombosis, (8)

2. Material and Methods

2.1 Study Group:

Between July 2012 and June 2014, 100 women, 20–38years of age, with at least 3 spontaneous consecutive miscarriages were referred to the Omdurman Maternity Hospital in Sudan .All women were healthy, and none had a history of thrombotic events.

2.3 Control Group:

Ninety four healthy women without previous miscarriage or pregnancy complications were enrolled as controls.

2.4 Blood collection for DNA extraction:

After consent was issued by the patients, 5 mL of blood were drawn in ethylene diamine tetra acetic acid–tubes for DNA extraction. Total genomic DNA was isolated from peripheral blood lymphocytes by the salting out technique (10,11)

2.5 Factor V Leiden, Molecular Methods:

Genomic DNA extraction blood was gathered in EDTA. DNA was extracted from blood by using a com-metrical kit (GF-1 Nucleic Acid Extraction Kits, Malaysia). Extracted genomic DNA will be quantified spectrophotometrically by measuring the absorbance at 260nm in GenQant spectrophotometer. The study required a minimum of 70 ng of genomic DNA. The samples were then aliquot into two 0.5 separate PCR tubes stored at-20ºC and will be assayed in batch.PCR was used to amplify a segment of the FV gene from 100 ng genomic DNA in a total reaction volume of 25lL. To detect the FVL point mutation, amplification refractory mutation system (ARMS) was used as described by Baglin et al (12).The detection of A4070G of factor V gene will be amplified according to the methods of Jenny et al (13), by polymerase chain reaction (PCR) followed by restriction enzyme digestion.

2.6 Statistical Method:

Data was processed by SPSS 16.0 software. Results were comparable by x2 test and p-value less than 0.05 assigned statistically significant. Odds ratio and 95% confidence intervals (CI) were calculated.

Ethics:

The study was approved by the Ethics Committee at Omdurman Maternity Hospital (Sudan).

3. Results

Participants included 100 women subjects. Their mean age SD was 24 3. The mean age of healthy women was 30 ± 4. In the case Out of them, 10 had a history of 2 or more events of recurrent fetal loss (abortion or miscarriage). 6% of cases were diabetic, 3% were infection with Toxoplasma Gondi, 1% hypertensive and 1% had a heart operation. The demographic data and clinical characteristics of study patients are presented in table I. The prevalence of factor V Leiden was higher in cases 8(8.0%) than control group 6(6.4%) that it was statistically insignificant (P=0.66). The prevalence of heterozygous FVL mutation in RM women was found to be 8% (Table 2). Wild-type G allele occurred with a frequency of 96. The percentage among cases mutant allele (A) was seen only in 4% of the cases. Frequency of the mutant allele (A) was 3.2% and G allele occurred with a frequency of 96.8% among controls. The difference in the prevalence of the mutant allele was statistically significant among cases and controls.

Table 1.Demographic distribution of patients:

characteristics		Patients N (%)	Controls N (%)
Age group	17-24	10(10.1)	13(13.8)
	25-29	29(29.3)	28(29.8)
	30-34	27(27.3)	36(38.3)
	35-39	21(21.2)	8(8.5)
	≥40	12(12.1)	9(9.6)
Area of resident	Khartoum	8(8.1)	5
	Omdurman	88(88.9)	85
	Bahri	3(3.0)	4
Blood group	O	69(69.7)	58(62.4)
	A	15(15.2)	9(9.7)
	B	9(9.1)	15(16.1)
	AB	6(6.1)	11(11.8)
History	10(10.1)		
Other diseases	Diabetic	6(6.0)	
	Toxoplasmosis	3(3.0)	
	Heart operation	1(1.0)	
	Hypertensive	1(1.0)	

Table 2. Frequency of normal genotype and genotypes with mutant allele in studied patients:

Genotype	Patients N (%)	Controls N (%)	P-value	OR (95%CI)
Heterozygous G/A	8(8.0)	6(6.4)	0.66	1.28(0.42 to 3.84)
Normal homozygous G/G	92(92.0)	88(93.6)		
Alleles G	192(96.0)	182(96.8)	0.67	0.76(0.27 to 2.33)
Alleles A	8(4.0)	6(3.2)		

Wild genotype: G/G
Mutant genotype (Heterozygote): G/A
Mutant genotype (Homozygote): A/A

4. Discussions

We have studied the frequency of thrombolytic mutations in women having recurrent abortions. Factor V Lei-den G20210A gene It has been thought to increase the risk of thrombotic events. (14, 15) The prevalence of these mutations and polymorphisms has also been viewed to differ between ethnic groups (16). Some studies reported the prevalence of factor V Leiden mutation varies depending on a nation to other nation and it's observed the lowest prevalence of the mutation among Asian nations especially in Indonesian and Japanese population (17-18). The results of the present study showed the factor V Leiden G1691A mutation is low frequently found in Sudanese women with RPL. It is not consistent with the hypothesis that factor V Leiden G1691A a play an important role in pregnancy loss. A study was done by Souza et al the frequency of factor V Leiden in Brazilian patients was 7.1% vs. 1.6% in controls (19). In contrast, none of the 52 Japanese women with RPL carried factor V Leiden mutation (20) Also, Another study reported a non-significant increase in FVL mutation was detected among Jewish women 16% compared with 5% in the control group, (P= 0.14) (21). Our finding was in agreement with those reported in a study among Iranian women in Teremmahi et who found FVL among cases was 2.5%, which was higher than controls (1.25%), but the difference was not significant.(22)

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