Heterozygosity of maternal factor V G1691A (Leiden) and relationship with times of pregnancy loss among unexplained recurrent pregnancy loss women

Abstract

Background: Recurrent pregnancy loss is defined by the consecutive loss of two or more pregnancies with the same partner. Recurrent pregnancy loss (RPL) or recurrent miscarriage (RM) affects from 1-5% of the reproductive age couples. This diagnosis is both emotionally challenging and confusing for most couples, as the definitive diagnosis using conventional evaluations is found in fewer than half of the couples experiencing repeated loss. The aim of this study was to evaluate the inherited heterozygosity of factor five Leiden (FVL) G1691A and Its relation to the time of recurrent spontaneous pregnancy loss.

Materials and methods: Retrospective case-control study, in which women with RPL were compared to healthy women without any evidence of spontaneous abortion. This study was undertaken at Omdurman maternity hospital in Khartoum state, Sudan. The case group consisted of one hundred women who experienced at least three or more consecutive recurrent spontaneous pregnancy loss that occurred before 20 weeks of gestation and the control group consisted of ninety five healthy women without any history of adverse pregnancy outcome. Questionnaire and direct interview were used to collect information. Genotyping was based on polymerase chain reaction. Data were entered and analyzed by SPSS program version 17.0.

Result: Heterozygosity for FVL alleles G/A was 8.0% in all cases and 6.4% was found in control group. Related to association with time of recurrent pregnancy loss our result shows three times (37.5%), four times (50.0%) and five times (12.5%).

Conclusion: Heterozygosity of FV Leiden G1691A could be one reason for recurrent pregnancy loss and pregnancy complications among women with unexplained pregnancy loss. Our study showed that there is an association between heterozygosity for FVL G1691A and time of recurrent pregnancy loss.

Keywords: factor V leiden, G1691A, pregnancy loss, heterozygosity, RPL, protein C

Introduction

Recurrent pregnancy loss (RPL), defined as the loss of three or more consecutive pregnancies, affects 1% of couples trying to conceive. It has been estimated that 1–2% of second-trimester pregnancies miscarry before 24 weeks of gestation. Recurrent pregnancy loss (RPL) is caused by various genetic and non-genetic factors. After chromosome abnormality, thrombophilia is one of the most important genetic factors that could cause RPL. Factor V Leiden and factor II G20210A mutation were the most common cause of thrombophilia in the world. Factor V is one of the essential clotting factors in the coagulation cascade. Its active form, factor Va, acts as a cofactor allowing factor X to stimulate the conversion of prothrombin to thrombin. Activated protein C is a natural anticoagulant it limits the extent of clotting by destroying factor V and reducing further thrombin formation. Heterozygosity for a mutation in the coagulation factor V gene leads to resistance to activated protein C and represents the most common cause of inherited thrombophilia. Recently several studies have suggested that FVL mutation, through the production of micro thrombosis on placental bed blood vessels, causes low placental perfusion, placental infarction, and is strongly associated with RPL and maternal and fetal complications. Some studies have demonstrated an association between recurrent pregnancy loss and prothrombotic states rendered by some genetic single nucleotide polymorphisms of factor V Leiden G1691A. Etiology is determined in approximately 50% of couples with RPL. Most of the diagnosed etiologies include endocrine abnormalities, autoimmune disorders, uterine anomalies, and genetic factors. Still, 50% of couples have no known etiology. The aim of the current study was to correlate heterozygosity of maternal factor V G1691A (Leiden) and relationship with times of pregnancy loss among unexplained recurrent pregnancy loss.

Materials and methods

Ethical approval was obtained from Omdurman Maternal hospital. Informed consent was obtained from all patients. Genomic DNA samples of 195 Sudanese women who recruited and followed at Omdurman Maternal hospital were screened from Aug 2012 to Dec 2014. Hundred females had a history of RPL and were considered the cases and they were compared to 95 healthy reproductive Sudanese women, as control group, who had a history of two or more successful live births. Cases and controls were tested for the FV Leiden G1691A and FII G20210A. Genomic DNA was extracted from 3–5ml of EDTA anti-coagulated blood by salting out using Master pure DNA purification...
kit for blood GF-1 Blood DNA Extraction Kit, 50 PREPS (cat. No. GF-
(bp) segment of the factor V gene was amplified using specific
forward primer (5′TCA GGC AGG AAC ACC AT-3′) and reverse primer 5′GGT TAC TTC AAG GAC AAA ATA CCT GTA
AAG CT-3′. The amplification program was as follows: Denaturation
at 94°C for 30seconds, annealing at 51°C for 30seconds, extension at
72°C for 30 seconds for 35cycles and 72°C for 5minute.19 Digestion
of the amplicon was performed by with MnI1 enzyme and digestion
products resolved on 2% agarose gel stained with ethidium bromide
(Figure 1).

![PCR amplification of FVL gene mutation](image)

**Figure 1** PCR amplification of FVL gene mutation: The 267bp DNA
products digested with MnI1 enzyme.

Lane 1: molecular weight marker 50bp, lane 2: undigested PCR product
(267bp); lane 3 and 5: heterozygous mutant (AG); Lane 4, 6, 7, 8, 9 and 10: Wild
type (AA)

### Data analysis

Data were statistically described in terms of mean±standard
deviation (± SD), median and range, or frequencies (number of
cases) and percentages when appropriate. Odds Ratio (OR) and the
95% confidence interval (95% CI) were calculated for the presence
of mutation between cases and controls and analyzed by SPSS
program (version: 17.0). Data were analyzed using the Chi-square test
for comparing the prevalence of MTHFR mutation between patients
and controls (The test considered significant when P. value <0.05).
The ethical committee approves the study; informed consent has to be
obtained from participants.

### Results

The participants included 195 women. Out of them, 100 had a
history of 3 or more events of recurrent fetal loss (abortion,
miscarriage or still birth); there was 25±4years and 95 women were
healthy; their age was 30±4years. Heterozygosity of maternal factor V
G1691A mutation distribution showed higher prevalence among cases
than controls group. The mutation was detected in 8 out of 100 cases
(8.0%) and in 6 out of 94 controls (6.4%); P=0.66, Odds Ratio=1.28,
95% CI (0.42 to 3.84) . Homozygous (G/G) among cases was 92%
but in controls it was 93.6%. G allele occurred with a frequency of
96.0% among cases and 96.8% in controls while mutant allele (A)
was seen only in 4 % of the cases and 3.2% of controls. The difference
is statistically insignificant (Table 1) (Figure 1). Heterozygosity of
maternal factor V (G1691A) related to times of recurrent pregnancy
loss (Table 2).

### Discussion

Most of the studies published on the association of factor V Leiden
and recurrent pregnancy loss were retrospective case- control studies.
Few studies in the literature were prospective case control studies;
they reported the outcome of treated pregnancies only or a comparison
between treated patients with untreated patients.11,12 The current
study is a retrospective analytical case control study designed to
investigate the relationship between heterozygosity of maternal factor V
(G1691A) and to number of recurrent pregnancy loss. Our results
agree with several studies conducted among women with unexplained
pregnancy loss.13-15 However, several studies did not report a
significant association between heterozygosity of maternal factor V
(G1691A) mutation and RPL.16-18 A difference in the prevalence of the
FVL mutation in individuals may be related to different ethnic
backgrounds. Some studies reported that the prevalence of factor V
Leiden mutation varies from one nation to another. FVL has a
certain distribution in different human populations, a fact
that may contribute to geographic and ethnic differences in the
prevalence.19 Our study also evaluated the heterozygosity of factor V
(G1691A) as related to times of recurrent pregnancy loss; we found
50% positive for FVL associated with four times loss of pregnancy
followed by 37.5% associated with three times and 12.5% with five
times. Our finding is supported by some studies that concluded that
women with FVL mutation are two to three times more likely to have
multiple (recurrent) miscarriages or pregnancy loss during the second
or third trimester.20 Some studies suggest that factor V Leiden mutation
may also increase the risk of other complications during pregnancy
including high blood pressure, preeclampsia, slow fetal growth, and
placental abruption.21 Available data suggest that Leiden variant
heterozygosity is, at most, a weak contributor to recurrent or late
pregnancy loss. A meta-analysis evaluating only prospective cohort
studies reported a slightly increased risk of pregnancy loss in women
with the Leiden variant.22 Our results show that heterozygosity of

### Table 1 Heterozygosity of maternal factor V (G1691A) mutation among
cases of recurrent pregnancy loss compared to controls

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

### Table 2 Heterozygosity of maternal factor V (G1691A) related to times of
recurrent pregnancy loss

<table>
<thead>
<tr>
<th>Times of RPL</th>
<th>Factor V G1691A heterozygous</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Positive</td>
</tr>
<tr>
<td>Twice</td>
<td>0</td>
</tr>
<tr>
<td>Three times</td>
<td>3 (37.5)</td>
</tr>
<tr>
<td>Four times</td>
<td>4 (50.0)</td>
</tr>
<tr>
<td>Five times</td>
<td>1 (12.5)</td>
</tr>
<tr>
<td>Six times</td>
<td>0</td>
</tr>
<tr>
<td>Seven times</td>
<td>0</td>
</tr>
<tr>
<td>Eight times</td>
<td>0</td>
</tr>
<tr>
<td>P= 0.66</td>
<td></td>
</tr>
</tbody>
</table>
factor V (G1691A) is associated with no significant trend towards risk of pregnancy complications; this may be due to the low frequency of factor V (G1691A) mutation among African people. Unfortunately, there is still an absence of adequate data to support or confirm the role of FVL mutation among unexplained pregnancy loss. Association between factor V Leiden mutation and pregnancy complications has not been confirmed and most women with factor V Leiden thrombophilia have normal pregnancies under good management and control.

**Conclusion**

Inherited heterozygosity of FV Leiden G1691A could be one reason for recurrent pregnancy loss and pregnancy complications among women with unexplained pregnancy loss. Our study shows that there is an association between heterozygosity for FVL G1691A and times of recurrent pregnancy loss.

**Acknowledgements**

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**Conflict of interest**

There was no conflict of interest, and the paper is not being considered by another journal. The paper was self-funded.

**References**