Objective: To investigate the effect of point mutation in FV Leiden G1691A and FII G20210A gene on coagulation and recurrent spontaneous abortion (RSA) among Sudanese women.

Study Design: This was retrospective case control study.

Place and Duration of Study: The study was carried out from Aug 2012 to Dec 2014 at Omdurman Maternal Hospital, Sudan.

Materials and Methods: The study included hundred pregnant females with a history of recurrent spontaneous abortion as (case group) and ninety five healthy reproductive Sudanese women as (control group). The data was collected with the help of structured questionnaire and direct interview to collect information. Identification of point mutation in factor V Leiden G1691A and factor II G20210A gene by polymerase chain reaction was performed; Coagulometer was used for the measurement of PT and APTT. Odds Ratio 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.

Results: The Heterozygous alleles G/A in factor V gene was 8.0% in all cases related with three, four and five times of recurrent abortion and 6% was found in control group. Heterozygous alleles of factor II G/A Prothrombin time (PT) and partial thromboplastin time (PTT) in women with Recusant Spontaneous Abortion (RSA) were not affected significantly (P=0.93 and P=0.69).

Conclusion: Based upon the results it is concluded that the point mutation in factor V Leiden G1691A and factor II G20210A might play a role in recurrent spontaneous abortion loss among Sudanese women. However these point mutations do not affect the coagulation profile.

Key Words: Factor V Leiden G1691A, Factor II G20210A, RSA, Sudanese Pregnant Women.
in a small absolute risk of clinically significant thrombosis. Factor V Leiden is a single point mutation involving a guanine to adenine transition at position 1691 in exon 10 of the factor V gene, which leads to the synthesis of a variant factor V molecule. The prothrombin G20210A mutation involves guanine to adenine substitution at nucleotide 20210 of the prothrombin gene. FV Leiden and factor II G20210A mutations are associated with increased production of thrombin and risk of venous thrombosis. Also Factor V Leiden mutation is found to be the most common inherited thrombotic risk factor associated with RPL its frequency in whites varies from 3% to 8% and 1 in 1000 are homozygous. It is rare in African Americans, Asians and Native Americans. The incidence of genetic prothrombotic mutations in women with unexplained pregnancy loss was examined in various studies: some of these studies supported the association. While others reported no association. The present retrospective case control study was conducted to evaluate the FV Leiden G1691A and FII G20210A mutations and their affect on some coagulation profiles (PT and PTT) among women with a history of three or more consecutive pregnancy losses and healthy controls. This is the first study that investigated FV Leiden G1691A and FII G20210A alleles and genotype distributions in the Sudanese females with habitual RPL.

Materials and Methods
This was retrospective case control study. The genomic DNA samples of one hundred and ninety five Sudanese women who recruited and followed at Omdurman Maternal Hospital were screened from Aug 2012 to Dec 2014. One hundred cases having a history of RPL were compared with ninety five healthy reproductive Sudanese women as control group with a history of two or more successful live birth. Cases and controls were tested for the FV Leiden G1691A and FII G20210A. Genomic DNA was extracted from 3–5 ml of EDTA anti-coagulated blood by salting. DNA was extracted from the blood samples using Master pure DNA purification kit for blood GF-1 Blood DNA Extraction Kit, 50 PREPS (cat. No. GF-BD-050, Vivantis Technologies Sdn. Bhd., Malaysia). FV Leiden G1691A and FII a 345-bp genomic DNA fragment encompassing a part of the prothrombin gene that contains the mutation was amplified by PCR using specific primers Forward (5’TCT AGA AAC AGT TGC CTG GC-3’) and Reverse primer (5’ATA GCA CTG GGA GCA TTG AAG C-3’). And 267-basepair (bp) segment of the factor V gene was amplified used specific forward primer (5’TCA GGC AGG AAC AAC ACC AT-3’) and reverse primer 5’GCT TAC TTC AAG GAC AAA ATA CCT GTA AAG CT3. The reaction program was as follows: Denaturation at 94°C for 30 seconds, annealing at 51°C for 30 seconds, extension at 72°C for 35 cycles and 72 °C for 5 minute. A master mix was prepared by adding Nuclease free water,10x buffer,dNTP,tow primers,Mgc12,Taq DNA polymerase and DNA, the mixture was loaded into thermocycler according to the specific Temperature profile. The working solution of 1X TBE is prepared from the stock solution (1 L) which contains the following: 89 mM Tris base (108 gm), 89 mM boric acid (55 gm) 40 ml of 0.5M EDTA, adjust pH to 8.0.1.5% agarose was prepared from 1x TBE, and 5μl PCR products was loaded by mixing PCR products with 1μl loading dye, run on the gel for 30 mins and visualized on UV transillimantor. Factor V Digested with 10 μl of DNA restriction enzyme MnlI at 37°C for 18 h, subjected to 2% low melting point agarose and Prothrombin product (10 μl) was digested with 20 U of Hind III, at 37°C for 16 h, and loaded into 2% low melting point agarose gel, eletropherosed at 90 volts for 60 mins.

Data were statistically described in terms of mean ± standard deviation (± SD), median and range, or frequencies (number of cases) and percentages where 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 prograrmme (version: 17.0). Data were analyzed using the Chi-square test to compareson the prevalence of MTHFR mutation between patients and controls (The test considered significant when Pvalue <0.05).

Ethical consent was obtained from ethical committee at Hospital of Omdurman Maternity Hospital (Sudan).

Results
The participants included 195 women subjects. Out of them, 100 had a history of 3 or more events of recurrent fetal loss (abortion, miscarriage or still birth). Their mean age ± SD was 25 ± 4. Ninety five
women were healthy the mean age of was 30 ± 4. The prothrombin time PT (p=0.93) and PTT (p=0.69) were normal among all women with RPL and controls. Factor V Leiden mutation distribution showed higher prevalence among study participant with RPL as compared to control group. The mutation was detected in 8 out of 100 (8.0%) and 6 out of 94 controls (6.4%). P- Value =0.66, Odds Ratio=1.28, 95% CI (0.42 to 3.84) The prevalence of heterozygous FVL mutation in recurrent miscarriage women was found to be 8% but in control it found to be 6.4%. Mutant allele (A) was seen only in 4% of the cases. Frequency of mutant allele (A) was 3.2% and G allele occurred with a frequency of 96.8% among controls. These results are statistically insignificant between the cases and controls group. Prevalence of the Prothrombin gene was 3% among cases with P- Value =0.091. but no mutant gene was detected among control group. According to the genotyping in cases showed (Heterozygotes, 3.0%; Homozygotes, 97.0%), Alleles G (98.5%) and Alleles A (1.5%) while in controls group show normal homozygous G/G (100%) and Alleles G (Alleles G). No significant association between cases carriage any of this mutation and risk with recurrent pregnancy miscarriage (Table II). The cases group was divided into subgroups based on time of recurrent abortion from second to eight times of repeated miscarriage. Our data indicates that factor V gene mutation was most frequent in women with recurrent miscarriage. Prothrombin mutation was found only among women with three time recurrent miscarriages with 100% and MTHFR present in three, four and five times of recurrent miscarriage women with equal percentage 33.3% for each (Table III).

**Table I: Frequency of factor V (Leiden) mutation among cases of recurrent pregnancy loss compared to controls**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Patients N (%)</th>
<th>ControlsN (%)</th>
<th>P-value</th>
<th>OR (95%CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heterozygous 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 homozygous G/G</td>
<td>92(92.0)</td>
<td>88(93.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles G</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>Alleles A</td>
<td>8(4.0)</td>
<td>6(3.2)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table II: Frequency of Prothrombin 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 G/A</td>
<td>3(3.0)</td>
<td>0</td>
<td>0.091</td>
<td>0</td>
</tr>
<tr>
<td>Normal homozygous G/G</td>
<td>97(97.0)</td>
<td>94(100)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alleles G</td>
<td>194(98.5)</td>
<td>188(100)</td>
<td>0.089</td>
<td>0</td>
</tr>
<tr>
<td>Alleles A</td>
<td>3(1.5)</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table III: Frequency of factor V (Leiden) and Prothrombin related to times of recurrent pregnancy loss**

<table>
<thead>
<tr>
<th>Times of RPL</th>
<th>Factor V</th>
<th>Prothrombin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Negative</td>
<td>Positive</td>
</tr>
<tr>
<td>Twice</td>
<td>0</td>
<td>8(8.8)</td>
</tr>
<tr>
<td>Three times</td>
<td>3(37.5)</td>
<td>57(62.6)</td>
</tr>
<tr>
<td>Four times</td>
<td>4(50.0)</td>
<td>16(17.6)</td>
</tr>
<tr>
<td>Five times</td>
<td>1(12.5)</td>
<td>6(6.6)</td>
</tr>
<tr>
<td>Six times</td>
<td>0</td>
<td>1(1.1)</td>
</tr>
<tr>
<td>Seven times</td>
<td>0</td>
<td>1(1.1)</td>
</tr>
<tr>
<td>Eight times</td>
<td>0</td>
<td>2(2.2)</td>
</tr>
</tbody>
</table>

**Fig 1: PCR amplification of VVL gene mutation**

Digestion of factor v gene with MnI1 enzyme on 2% agarose gel dissolved in 1X TBE buffer, stained with ethidium bromide, Lane 1 molecular weight marker 50 bp, lane2 undigested PCR products lane 3 and 5 were heterozygous mutant (AG), Lane 4,6,7 and 8,9 and 10 were Wild typ (AA), The 267 bp DNA products digested with MnI1.
Digression of prothrombin gene with Hind III on 2\% agarose gel dissolved in 1X TBE buffer, stained with ethidium bromide, Lane 1 molecular weight marker 100 bp, lane 2 (322 bp), mutant(AA), control, lane 3 and 5 were hemizygous mutant (GA), Lane 4, 6 and 7 were Wild type (GG), lane 8 undigested(345 bp).

Discussion
One hundred Sudanese women suffering from RPL as compared to ninety five healthy women. Because inherited thrombophilia has been implicated as a possible cause of RPL. Gene defects frequently associated with RPL were prothrombin A20210G and factor V Leiden reported in many studies. Due to their important roles in the coagulation pathway, this study was conducted to investigate the association between genetic polymorphisms of Factor V and Factor II G20210A among women experiencing RPL. The frequency of polymorphic A allele was more prevalent in RPL patients (8%) than in controls (6.4%) and the G allele was less prevalent in RPL patients (98%) than in controls (100%). The prothrombin G20210A mutation our result revealed that the mutation not common among recurrent spontaneous aborted Sudanese women they were found Heterozygous G/A alleles with frequency 3\% and did not found any mutated gene among control group. The frequency of polymorphic A allele was prevalent in RPL patients (1.5\%) and the G allele was less prevalent in RPL patients (98.5\%) than in controls (100\%). Our finding was consistence with Altintas et al, 2007, Freire et al, Sottilotta et al and Dalmaz et al but it was inconsistent with Mello et al, Behjati et al, and Bagheri et al. Prothrombin time (PT) and partial thromboplastin time (PTT) in women with RPL in this study were not affected significantly (P=0. 93 and P=0.69) respectively this is similar to the normal results reported by Ghulam, et al., (2014) among Sixty three pakistani women with history of three spontaneous abortions in their first three months of pregnancy. Also our finding in PT and PTT were consistence with Salamat et al and Shahida et al. The normal result of PT and PTT in women with V Leiden G1691A, factor II G20210A because the patient with these mutations makes fibrin at same rate as a person with normal factor V. It's just that later on, when the body tries to turn factor V off, the factor V Leiden patient will keep on making fibrin. In future sample should be increased and study may extent to other provinces in Sudan to cover up more races and tribes and make the study more accurate and precise.

Conclusion
In our study we found that the Polymorphism in V Leiden G1691A and FII G20210A mutation do not increase risk for RPL in tested population and there are no any affect of these mutations in the prothrombin time (PT) and partial thromboplastin time (PTT).

Acknowledgement
We are grateful to the patients and healthy individuals for participating in our study.

REFERENCES
11. Rey E, Kahn SR, David M, Shrier I. Thrombophilic Disorders