Original Article

Molecular Characterization of Prothrombin G20210A gene Mutations In pregnant Sudanese women with spontaneous recurrent abortions

Asaad Mohammed Ahmed Abd Allah Babker, Fath Elrahman Mahdi Hassan Gameel

Department of Hematology and Immunohaematology, College of Medical Laboratory Science, Sudan University of Science and Technology

Objective: This study aimed at analyzing the association between recurrent spontaneous abortion and prothrombin G20210 gene mutation in Sudanese women.

Methodology: This prospective analytical case control study was carried out at Omdurman Maternity Hospital, Sudan and included 100 Sudanese women who experienced three or more of the adverse pregnancy outcomes during their reproductive age. 96 controls were also included. Cases and controls were tested for the above mutations.

Results: Out of 100 case, 3 (3%) had gene mutation and none was found control group (P =0.091).

Conclusion: We found limited importance of prothrombin mutation as genetic risk factor among women with recurrent spontaneous abortion. There was no significant association between cases carriage any of this mutation and risk with recurrent pregnancy miscarriage. (Rawal Med J 201;40: 207-209).

Keywords: Prothrombin, mutation, Sudanese pregnant women, recurrent spontaneous abortion.

INTRODUCTION

Recurrent miscarriage (RM), which is also referred to as repeated pregnancy loss (RPL) and habitual abortion, is defined as three or more consecutive spontaneous miscarriage.[1,2] Miscarriages are the most common complication of pregnancy, affecting approximately 15% of all clinically recognized pregnancies in the general population. Thrombophilia is considered still a debated problem that may be common in women with unexplained RPL, with prevalence as high as 65% in selected populations.[3]

The prothrombin G20210A gene mutation is one of inherited factor that can cause recurrent miscarriage. If somebody has too little prothrombin, he or she has a bleeding tendency.[4] Having too much prothrombin makes the blood more likely to clot. People with this condition are said to have a prothrombin mutation, also called the prothrombin variant, prothrombin G20210A, or a factor II mutation. Prothrombin gene (G20210A) mutation is associated with an increased risk of thrombosis and it is the most identifiable risk factor for venous thrombosis.[5] The prothrombin gene mutation (PT) is signaled by a defect in clotting factor II at position G20210A and the human prothrombin gene spans 21 kb on chromosome 11p11-q12 and consists of 14 exons and 13 introns, which account for 90 percent of the sequence. This mutation occurs as a result of the G to A transition at nucleotide 20210 in the prothrombin gene. The reported prevalence in Europe is around 2-6% and the risk of venous thrombosis to heterozygous carriers is three times the normal population.[6]

Inherited thrombophilic conditions are increasingly being implicated in these pregnancy outcomes, yet paradoxically, the majority of patients harboring the most common mutations, such as Factor V Leiden, methylene tetrahydrofolate reductase MTHFR) gene and prothrombin gene mutation G20210A are asymptomatic.[7] Majority of patients harboring Factor V Leiden, methylene tetrahydrofolate reductase MTHFR) gene and prothrombin gene mutation G20210A are asymptomatic.[8] Acquired or hereditary thrombophilia have been related to adverse pregnancy outcome and a higher incidence of early recurrent abortion is associated with factor V Leiden, the methyleneterhydrofolate reductase (MTHFR) C677T and to the prothrombin G20210A variant.[9] The aim of this study was to analyze the association between RPL and prothrombin G20210 gene mutation in Sudanese women.

METHODOLOGY

Between January 2013 and August 2014, 100 women, with 20-38 years of age, with at least 3 or more recurrent spontaneous abortion were referred to the Omdurman Maternity Hospital, Sudan. Control Group comprised of 96 women with at least 2 normal pregnancies and without any history of adverse pregnancy outcome or recurrent miscarriages. The study was approved by the National Health Service and Ethics Committee at the Omdurman Maternity Hospital, Sudan and an informed consent was taken.

Prothrombin Time was measured by coagulometer (MSLBA13) and result was reported in seconds or as a ratio compared to the laboratory mean normal control (prothrombin ratio, PTR). [10] Partial Prothrombin Time was measured by coagulometer (MSLBA13) and aPTT result was reported as the time required for clot formation after the addition of $CaCl_2$.[10] For detection of G20210 prothrombin gene mutation, we used a PCR method 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).[6]

Data were analyzed by SPSS version 17.0. All demographic data of the study population are presented as mean±SD in the text and Odds Ratio was used for detecting the power of relationship between the determinant and the outcome and 95% confidence interval was calculated. Chi-square test was used for comparison the prevalence of MTHFR, Prothrombin *gene* and FVL mutation between patients and controls. P <0.05) was considered significant.

RESULTS

The general mutation prevalence of the Prothrombin gene was 3% among cases (P=0.091) and there are no mutant gene detected in control group. Genotyping in cases showed Heterozygotes, 3.0% and 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) (Table 1). There was no significant association between cases carriage any of this mutation and risk with recurrent pregnancy miscarriage.

Table 1. Prothrombin mutation among cases of recurrent pregnancy loss compared to controls.

Genotype	Patients (%)	Controls (%)	P-value	OR (95%CI)
Heterozygous G/A	3(3.0)	0		
Normal homozygous G/G	97(97.0)	94(100)	0.091	0
Alleles G	194(98.5)	188(100)		
Alleles A	3(1.5)	0	0.089	0

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

Table 2. Average distribution of PT and PTT in patients and controls.

Risk factor	Patients Mean±SD	Controls Mean±SD	P-value
PT	13.82±1.13	13.84±1.17	0.93
PTT	32.47±3.26	32.67±3.54	0.69

Prothrombin time (p=0.93) and PTT (p=0.69) were normal among all women with RM and controls (100%) had normal PT and PTT (Table 2). There were no significant correlation between the PT, PTT and recurrent miscarriage.

DISCUSSION

In 3 out of 100 women had prothrombin mutation and did not have any mutating gene in control group. We found there was no significant association between the prothrombin G20210A mutation and repeated spontaneous abortion among Sudanese women. This is in agreement with study by Silver and Zhou[11] and Behjati et al.[12] In another study from Iran, no factor II mutation was found in Iranian women.[13]

The result of this study were different from one reported by Sehirali et al who found FII mutation significantly higher in Turkish women with RM compared to controls (10.9% vs. 2.04%, P<0.05).[14] Also Gihan and Osama,(2013) were found that FII was [50.1% vs. 38%, P<0.0001] , FV (27% vs. 25% , P>0.0001) compared to control group and concluded that recurrent pregnancy loss among Saudi pregnant women was strongly associated with thrombophilic mutations related to both FVL and FII. Characteristically, these cases showed a high frequency of factor II mutation.

Prothrombin time (PT) and partial thromboplastin time (PTT) in women with RM in this study were not affected significantly. This is similar to the results

reported by Nizamani et al in Pakistani women with history of three spontaneous abortions in their first three months of pregnancy.[15] Also our results agree with those reported Salamat et al[16] and Mohsin et al.[17] In general, not all retrospective studies showed a relationship between the prothrombin G20210A mutation and obstetric complications. Several case control studies failed to show an association between this mutation and abruption,[18] recurrent pregnancy loss,[19] preeclampsia,[20] and other adverse obstetric outcomes.[21]

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Conception and design: Assad and Fathelrahman Mahdi
Collection and assembly of data: Assad Mohammed Ahmed Abd Allah Babker
Analysis and interpretation of data: Fathelrahman Mahdi and Asaad
Drafting of the article: Assad Mohammed Ahmed Abd Allah Babker
Critical revision of the article for important intellectual content: Fathelrahman Mahdi
Statistics expertise: Asaad and Fathelrahman Mahdi
Final approval and guarantor of the article: Fathelrahman Mahdi
Corresponding author email: Azad.88@hotmail.com
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REFERENCES

1. Jauniaux E, Farquharson RG, Christiansen OB, Exalto N. Evidence-based guidelines for the investigation and medical treatment of recurrent miscarriage. Hum Reprod 2006;21:2216-22
2. Aruna M, Reddy BM. Recurrent spontaneous abortions: An overview of the Genetic and Non-Genetic backgrounds. Int J Hum Genet 2006;6:109-17.
3. Blickstein I. Thrombophilia and women's health: An overview. Obstet Gynecol Clin North Am 2006;33:347-56.
4. Varga EA, Moll S. Prothrombin 20210 Mutation. Am Heart Assoc 2013;110:e15-e18.
5. Zoller B, Garcia de F, Hillarp PA, Dahlback B. Thrombophilia as a multigenic disease. Haematologica 1999;84:59-70.
6. Poort SR, Rosendaal FR, Reitsma PH, Bertina RM. A common genetic variation in the 3' untranslated region of the prothrombin gene is associated with elevated plasma prothrombin levels and an increase in venous thrombosis. Blood 1996;88:3698-703.
7. Ehrenforth S, Nemes L, Mannhalter C, Rosendaal FR, Koder S, Zoghlami-Rintelen C, et al. Impact of environmental and hereditary risk factors on the clinical manifestation of thrombophilia in15 homozygous carriers of factor V:G1691A. J Thromb Haemost 2004;2:430-6.
8. Aleman MM, Walton BL, Byrnes JR, Wang JG, Heisler MJ, Machlus KR, et al. Elevated prothrombin promotes venous, but not arterial, thrombosis in mice. Arterioscler Thromb Vasc Biol 2013;33:1829-36.
9. Vossen CY, Preston FE, Conard J, Fontcuberta J, Makris M, van der Meer FJ, et al. Hereditary thrombophilia and fetal loss:a prospective follow-up study. Thromb Haemost 2004;2:592-6.
10. Dacie JV, Lewis SM. Practical Haematology, 8th edition, Churchill Living Stone, Edinburgh, 1995; p.p. 212-185-
11. Silver RM, Zhao Y. Prothrombin Gene G20210A Mutation and Obstetric Complications: Obstet Gynecol 2010;115:14-20.
12. Behjati R, Modarressi MH, Jeddi-Tehrani M, Dokoohaki P, Ghasemi J, Zarnani AH, et al. Thrombophilic mutations in Iranian patients with infertility and recurrent spontaneous abortion. Ann Hamatol 2006;85:268-71.
13. Ardestani M, Nodushan H, Aflatoonian A, Ghasemi N, Sheikhha M. Case control study of the factor V Leiden and factor II G20210A mutation frequency in women with recurrent pregnancy loss: Iran J Reprod Med 2013;11:61-4.
14. Sehirali S, Inal MM, Yildirim Y, Balim Z, Kosova B, et al. Prothrombin G20210A mutation in cases with recurrent miscarriage: a study of the mediterranean population. Arch Gynecol Obstet 2005;273:170-3.
15. Nizamani GS, Memon RA, Khan A, Shaikh SS, Ujjan IU. Analysis of anti phospholipid antibodiesin women with recurrent spontaneous abortion. Isra Med J 2014;6:
16. Salamat N, Saleem M, Ahmed T. Lupus coagulant and anticardiolipin antibodies in patients with recurrent fetal loss: A case control Study. Ann Saudi Med 2000;20:450-3.
17. Mohsin S, Ilyas A, Dar LR, Rasool G, Hussain S, Ullah I. Levels of Anti Phospholipid antibodies in females with recurret abortions. Ann Pak Inst Med Sci 2011;7:156-9.
18. Nath CA, Ananth CV, DeMarco C, Vintzileos AM. New Jersey-Placental Abruption Study Investigators Low birth-weight in relation to placental abruption and maternal thrombophilia status. Am J Obstet Gynecol 2008;198:293.e15.
19. Altintas A, Pasa S, Akdeniz N, Cil T, Yurt M, Ayyildiz O, et al. Factor V Leiden and *G20210A* prothrombin mutations in patients with recurrent pregnancy loss: data from the southeast of Turkey. Ann Hematol 2007;86:727-31.
20. Lin J, August P. Genetic thrombophilias and preeclampsia: a meta-analysis. Obstet Gynecol 2005;105:182-92.
21. Zahed LF, Rayes RF, Mahfouz RA, Taher AT, Maarouf HH, Nassar AH. Prevalence of factor V Leiden, prothrombin and methylene tetrahydrofolate reductase mutations in women with adverse pregnancy outcomes in Lebanon. Am J Obstet Gynecol 2006;195:1114-8.