PROTHROMBIN G20210A GENE MUTATION IN PREGNANT FEMALES WITH THROMBOTIC OBSTETRIC COMPLICATIONS

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ABSTRACT

Objective: To determine the frequency of prothrombin *G20210A* gene mutation in pregnant females with adverse thrombotic obstetric complication and to compare it with prothrombin *G20210A* gene's frequency in control population.

Study Design: Case control study.

Place and Duration of Study: Department of Haematology, Army Medical College Rawalpindi and Military Hospital Rawalpindi, from Nov 2013 to Oct 2014.

Material and Methods: Sixty pregnant females were included in the study; 30 were cases with adverse thrombotic obstetric complication, while 30 were controls. Detailed history was obtained and 3 ml blood in EDTA tube was collected. DNA was extracted from whole blood and through RT-PCR, presence of prothrombin *G20210A* gene mutation was looked for in patients and controls. Data was analyzed using SPSS 21.

Results: A total of 60 women-30 cases with thrombotic obstetric complications as 'cases' and 30 as 'controls'- were included in the study. Mean age of 'cases' was 28.70 ± 4.23 years while that of 'controls' was 27.33 ± 4.49 years. There was no statistically significant difference among the two groups (p=0.54). In case group only one of 30 (3.3%) patients had heterozygous F2 *G20210A* mutation while 29 (96.7%) patients had wild type allele. In control group, all the 30 (100%) subjects had wild type allele. The odds of finding the mutation in cases was 1:29 i.e. 0.03 as compared to zero in the control group. The difference was statistically insignificant (p= 0.5).

Conclusion: Our study shows that the frequency of F2 *G20210A* gene mutation in pregnant females having adverse thrombotic obstetric complications was not significantly different from its frequency in control population.

Keywords: Prothrombin *G20210A* gene mutation, Pregnant females, Thrombotic obstetric complications.

INTRODUCTION

Inherited thrombophilia are group of conditions in which there is increased thrombo-embolic tendency. The common hereditary thrombophilic conditions include factor V Leiden mutation, methylene-tetra-hydro-folate reductase mutations, prothrombin gene mutation, plasminogen activator inhibitor-1 (PAI-1) mutation and deficiencies of anticoagulant proteins such as protein C, protein S and anti-thrombin[1].

Among the hereditary thrombophilic conditions, factor V Leiden mutation and prothrombin gene mutation are more common with a risk of venous thrombosis in terms of odds ratio (OR) of more than 2. Prothrombin or the factor II, a 72 kDa zymogen precursor of thrombin, consists of 579 amino acids and has a plasma concentration of 2 µmol/l. It is a vitamin K-dependent protein, synthesized in the liverand its gene is located on chromosome[2]. The gene contains 14 exons spread over 21 kb[2]. A single nucleotide mutation in which guanine is replaced byadenine at position20210 in the 3′ untranslated region of the prothrombin gene (denoted as F2 *G20210A*), is associated with elevated plasma prothrombinlevels and an increased risk of venous thrombosis. In Europeprevalence of the F2 *G20210A* mutation is about 2% in whites. The mutation increases prothrombin levels in plasmaby about 30% through an unknown mechanism[1].

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Pregnancy in itself is a hypercoagulable condition. Various studies have shown different results about the association of hereditary thrombophilic conditions including F2 *G20210* Amutation with complications of pregnancy such as eclampsia/pre-eclampsia, abruptio-placentae (AP), placenta-previa (PP), recurrent abortions, still births, gestational hypertension (GHTN), intrauterine growth retardation (IUGR), intra-uterine death (IUD) and hemolysis, elevated liver enzymes, and low platelet (HELLP) syndrome. The link between thrombophilia and these complications of pregnancy is thought to be due toimpaired foeto-placental circulation because of thrombosis[3].

In Pakistan an earlier study detected only 2(1%) carriers of F2 *G20210A* mutation in a sample population of two hundred[4]. However its association with thrombotic complications in pregnancy or obstetrics has not been studied. We carried out this study to compare the frequency of F2 *G20210A* gene mutation in pregnant females having adverse thrombotic obstetric complications with its frequencyin control population.

PATIENTS AND METHODS

This case-control study was conducted in haematology section of the Clinical Pathology Laboratory (CPL) of Army Medical College Rawalpindi in collaboration with Gynaecology and Obstetrics Department of Rawalpindi's Military Hospital, after obtaining institutional permission. The study was conducted from November 2013 to October 2014. A total of 30 pregnant females with thrombotic obstetric complications were included as cases; and another 30 healthy females with normal single pregnancies, each ending at full term in an uneventful normal vaginal delivery of a normal healthy baby, were selected as control.

The cases were selected by non-probability convenient sampling. These included patients diagnosed with eclampsia / pre-eclampsia, oligo-hydramnios, abortions/still births, preterm labour, PP, GHTN, AP, IUD, IUGR and HELLP syndrome.

The patients having chronic hypertension, diabetes mellitus, pre-existing renal disease and malignancy, multiple pregnancies and patients with a previous history of a coagulation disorder were excluded from the study. Pregnancies with fetal congenital anomalies detected on ultrasound examination and women with history of oral contraceptive use were also excluded.

The study was commenced after approval from Ethical Review Committee of Army Medical College. Informed written consent was taken in each case. Sampling was done on the 1st post-partum day in case of controls while in the case patients it was performed after the diagnosis of thrombotic obstetric complication. Detailed history was obtained from the participants and 3 ml venous blood samples was collected in EDTA tubes. DNA extraction was carried out from whole blood samples with QIAamp DSP DNA blood mini kit (QIAGEN GmbH, Hilden, Germany) following the instructions of the manufacturer. The Gene Proof Factor II prothrombin PCR kit (Brno, Czech Republic) was used for the detection of F2 *G20210A* mutation in the prothrombin gene using real-time PCR method following the instructions of the manufacturer of the kit. The test procedure is based on the PCR amplification of a DNA fragment harboring the site of possible F2 *G20210A* mutations, and the presence of allele-specific fluorescently labelled hybridization probes.

Data analysis was carried out using SPSS version 21. Mean and standard deviation were calculated for quantitative variables while frequencies and percentages were calculated for qualitative variables. Odds of finding mutation in cases and controls were compared. Fisher exact test and t-test was applied and *p*-value of <0.05 was considered as statistically significant.

RESULTS

A total of 60 women-thirty cases with thrombotic obstetric complications as 'cases' and

thirty as 'controls'- were included in the study. Mean age of the participants was 28.02 ± 4.38 years with minimum age of 19 years and maximum age of 37 years. Mean age of 'cases' was 28.70 ± 4.23 years while that of 'controls' was 27.33 ± 4.49 years. There was no statistically significant difference among the two groups (p= 0.229).

Thrombotic obstetric complications and their frequencies seen in the in the patients of group comprising 'cases' are given in table.

In patients' group only one of 30 (3.3%) patients had heterozygous F2 *G20210A* mutation while 29 (96.7%) patients had wild type allele. In control group, all the 30 (100%) subjects had wild type allele. The odds of finding the mutationin cases was 1: 29 i.e. 0.03 as compared to zero in the control group. The difference was statistically insignificant (p=0.5).

Table: Frequency of various obstetric complications seen in the patients grouped as 'cases'.

S No.	Obstetric complications	Frequency (%)
1	Placenta-previa	6 (20%)
2	Intra-uterine growth retardation	4 (13.3%)
3	Oligohydramnios	4 (13.3%)
4	Unexplained stillbirth	3 (10%)
5	Abortion	3 (10%)
6	Gestational hypertension	3 (10%)
6	Abruptio-placentae	2 (6.6%)
7	Eclampsia / pre-eclampsia	2 (6.6%)
8	Preterm labour	2 (6.6%)
9	HELLP syndrome	1 (3.3%)
	Total	30 (100%)

DISCUSSION

Increased thrombotic risk associated with the hereditarily thrombophilic conditions such as F2 *G20210A* mutation and obstetric complications suggest a cause and effect relationship between former and latter. Internationally, the publication of the European Prospective Cohort on Thrombophilia study in 1996 was among the first to provide evidence of a link between hereditary thrombophilia and recurrent miscarriages[5].

Kupferminc et al carried out a case-control study in Israel and concluded that thrombophilia and specifically F2 *G20210A* mutation was more frequently associated with AP and early severe pre-eclampsia. It was recommended in the study that pregnant women with complications such as severe pre-eclampsia, AP or unexplained IUGR, and in those with unexplained recurrent stillbirths should undergo testing for acquired and genetic indicators for thrombophilia[6].

There have been other studies too which show association of F2 *G20210A* mutation with thrombotic obstetric complications. Grandone et al in an Italian study found F2 *G20210A* carrier status as a risk factorfor GHTN with or without proteinuria[7]. In a case control study, Gerhardt et al demonstrated that onset of severe pre-eclampsia was significantly early in patients with F2 *G20210A* mutation than in the control group[8]. Kupferminc et al in another study published in 2000, showed that as compared to the control women, F2 *G20210A* mutation was significantly more prevalent in women with IUGR, AP, and second trimester loss but not in women with mild or severe preeclampsia, stillbirth and habitual abortion[9].

Martinelli et al concluded in their study that both the factor V and F2 *G20210A* mutations were associated with tripling of the risk of late foetal loss[10]. Similar conclusion was drawn by Foka et al who showed that F2 *G20210A* mutation was associated with recurrent miscarriages[11]. Reznikoff-Etievan et al drew similar results from

their research and showed that F2 *G20210A* mutation was associated with early recurrent spontaneous miscarriage before 10 weeks of pregnancy, with an OR of 2.7 (95% CI 1-7) when compared with the control group[12]. Alfirevicet al conducted a systematic review and showed that placental abruption, pre-eclampsia/eclampsia and IUGR were associated with F2 *G20210A* mutation[13]. In addition to above, research by Kutteh et al while evaluating the role of F2 *G20210A* mutation in adverse obstetrical outcome also established a strong association between the two[14].

We found only one (3.3%) carrier of F2 *G20210A* mutation among our 30 cases. In contrast to the above studies, we did not find statistically significant difference in the frequency of F2 *G20210A* mutation in the cases with obstetrical complications from that of the control group. Sample size of our study is small but there are many, more recent studies with larger sample size which also did not show any association between F2 *G20210A* mutation and adverse obstetric outcome.

Infante-Rivard et al did not find any association between different thrombophilia polymorphisms including F2 *G20210A* mutation with IUGR[15]. Nath et al showed there was no association between F2 *G20210A* and AP[16]. A meta-analysis by Lin et al showed that the OR for F2 *G20210A* mutation and pre-eclampsia was 1.37 (95% CI 0.72-2.57)[17]. A Lebanese study carried out by Zahed et al failed to establish association between F2 *G20210A* and recurrent foetal loss[18]. A large case-control study which recruited 301 women conducted by Giovanni et al in Italy did not find increased incidence of adverse obstetric outcome in patients with F2 *G20210A* mutation[3].

Most of the above mentioned studies were case-control studies. A prospective study carried out by Silver et al on a large cohort of women revealed 157 (3.8%) out of 4167 women were positive for F2 *G20210A* mutation. However they did not find any association between F2 *G20210A* mutation and obstetric complications such as abortions, stillbirths, pre-eclampsia, AP, IUGR and preterm labour[19].

We see a lot of inconsistency in the results of various studies. This could be due to varied prevalence of mutation in different countries, differences in the size of samples, and type of study design i.e. case control vs cohort studies. The review of literature shows that the debate on the cause and effect relationship of F2 *G20210A* and thrombotic obstetric complications is far from over. However it is important to establish a consensus in order to draw the guidelines for the recommendation or otherwise of routine antenatal screening for F2 *G20210A* mutation mutation and management of carriers during pregnancy.

CONCLUSION

Our study shows that the frequency of F2 G20210A gene mutation in pregnant females having adverse thrombotic obstetric complications was not significantly different from its frequency in control population.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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