Is There an Association between Maternal Thrombophilia and Oligohydramnios During the Gestational Age of 18-23 Weeks? A Case-Control Study

Asma Basha [1]*

Abstract

Objective: To study the prevalence of genetically related thrombophilic Factor V Leiden, Factor V Cambridge, prothrombin gene mutation G20210A, factor XIII, methylenetetrahydrofolate reductase (MTHFR) C677T and A1298C mutations in pregnant women with oligohydramnios at the gestational age of 18-23 weeks.

Methods: A case-control study was conducted in Jordan, at Jordan University Hospital and Farah Hospital between 2007 and 2011. Sixty three pregnant women with oligohydramnios were compared with 85 pregnant women with normal amniotic fluid volume at the gestational age of 18-23 weeks. All the fetuses have satisfactory fetal anomaly scan and there was no history suggestive of preterm premature rupture of the membranes. All subjects were investigated for 6 genetically related thrombophilic factors.

Results: The presence of MTHFR C677T was found to be significantly higher in the oligohydramnios group compared to the control group, 61.9% vs. 37.6% (p value 0.01), and The presence of heterozygosity for pregnant women with oligohydramnios (49.2%) was also significant (p value 0.005) compared to the control (29.4%). Factor XIII, Factor II G20210A, MTHFR A1298C, Factor V Leiden were not found to have a statistically significant difference between the two groups. Factor V Cambridge was found in none of the cases, but was found in 3% of the control group (p value 0.32).

Conclusions: This study suggests that the presence of MTHFR C677T in women with oligohydramnios at the time of fetal morphology scan may indicate a placental mal-function; however, larger studies are needed to validate this association.

Keywords: Pregnancy, Thrombophilia ,Pregnant women and Oligohydramnios.

(J Med J 2016; Vol. 50 (2):115-121)

Received	***Accepted***
Nov. 9, 2015	*Feb. 24, 2016*

Introduction

Thrombophilia refers to disorders associated with a persistent hypercoagulable state and a tendency towards thrombosis. It may be inherited, acquired, or complex when both genetic and environmental factors interact[(1)]. Pregnancy, per se, predisposes to hypercoagulability[(2)]. Current understanding indicates that a combination of risk factors,

1. Department of Obstetrics and Gynecology, Faculty of Medicine, Jordan University Hospital, The University of Jordan.

The study was conducted in Jordan, at Jordan University Hospital and Farah Hospital between 2007 and 2011.

**** Correspondence should be addressed to:***
Asma Basha;
E-mail: bashaasma@yahoo.com
P. O. Box: 13046 Amman 11942, Jordan.

including multiple inherited thrombophilic defects associated with secondary hypercoagulable states, have a particularly strong association with adverse pregnancy outcome[2] A successful outcome of pregnancy is dependent upon the development of efficient uteroplacental circulation and the establishment of an effective feto-maternal circulatory system which may be compromised by disturbances of haemostasis leading to prothrombotic state[3].

Studies have shown that there is an association between thrombophilia and adverse obstetric outcomes such as recurrent miscarriage, intrauterine growth restriction, pre-eclampsia, and placental abruption[1, 2]. Among the proposed hypotheses to explain the occurrence of oligohydramnios is chronic uteroplacental insufficiency that is caused by decreased renal blood flow in the fetus[4]. It is possible that oligohydramnios is an early signal of placental insufficiency[4].

Recent attention has focused on a group of mostly inherited autosomal dominant gene mutations that included Factor V Leiden (FVL), Factor V Cambridge (FVC Arg306àThr), Prothrombin gene mutation G20210A (F II), Factor XIII a (Val34àLeu) methylenetetrahydrofolate reductase (MTHFR) C677T and MTHFR (A1298C).

The association of these genetically thrombophilic factors with adverse pregnancy outcome have been studied in the third trimester[5], but not in the mid second trimester.

During routine detailed fetal anomaly scan between 18 and 23 weeks gestation some pregnant women are found to have oligohydramnios, as evidenced by an obvious lack of amniotic fluid, poor fluid–fetal interface, and marked crowding of fetal parts[6] with deepest amniotic fluid less than 4x4 cm.

This study aimed at investigating the association between 6 common genetically related thrombophilic factors, namely FVL, FVC, FII, F XIII and MTHFR (C677T, A1298C), and oligohydramnios in pregnant women between 18 and 23 weeks gestation.

Materials and Methods

A case–control study was conducted at a tertiary university hospital (Jordan University Hospital) and a private obstetric hospital (Farah Hospital) in Jordan during the period 2007 to 2011. Two hundred pregnant women agreed and signed to participate in the study, but we were left with only 148 candidate. A total of 63 pregnant women with oligohydramnios at 18-23 weeks gestation were compared with 85 pregnant women with normal amniotic fluid volume at the same gestational age. All of these women were medically free .The diagnosis of oligohydramnios was made during routine detailed fetal anomaly scans performed between 18-23 weeks gestation (Table 1). All the fetuses have satisfactory fetal anomaly scan and there was no history suggestive of preterm premature rupture of the membranes.

All women were scanned by one certified fetomaternal medicine sub-specialist (using GE Voluson Expert, GE E-8 or Philips HD-11 ultrasound machine). All subjects were negative for known thromboembolic disease.

The prevalence of FVL, FVC, F II, F XIII, MTHFR C677T and MTHFR A1298C were Investigated using primer-engineered multiplex polymerase chain reaction–

restriction fragment length polymorphism (PCR-RFLP). The DNA was isolated from EDTA anticoagulated whole blood using the Wizard Genomic DNA Purification kit (Promega, Madison, WI, USA) according to manufactures instructions. Genomic DNA was tested for the polymorphisms by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method where the PCR products were digested with restriction endonucleases, and the restriction fragments were separated by agarose gel electrophoresis using polymorphisms as described in the literatur. All the blood samples were sent to Faculty of Medicine, Coagulation and thrombosis laboratory.

Table 1. Baselines and Clinical Characteristics of cases and control subjects

Age and pregnancy distribution	pregnant women with Oligohydramnious, N = 63	pregnant women with normal amniotic fluid volume, N = 85
Age in years {mean ±SD}	28.05 ± 5.41	28.47 ± 5.7
Age range in years	16-44	19-42
Race	100% Jordanians	100% Jordanians
	18-23	18-23
Gestational age (wks) {mean ±SD}		
Gravida{mean ±SD}	3.02 ± 1.95	2.68 ± 1.82
Gravida range	1-10 gestation	1-8 gestation
Parity {mean ±SD}	1.17 ± 1.19	1.53 ±1.63
Parity range	0 – 4 deliveries	0 – 7 deliveries
Miscarriage {mean ±SD}	0.92 ± 1.35	0.14 ± 0 .38
Miscarriage range	0 – 6	0 – 2

Institutional Review Board (IRB) approval was obtained from both hospitals and a consent form was signed by all women.

Statistical analysis:

We used a case-control design to allow quantitative comparison between groups. All statistical tests were independent t-test and Pearson Chi square was used to test for association in the distribution of categorical variables (Thrombophilia genetic factors (FVL, FII, MTHFR). P-values of < 0.05 were considered statistically significant. The analyses were performed using the SPSS package version 16.0.

Results

All 184 invited pregnant women, agreed to participate in the study. The baseline characteristics of women in both groups are shown in Table 1. There was no difference in profiles between the two groups with respect to age, gravidity, parity and gestational age at the time of ultrasound scanning. All women denied any medical illnesses.

The data analyses of the 6 studied thrombophilic factors are summarized in Table 2.

The frequency of MTHFR C677T variant in pregnant women with oligohydramnios was 39 (61.9%), of which 31 (49.2%) were heterozygous and 8 (12.7%) were

homozygous, as compared to 32 cases (37.6%) in the control group, of which 25 (29.4%) were heterozygous and 7 cases (8.2%) were homozygous, with p value of 0.01.The presence heterozygosity for MTHRR C677T in pregnant women with oligohydramnios (49.2%), was significant with p value of 0.005) compared to the control (29.4%).

For the MTHFR A1298C variant, among all pregnant women with oligohydramnios, there were 33 (52.4%) cases, 24 (38.1%) of which were heterozygous, the other 9 (14.3%) were homozygous. In the control group there were 48 cases (56.5%), of which 31 (36.5%) were heterozygous and 17 (20.0%) were homozygous, the p value was 0.66.

Table 2. The Frequency of genetically related thrombophilic factors in all pregnant women with oligohydramnios and control group

Mutation	Cases No =66	Control No= 85	Likelihood Ratio	P-value
Factor V Leiden	13.0 (20.6%)	18.0 (21.2%)	0.94	0.94
Factor II G20210A	4.0 (6.4%)	3.0 (3.5%)	0.39	0.47
MTHFR C677T	39.0 (61.9%)	32.0 (37.6%)	0.01	0.01
MTHFR A C1298	25.0 (52.4%)	38.0 (56.5%)	0.66	0.66
Factor XIII	17.0 (27.0%)	13.0 (15.3%)	0.22	0.21
Factor V Cambridge	0.0 (0.0%)	3.0 (3.6%)	0.19	0.32

Among all pregnant women with oligohydramnios, 13 (20.6%) had Factor V Leiden, all were heterozygous. In the control group there were 18 (21.2%) cases, all of which were heterozygous with p value of 0.94.

There were 4 cases (6.4%) with Factor II G20210A mutation in the group of pregnant women with oligohydramnios, 3 (4.8%) of them were heterozygous and 1 (1.6%) was homozygous, however there were 3 (3.5%) cases in the control group, all were heterozygous, with p value of 0.47.

The frequency of Factor XIII variant was 17 (27%) in women with oligohydramnios, of which 14 (22.2%) were heterozygous and 3.0 (4.8%) were homozygous, as compared to 13 (15.3%) cases in the control group, of which 11 (12.9%) were heterozygous and 2 (2.4%) were homozygous, with p value of 0.21.

Factor V Cambridge was present in no cases in the oligohydramnios group , while there were 3 (3.6%) cases in the control group, of which 2 (2.4%) were heterozygous and 1 (1.2%) was homozygous, with none in the oligohydramnios group, with p value 0.32.

The P values were not significant for the presence of homozygosity or heterozygosity for the other thrombophilia factors.

Discussion

Thrombophilic defects have been shown to be associated with an increased risk of venous thrombosis, fetal loss, and gestational complications[7]. Pregnancies with adverse

neonatal outcomes were more likely to present with idiopathic oligohydramnios at an earlier gestational age than those without adverse outcomes [4]. It has been stated that patients with preterm appropriate-for-gestational-age fetal biometry who present with idiopathic oligohydramnios at an earlier gestational age are at risk for adverse perinatal outcomes compared with those presenting later in gestation[4].

Ogunyemi et al. evaluated the association between obstetrical complications and thrombophilic factors in 66 pregnant women with live births and no obstetrical complications and 75 pregnant women with obstetrical complications such as unexplained oligohydramnios, IUGR, preeclampsia, recurrent abortions, fetal demise, abruption and hypercoagulable disorders. They found that factor V Leiden mutation was present in 7 (10%) of women with obstetric complications versus 1 (2%) among controls (p-value was 0.064). They suggested an association between non-thromboembolic pregnancy complications and hypercoagulable disorders including unexplained oligohydramnios.

Magriples et al. reviewed the ultrasound findings of treated and untreated pregnant women with genetic thrombophilia and anticoagulant therapy that were referred for growth restriction, oligohydramnios and abnormal Doppler results. They found that anticoagulant treatment markedly improved ultrasound parameters of growth, amniotic fluid volume and feto-placental blood flow in patients with thrombophilia[8]. Those findings could explain the results of this study, albeit at an earlier gestation.

Simchen et al studied the characteristics of placental stillbirth and the possible contribution of thrombophilic risk factors. In their prospective cohort study; they found the prevalence of maternal thrombophilia was higher for placental stillbirth women (63.6%). Factor V Leiden and/or prothrombin G20210A mutation were much more prevalent in placental versus non-placental stillbirth women (OR 3.06, 95% CI 1.07–8.7), and they conclude that maternal thrombophilia is highly prevalent, especially in preterm placental stillbirth[9]. In comparison to this study, we still believe that thrombophilia plays a part in placental dysfunction which in turn is reflected by oligohydramnios at earlier gestational age.

The drawbacks of this study is that we did not investigate the paternal or the neonatal status for the 6 genetically related thrombophilia factors and also the level of plasma homocysteine in women with oligohydramnios who had the gene for MTHFR C677T.

So the conclusion is, oligohydramnios at the time of detailed fetal morphology scan could be explained by the presence maternal thrombophilia factors as MTHFR C 677; which could have affected placental function at an earlier gestational age; however, additional patients would be needed to reach a better significance and to evaluate this preliminary finding.

Acknowledgments

The author would like to thank all the people who helped, Proff Abdalla Abadi, Dr Zaid Kelani, Proff Zuhair Ammarin, Dr Lamia Al Haj Hasan, Miss Elham Abu Kadra and technician Mohammad Al Kateeb.

References

1. Tan JY, Thrombophilia in pregnancy, Ann Acad Med Singapore. 2002 May; 31 (3): 328-34.
2. Kutteh WH, Triplett DA. Thrombophilias and recurrent pregnancy loss. Semin Reprod Med. 2006 Feb; 24 (1): 54-66.
3. Hossain N, Shamsi T, Soomro N. Frequency of thrombophilia in patients with adverse pregnancy outcome. J Pak Med Assoc. 55 (6): 245-7.
4. Vink J, Hickey K, Ghidini A, Deering S, Mora A, Poggi S. Earlier gestational age at ultrasound evaluation predicts adverse neonatal outcomes in the preterm appropriate-for-gestational-age fetus with idiopathic oligohydramnios. Am J Perinatol. 26 (1): 21-5. Epub 2008; 8.
5. Ogunyemi D, Ku W, Arkel Y. The association between inherited thrombophilia, antiphospholipid antibodies and lipoprotein A levels with obstetrical complications in pregnancy. J Thromb Thrombolysis. 2002; 14 (2): 157-62.
6. Philipson EH, Sokol RJ, Williams T: Oligohydramnios: Clinical associations and predictive value for intrauterine growth retardation. Am J Obstet Gynecol, 146: 271, 1983.
7. Lindhoff-Last E, Luxembourg B. Evidence-based indications for thrombophilia screening. Vasa. 2008; 37 (1):19-30.
8. Magriples U, Ozcan T, Karne A, Copel JA. The effect of anticoagulation on antenatal ultrasound findings in pregnant women with thrombophilia. J Matern Fetal Neonatal Med. 2006; 19 (1): 27-30.
9. Simchen MJ1, Ofir K, Moran O, Kedem A, Sivan E, Schiff E . Thrombophilic risk factors for placental stillbirth. Eur J Obstet Gynecol Reprod Biol. 2010 Dec; 153 (2): 160-4. doi: 10.1016/j.ejogrb.2010.07.031. Epub 2010 Aug 12.

هل هناك ترابط بين تخثر الدم لدى النساء الحوامل وقلة الصاء الذاتي خلال العمر الحملي (للجنين) 18–23 أسبوع؟ دراسة استباقية

أسماء الباشا[1]

1– قسم أمراض النساء والولادة، كلية الطب، مستشفى الجامعة الأردنية، الأردن.

الملخص

الهدف: دراسة تكرار تغيرات العوامل الوراثية لتخثر الدم وهي عامل ليدن الخامس FVL، وعامل كامبردج الخامس FV Cambridge، والعامل الثاني G20210A (FII) وعاملا مختزلة الميثيلين تترا هيدروفولات C677T وA1298 (MTHFR) والعامل الثالث عشر XIII F لدى النساء الحوامل مع قلة الصاء الذاتي خلال العمر الحملي (للجنين) 18–23 أسبوع.

الطريقة: أجريت الدراسة استباقيا لحالات وشواهد في مستشفى الجامعة الأردنية، ومستشفى فرح بين الأعوام 2007–2011 على ثلاث وستون امرأة حامل مع قلة الصاء مقارنة مع 85 شواهد حوامل لديهم صاء طبيعي خلال العمر الحملي (للجنين) 18–23 أسبوع حيث كان فحص سلامة الجنين طبيعيا ولم تعاني هؤلاء السيدات من نزول مبكر للصاء. تم إجراء فحص عوامل تخثر الدم الوراثي لجميع الحالات والشواهد.

النتائج: إن تكرار العامل الوراثي لمختزلة الميثيلين تترا هيدروفولات (MTHFR) C677T لدى النساء الحوامل مع قلة الصاء هي أعلى 61.9% مقارنة مع الشواهد 37.6% وبدلالة إحصائية P=0.dm، وكان تكرار وجود متغاير الزيجوتيد (MTHFR) C677T له دلالة إحصائية لدى النساء الحوامل مع قلة الصاء (49.2%) مقارنة مع الشواهد (29.4%) P=0.005 ولم يكن الاختلاف ذو دلالة احصائياً لكل من عامل ليدن الخامس FVL، والعامل الثاني FII(G20210A) وعامل مختزلة الميثيلين تترا هيدروفولات (MTHFR) A1298 والعامل الثالث عشر FXIII لدى النساء الحوامل مع قلة الصاء مقارنة مع الشواهد. عامل كامبردج الخامس FV Cambridge لم يتواجد لدى الحوامل مع قلة الصاء وكان لدى الشواهد بنسبة 3% وبدلالة إحصائية P=0.32.

الخاتمة: أن هذه الدراسة تقترح ان تكرار وجود العامل الوراثي لمختزلة الميثيلين تترا هيدروفولات (MTHFR C677T) وخصوصاً متغاير الزيجوتيد لدى النساء الحوامل مع قلة الصاء عند فحص الجنبن التفصيلي قد يدل على خلل وظيفي للمشيمة ولكن نحن بحاجة إلى دراسات أكبر إثبات هذه العلاقة.

الكلمات الدالة: الحمل، تخثر الدم، النساء الحوامل.