First and Second Trimester Biochemical Markers in Familial Mediterranean Fever

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Abstract

Familial Mediterranean Fever (FMF) is the most common hereditary monogenic auto-inflammatory disease. In this study we aimed to document whether FMF affects the biochemical components of first and second trimester combined aneuploidy screening tests including serum free beta human chorionic gonadotropin (β-hCG), placenta associated plasma protein-A (PAPP-A), unconjugated estriol (uE3), total hCG and alfa-fetoprotein (AFP). In this prospective case control study, a total of consecutive 59 singleton pregnancies, 29 of with FMF and other 30 healthy women, were followed from the first trimester to end of the pregnancy. Most of pregnant women with FMF (86.2%) were under treatment with colchicine. The markers PAPP-A, free β-hCG and the nuchal translucency (NT) thickness were examined at 11-13 weeks. Serum samples for AFP, uE3 and total hCG were obtained at 16-19 weeks after detailed examination. There were no cases of fetal chromosomal anomaly in neither of the groups. Both first-and second trimester marker levels were not significantly different in absence of aneuploidy or neural tube defects in FMF and control group. No difference was seen for NT measurements. False-positive rate for Down syndrome was comparable between the groups using a term risk cut-off level of 1/250. Pregnant with FMF should be reassured about the not altered levels of first-and second trimester marker. Therefore readjusting the serum markers used for Down syndrome screening is not advised. Future trials of larger scale are needed to assess any need for readjustment of the risk in the patient population with FMF.

Keywords: Pregnancy, colchicine, nuchal translucency, PAPP-A, AFP, HCG

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Introduction

Familial Mediterranean Fever (FMF) is an inherited auto-inflammatory disease characterized by recurrent and self-limited attacks of fever and serositis. FMF is caused by mutations in Mediterranean Fever Gene that encodes a pyrin protein. Pyrin is expressed in mainly neutrophils, serosal and synovial fibroblasts. It is responsible for the regulation of apoptosis, inflammation and cytokine production [1]. The diagnosis of the disease is still based on clinical criteria since there is no specific diagnostic test. Tel Hashomer criteria are widely used for diagnosis of FMF. Colchicine, an ancient anti-inflammatory drug is the first-line therapy to treat pain and prevent amyloidosis in FMF. It has been reported that colchicine can be used safely in pregnancy [1,2].

Aneuploidy is one of the leading causes of perinatal deaths and childhood handicaps. In a conventional fetal aneuploidy screening today, various products are used such as Alpha Fetoprotein (AFP), free beta human Chorionic Gonadotrophin (β-hCG), Inhibin A, Pregnancy-Associated Plasma Protein A (PAPP-A), and unconjugated Estriol (uE3), which are derived from the feto-plasental unit are assessed in maternal blood. The parameters in maternal serum biochemistry are combined ultrasonography measurements and they are used for screening purposes. These screening tests are named as double test (free β-hCG and PAPP-A) in the 1st trimester and as triple test (AFP, free β-hCG and uE3) and quadruple test (AFP, uE3, free β-hCG and Inhibin A) in the 2nd trimester. Once the risky group is identified, invasive tests administered in an attempt to obtain the fetal genetic material for a definitive diagnosis. An assessment of NT in combination with maternal age, free β-hCG and PAPP-A is called a combined test can detect Trisomy 21 at a rate of 85-95% in the first trimester with a 5% false positivity rate [3,4]. In a previous study, low levels of PAPP-A were reported in a group of pregnant women with FMF [5].

In this study we aimed to document whether biochemical markers used in first and second trimester screening for aneuploidies, including maternal serum free β-hCG, PAPP-A, uE3, total HCG and AFP, are altered in pregnant with FMF. We also documented perinatal outcomes of pregnant with FMF in a tertiary care hospital prospectively.

Material and Methods

This case–control study was conducted at Zekai Tahir Burak Women's Health Education and Research Hospital, Ankara, Turkey between February 2014 and January 2015. Ethical approval was obtained from the local research ethics committee. Informed consent was obtained from all of the participants. This study was conducted in accordance with the basic principles of the Helsinki Declaration.

The diagnosis of FMF was made according to the Tel Hashomer criteria [1]. The definitive diagnosis of FMF was made in the presence of two major criteria or one major and two minor criteria. (The major criteria are recurrent febrile episodes accompanied by serositis, AA type amyloidosis without apparent predisposing disease and favorable response to colchicine test. Minor criteria are recurrent febrile episodes, erysipelas-like skin lesions, and family history of FMF).

All of pregnant had Turkish ethnicity derived by questionnaires and country of origin. Proteinuria, suggestive of renal amyloidosis, was excluded on the basis of repetitive urine testing. Patients with any of the following criteria were not eligible to participate in the study: late registration (beyond 14th weeks of gestation), multiple pregnancy, detection of fetal anomalies by USG, history of any other systemic diseases. We also excluded pregnancies with missing outcome data, those ending with miscarriage and those with fetal defects.

In the end, a total of 59 pregnant women in the first trimester, 29 of who with FMF and other 30 healthy women, matched for maternal age and BMI were invited to participate in this prospective case–control study. All pregnant were followed from the first trimester to end of the pregnancy according to the standard antenatal follow-up protocol. *G*estational age was determined based on the first day of the last menstrual period (LMP) and first trimester ultrasonography measurement of the crown–rump length (CRL) [6]. If the ultrasound dating differed from the LMP dating by more than seven days, the estimated due date was changed to correspond with the ultrasound dating. If the patient was unsure of her LMP, dating was based on ultrasound estimates by using the earliest CRL measurement during the first trimester. Ultrasonography measurements were performed using Voluson 730 (GE Medical Systems, Zipf, Austria) machines with a 5-MHz transabdominal and/or an 8-MHz transvaginal probe. Serum samples were obtained between 11+0 to 13+6 (CRL between 45 to

84 mm), and 16 to 19 weeks gestation. NT measurement was performed according to the published guidelines [7].

Levels of PAPP-A, free B-hCG, AFP, total hCG and unconjugated E3 were measured with the Immulite 2000 Analyzer (EURO/ DPC Ltd, Llanberis, UK). The measured marker values were converted to a gestation-specific multiple of the medians (MoM), after appropriate correction for gestational age, smoking status and maternal weight, as needed using Prisca 4.0 software (Typolog, Tornesch, Germany) and using reciprocal-linear regression [8]. Invasive tests was recommended when combined risk index was higher than 1/250 for a definitive diagnosis.

All other serum analyses were conducted in the central hematology laboratory of the hospital within two hours of collecting the blood samples, using a Gen-S automated analyzer (Beckman Coulter, High Wycombe, UK). Plasma concentrations of hs-CRP were determined with a Tinaquant CRP (Latex) high-sensitive particle-enhanced immuno turbidimetric assay on a Roche Modular P analyzer (Roche kit; Roche Diagnostics) according to manufacturer instructions.

The maternal age, body mass index (BMI), FTS and STS results, hs-CRP, CBC results of each participant were recorded at first and second trimester. The diagnosis of preeclampsia was based on a systolic blood pressure of ≥140 mmHg or diastolic blood pressure ≥90 mmHg, measured twice in four-hour intervals while resting, after the 20th gestational week, as well as 300 mg/dL proteinuria detected in a 24-hour urine sample [9]. Preterm birth was defined as delivery before 37 weeks of pregnancy were completed. The diagnosis of fetal growth restriction was made when estimated fetal weight was below the tenth percentile and was associated with abnormal fetal Doppler parameters, with low birth weight defined as <2500 g and very low birth weight as <1500 g [10]. The diagnosis of gestational diabetes mellitus (GDM) was made according to American Diabetes Association criteria, using the two-step method [11]. The perinatal outcome parameters, including gestational age at delivery, mean birth weight, perinatal complications including preterm delivery, preeclampsia, low 5-minute Apgar score (<7) and neonatal intensive care unit admission were also recorded.

Results

Fifty one pregnant diagnosed with FMF were followed throughout pregnancy during the study period at our hospital. A total of 22 pregnant with FMF were excluded from the study due to the fact that 3 had multiple pregnancy and 3 had IVF pregnancy, 2 had concomitant connective tissue diseases, 2 had diabetes mellitus, and 10 were late (after 14th weeks of gestation) registration and 2 patients didn't accept the screening test.

In the end, the data of 29 pregnant with FMF and 30 healthy pregnant women were evaluated for the final analysis. The mean duration of FMF during pregnancy was 6.5 years in study group and most of them (86,2%) were under treatment with colchicine 1-2 g daily. The demographic characteristics of the groups are presented in Table 1.

Table 1. Maternal characteristics of the FMF and control groups

Parameters	FMF (n= 29)	Control (n= 30)	p value
Maternal age, years	25 (18-39)	28.5 (19-36)	0.42
Parity (range)	0 (0-3)	1 (0-2)	0.49
Pre-pregnancy BMI, kg/m^2	24.78+2.59	23.10+2.62	0.05
Maternal weight gain during pregnancy, kg	9.9±3	11.8±4.2	0.72
Smoking, n (%)	3 (10,3%)	2 (6,6%)	0.11
Duration of disease, years	6.5 ± 2.2	-	-
Colchicine treatment during pregnancy, n (%)	25 (86,2%)	-	-
0,5 mg x 2 day	13 (52%)	-	-
0,5 mg x 3 day	10 (40%)	-	-
0,5 mg x 4 day	2 (8%)	-	-

Data expressed as number (%), mean ± SD, median (minimum - maximum).
BMI, Body mass index.

The results of the FTS and some inflammation related markers (that was obtained when all pregnant were asymptomatic) in FMF and control group are showed in Table 2. There were no differences in B-HCG, µE3, and AFP values between the groups at second trimester (p=0.98, p=0.26 and p=0.9, respectively**)** (Table 3).

While fibrinogen and WBC values were not significantly different, the mean hs-CRP levels were significantly higher in FMF group compared with control group at first and second trimester (p=0.05 and p<0.01, respectively).

There were no significant differences in obstetric outcomes found between the groups. There

were no cases of fetal chromosomal anomaly or perinatal mortality in neither of the groups.

The comparison of the perinatal outcomes of the FMF and control groups is presented in Table 4.

Table 2. First trimester maternal serum marker levels for aneuploidy in the absence of aneuploidy or neural tube defects in FMF and control group.

	FMF (n=29)	Control (n=30)	p value
Gestational age at FTS, weeks	11.6 (11.2-13.1)	12 (11-13.4)	0.36
CRL at screening, mm	55.74±6.94	58.19±9.46	0.26
WBC count (x10^3/mm^3)	9.9±2.5	9.33±2.03	0.83
hs-CRP (mg/ L)	8.40± 5.1	6.04 ± 3.5	0.05
Fibrinogen (gr/L)	465± 93	433 ± 105	0.24
NT (MoM)	0.93 (0.3-2.2)	0.97 (0.57-1.47)	0.9
PAPP-A (MoM)	1 (0.41-3.41)	0.96 (0.23-3.40)	0.17
Free B-HCG (MoM)	0.90 (0.22-4.88)	0.97 (0.27-2.70)	0.92
FPR*	2 (6,8%)	2 (6,6%)	1.0

Data expressed as number (%), mean ± SD, median (minimum - maximum).
FTS, first trimester screening; MoM, multiple of the medians; NT, nuchal translucency; PAPP-A, Pregnancy associated-plasma protein-A.
* FPR, False Positive Rate is defined as combined risk exceeded the cut-off level risk of 1 in 250.

Table 3. Second trimester maternal serum marker levels for aneuploidy in the absence of aneuploidy or neural tube defects in FMF and control group.

	FMF (n=29)	Control (n=30)	p value
Gestational age at STS, weeks	17.1 (16-19)	17.5 (16-18,5)	0.13
hs-CRP (mg/ L)	10 ± 4.6	6.01 ± 2.1	<0.001
Fibrinogen (gr/L)	477 ± 101	461 ± 92	0.66
WBC count (x10^3/mm^3)	10.1±2.96	10.3±2.46	0.64
Free B-HCG (MoM)	0.97 (0.3-2.12)	0.96 (0.37-2.31)	0.98
µE3 (MoM)	0.95±0.3	1.03±0.29	0.26
AFP (MoM)	0.83 (0.49-2.90)	0.94 (0.48-1.81)	0.90
FPR	2 (6,8%)	2 (6,6%)	0,97

Data expressed as number (%), mean ± SD, median (minimum - maximum).
AFP, alfa-fetoprotein; MoM, multiple of the medians; STS, second trimester screening; µE3, Unconjugated estriol

The false-positive rate for Down syndrome was not significantly different between the groups using a term risk cutoff level of 1/250 at both FTS and STS (p=1, p=1, respectively).

Table 4. Obstetric outcomes of pregnant with and without FMF in the absence of aneuploidy or neural tube defects.

	FMF (n=29)	Control (n=30)	p value
Gestational age at delivery, weeks	38.9±1.76	40.4±0.6	0.59
Mean Birth weight, gram	3148± 408	3367± 219	0.83
Mode of Delivery			0.78
Vaginal	18 (62%)	20 (66,6%)	
C/S	11(38%)	10 (33,4%)	
Preterm Delivery, n(%)	7 (24%)	5 (16,6%)	0.73
GDM, n (%)	1 (3,4%)	1 (3,3%)	1.0
FGR, n (%)	1 (3,4%)	1 (3,3%)	1.0
Preeclampsia, n (%)	1(3,4%)	1 (3,3%)	1.0
5 minute Apgar ≤7, n (%)	2 (6,8%)	3(10%)	1.0
NICU admission, n (%)	3 (10,3%)	3(10%)	1.0
Perinatal mortality, n (%)	-	-	-

Data expressed as number (%), mean ± SD, median (minimum - maximum).
FGR; Fetal growth restriction; GDM, Gestational Diabetes Mellitus; NICU, Neonatal intensive care unit.

Discussion

In this prospective study, we demonstrated that maternal serum PAPP-A, free β-hCG and NT values at 1st trimester, and μE3, total hCG and AFP levels at 2nd trimester were not altered in pregnant with FMF in the absence of aneuploidy or neural tube defects when compared with the healthy subjects. Furthermore, we showed that perinatal outcome of pregnant with FMF was comparable to the healthy pregnant without FMF.

Every woman has the risk of giving birth to a child with aneuploidy. Therefore, all pregnant women should be advised to go through a fetal aneuploidy screening routinely regardless of their age and risk status in their previous pregnancies [4].

Colchicine is one of the oldest known drugs used for prophylaxis of FMF attacks, and prevention of the development of amyloidosis. Its anti-inflammatory mechanism differs from the other anti-inflammatory agents like nonsteroidal anti-inflammatory drugs and glucocorticoids. Instead of being involved in the arachidonic acid pathway, colchicine promotes microtubule depolymerization leading to cytoskeletal changes through cell mitosis, exocytosis, and neutrophil motility [2].

It has been reported that colchicine was a teratogen in animals. The potential teratogenic effect of colchicine arises from its effect on mitotic activity and cytoskeletal structure as a

result of ultrastructural changes in spindle microtubules, leading to impaired mitotic function. Although colchicine crosses the human placenta it has been reported no unusual frequency of fetal abnormality among women taking colchicine before or during human pregnancy. Studies suggest that it does not appear to be a major human teratogen, and, probably, has no cytogenetic effect [2, 12]. In our hospital, our current policy is to recommend continuous colchicine before conception and during pregnancy and where feasible, it is advisable to perform FTS and STS. Although in a previous study it has been showed that pregnant women with FMF had low levels of PAPP-A [5], we didn't found any statistically significant difference between in pregnant with and without FMF in terms of FTS or STS markers.

In current study we documented pregnancy outcomes of pregnant with FMF. We showed that their perinatal results were comparable with healthy pregnant. In this cohort, all pregnant continued their colchicine treatment during pregnancy from first trimester safely, and no significant differences were noted regarding congenital malformations.

The strengths of our study are the homogeneity of the characteristics of the women in the study groups and its prospective design. Moreover in the present study, since the BMI, the maternal age and the ethnicity were comparable between the groups; the possible confounding effects were also being eliminated.

In conclusion, main findings of our study that both first-and second trimester serum aneuploidy screening marker levels were not significantly different in absence of aneuploidy or neural tube defects in FMF and control group. No difference was seen for NT measurements. False-positive rate for Down syndrome was comparable between the groups using a term risk cut-off level of 1/250. Furthermore perinatal outcome of pregnant with FMF was comparable to the pregnant without FMF.

On the basis of this observation, pregnant with FMF should be reassured about the not altered levels of first-and second trimester marker. Therefore readjusting the serum markers used for Down syndrome screening is not advised. Future trials of larger scale are needed to assess any need for readjustment of the risk in the patient population with FMF.

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References

1. Berkun Y, Eisenstein EM. Diagnostic criteria of familial Mediterranean fever. Autoimmun Rev. 2014;13(4-5):388-90.
2. Diav-Citrin O, Shechtman S, Schwartz V, Avgil-Tsadok M, Finkel-Pekarsky V, Wajnberg R, Arnon J, Berkovitch M, Ornoy A. Pregnancy outcome after in utero exposure to colchicine. Am J Obstet Gynecol. 2010;203(2):144.e1-6.
3. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn. 2011;31(1):7-15.
4. American College of Obstetricians and Gynecologists. ACOG Practice Bulletin No. 77: screening for fetal chromosomal abnormalities. Obstet Gynecol. 2007;109(1):217-27.
5. Turkcapar F, Engin-Üstün Y, Simsek OY, Deveer R, Danisman N, Dilmen U, Mollamahmutoglu L. First and second trimester biochemical markers in familial mediterranean fever. Eur Rev Med Pharmacol Sci. 2013;17(13):1820-3.
6. Committee opinion no 611: method for estimating due date. Obstet Gynecol. 2014;124(4):863-6.
7. Salomon LJ, Alfirevic Z, Bilardo CM, Chalouhi GE, Ghi T, Kagan KO, Lau TK, Papageorghiou AT, Raine-Fenning NJ, Stirnemann J, Suresh S, Tabor A, Timor-Tritsch IE, Toi A, Yeo G. ISUOG practice guidelines: performance of first-trimester fetal ultrasound scan. Ultrasound Obstet Gynecol. 2013;41(1):102-13.
8. Spencer K, Bindra R, Nicolaides KH. Maternal weight correction of maternal serum PAPP-A and free beta-hCG MoM when screening for trisomy 21 in the first trimester of pregnancy. Prenat Diagn. 2003;23(10):851-5.
9. American College of Obstetricians and Gynecologists; Task Force on Hypertension in Pregnancy. Hypertension in pregnancy. Report of the American College of Obstetricians and Gynecologists' Task Force on Hypertension in Pregnancy. Obstet Gynecol. 2013;122(5):1122-31.
10. Miller J, Turan S, Baschat AA. Fetal growth restriction. Semin Perinatol. 2008;32(4):274-80.
11. American Diabetes Association. Standards of medical care in diabetes--2012. Diabetes Care. 2012;35(Suppl 1):S11-63.
12. Rabinovitch O, Zemer D, Kukia E, Sohar E, Mashiach S. Colchicine treatment in conception and pregnancy: two hundred thirty-one pregnancies in patients with familial Mediterranean fever. Am J Reprod Immunol. 1992;28(3-4):245-6.