Thieme

Methylenetetrahydrofolate Reductase Polymorphisms and Pregnancy Outcome

Methylentetrahydrofolatreduktase-Polymorphismen und Schwangerschafts-Outcome

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ABSTRACT

Introduction Aim of the study was to evaluate the effect of methylenetetrahydrofolate reductase (MTHFR) polymorphisms on pregnancy outcome.

Materials and Methods A total of 617 pregnancies of women who were investigated for MTHFR C677T and A1298C polymorphisms prior to pregnancy were included in the study. Cases were classified into "homozygous polymorphisms" (Group I), "heterozygous polymorphisms" (Group II), and patients without polymorphisms who functioned as controls (Group III). Patients with polymorphisms were assigned to a specific protocol at least 3 months before becoming pregnant. Administration of low molecular weight heparin (LMWH) was started very early during pregnancy. The Beksac Obstetrics Index (BOI) was used to estimate the obstetric risk levels for the different groups.

Results We found that the early pregnancy loss (EPL) rate increased as MTHFR polymorphism complexity increased and that the early EPL rate was significantly higher in patients with MTHFR C677T polymorphism compared to patients with MTHFR A1298C polymorphism ($p = 0.039$). There were significant differences between the previous pregnancies of the patients in the 3 study groups in terms of perinatal complications and EPLs ($p = 0.003$ and $p = 0.019$). The BOI decreased as the severity of polymorphisms increased. An association between MTHFR polymorphisms and congenital malformations and chromosomal abnormalities was observed. We could not demonstrate any statistically significant difference between study groups when the 3 groups were compared with regard to the pregnancy outcomes under specific management protocols.

Conclusion MTHFR polymorphisms are potential risk factors for adverse pregnancy outcomes.

ZUSAMMENFASSUNG

Einleitung Ziel dieser Studie war es, die Auswirkungen von Methylentetrahydrofolatreduktase-(MTHFR-)Polymorphismen auf das Schwangerschafts-Outcome zu untersuchen.

Material und Methoden Es wurden insgesamt 617 Schwangerschaften von Frauen, bei denen vor der aktuellen Schwangerschaft eine Untersuchung auf MTHFR-C677T- und -A1298C-Polymorphismen durchgeführt wurde, in die Studie aufgenommen. Die Frauen wurden in 3 Gruppen eingeteilt: „homozygote Polymorphismen" (Gruppe I), „heterozygote Polymorphismen" (Gruppe II) sowie Frauen ohne Polymorphismen, die als Kontrolle fungierten (Gruppe III). Patientinnen mit Polymorphismen wurden mindestens 3 Monate vor ihrer Schwangerschaft einem spezifischen Protokoll zugeordnet. Mit der Verabreichung von niedermolekularem Heparin (LMWH) wurde bereits sehr früh während der Schwanger-

schaft begonnen. Der Beksac Obstetrics Index (BOI) wurde zur Schätzung des geburtshilflichen Risikos der 3 Gruppen verwendet.

Ergebnisse Es zeigte sich, dass der Anstieg der Frühabortrate mit einem Anstieg an MTHFR-Polymorphismus-Komplexität einherging und dass die Frühabortrate bei Frauen mit MTHFR-C677T-Polymorphismen signifikant höher war als bei Frauen mit MTHFR-A1298C-Polymorphismen (p = 0,039). Es gab signifikante Unterschiede zwischen den früheren Schwangerschaften der Patientinnen in den 3 Untersuchungsgruppen in Bezug auf perinatale Komplikationen und Frühaborte (p = 0,003 bzw. p = 0,019). Der BOI nahm mit zunehmendem Schweregrad der Polymorphismen ab. Es wurde auch eine Assoziation zwischen MTHFR-Polymorphismen und angeborenen Fehlbildungen sowie Chromosomenanomalien beobachtet. Beim Vergleich der 3 Studiengruppen in Bezug auf Schwangerschafts-Outcome und Managementprotokoll konnten wir keinen statistisch signifikanten Unterschied feststellen.

Schlussfolgerung MTHFR-Polymorphismen sind ein potenzieller Risikofaktor für einen ungünstigen Schwangerschaftsverlauf.

Introduction

Methylenetetrahydrofolate reductase (MTHFR) polymorphism(s) and adverse "obstetrical/perinatal outcome" relationship has been a matter of interest for a long time [1, 2]. Likewise, the connections between MTHFR polymorphism(s) and repeated miscarriage, intrauterine growth retardation (IUGR), preterm delivery, preeclampsia, congenital abnormalities, fetal aneuploidies and ablation placenta has been reported already by various authors [3 – 7]. On the other hand, one must consider the chaotic nature and robustness of these relationship(s) in order to understand the biological rationale behind this "inherited folate metabolism disorder" and to have better management protocols [8 – 11].

The MTHFR polymorphisms are relatively common. Several studies reveal that estimated population frequency changes between 5 to 14 percent [2, 9, 11]. MTHFR polymorphisms are more frequent than it has been expected and one should be careful before having epidemiological comments [9, 12]. We believe that MTHFR polymorphisms are genetic risk factors which necessitate some other pathological conditions in order to trigger "chained biological events" that will cause metabolic placental inflammation and intrauterine hypoxia.

MTHFR is an enzyme converting dietary folate (methylenetetrahydrofolate) to its active form (methyltetrahydrofolate) which is the co-enzyme of methionine synthase together with vitamin B_{12} [13, 14]. Methionine synthase is a critical enzyme taking role in the conversion of homocysteine to methionine which also takes an important role in the DNA methylation process [15]. Homocysteine-Methionine and MTHFR related biochemical pathways are required for various biological processes such as the synthesis of various nucleotides (methylation of cytosine) and DNA methylation [16]. We must also keep "the hazardous structure of dietary folate (methyleneTHF)" in mind and arrange specific dietary protocols not to have impaired pyrimidine (timidilate; uracil → thymine) and DNA synthesis and co-factor deficiencies (vitamin B_1, B_6, B_2, B_3) [15, 17]. At the presence of MTHFR polymorphisms, tetrahydrofolate is converted to dihydrofolate, which in turn is converted to monohydrofolate. This is subsequently reconverted to methyltetrahydrofolate for the clearance of MTHFR. During this process, translational autoregulation of thymidilate synthase and dihydrofolate reductase, and uridine monophosphate conversion to thymidine monophosphate occur, resulting in the formation of tetrameric DNA and related complications.

MTHFR polymorphism(s) is generally accepted as a risk factor for thrombus formation and venous thromboembolism, and has been associated with cardiovascular diseases such as coronary artery disease [18, 19]. Hyperhomocysteinemia and homocysteine related pathway disorders seem to be the critical actors behind these unwanted obstetrical complications and thrombotic events [19 – 21]. Balanced homocysteine remethylation and trans-sulfuration are critical metabolic pathways because homocysteine is a vascular toxin which may cause endothelial injury at various organs [22]. And most probably, elevated homocysteine level is responsible from the injury of "intervillous space cellular structures" (endothelial cells of spiral veins, endovascular trophoblasts at the tip of spiral arteries, syncytotrophoblasts, superficial/glandular epithelial cells of decidua, decidual/sertoli cells, etc.) and cause placental inflammation going together with impaired fetal perfusion [23 – 25].

The aim of this retrospective study was to evaluate prenatal, obstetrical and perinatal complications in pregnancies with MTHFR C677T and A1298C gene polymorphisms.

Methods

In this study, we have screened "Perinatal Medicine Databank" of the Department of Obstetrics and Gynecology, Hacettepe University between January 2002 and December 2012 and selected "pregnancies" (aged between 18 and 45 years old) who had undergone MTHFR C677T and A1298C polymorphism investigations "before their subsequent pregnancies" due to various reasons (obstetrical, medical, family history etc.) (Hacettepe University Ethical Committee approval number: GO 14/443-34). Polymorphism analyses for MTHFR were performed by a single researcher (FG) with real time polymerase chain reaction (PCR) method over the ten years.

Patients with Factor V Leiden and Prothrombin 20210A mutations, and Antithrombin III, Protein C & S deficiencies, were excluded from the study. Patients with known autoimmune disorders and severe systemic disorders which may affect the study results were also excluded from the study. For the final analysis, a total of 617 patients were recruited for the evaluation in 3 groups. Group I consisted of 227 cases with homozygous or compound heterozygous MTHFR polymorphisms, Group II comprised 257 cases with heterozygous MTHFR polymorphisms (C677T or A1298C), and 133 patients (with various risk factors necessitating MTHFR

polymorphism investigation) were recruited for the control group in which MTHFR polymorphisms were negative at all (Group III).

These 617 patients were examined and reevaluated before their subsequent pregnancies. Patients with MTHFR polymorphisms were carefully assigned to a specific treatment/management protocol (methionine restricted diet, 100 mg/day salicylic acid, vitamins B_1, B_2, B_3, B_6, B_9, and B_{12} daily, and 5 mg folic acid twice a week) at least 3 months before getting pregnant and this protocol was continued during pregnancy. The daily intake of methionine in the general population varies from 1 to 4 g/day. Methionine restricted diet is the diet having less than 1 g/day methionine. Additionally, low dose low molecular weight heparin (LMWH; enoxaparine 1 × 2000 Anti-XA IU/0.2 ml/day) was started very early in their subsequent pregnancies.

The demographic and clinical characteristics of the patients were achieved through patient files and electronic records of the hospital and Division of Perinatal Medicine. Patient demographics (age, parity, gravida, etc.), obstetrics history, maternal serum vitamin and minerals levels, diagnostic laboratory parameters, pregnancy outcome such as gestational weeks at delivery, birth weight (gram), perinatal complications (miscarriage, chromosomal abnormalities, malformations, preeclampsia, intrauterine growth retardation, preterm rupture of membranes, ablation placenta, and stillbirth) were recorded. In this study, we have used "composite perinatal complications" (CPC) in the comparison of the study groups. CPC is consisted of "IUGR, PPROM/preterm delivery, preeclampsia, ablation placenta and stillbirths". The data about outcomes of the newborns were kindly donated from the records of Neonatology Unit. Necessary consent forms were obtained at each step of the antenatal care program.

Beksac Obstetric Index

Beksac Obstetrics Index (BOI) is used for the evaluation of "risk level" in "pregnancy/patient" groups going together with different types of medical problems and perinatal complications. (BOIp is the calculation of the index during the course of current pregnancy) [26]. This obstetric index was calculated as: ("number of live born children" + "π/10")/Gravida. In this study, BOIp was calculated and recorded separately during the last pregnancies of the patients in order to compare the study groups in terms of obstetrical history and obstetrical performances.

Statistical analysis

Descriptive statistics were used to describe and compare the baseline characteristics of the groups. Continuous variables were compared using One-Way Anova or Kruskal Wallis tests, and categorical variables were compared using the Pearson Chi-Square test. A p-value of < 0.05 was accepted as statistically significant. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) v. 20.0 for Windows (SPSS, Inc., Chicago, IL).

Results

Patients demographics

Obstetrical and demographical features of the 3 groups are shown at ▶ **Table 1**. There were statistically significant differences between 3 groups in terms of miscarriage and "number of live born children" (p = 0,035 and p = 0,000, respectively) (▶ **Table 1**).

▶ **Table 1** Demographic features and maternal serum vitamin and mineral levels.

	Group I (n = 227) (n [%])	Group II (n = 257) (n [%])	Group III (n = 133) (n [%])	p value
Maternal age (years)	31.40 ± 5.06	31.49 ± 5.06	31.83 ± 5.02	0.729[a]
Gravida (n = 1799)	3.0 (2.0–4.0) (665 [36.4%])	3.0 (2.0–4.0) (779 [43.3%])	2.0 (2.0–3.0) (355 [19.7%])	0.095[b]
Parity (n = 494)	1.0 (0–1.0) (167 [33.8%])	1.0 (0–1.0) (216 [43.7%])	1.0 (0–1.0) (111 [22.5%])	0.057[b]
Miscarriage (n = 571)	0 (0–2.0) (224 [39.2%])	0 (0–1.5) (252 [44.1%])	0 (0–1.0) (95 [16.7%])	0.035[b]
Live born (n = 349)	0 (0–1.0) (97 [27.8%])	0 (0–1.0) (154 [44.1%])	1.0 (0–1.0) (98 [28.1%])	0.000[b]
Blighted ovum (n = 51)	0 (0–0) (18 [35.3%])	0 (0–0) (27 [52.9%])	0 (0–0) (6 [11.8%])	0.428[b]
Postpartum death (n = 90)	0 (0–0) (38 [42.2%])	0 (0–0) (39 [43.3%])	0 (0–0) (13 [14.5%])	0.472[b]
Intrauterine death (n = 66)	0 (0–0) (30 [45.5%])	0 (0–0) (29 [43.9%])	0 (0–0) (7 [10.6%])	0.212[b]
Ectopic pregnancy [n = 10]	0 (0–0) (2 [20%])	0 (0–0) (7 [70%])	0 (0–0) (1 [10%])	0.186[b]
Molar pregnancy(n = 4)	0 (0–0) (0 [0%])	0 (0–0) (3 [75%])	0 (0–0) (1 [25%])	0.276[b]
History of fetal anomaly (n = 103)	0 (0–0) (33 [32.0%])	0 (0–0) (47 [45.6%])	0 (0–0) (23 [22.4%])	0.532[b]
Folic acid (ng/mL)	12.98 ± 5.18	33.03 ± 189.70	13.12 ± 5.20	0.173[a]
Vitamin B_{12} (pg/mL)	307.08 ± 158.22	327.41 ± 216.59	344.20 ± 386.29	0.407[a]
Zinc (pg/mL)	108.20 ± 24.72	106.24 ± 9.13	104.67 ± 32.19	0.814[a]
Copper (pg/mL)	26.14 ± 17.62	28.86 ± 19.57	25.42 ± 11.82	0.461[a]

Data are given as means ± standard deviation or median (IQR 25–75th centiles).

[a] Values calculated by One-Way ANOVA.

[b] Values calculated by Kruskal Wallis test.

IQR: Interquartile range.

▶ **Table 2** Comparison of multigravida pregnant women in our three study groups in terms of their previous pregnancy complications.

	Group I (n = 193)	Group II (n = 221)	Group III (n = 114)	p value		
				Group I vs. II*	Group I vs. III*	Group II vs. III*
Early pregnancy complications	118 (61.1%)	125 (56.6%)	51 (44.7%)	0.313	0.005	0.04
Composite perinatal complications	103 (53.4%)	103 (46.6%)	38 (33.3%)	0.169	0.006	0.001
Fetal genetic problems	36 (18.7%)	42 (19.0%)	18 (15.4%)	0.997	0.524	0.467

* Pearson Chi-Square test

▶ **Table 3** Comparison of MTHFR mutation groups in terms of previous pregnancy complications.

	MTHFR 677 homozygous (n = 46) + heterozygous (n = 128) (n = 174)	MTHFR 1298 homozygous (n = 32) + heterozygous (n = 93) (n = 125)	p value
Early pregnancy complications (homozygous + heterozygous)	(n = 31 + 68) 99 (56.9%)	(n = 16 + 40) 56 (44.8%)	0.039*
Composite perinatal complications (homozygous + heterozygous)	(n = 20 + 66) 86 (49.4%)	(n = 11 + 43) 54 (43.2%)	0.287*
Fetal genetic problems (homozygous + heterozygous)	(n = 6 + 27) 33 (19.0%)	(n = 5 + 15) 20 (16.0%)	0.508*

* Pearson Chi-Square test

Maternal serum folic acid, vitamin B_{12}, zinc and copper plasma levels were not statistically significantly different in between the 3 groups probably because blood samples were obtained during the beginning of their last pregnancies under medical treatment (▶ **Table 1**).

Previous pregnancies

We have demonstrated that there are statistically significant differences between the previous pregnancies of the multigravid pregnant women of the 3 study groups in terms of the presence of "composite perinatal complications" (p values = Group I vs. III: 0.006, Group II vs. III: 0.001) and composite "early pregnancy complications" (miscarriage/abortion, blighted ovum, ectopic pregnancy and molar pregnancy) (p values = Group I vs. III: 0.005, Group II vs. III: 0.04) (▶ **Table 2**).

We have shown that early pregnancy loss rate is statistically significantly increased at patients with MTHFR C677T polymorphism compared to patients with MTHFR A1298C polymorphism (p = 0.039) (▶ **Table 3**). There is no statistically significant difference in between these two polymorphisms in terms of composite perinatal complications (CPC) and genetic disorders. On the other hand, there were no statistical differences between homozygous and heterozygous patients in these three outcomes.

Previous obstetrical performance (obstetrical backgrounds/histories) of the three groups were compared in terms of a new index, heretofore named BOIp and statistically significant differences were found in between the 3 groups (p < 0.001) (BOIp values were 0.2886 ± 0.2089, 0.3290 ± 0.2102 and 0.4147 ± 0,2274 for Group I, Group II and Group III respectively). Statistically significant differences were detected between Group I versus III and Group II versus III (p < 0.001 and p = 0.001 respectively). Reduced MTHFR activity (Group I > II > III) was associated with lower BOIp values.

Previous pregnancies of 528 multigravid patients (89 primigravid patients were excluded) were also evaluated in terms of the genetic problems of the fetus. One hundred and eleven pregnancies in terms of genetic disorders (chromosomal abnormalities, congenital malformations and gene disorders) were detected in 1193 pregnancies of 528 multigravid patients (9%) (412 pregnancies of 193 patients in Group I, 517 pregnancies of 221 patients in Group II and 264 pregnancies of 114 patients in Group III). It has been demonstrated that 72.1% of these 114 cases were congenital malformations while 11.5% and 7.7% of cases were chromosomal abnormalities and single gene mutations respectively (8.7% of the cases were excluded from statistical analysis due to lack of sufficient information or finding) (▶ **Table 4**). There were no statistical differences between all study groups (Group I vs. II, I vs. III, and II vs. III). Nineteen cases with chromosomal abnormalities were detected and the most common chromosomal anomalies were Turner syndrome (n: 4) and Down's syndrome (n: 4). There was no statistically significant difference between study groups in terms of genetic disorders though 18 of 19 "chromosomal abnormality cases" belonged to polymorphism groups.

▶ **Table 5** shows the distribution of congenital malformations. The most common malformations were NTD (25,3%) and cardiac anomalies (25,3%). About 90% of NTDs were observed at patients

▶ **Table 4** Distribution of congenital malformations, chromosomal anomalies and single gene disorders for the three study groups in previous pregnancies.

	Group I (n = 412)	Group II (n = 517)	Group III (n = 264)	p values		
				Group I vs. II*	Group I vs. III*	Group II vs. III*
Congenital malformations (n = 75)	24 (32%)	33 (44%)	18 (24%)	0.724	0.601	0.815
Chromosomal anomalies (n = 19)	9 (47.3%)	9 (47.3%)	1 (5.4%)	0.626	0.057	0.109
Single gene disorder (n = 8)	2 (25%)	6 (75%)	0	0.268	NA	NA
Unidentified cases (n = 9)	2 (22.2%)	3 (33.3%)	4 (44.4%)	0.844	0.163	0.271
Total (n = 111)	37 (100%)	51 (100%)	23 (100%)	0.647	0.904	0.602

* Pearson Chi-square test
NA = not applicable

▶ **Table 5** Congenital malformations observed in fetuses.

	Group I (n = 24)	Group II (n = 33)	Group III (n = 18)
Neural tube defects (n = 19)	6 (31.5%)	11 (57.8%)	2 (10.5%)
Cardiac anomalies (n = 19)	7 (36.8%)	5 (26.3%)	7 (36.8%)
Urinary tract anomalies (n = 11)	7 (63.6%)	3 (27.2%)	1 (9.0%)
Congenital diaphragm hernia (n = 8)	2 (25%)	4 (50%)	2 (25%)
Multiple anomalies (n = 7)	0	6 (85.7%)	1 (14.3%)
Nonimmune hydrops fetalis (n = 5)	1 (20%)	2 (40%)	2 (40%)
Agenesis of the corpus callosum (n = 3)	0	2 (66.6%)	1 (33.3%)
Omphalocele (n = 2)	1 (50%)	0	1 (50%)
Cleft lip/palate (n = 1)	0	0	1 (100%)

with reduced MTHFR activity (Group I and II). Urinary system anomalies are more frequent in Group I and about 90% of cases concern patients with reduced enzyme activity (Group I and II).

First pregnancies of the study groups were specifically compared in terms of 4 items such as

1. early pregnancy losses,
2. composite perinatal complications (IUGR, PPROM/preterm delivery, preeclampsia, placental abruption and perinatal mortality/stillbirth),
3. term delivery ratio and
4. genetic problems of the newborn (chromosomal abnormalities, congenital malformations and gene defects).

Therefore, 89 of the all patients were removed from the analysis due to primigravidity (whose MTHFR polymorphisms were detected due to various medical problems before getting pregnant). Term delivery rates were 25,3, 26.7 and 41.6% in Group I, Group II and Group III respectively and the differences were statistically significant (p < 0,001) even though Group III itself is a kind of high risk pregnancy group. We have also found increasing "early pregnancy loss" rates going together with MTHFR polymorphism complexity (Group I > II > III).

Pregnancies under treatment

When the 3 groups were compared in terms of their last pregnancies (who were under careful antenatal care), it has been demonstrated that gestational weeks at delivery were 36.67 ± 2.06, 36.91 ± 1.77 and 37.09 ± 2.01 for Group I, II and III respectively with no statistical difference between groups. The birthweights of the neonates in Group I, II and III were found to be 2839.6 ± 519.3 g, 2895.6 ± 475.3 g and 2988.2 ± 566.5 g respectively. We have shown a statistically significant difference only in between Group I and III in terms of birthweight (p = 0.030). The mortality rates were 0.9, 1.2 and 1.1% in Group I, II and III respectively (p = 0.863). We have found low rates of IUGR, oligohydramnios, PPROM, preeclampsia and ablation placenta at the last pregnancies of the study groups probably because last/current pregnancies were under careful antenatal care and specific treatment (▶ **Table 6**). We could not demonstrate any statistical difference between study groups in terms of these complications. On the other hand, early pregnancy loss rate in these patients (patients with MTHFR polymorphisms) was determined as 10.8% which is very much like normal population again probably because last pregnancies of these patients were under intensive care and medical treatment (vitamin B supplementation, low dose low molecu-

▶ **Table 6** Comparison of the outcomes of the last pregnancies of the patients of 3 different groups in terms of obstetrical complications and perinatal outcomes including genetic problems and congenital malformations.

	Group I (n = 227)	Group II (n = 257)	Group III (n = 133)	p value
Preeclampsia (n = 20 [%])	5 (2.2%)	10 (3.9%)	5 (3.8%)	0.538
PPROM (n = 18 [%])	7 (3.1%)	7 (2.7%)	4 (3.0%)	0.970
IUGR (n = 11 [%])	1 (0.4%)	8 (3.2%)	2 (1.5%)	0.820
Ablatio placenta (n = 1 [%])	1 (0%)	0 (0%)	0 (0%)	0.423
Oligohydramnios (n = 11 [%])	5 (2.2%)	6 (2.3%)	0 (0%)	0.213
Stillbirth (n = 6 [%])	2 (0.9%)	3 (1.2%)	1 (0.8%)	0.863

PPROM: Preterm premature rupture of membranes
IUGR: Intrauterine growth restriction

lar weight heparin and low dose salicylic acid treatment together with methionine restricted diet).

Discussion

The question is the explanation of the mechanisms involved in increased perinatal morbidity and mortality in pregnant women with MTHFR gene polymorphisms (C677T and A1298C). It has been reported that several biological processes are disturbed due to the decreased MTHFR enzyme activity related to these mutations [20, 21, 25, 27]. Thus, increased homocysteine (injury of the cellular components of intervillous space of the placenta which results in impaired implantation and decreased fetal perfusion) and decreased methionine (impaired DNA methylation) levels may be responsible from various perinatal and obstetrical complications as well as the unwanted complications due to the accumulation of inactive dietary folic acid (activation of tetrameric DNA, timidilate) [16, 20, 24].

MTHFR enzyme polymorphisms are associated with increased risk for chromosomal abnormalities [28]. These polymorphisms can even cause consequent trisomic fetuses in the same patient [28, 29]. Association of MTHFR polymorphism(s) and congenital malformations were also reported by several authors (neural tube defects (NTD), cleft lip/palate, congenital heart defects etc.) [4, 30 – 33]. We have demonstrated similar findings in our series, 18 of the 19 (94.7%) pregnancies with chromosomal anomalies were in the MTHFR homozygous or heterozygous group. We have also demonstrated that the most common malformations were NTD (25.3%) and cardiac anomalies (25.3%), and about 90% of NTDs affected patients with reduced MTHFR activity.

Additionally, recurrent miscarriages and adverse perinatal outcomes such as intrauterine growth restriction, preeclampsia, preterm labor, preterm premature rupture of membranes (PPROM), ablation placenta and stillbirth were reported in pregnancies with MTHFR polymorphisms [5, 6, 34, 35]. We have found increasing "early pregnancy loss" rates going together with MTHFR polymorphism complexity in terms of enzyme activity (Group I > II > III). We have also demonstrated that there are statistically significant differences in between the previous pregnancies of the multigravid pregnant women of the 3 study groups in terms of the presence of "composite perinatal complications" and "early pregnancy complications" (miscarriage/abortion, blighted ovum, ectopic pregnancy and molar pregnancy) ($p = 0.003$ and $p = 0.019$). However, there are conflicting publications in this field due to the complexity of the problem [27, 36, 37].

The association between MTHFR polymorphisms and recurrent pregnancy loss has already been known for a long time, and it has been reported that low-dose acetylsalicylic acid (ASA) and low molecular weight heparin (LMWH) have a positive impact on pregnancy outcome [36, 38]. Our findings are consistent with the literature. We have also demonstrated that "MTHFR homozygous group" (Group I) had the highest early pregnancy loss rate in their first pregnancies (untreated pregnancies) when compared to the others.

Our study group consisted of patients that were preconceptionally evaluated and prepared for their subsequent pregnancies. Low dose LMWH, low dose ASA, folic acid, pyridoxine and cobalamin treatments and diet containing lower methionine levels were started as early as possible when they got pregnant under intensive antenatal care program. We believe that this treatment modality improved the pregnancy outcomes.

In clinical practice, we are dealing with a wide spectrum of patients with various health disorders causing different perinatal complications. The critical issue is the existence of multiple variables behind the maternal health disorders and perinatal complications [1, 34, 39]. This reality makes life difficult for physicians in the categorization and comparison of risk levels for different patient groups. Therefore, in this study we have used a new obstetric index which is named "Beksac Obstetrics Index" (BOI) which is defined as (number of living children + [Π/10])/gravida [26]. We believe that BOI is especially profitable during pregnancy and critical for the planning/envisioning of the management of risk factors. In this perspective, when comparing all of the groups in terms of BOI, increasing MTHFR polymorphism severity (Group I > II > III) was associated with lower BOI values and these results were statistically significant.

In conclusion, MTHFR polymorphisms and impaired "homocysteine/methionine" metabolism disorders are potential risk factors for "placental inflammation and impaired fetal perfusion" going together with obstetrical/perinatal complications. The same mechanisms are also responsible for the "impaired nucleotide

and DNA synthesis" and most probably the reason of increased incidence of congenital abnormalities and fetal aneuploidies.

We believe that pregnancies with poor obstetrical history concerning repeated miscarriages and obstetrical complications might be screened for MTHFR polymorphisms, and we recommend methionine restricted diet, 100 mg/day salicylic acid, vitamins B_1, B_2, B_3, B_6, B_9, and B_{12} daily, and 5 mg folic acid twice a week together with enoxaparine (1 × 2000 Anti-XA IU/0.2 ml/day) for pregnant women with these polymorphisms.

Conflict of Interest

The authors declare that they have no conflict of interest.

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