Ege Tıp Dergisi / *Ege Journal of Medicine* 51(1): 37-42, 2012

Evaluation of mothers' MTHFR genotype and other risk factors of neural tube defects in their children

Nöral tüp defektlerinde annelerde MTHFR gen polimorfizmleri ve diğer risk faktörlerinin değerlendirilmesi

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Summary

Aim: Neural tube defects (NTDs) are common congenital anomalies of the nervous system, comprising primarily of spina bifida and anencephaly. The etiology of NTDs is complex and the genetic and enviromental factors which appear to be involved in the etiology are imperfectly understood. This study was conducted to investigate the influence of genetic and enviromental factors, namely parental consanguinity, maternal obesity, maternal folate and vitamin B12 levels, parental age and the maternal MTHFR genotype on the risk of NTD in the child. Additionally, maternal multivitamin intake, maternal education and occupation status, socioeconomic levels and the type of delivery were also evaluated.

Materials and Methods: Forty-five mothers who had children affected with NTD and forty-one mothers who had healthy children were included in the study. All mothers' serum folate and vitamin B12 levels were measured, and the 677C>T and the 1298A>C polymorphisms of the MTHFR gene were analysed, and body mass index was calculated. A questionnaire was administered to detect the sociodemographic and socioeconomic status in both groups.

Results: Comparison of the parameters of both groups revealed that frequencies of maternal obesity, consanguineuos marriages are higher, multivitamin intake and serum vitamin B12 levels are lower, and frequency of MTHFR 677TT genotype is higher in mothers of children with NTD.

Conclusion: Parental consanguinity, inadequate multivitamin intake during pregnancy, maternal obesity and the MTHFR 677TT genotype may play a role in the etiology of NTDs.

Key Words: Neural tube defects, obesity, MTHFR.

Özet

Amaç: *Nöral tüp defektleri, başlıca spina bifida ve anensefali olmak üzere, sinir sisteminin sık görülen konjenital anomalileridir. Nöral tüp defekti etyolojisi tam anlaşılamamakla beraber, etyolojide genetik ve çevresel faktörler sorumlu tutulmaktadır. Bu çalışmada akraba evliliği, annedeki obesite, annenin folat ve vitamin B12 düzeyleri, ebeveyn yaşı ve annenin metilentetrahidrofolat redüktaz gen polimorfizmleri gibi genetik ve çevresel risk faktörlerinin çocukta nöral tüp defekti gelişimi üzerine etkisinin araştırılması hedeflenmiştir. Ek olarak, annelerin multivitamin kullanımı, eğitim düzeyi, meslek durumu, sosyoekonomik düzey ve doğum şekilleri de incelenmiştir.*

Gereç ve Yöntem: *Nöral tüp defektli çocuğu olan 45 ve sağlıklı çocuğu olan 41 anne çalışmaya alınmıştır. Tüm annelerde serum folat ve vitamin B12 düzeyleri ile metilentetrahidrofolat redüktaz 677C>T ve 1298A>C polimorfizmleri çalışılmıştır. Vücut kitle indeksi ölçülmüştür. Sosyodemografik ve sosyoekonomik özellikler anket uygulanarak değerlendirilmiştir.*

Bulgular: *Nöral tüp defektli çocuğu olan annelerde obesite ve akraba evliliği daha sık, gebelikte multivitamin kullanımı daha az, serum vitamin B12 düzeyi daha düşük ve metilentetrahidrofolat redüktaz 677TT genotipinin daha sık olduğu görülmüştür.*

Sonuç: *Nöral tüp defekti etyolojisinde gebelikte yetersiz multivitamin alımı, akraba evliliği, maternal obesite ve metilentetrahidrofolat redüktaz 677TT genotipinin rol aldığı saptanmıştır.*

Anahtar Sözcükler: *Nöral tüp defektleri, obesite, MTHFR.*

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Makalenin Geliş Tarihi: 22.08.2011 Kabul Tarihi: 25.10.2011

Introduction

Neural tube defects (NTDs) are malformations secondary to abnormal neural closure that occurs between the third and fourth weeks of gestational age. The birth prevalance is dependent on country and socioeconomic and ethnic groups, 1:1000 America, 3:1000 in Turkey, 1:2500 in Finland, 2:1000 in Poland and 1:700 in Netherlands (1-5).

NTDs have complex and imperfectly understood etiology in which genetic and enviromental factors appear to be involved. Recognized genetic causes of NTD include multifactorial inheritance, single-gene mutations (eg: Meckel's syndrome), and chromosomal abnormalities. Some possible enviromental factors are diabetes mellitus, hypertermia, use of teratogens like aminopterine, thalidomide, valproic acid and other antiepileptic medications, alcohol, inadequate folic acid intake, maternal age, birth order, socioeconomic status, and maternal obesity. It has been suggested that periconceptional folic acid suplementation can prevent both the occurrence and recurrence of NTDs by approximately 50-70% (6).

Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in one-carbon metabolism and folate pathway. The enzyme catalyses the conversion of 5,10-methylenetetrahydrofolate into 5-methyletetrahydrofolate, the predominating circulating form of folate. MTHFR takes a part in S-adenosylmethionine synthesis that serves as a methyl group donor in the methylation of DNA, proteins, neurotransmitters and phospholipids (7).

A number of studies have reported that polymorphisms in the MTHFR gene are associated with increased risk of NTDs (1, 3, 5, 9, 11, 20, 21). The best characterized MTHFR gene polymorphism consists of a 677C>T transition which results in an alanine to valine substitution in the catalytic domain of MTHFR. This substitution tenders the enzyme thermolabile, and homozygotes and heterozygotes have about 70% and 55% reduced activity in vitro, respectively (8). A second common polymorphism in the MTHFR gene is a 1298A>C transition which results in a glutamate to alanine substitution in the regulatory domain of MTHFR (9).

In the present study, a number of risk factors which are considered to play a role in the etiology of NTDs were investigated.

Materials and Methods

Forty-five mothers who have a child with NTD and 41 mothers of healthy children were included into the study. Mothers who have a child with NTD were selected from Obstetry, Pediatric Urology and Neurochirurgy Clinics which were invited to participate in the study. Informed consent was obtained from all participants. The Ethics Committee of Ege University Faculty of Medicine approved this study.

A questionnaire was administered to all mothers from both groups by the same doctor. This questionnare included questions on parental age, maternal reproductive history, family history, maternal nutrition, and drug usage during pregnancy, parental occupation and parental education, maternal chronic diseases and socioeconomic status. To detect the maternal nutrional status during pregnancy, all mothers were asked how often they consumed the following foods: cheese, eggs, bread, milk, fruits, vegetables, meat and grains. There were six possible answers ranging from "never or less than once per month" to " at least once per day". The maximum score was 50. If the final score was less than 41, the mother was considered to be poorly nourished during pregnancy (10).

Parental ages were grouped in 4 classes as shown in (Table-1).

Table-1. Parental ages in study and control group at time of study.

		Case (n:45)		Control (n=41)	
		n	**%**	**n**	**%**
Maternal age (years)	*20-24*	7	15.6	1	2.4
	25-29	10	22.2	14	34.1
	30-34	15	33.3	11	26.8
	≥35	13	28.9	15	36.7
Paternal age (years)	*20-24*	2	4.4	-	-
	25-29	6	13.3	10	24.4
	30-34	13	28.9	13	31.7
	≥35	24	53.4	18	43.9

Two different blood samples (without anticoagulant and with EDTA) were taken from mothers after an 8-hour fasting period. Serum was separated and stored at -20 °C until analysis. Serum folate levels were measured using chemiluminescent assay (normal level 7.0-39.7 nmol/L). Serum vitamin B12 levels were measured by radioimmune assay (normal level 22-1476 pmol/L) (Roche Elecsys 2010 and Moduler Analytics E170 Elecsys Module, Electochemilıminescense) (11).

The 677C>T and 1298A>C polymorphisms of the MTHFR gene were analyzed using the polymerase chain reaction (PCR) according to the methods previously described (8,9).

NTDs were classified as being either “low” (defects at or below the 12th thorasic vertebra) or “high” (dfecets above the 12th thorasic vertebra) (12).

All statistical analysis were performed using the Statistical Program for Social Sciences (SPSS) software, version 10.01. Statistical significance was established at $p<0.05$.

Results

Sociodemografic factors, parental age, parent’s occupation at the time of the study, consanguineous marriage, maternal nutrition and vitamin intake during pregnancy are depicted in (Table-2). The percentage of the housewife mothers in study group (66.7%) was significantly higher than that was found in control group (19.5%) ($p<0.05$). No significant difference was observed between parental education and socioeconomic status in the two groups. The percentage of consanguineous marriages was found to be 20.0% in study group, whereas it was 4.9% in the control group ($p=0.052$).

The overweight and obesity ratio were significantly higher in the mothers of NTD children ($p<0.05$). In the study group four mothers had diabetes mellitus, three had both diabetes mellitus and obesity, one hypertension, one hypothyroidism, one arrhytmia; in control group two mothers had hypertension, one hypothyroidism, and one asthma. There was no significant difference between the two groups regarding chronic illnesses.

Only 24.4% of the mothers in the study group and only 36.6% of the mothers in the control group took folic acid supplementation during pregnancy. The number of mothers taking multivitamins throughout pregnancy was significantly higher in the control gruop ($p<0.05$). Nutritional intake was inadequate in both groups. The most frequently consumed foods on a daily basis were bread, cheese and fruits. Control group mothers consumed more grains and vegetables than NTD mothers did.

Among 45 NTD patients, 9 had anencephaly, 10 encephalocele, 18 meningomyelocele, 2 myeloschisis, and 6 spina bifida occulta. 42.2% of patients had NTDs above the 12th thorasic vertebra, 57.8% of the patients had NTDs lower than 12th thorasic vertebra.

Serum folic acid and vitamin B12 levels are given in (Table-3). No difference was found between the folate levels in both groups ($p>0.05$). However, serum B12 levels were significantly lower in the mothers of NTD patients ($p<0.05$).

The genotype frequencies for the polymorphisms 677 C>T and 1298 A>C in the MTHFR gene in both groups and are shown in (Table-4). The frequency of the TT genotype for the polymorphism 677 C>T was found to be significantly higher in the mothers of NTD children ($p=0.001$). Genotype frequencies were not significantly different for the polymorphism 1298 A>C in both groups.

At the time of study there were no differences between the serum folic acid and vitamin B12 levels of the mothers having different genotypes of 677 C>T polymorphism ($p>0.05$) (data not shown). Vitamin B12 levels were found to be significantly low in the mothers carrying AC genotype of the polymorphism 1298 A>C ($p=0.003$) (data not shown).

No relationship was observed between the MTHFR genotypes and NTD lesion levels ($p>0.05$) (data not shown).

Discussion

Neural tube defects are one of the most common congenital malformations and are the main cause of disability or death of new-borns (1). NTDs commonly have polygenic inheritance. Furthermore, enviromental factors are the risk factors for NTD. NTD rates vary from one population to another, and have also been found to vary by geography, time, and selected maternal characteristics.

NTDs can be detected antenatally through maternal serum alpha-fetoprotein and prenatal ultrasound tests. These screening methods and periconceptional folic acid supplementation have decreased the incidence of NTDs. The incidence of NTDs was found to be 2.12 per 1.000 births in northeast of Turkey for the period of 1981-1986, 2.7 for the period of 1988-1995, and 3.01 for the period of 1993-1994 throughout Turkey, and 1.5 per 1.000 births in 2000, in Izmir, western Turkey (4, 10, 13, 14).

Maternal age, race, education, socioeconomic status, nutritional status before and during pregnancy have a significant impact on maternal and infant deaths. Tuncbilek et al. (4) reported that maternal illiteracy, advanced maternal age and being settled in northern or eastern regions of Turkey were the risk factors for NTDs. In our study, all the mothers in the study group and in the control group gave birth before the age of 35. The age of the mothers in the study group was significantly higher ($p=0.023$) than the age of the mothers in the control group (Table-2).

Farley et al. (15) have reported that maternal illiteracy is associated with NTD in children. Low socioeconomic levels have been found as risk factors for NTD (16). In our study, however, the educational levels of NTD mothers seem to be lower than that of the control mothers’, the difference between the two groups was not statistically significant. There was also no difference between the socioeconomic levels of families from both groups. Higher percentage of housewives among the cases is the result of women leaving their jobs to care for an ill child.

Table-2. Parental Characteristics According to Case-Control Study Group.

		Case (n:45)		Control (n=41)			
		n	**%**	**n**	**%**	**χ^2**	**p**
Maternal age at the birth of NTD child (years)	*20-24*	20	44.4	18	43.9	7.566	0.023
	25-29	14	31.2	21	51.2		
	30-34	11	24.4	2	4.9		
Maternal education	*Primary school*	20	44.4	16	39.1	4.936	0.085
	High school	21	46.7	14	34.1		
	University	4	8.9	11	26.8		
Paternal education	*Primary school*	19	42.2	15	36.6	0.601	0.740
	High school	16	35.6	14	34.1		
	University	10	22.2	12	29.3		
Maternal occupation at the time of study	*Housewife*	30	66.7	8	19.5	19.536	0.000
	Worker	7	15.5	13	31.7		
	Civil servant	8	17.8	20	48.8		
Socioeconomic status	*Good*	18	40.0	20	48.8	1.562	0.548
	Moderate	19	42.2	12	29.2		
	Poor	8	17.8	9	22.0		
Body mass index	25-28.9	14	31.1	6	14.6	6.774	0.034
	>29	8	17.8	3	7.3		
Delivery by	*Vaginal*	11	24.4	26	63.4	23.979	0.000
	Cesarian section	16	35.6	15	36.6		
	Prenatally diagnosis of NTD and therapeutic abortion	18	40.0	-	-		
Maternal nutrition during pregnacy	*poor*	28	62.2	21	51.2	1.059	0.209
	good	17	37.8	20	48.8		
Multivitamin intake during pregnacy		22	48.9	31	75.6	6.477	0.015
Folic asid intake during pregnacy		11	24.4	15	36.6	1.499	0.248

The effect of maternal age on risk for NTDs is generally considered to be small. When an association with age can be found, the risk tends to be elevated in older or very young mothers (17). In our study, no mother had a child before age 18 or after 35.

Studies have shown a moderate risk in mothers of three or more children and an increased risk in primiparous mothers (17). All NTD affected children in our study were the first child of their families. It is well known that poor maternal nutrition and low dietary folate intake increase the risk of NTDs. In our study, all mothers in both groups had inadequate nutritional intake during pregnancy and there was no association between NTDs and maternal nutritional status.

The rate of consanguineous marriage is 25.1% according to a Turkish Demographic and Health Survey in 1998 (18). The percentage of consanguineous marriage (20%) was found to be significantly higher in case group ($p<0.05$).

Table-3. Serum levels of folate and vitamin B12 in the case and control groups.

		Study group n: 45	Control group n: 41	p
Vitamin B_{12} (pmol/L)	Mean	222,74 ± 67,69	280,03 ± 97,71	0,001
	Min-max	95.15-427.7	106.4-511.1	
Folate (nmol/L)	Mean	8,63 ± 4,23	7,42 ± 2,69	0,06
	Min-max	2.78-20.01	4.41-18.8	

Table-4. MTHFR 677C>T and 1298A>C genotype distribution in study and control groups.

Genotype		Study group n: 45		Control group n: 41			
		n	%	n	%	χ^2	p
677C>T	CC	9	20.0	22	53.7	13.359	0.001
	CT	22	48.9	16	42.1		
	TT	14	31.1	3	7.3		
1298A>C	AA	21	46.7	25	61.0	1.766	0.414
	AC	21	46.7	14	34.1		
	CC	3	6.7	2	4.9		
C677T/ A1298C		9	20	7	17	1.787	0.121

Twenty-two percent of NTD-mothers and 9.8% of control mothers had chronic diseases like diabetes mellitus, hypertension, hypothyroidism, asthma but no correlation was obtained between these diseases and NTDs.

Maternal obesity and elevated body mass index have been consistently associated with an increase risk for NTDs. Body mass index >29 doubles the risk (19). In our study the rate of obesity and overweight was significantly higher in mothers of NTD affected children.

Recently the incidence of NTDs is on the decrease owing to the screening tests performed prenatally (2,6). In our study, 38.8% of mothers in the study group had no antenatal follow-up, whereas 90.2% of the control mothers had regular control visits during pregnancy.

The MTHFR 677C>T and 1298A>C polymorphisms are risk factors for NTDs in some populations. Studies conducted in various parts of the world considering these two polymorphisms have yielded conflicting results. This may be attributed to ethnic differences in genotype distribution, heterogenity of the phenotype, and the multifactorial aetiology of NTDs. McDermott et al. (20) reported that they genotyped 276 complete NTD triads (mother, father, and child affected with NTD) and 256 controls. Their findings did not support a role for the 1298A>C and 677C>T polymorphisms in NTDs. Felix et al. (3) analyzed the 677C>T and 1298A>C polymorphisms in 41 NTD child-mother pairs and 44 normal child-mother control pairs. They obtained no difference in the genotype distrubition for 677C>T and 1298A>C polymorphisms in MTHFR in the case and control pairs. In our study, the 677TT genotype was significantly more prevalant in mothers with a NTD child. However, there were no significant difference in the 1298A>C and 677CT/1298AC compound heterozygous polymorphisms in case and the control groups.

The MTHFR polymorphisms were not able to be analysed in NTD affected children in this study. The reasons were that 40% of pregnancies were terminated after the diagnosis of NTD prenatally and most of the parents having a NTD child refused genetic analyses of their children.

A study from India revealed that MTHFR 677TT genotype is considered to be a definite risk factor for development of NTDs. The study group consisted of children with NTDs, their mothers and healthy controls. There was a significant difference in the prevalence of MTHFR 677 C>T mutation among the 3 groups (p = 0.002). The risk conferred by the TT genotype in the child was statistically significant (OR 12.625, 95% CI 1.430-111.465). In the mothers, however, although there was an increased prevalence of the mutation compared with the control individuals, although the difference was not statistically significant (p = 0.152). They concluded that it was the TT genotype status of the developing embryo, rather than the TT genotype status of its mother (21).

Volcick et al. (11) reported that 677TT genotype has been associated with high level NTDs (defects above the 12th thorasic vertebra) rather than low level NTDs. We found no association between polymorphisms and the levels of lesion levels.

We investigated the association between serum folate and vitamin B12 levels and MTHFR 677C>T and 1298A>C polymorphisms. There were no significant differences between serum folic acid levels in case and the control groups and no association between TT genotype and folic acid and vitamin B12 levels. Low levels of vitamin B12 were observed in the mothers of children with NTD. Low levels of vitamin B12 reduce the remethylation of homocystein to methionin which may result in high levels of homocystein. No correlation was found between the levels of vitamin B12 and 677TT

genotype. The mean level of vitamin B12 was observed to be lower in mothers who had 1298AC heterozygous polymorphism in this case group.

In another study from Turkey, serum folate, vitamin B12 and homocysteine serum concentrations and polymorphism of the 677C>T MTHFR gene were investigated in 33 Turkish children with neural tube defects (22). They found that 677C>T MTHFR gene polymorphism does not affect folic acid, vitamin B12 and homocysteine metabolism in children with NTDs.

In conclusion, although the number of the subjects in our study group is small, obesity, parental consanguinity, and MTHFR 677TT genotype, inadequate multivitamin intake and insufficient antenatal follow-up were found to be risk factors for NTD. Such findings are important in order to design a program for prevention of NTDs. Women should be advised to maintain a healthy nutritional diet, obesity must be prevented, and women who can become pregnant should be advised to take a multivitamin containing 0.4 mg folic acid daily, at least during periconceptional period, and should be recommended to be regularly followed-up during pregnancy.

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