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ORIGINAL ARTICLE
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Nöral tüp defekti etkilenen çocuklarda MTHFR enziminin kantitatif değerlendirilmesi

Quantitative Evaluation of MTHFR Enzyme in Neural Tube Defect Affected Children

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ABSTRACT

Background: Neural tube defect pathogenesis is still not clear and controversial. Maternal Methylene Tetrahydrofolate Reductase is an important enzyme in the cycle of folate metabolism which has a considerable relationship with defect development, while the role of fetal enzyme was yet not known.

Objective: To evaluate the serum level of Methylene Tetrahydrofolate Reductase in neural tube defect affected children and its relationship to pathogenesis.

Patients and methods: A cohort study was performed at Sept 2017- Aug 2018 in Al- Batool hospital. A newly delivered forty six neonates had neural tube defects (cases group) were included, serum level of the enzyme was measured by ELISA, results were compared to that of healthy neonates (control group). SPSS version 22 was used for statistical analysis.

Results: Cases group included spina bifida defect type (n=39, 84.8%) and cranial type (7, 15.2%), males to females ratio was 1.35:1. Methylene Tetrahydrofolate Reductase enzyme readings showed wide range in both cases and control groups, generally, for cases they were insignificantly higher than that of control (p value= 0.115); they were unrelated with the defect whether cranial or spinal, p value (0.264). Enzyme level significantly higher in cases whom mothers aged more 35 yrs old (p value= 0.00) and insignificantly higher in babies whom mothers received folic acid than others.

Conclusion: There was high enzyme level in most of cases, which might reflect a compensatory mechanism to defect formation. Enzyme deficiency was not related to defect pathogenesis.

Keywords: MTHFR, Neural tube defect, Congenital anomaly, Folate.

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Introduction:

Neural tube defect pathogenesis has multi-factorial and complicated mechanism, including environmental factors, in addition to genetic and life style causes [1]. A considerable review was available on close relationship of folic acid and NTDs pathogenesis [2]. During embryogenesis, folic acid will be supplied from maternal intake of dietary or synthetic folic acid. Once metabolized, 5-methyl-tetrahydeofolate will be the major type of folic acid in tissues and serum and it is the main donor of methyl group to produce methionine from homocysteine. Maternal 5–10 methylene tetrahydrofolate reductase (MTHFR) will replenish 5-methyl-tetrahydeofolate by 5-10-methyl-THF [3,4].

MTHFR enzyme effect is an important and significant step that control the metabolism of methionine and folic acid, a significant factors in nucleotide synthesis DNA methylation [3,4]. Many previous studies showed that the entire methylation process might be implicated in the etiology of neural tube defects and not just folic acid role [5]. Deficiency of folic acid or decreased MTHFR activity will reduce serum methionine or raise homocysteine levels [3,4]. In early pregnancy, increased maternal homocysteine and decreased folic acid concentrations are predisposing factors for NTDs, both derangements can be corrected via folate supplementation [6-9].

Over 20 different mutations in MTHFR gene have been reported, most of these mutations are mainly associated with inactivation or decreasing MTHFR activity [10-12]. Common maternal MTHFR gene polymorphisms are 1298 A C and 677C T alleles, they decrease the enzyme activity. 677C→T mutation associated with mild MTHFR deficiency, it is common in many Asian states, in addition to European and North American countries, with frequency of homozygosity ranging between 5- 25% and it has been related to NTDs and many other diseases [13,14].

All previous studies in the world demonstrated the maternal genetic risk factors for NTD, including MTHFR mutations, this article was aimed to evaluate the serum level of MTHFR in NTDs affected children to look for its relationship with NTD pathogenesis, in addition to study its demographic risk factor.

Patients and Methods

This study was cohort cross- sectional designed, It was performed at Sept 2017- Aug 2018 in neonatal care unit of Al- Batool Hospital for Maternity and Children in Iraq/ Diyala.

Inclusion/ exclusion criteria

The study include **cases group** which comprise all delivered 2-3 weeks old babies with neural tube defects of different types, in addition to **control group** which included healthy neonates of the same age group (2-3 weeks) who were a product of healthy mothers. Neonates with congenital anomalies other than NTDs were excluded from the study.

Procedure

Blood sample (3 ml) were aspirated from case and control group's neonates, the serum was separated by centrifugation, then carefully withdrawn into plan tube to be kept frozen at -20° C at laboratory of the hospital. Serum level of MTHFR was measured at immunology lab of College of Medicine/ Diyala university according to manufactures' instructions by using Human Methylene Tetrahydrofolate Reductase (MTHFR) ELISA Kit (SunRedBio SRB Technology Company, catalog number 201- 12- 4254, Shanghai, https://sunredbio.en.ecplaza.net/).

International reference value of MTHFR enzyme was not registered, hence its level in the NTD affected infants was evaluated by comparison with control group which were delivered from healthy women in the same hospital.

Level of the enzyme were evaluated in respect to many descriptive criteria, including gender, gestational age, birth weight, type of NTD, maternal age, consanguinity marriage, and folic acid intake.

Ethical consent

An knowledgeable oral consent was achieved by enrolled woman before the study and an ethical agreement was taken from administration of the hospital prior to perform the data collection. Reviewing and approving of the study protocol were performed by the Ethical Research Committee at College of Medicine/ Diyala university.

Statistical analysis

It was carried out by using SPSS version 22, student t- test was employed to measure the variables association, significance was taken at level of χ =0.05.

Results

Total number of enrolled children with neural tube defect was (46), most of these defects were spina bifida (n=39, 84.8%) and the remaining were having cranial NTD, distributed between anencephaly (n=4, 8.7%) and encephalocele (n=3, 6.5 %), p value (.000). NTD affected females babies were higher than males with a ratio of 1:1.35, p value= 0.376.

The measured serum MTHFR enzyme level showed a wide range in both cases and control group, in general, its levels in cases of NTD were higher than that of control group, but this discrepancy was statistically not significant (p value= 0.115). A noteworthy, one case showed a level of 146.97, which was extremely higher than others. The level of the enzyme in cases was insignificantly higher in cranial types than spina bifida (p value=0.264) and neither cases of spinal nor cranial type of NTD had significant differences of MTHFR measures than control group, (p values = 0.072 and 0.450, respectively), table 1.

Table (1): Methyline Tetrahydrofolate Reductse enzyme level in groups of the study.

Groups [a]	Mean (ng/ml)	Standard Deviation	Maximum	Minimum
Cases [b]				
Spina Bifida [c]	2.23	2.88	146.97	2.98
Cranial defects [d]	9.85	6.67	20.70	2.25
Total	2.04	26.67	146.97	2.25
Control	1.26	16.22	71.72	0.44

[a] p value= 0.115, [b] p value= 0.264, [c] p value= 0.072, [d] p value= 0.450

Table (2) showed that female babies with NTD had insignificantly higher MTHFR level than male. One third of NTD patients had history of preterm delivery and one half of NTD cases were delivered with low birth weight, anyhow, both of these criteria were statistically unrelated to MTHFR measures.

Table (2): Relationship of Methyline Tetrahydrofolate Reductse enzyme level with neural tube defect affected infantile criteria.

Criteria	n	Mean of enzyme level (ng/ml)	Std. Deviation	P value
Gender				
Male	20	1.44	13.92	0.148
Female	26	2.51	33.31	
Maturity				
Term	29	2.44	3.18	0.126
Preterm	17	1.37	1.40	
Birth weight				
Normal	23	2.49	35.57	0.248
Low	23	1.55	13.41	

Most of mother of NTD affected children were 25- 35 yrs old, MTHFR enzyme level was significantly higher in mothers aged more 35

yrs old than those below 25 yrs old (p value= 0.010) and 25 yrs- 35 yrs old (p value= 0.039). More than half of women whom babies delivered with NTD had no or irregular intake of folic acid, MTHFR enzyme level was insignificantly higher in babies whom mothers received folic acid than others. Consanguinity marriage was unrelated with level of MTHFR, table 3.

Table (3): Relationship of Methyline Tetrahydrofolate Reductse enzyme level with maternal/ parental criteria whose babies had neural tube defects.

Criteria	n (%)	Mean of enzyme level (ng/ml)	Std. Deviation	P value
Maternal age				
Below 25 yrs	17 (37)	1.25	1.48	
25- 36 yrs	24 (52)	2.05	2.08	.000
Above 35 yrs**	5 (11)	4.73	6.04	
Folic acid intake				
Regular	20 (43.5)	2.56	34.73	.261
No/ irregular	26 (56.5)	1.65	18.83	
Consanguinity marriage				
Positive	22 (47,8)	1.5	16.08	0.170
Negative	24 (52.2)	2.85	36.9	

** highly significant association.

Discussion

World widely, it was found that around 300,000 babies with neural tube defects were born annually [15], ensuing about 8.6 million disable child and 88,000 deaths each year [16,17]. Many studies support the observations implicating the genetic factors in neural tube defects pathogenesis: firstly, the increased recurrent risk of the defect for siblings of affected patients and secondly, an augmented risks in certain ethnics (e.g., Irish and Mexican) [15,17,18]. Maternal MTHFR is one of the implicated steps in pathogenesis of NTDs and over 20 different mutations in MTHFR have been reported, most of these mutations are mainly associated with inactivation or decreasing MTHFR activity [10-12].

This is the first study which search for infantile causes of NTD as all the previous studies implicated maternal factors, including genetic causes. In the current study, we evaluated on level of MTHFR of NTD affected children, it showed no MTHFR enzyme deficiency was reported in the data of the study, on the contrary, higher enzyme level was registered in the NTD affected babies, this might be due to compensatory mechanism due to the defect development, however this variation statistically was not significant. A noteworthy that there was wide variation of MTHFR level in both healthy and NTD affected children, this might make the disparity between the two groups negligible, unfortunately, standard international reference value was not registered to compare with.

There were no previous studies searched for enzyme level in victims of NTD and this is the first study in the world. However, it was found that women who are MTHFR TT homozygous, who had low and inactive MTHFR do have a modestly increased chance of born a child with a NTDs and this risk increases if the fetus is also homozygous [19]. However, limited sample of this study might make these specific mutations less involved in data, or these mutations might not be present in Iraq/ province community itself.

Most of the NTDs in the study was spina bifida, this was going with other study which showed that spina bifida is the commonest type [20,21]. Serum MTHFR level was lower in spina bifida than cranial types, but this difference was also statistically not significant.

Regarding maternal age at pregnancy of NTD affected baby, most of mothers were 25-35 yrs old (no=24, 52%). whereas maternal age of over 40 years in Texas and maternal age of over 30 years in Russia were associated with NTDs [22,23], in a study in northern Iran

reported that there was insignificant variation among mothers' ages and neural tube defects formation [24]. Regarding MTHFR enzyme level, it was directly associated with maternal age, it is highly statistically measured in those whom mothers aged more than 35 yrs old.

In spite of the advises to fortify the pregnant women with folic acid, a significant percent (n=26, 56.5%) of women whom babies affected with NTD had no or irregular intake of folic acid. MTHFR enzyme level was higher in those receiving folic acid than others, but this was statistically insignificant. This might suggest other etiology for NTD than maternal folic acid intake or fetal MTHFR deficiency.

Most of the inherited diseases were passed by recessive mode of mendelian inheritance, including MTHFR deficiency, where consanguinity marriage considered as the main cause of inheritance of this disorder [19]. In the current study, consanguinity marriage was reported in about 50% of NTD cases, it was statistically not associated with development of NTD, A study in Anbar/ Iraq reported that 63.6% of neural tube defect were resulting from consanguinity marriages [25]. Results of this study was going with a study in Riyadh, Saudi Arabia [26], furthermore, it was observed as predisposing factor in many studies in Egypt, Palestine, and India [27-29], nevertheless, none of these studies was designed as a case- control study to test seriously the consanguinity effect on neural tube defect development. Regarding relationship with MTHFR level, it was found that is unrelated with consanguinity, this support the concept of absence of the implicated gene mutations in the study.

Many other variables were analyzed in the study, including gender, birth weight, and preterm delivery, all these showed no statistical differences with variation of serum MTHFR level.

The major limitation of the study was inaccessibility of the genetic analysis to detect MTHFR gene mutation for both parents and offspring, in addition to the evaluation of activity of the enzyme, which had the most deleterious effect of these mutations in many previous studies.

Conclusion and Recommendations

In the light of results of the study, it was found no observed role of fetal MTHFR deficiency in pathogenesis of NTD, in contrast, there was high enzyme level in affected children, which might reflect a compensatory mechanism to the defect development. This study re-directed the view of researches to overlooked fetal causes of congenital anomalies like NTD.

Disclosure

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References:

1. Lynch S.A. Non-multifactorial neural tube defects. Am. J. Med. Genet. C Semin. Med. Genet. 2005;135C:69–76.

2. Blom H.J. Folic acid, methylation and neural tube closure in humans. Birth Defects Res. 2009;85:295–302.

3. Botto LD, Yang Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: a HuGE review. Am J Epidemiol. 2000;151:862–877.

4. Algasham A, Ismail H, Dewaidar M, Settin AA. Methylenetetrahydrofolate reductase and angiotensin-converting enzyme gene polymorphisms among saudi population from qassim region. Genet Test Mol Biomarkers2009;13:817-20.

5. Apolline Imbard, Jean-François Benoist, Henk J. Blom. Neural tube defects, folic acid and methylation. Int J Environ Res Public Health. 2013 Sep; 10(9): 4352–4389.

6. Van der Put NM, Blom HJ. Neural tube defects and a disturbed folate dependent homocysteine metabolism. Eur J ObstetGynecolReprod Biol. 2000;92:57–61.

7. Van der Put NM, van Straaten HW, Trijbels FJ, Blom HJ. Folate, homocysteine and neural tube

defects: an overview. ExpBiol Med (Maywood) 2001;226:243–270.

8. Kruger WD, Evans AA, Wang L, Malinow MR, Duell PB, Anderson PH, et al. Polymorphisms in the CBS gene associated with decreased risk of coronary artery disease and increased responsiveness to total homocysteine lowering by folic acid. Mol Genet Metab. 2000;70:53–60.

9. Peadar N Kirke, James L Mills, Anne M Molloy, Lawrence C Brody, Valerie B O'Leary, Leslie Daly, et al. Impact of the MTHFR C677T polymorphism on risk of neural tube defects: case-control study. BMJ. 2004 Jun 26; 328(7455): 1535–1536.

10. Goyette, P., Sumner, J. S., Milos, R., Duncan, A. M. V., Rosenblatt, D. S., Matthews, R. G., et al. Human methylenetetrahydrofolatereductase: isolation of cDNA, mapping and mutation identification. Nature Genet. 1994;7:195–200.

11. Kluijtmans, L. A. J., Wendel, U., Stevens, E. M. B., van den Heuvel, L. P. W. J., Trijbels, F. J. M., and Blom, H. J. Identification of four novel mutations in severe methylene tetrahydrofolate reductase deficiency. Eur. J. Hum. Genet. 1998;6: 257–265.

12. Sibani, S., Christensen, B., O'Ferrell, E., Saadi, I., Hiou-Tim, F., Rosenblatt, D. S., et al. Characterization of six novel mutations in the methylene tetrahydrofolate reductase (MTHFR) gene in patients with homocystinuria. Hum. Mutat. 2000;15:280–287.

13. Volcik KA, Shaw GM, Lammer EJ, Zhu H, Finnell RH. Evaluation of infant methylene tetrahydrofolate reductase genotype, maternal vitamin use, and risk of high versus low level spina bifida defects. Birth Defects Res Part. A 2003;67:154-157.

14. Botto, L. D. and Yang, Q. 5,10-Methylenetetrahydrofolate reductase gene variants and congenital anomalies: A HuGE review. Am. J. Epidemiol.2000;9:862–877.

15. Christianson AL, Howson CP, Modell B. Global report on birth defects: the hidden toll of dying and disabled children. White Plains (NY): March of Dimes Birth Defects Foundation; 2006.

16. World Health Organization. Global health estimates (GHE)–Cause-specific mortality. 2015.

Available:http://www.who.int/healthinfo/global_burden_disease/estimates/en/index1.html. Accessed 2015 Apr 14.

17. World Health Organization. Global health estimates (GHE)–Disease burden. 2015. Available: http://www.who.int/healthinfo/global_burden_disease/estimates/en/index2.html. Accessed 2015 Apr 14.

18. Blencowe H, Cousens S, Modell B, Lawn J. Folic acid to reduce neonatal mortality from neural tube disorders. International Journal of Epidemiology. 2010; 39 (Suppl 1): i110–i121.

19. Laura Dean. Methylene tetrahydrofolate Reductase Deficiency. In: Medical Genetics Summaries Editors: Victoria Pratt, Howard McLeod, Wendy Rubinstein, Laura Dean, Brandi Kattman, Associate Editor, and Adriana Malheiro, Editor-in-chief. Bethesda (MD): National Center for Biotechnology Information (US); 2012

20. Schoner K, Axt-Fliedner R, Bald R, Fritz B, Kohlhase J, Kohl T, et al. Fetal Pathology of Neural Tube Defects - An Overview of 68 Cases. Geburtshilfe Frauenheilkd. 2017 May;77(5):495-507

21. Asindi A, Al-Shehri A. Neural tube defects in the Asir Region of Saudi Arabia. Ann Saudi Med. 2001 Jan-Mar; 21(1-2):26-9

22. Canfield MA, Marengo L, Ramadhani TA, Suarez L, Brender JD, Scheuerle A. The prevalence and predictors of anencephaly and spina bifida in Texas. Paediatr Perinat Epidemiol. 2009 Jan; 23(1):41-50.

23. Petrova JG, Vaktskjold A. The incidence of neural tube defects in Norway and the Arkhangelskaja Oblast in Russia and the association with maternal age. Acta Obstet Gynecol Scand. 2009; 88(6):667-72.

24. Mohammad Jafar Golalipour, Mostafa Qorbani, Arezo Mirfazeli, and Elham Mobasheri. Risk Factors of Neural Tube Defects in Northern Iran. Iran Red Crescent Med J. 2014 Jun; 16(6): e7940.

25. Al-Ani ZR, Al-Hiali SJ, Al-Mehimdi SM. Neural tube defects among neonates delivered in Al-Ramadi Maternity and Children's Hospital, western Iraq. Saudi Med J. 2010 Feb; 31(2):163-9.

26. Mustafa A M Salih, Waleed R Murshid, Ashry Gad Mohamed, Lena C Ignacio, Julie E de Jesus, Rubana Baabbad, et al. Risk factors for neural tube defects in Riyadh City, Saudi Arabia: Case-control study. Sudan J Paediatr. 2014; 14(2): 49–60.

27. Teebi AS, Talaat F I. Genetic disorders among the Egyptians. In: Genetic disorders among Arab populations New york: Oxford University Press; 1997. page 191–207.

28. Zlotogora J. Genetic disorders among Palestinian Arabs: 1. Effects of consanguinity. Am J Med Genet. 1997 Feb 11; 68(4):472-5.

29. Mahadevan B, Bhat BV. Neural tube defects in Pondicherry. Indian J Pediatr. 2005 Jul; 72(7):557-9.