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Research Article

Evaluation of Plasma Levels of Folic Acid and Homocysteine in Babies Born With Various Types of Neural Tube Defects: A Single Center Study in South India

Aravinthan Singaravelu [1], Swetha Rajan Vasantharajan [2], Rajesh Raveendra [3], Anitha Nancy [4]*

1. Department of Anatomy, PSP Medical College, Dr. M.G.R. University, Tamil Nadu, Chennai, India
2. Department of Obstetrics & Gynecology, St.Peter's Medical College Hospital & Research Institute, Hosur, Tamil Nadu, India
3. Department of Anatomy, Phulo Jhano Medical College, Dumka, Jharkhand, India
4. Department of Anatomy, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth, Puducherry-607402, India

*Correspondence: Anitha Nancy, Associate Professor, Department of Anatomy, Mahatma Gandhi Medical College & Research Institute, Sri Balaji Vidyapeeth, Puducherry-607402, India
Email: ancyjean2010@gmail.com

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ABSTRACT

Background and objectives: Neural tube defects (NTDs) are common congenital anomalies caused by genetic, environmental, or nutritional factors. Normal plasma folic acid levels in the fetus are required for proper development of the neural tube. Plasma folic acid level has an inverse relationship with homocysteine level. This study aimed to determine plasma folic acid and homocysteine levels in babies born with NTDs and healthy controls.

Methods: The study included 30 clinically diagnosed NTD cases and 30 healthy age- and sex-matched control subjects. Plasma levels of folic acid and homocysteine were measured using a direct chemiluminescence method. Data were compared using the independent t-test. Statistical analysis of data was performed using GraphPad InStat 3.0 at significance of 0.05

Results: The mean plasma level of folic acid in NTD cases (5.1±4.9 mol/l) was significantly lower than that in healthy controls (19.5±2.1 mol/l) (p<0.05). The mean plasma level of homocysteine in NTD cases (14.3 ± 2.4 ng/ml) was significantly higher than that in healthy controls (4.9±1.8 ng/ml)(p<0.05). The mean plasma level of folic acid was 20.1±1.5 µmol/l, 8.5 ±2.9 µmol/l, and 1.9 ±0.4 µmol/l in mild, moderate, and severe cases of NTD, respectively. The mean plasma level of homocysteine was 10.7±3.4 ng/ml, 15.4±1.2 ng/ml, and 18.5±0.8 ng/ml in mild, moderate, and severe cases of NTD, respectively.

Conclusion: Low level of folic acid and high level of homocysteine are directly associated with the development of neural tube abnormalities. Moreover, the severity of the NTD is inversely related to plasma level of folic acid and directly related to plasma level of homocysteine.

Keywords: Neural tube defects; Folic acid; Homocysteine

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INTRODUCTION

The incidence of neural tube defects (NTDs) has been established to be 16 per 10,000 live births in the United States. There are various types of NTDs such as cranium bifidum, holoprosencephaly, meningocele, anencephaly, etc. A population-based study in remote India reported the incidence of NTDs as 6.5-8.2 per 1,000 live births, which is among the highest in the world (1). Many factors such as teratogenic drugs, nutritional factors, genetic mutations, and environmental factors play a role in the development of NTDs (2).

Folic acid is necessary for cell division and development because it aids purine, pyrimid ine, and nucleoproteins formation as well as methylation processes. Folic acid deficiency hinders DNAsynthesis, which mainly affects rapidly dividing cells, such as bone marrow and the gut.

Reduced folic acid levels and higher homoc ysteine levels in pregnancy are also linked to the development of NTDs (3). Tetrahydrofol ate acts as an acceptor of one carbon unit, producing a variety of other folates, which are specific co-enzymes in intracellular reactions. A source of tetrahydrofolate is 5-methyl tetrahydrofolate, formed from 5,10-methylene tetrahydrofolate by the action of the enzyme 5,10-methylene tetrahydrofolate reductase (MTFHR). A mutation in the *MTFHR* gene lowers serum folic acid levels. On the other hand, 5-methyl tetrahydrofolate also act as a methyl donor in cells, which is required for the conversion of homocysteine to methionine; hence, this gene mutation causes a rise in blood homocysteine levels (4). The *MTHFR* gene mutation is the most common cause of NTDs (5). According to Patterson et al., the proper neural tube development requires the co-operation of *Trp53* and *Gadd45a* genes (6).

There is possibility of various genomic instabilities in NTDs. Increased homocysteine levels itself is a risk factor for the development of NTDs (7). The plasma levels of folic acid also play a key role in the proper neural tube formation (8,9). Although it is not realistic to alter genetic variables, we can increase the mother's serum folic acid status and thereby avoid both the incidence and recurrence of NTDs (10). The aim of this study was to evaluate plasma levels of folic acid and homocysteine and their association with development of neural tube abnormalities.

MATERIALS AND METHODS

The study population included 30 clinically diagnosed NTD cases and 30 age- and sex-matched healthy control subjects. The study was approved by the local ethics committee, and informed consent was obtained from parents or legal guardians of the subjects. Based on the type and severity of the disease, the patients were categorized into mild (n=5), moderate (n=6), and severe (n=19) cases. Mild cases included spina bifida, lipomeningocele, and hydromyelia. Moderate cases included encephalocele and meningocele, while exencephaly, anencephaly, and meningomyelocele were considered as the severe forms of NTDs (11). Two ml of heparinized blood were collected from the subjects. Plasma was separated and folic acid and homocysteine levels were measured by using the ADVIA Centaur XPT Immunoassay System (Siemens, Germany).

Data of cases and controls were compared using the independent t-test. Statistical analysis of data was performed using GraphPad InStat 3.0 at significance of 0.05.

RESULTS

The plasma folic acid level ranged from 1.9 to 20.1 µmol/l in patients with NTD and from 15 to 22.4 µmol/l in healthy controls. The mean plasma folic acid level in patients with NTD (5.1 ± 4.9 µmol/l) was significantly lower than that in healthy controls (19.5 ± 2.1 µmol/l)(p<0.05). The plasma homocysteine level ranged from 10.7 to 18.5 ng/ml in patients with NTD and from 1.5 to 8.7 ng/ml in healthy controls. The mean plasma homocysteine level in patients with NTDs (14.3 ± 2.4 ng/ml) was significantly higher than that in healthy controls (4.9 ± 1.8 ng/ml) (p<0.05).

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We also found that the plasma levels of folic acid and homocysteine varied based on the severity and type of NTDs. The plasma level of folic acid in mild cases was close to that of normal controls. However, in moderate and severe cases, the plasma levels of folic acid were significantly lower than in healthy controls (p<0.05). Homocysteine levels were significantly higher in all NTD cases compared with healthy controls (p<0.05) (Table 1).

Table 1. Plasma level of folic acid and homocysteine in healthy subjects and patients with different types of NTD

Severity of NTD	**Plasma Folic acid level** Controls (19.5 ± 2.1 µmol/l)	**Plasma homocystiene level** Control (4.9 ± 1.8 ng/ml)
Mild (A)	20.1±1.5 µmol/l	10.7±3.4 ng/ml *
Moderate (B)	8.5±2.9 µmol/l *	15.4±1.2 ng/ml *
Severe (C)	1.9±0.4 µmol/l *	18.5±0.8 ng/ml *

All values are shown as mean ± standard deviation. * Denotes statistical significance (p<0.05)

DISCUSSION

Folic acid is essential for the generation of tetrahydro folate, which serves as a carbon donor in the synthesis of DNA and RNA. Purines and pyrimidines are biosynthesized by the *MTHFR* gene and its product enzyme (5). Various studies have demonstrated that the plasma level of folic acid plays a critical role in proper formation of the neural tube as well as its low levels in cases with NTDs (7,8). In our study, we also found that the mean plasma level of folic acid was lower in NTD cases than in controls. The enzymes methionine synthase and 5,10-MTHFR as well as vitamin B12 play a key role in converting homocysteine to methionine (9). Mutations in the genes that code for these enzymes result in an increase in plasma homocysteine levels. In general, NTD is caused by several mutations affecting folic acid and homocysteine metabolism including mutations of the *MTHFD1*, *MTHFR*, *MTR/MS*, *MTRR*, and *RFC1* genes (10,11). According to Felkner et al., excess homocysteine levels may play an independent role in the development of NTDs (7). Mills et al. demonstrated that homocysteine metabolism might be the critical pathway affected by folic acid. It has been also demonstrated that women carrying fetuses with NTDs had elevated plasma homocysteine levels (12,13). In the present study, the plasma homocysteine level was considerably higher in NTD patients compared with the controls. The plasma folic acid level in cases with spina bifida occulta (mild form) was within the normal range. This may be due to the heterogeneity of the affected chromosome carrying the gene. The plasma level of folic acid in patients with moderate and severe NTDs was lower than the normal values, while the plasma level of homocysteine was higher than the normal values in all types of NTDs.

CONCLUSION

The appropriate development of the neural tube in the fetus requires a normal plasma folic acid status, which is inversely related to plasma homocysteine levels. According to the findings of this study, increased plasma homocysteine and reduced plasma folic acid levels are directly associated with development of neural tube abnormalities. The severity of NTDs is inversely related to the plasma level of folic acid and directly related to the plasma level of homocysteine.

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Ethics approvals and consent to participate

The study was approved by the local ethics committee, and informed consent was

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obtained from parents or legal guardians of the subjects.

Conflict of interest

The authors declare that there is no conflict of interest regarding publication of this article.

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