Cukurova Medical Journal
ÇUKUROVA ÜNİVERSİTESİ TIP FAKÜLTESİ

Cukurova Med J 2018;43(4):792-796
DOI: 10.17826/cumj.383363

ARAŞTIRMA / RESEARCH

Vitamin B12 and folic acid levels in childhood cancers

Çocukluk çağı kanserlerinde vitamin B12 ve folik asit seviyeleri

Serhan Küpeli[1], Ayşe Özkan[1], Tuna Eker[1], Gülay Sezgin[1], İbrahim Bayram[1]

[1]Cukurova University, Faculty of Medicine, Department of Pediatric Oncology/Pediatric Bone Marrow Transplantation, Adana, Turkey.

Cukurova Medical Journal 2018;43(4):792-796

Abstract

Purpose: Vitamin B12 and folic acid have some basic functions in the synthesis, repair and expression of DNA of which susceptibility to damage is a major concern in carcinogenesis. We have examined the relationship between childhood cancers and vitamin B12 and folic acid levels in the present study.

Materials and Methods: Between November 2013 and December 2015 newly diagnosed 125 patients with solid tumors and 113 patients with lymphoproliferative/ myeloproliferative malignant diseases and 63 controls were enrolled into the study. Vitamin B12, folic acid and homocysteine levels were measured as a part of diagnostic evaluation at the time of diagnosis.

Results: Vitamin B12 and folic acid levels were found to be significantly lower in children with malignant diseases compared to the control group. Homocysteine levels however were statistically higher than those of the control group. Folic acid levels were significantly lower in lymphoproliferative/myeloproliferative malignant diseases group compared to the solid tumor group.

Conclusion: Lower vitamin B12 and folic acid levels can be used as supportive markers at the time of diagnosis of cancer. This relationship needs to be proved in future studies with larger series.

Key words: Vitamin B12, folic acid, homocysteine, childhood cancers

Öz

Amaç: Vitamin B12 ve folik asit DNA sentezi, tamiri ve ekspresyonunda önemli görevler alırlar. Bu çalışmada çocukluk çağı kanserleri ile vitamin B12 ve folik asit düzeyleri arasındaki ilişki araştırıldı.

Gereç ve Yöntem: Kasım 2013 ve Aralık 2015 arasında yeni tanı alan solid tümörlü 125, lenfoproliferatif/miyeloproliferatif malign hastalığı olan 113 hasta ve 63 kontrol çalışmaya dahil edildi. Vitamin B12 ve folik asit düzeyleri tanısal çalışmaların bir parçası olarak tanı anında ölçüldü.

Bulgular: Vitamin B12 ve folik asit düzeyleri malign hastalığı olan çocuklarda kontrol grubuna oranla anlamlı olarak düşük bulundu. Homosistein düzeyleri ise kontrol grubuna göre istatistiksel olarak daha yüksekti. Folik asit düzeyleri lenfoproliferatif/miyeloproliferatif malign hastalığı olanlarda solid tümör grubuna oranla daha düşüktü.

Sonuç: Düşük vitamin B12 ve folik asit düzeyleri kanser tanısında destekleyici belirteçler olarak kullanılabilir. Bu ilişkinin gelecekte daha geniş serilerle ortaya konması gerekmektedir.

Anahtar kelimeler: Vitamin B12, folik asit, homosistein, çocukluk çağı kanserleri

INTRODUCTION

Cancer is among the most common causes of death in children. Earlier diagnosis and more effective treatment of malignant diseases can be possible with a better understanding of the pathogenesis of cancer. Little is known about the etiology of childhood cancers and these malignancies probably have a complex interaction between genetic predisposition and exposures to environmental carcinogenic agents. Vitamin B12 and folic acid play several fundamental roles in the synthesis, repair and expression of DNA of which susceptibility to damage is a major concern in carcinogenesis[1]. DNA methylation is among the prominent mechanisms of

Yazışma Adresi/Address for Correspondence: Dr. Serhan Kupeli, Çukurova University, Faculty of Medicine, Department of Pediatric Oncology and Pediatric Bone Marrow Transplantation Unit, Adana, Turkey. E-mail: serhankupeli@cu.edu.tr
Geliş tarihi/Received: 05.09.2017 Kabul tarihi/Accepted: 11.10.2017

epigenetic alteration[2]. Hyperhomocysteinemia can be encountered in case of suboptimal intake of vitamins involved in its metabolism like vitamin B12 and folic acid. Beside this, hyperhomocysteinemia is a biologic marker of oxidative stres and has a toxic potential for endothelial cells[3]. There is only a few studies dealing with the association of deficiencies of these vitamins with malignant diseases in pediatric age group. In this study we have examined the relationship between childhood cancers and vitamin B12 and folic acid levels. To our knowledge, for the first time in literature, a large cohort of pediatric patients with malignant diseases and control group were compared according to vitamin B12, folic acid and homocystein levels in the present study.

MATERIALS AND METHODS

In a 2-year-period (between November 2013-December 2015) newly diagnosed 125 patients with solid tumors and 113 patients with lymphoproliferative/myeloproliferative malignant diseases (LP/MP) (a total of 238 patients) and 63 controls were enrolled into the study. In two years 272 new pediatric patients with a malignant disease visited our department. Among them, written informed consent was taken from parents of 238 patients and these patients were included in the study. Vitamin B12, folic acid and homocysteine levels were measured before the treatment in patient group after taking written informed consent. The children in control group were without a malignant or chronic disease from the outpatient clinic of the Department of Pediatrics. Among the patients visited the outpatient clinic of the Department of Pediatrics those without a chronic disease and accepted to sign informed consent were enrolled into the study as control group. Blood samples of 63 controls whose parents signed informed consent were evaluated. The study was approved by the Ethical Committee of the Faculty of Medicine (date: 08.11.2012, number: 13), and conducted in accordance with ethical standards.

Vitamin B12, folic acid and homocysteine levels of the patients were measured at the time of diagnosis before the initiation of treatment. Blood samples were drawn as a part of diagnostic evaluation at the time of diagnosis and were studied at the same time with other parameters of serum biochemistry. Venous blood samples were put into anticoagulant-free test tubes for measurement of vitamin B12 and folic acid and into tubes with EDTA for measurement of homocysteine. Serum vitamin B12 and folic acid levels were simultaneously determined by chemiluminescent detection technology by using Beckman Coulter kits in UniCel DXI 800 autoanalyser (Beckman Coulter Inc. CA, USA), in accordance with the manufacturer's recommendations. Plasma homocystein levels were tested by HPLC technology using ImmuChrom GmbH kits (Heppenheim) in Shimadzu HPLC Prominence System (Shimadzu Inc., Kyoto, Japan) autoanalyser. Laboratory reference range was 126.5-505 pg/ml for vitamin B12, 3.1-19.9 ng/ml for folic acid and 4.3-9.9 μmol/L.

Statistical analysis

All statistical analyses were performed using SPSS statistical software (SPSS Inc, Chicago, IL). Descriptive data are given as percentages. The comparison between parammetrical variables were made using *t* test and that of nonparammetrical variables with Mann-Whitney *U* test. Chi-square and Fisher's exact test was used for dichotomal variables. A two-tailed $P<.05$ was considered statistically significant.

RESULTS

Clinical characteristics and diagnoses of 238 patients with malignant diseases are summarized in Table 1. Thirty five (55.5%) males and 28 (44.5%) females served as control group For comparison, patients with malignant disorders were divided into two groups, namely solid tumors (n=125) and LP/MP malignant tumors (n=113). We did not find any statistically significant differences between mean values of vitamin B12, folic acid and homocysteine levels in pediatric cancer patients and in healthy controls ($p>0.05$). Similarly there was no significant difference in comparison of mean values of vitamin B12, folic acid and homocysteine levels in patients with solid tumors and lymphoproliferative/myeloproliferative malignant diseases ($p>0.05$) (Tables 2 and 3). Vitamin B12 and folic acid levels, however, were found to be significantly lower in children with malignant diseases compared to the control group ($p=0.002$ and 0.007 respectively).Proper vitamin supplementation was recommended to the children with deficiencies.

Table 1. Clinical characteristics of the patients with malignant diseases

Characteristics	N (%)
Gender	
Male	142 (59)
Female	96 (41)
Median age, years, (range)	7 (0.1-18)
Solid tumors	125 (53)
Neuroblastoma	28 (11.8)
Wilms' tumor	22 (9.2)
Brain tumors	19 (7.9)
Germ-cell tumors	16 (6.7)
Rhabdomyosarcoma	12 (5.0)
Ewing/PNET family tumors	11 (4.7)
Osteosarcoma	6 (2.6)
Nasopharyngeal carcinoma	5 (2.2)
Hepatoblastoma	2 (0.9)
Pancreatoblastoma	1 (0.5)
Adrenocortical carcinoma	1 (0.5)
Retinoblastoma	1 (0.5)
Papillary thyroid carcinoma	1 (0.5)
LP/MP malignant diseases	113 (47)
Acute lymphoblastic leukemia	48 (20.1)
Non-Hodgkin lymphoma	27 (11.3)
Histiocytosis	11 (4.6)
Hodgkin lymphoma	17 (7.0)
Acute myelogenous leukemia	10 (4.0)

LP/MP: lymphoproliferative or myeloproliferative malignant diseases, PNET: primitive neuroectodermal tumor.

Table 2. Vitamin B12, folic acid and homocysteine levels in both groups

	Patients	Controls	*P*
B12 (pg/ml)	142.70	181.05	0.283
Folic acid (ng/ml)	143.72	177.08	0.265
Homocysteine (µmol/L)	99.85	71.69	0.177

Table 3. Vitamin B12, folic acid and homocysteine levels in patients with solid tumors and lymphoproliferative/myeloproliferative malignant diseases

	Solid tumors	Lymph/myelo malignancies	P
B12 (pg/ml)	120.94	117.90	0.324
Folic acid (ng/ml)	132.80	104.78	0.278
Homocysteine (µmol/L)	69.50	65.88	0.381

Table 4. Number of cases with low levels of vitamin B12, folic acid and homocysteine.

	Patients (%)	Controls (%)	P
↓B12	**13.0**	**3.2**	**0.002**
↓Folic acid	**5.4**	**0**	**0.007**
↑Homocysteine	**59.5**	**31.2**	**0.002**

Table 5. Number of cases with low levels of vitamin B12, folic acid and homocysteine levels in patients with solid tumors and lymphoproliferative/myeloproliferative malignant diseases.

	Solid tumors (%)	Lymph./myelo. malignancies (%)	*P*
↓B12	11.3	15.0	0.054
↓Folic acid	1.6	9.7	0.002
↑Homocysteine	56.9	62.5	0.162

Homocysteine levels however were statistically higher than those of the control group (p=0.002). Only folic acid levels were significantly lower in LP/MP malignant disease group compared to the solid tumor group (p=0.002). Comparison of B12, folic acid and homocysteine levels in pediatric cancer patients and in healthy controls are shown in Tables 4 and 5.

DISCUSSION

We have limited knowledge about the etiology of cancers in pediatric age group. Many genetic and environmental factors can play a role in transformation from a normal cell into a malignant one. The relationship between childhood cancers and vitamin B12 and folic acid has not yet been confirmed. Vitamin B12 is important in regeneration of tetrahydrofolate (TF) and methionine which are required in nucleotide biosynthesis. Disorders or deficient oral intake related to vitamin B12 and/or folic acid can lead to hyperhomocystenemia.

In the present study we have tried to determine whether an association exists between vitamin B12, folic acid and homocysteine levels and childhood cancers or not. Vitamin B12 and folic acid levels were found significantly lower in children with malignant diseases, accordingly homocysteine levels were statistically higher than those of the healthy children. Inadequate consumption of animal proteins especially in developing countries due to low socioeconomic status could be a significant risk factor for vitamin B12 deficiency. In a study from Turkey, vitamin B12 deficiency has been reported in 81.6% of the mothers and 42% of the infants[4].

The role of folate metabolism in cancer development is a topic of much current interest, with maintenance of adequate folate status tending to show a protective effect. Folate deficiency induces DNA breaks and may alter cellular capacity for mutation and epigenetic methylation. Similarly some researchers proposed an association between low serum folic acid levels and ALL[5]. An additional finding in our study was significantly lower levels of folic acid in LP/MP malignant disease group compared to the solid tumor group. Thompson et al. have reported that folic acid intake among pregnant women was associated with a reduced risk of ALL in early childhood[6]. Recently, higher dietary intake of folate during childhood has been shown to reduce the risk of childhood brain tumors[7]. In the same study, however, the authors have not found an association between vitamin B6 and vitamin B12 intake and brain tumors in pediatric age.

On the other hand, in recent years, investigators heve searched possible protective role of maternal vitamin intake before and during pregnancy against childhood cancers. Milne et al. have reported that folic acid supplements before and during pregnancy may protect against childhood brain cancers[8]. Accordingly, Bailey et al. have found a protective effect of higher dietary intake of folic acid and vitamin B12 against ALL in the offspring[9]. In another study, maternal gestational intake of folic acid has been found to be associated with reduced risk of brain tumors in childhood[10].

Recently, Singer et al. have suggested that higher maternal intake of one-carbon metabolism nutrients including folate, vitamins B12 and B6, riboflavin and methionine may reduce risk of childhood leukemia[11]. However, researchers have not found consistent associations between paternal dietary intake of folate, vitamins B12 and B6 and ALL or brain tumors in childhood[12,13].

Suboptimal intake of vitamins involved in its metabolism like vitamin B12 and folic acid is a known cause of hyperhomocysteinemia. Higher homocysteine levels in our study could be a reflection of decreased levels of vitamin B12 and folic acid in patient group. Some investigators have recommended hyperhomocysteinemia as a marker of malignant diseases in children, and it is linked with tumor growth and progression[14]. In adult studies, an association between hyperhomocysteinemia and some types of malignant tumors especially with colorectal cancers was reported[15]. We did not observe any thromboembolic events in patients with high homocysteine levels.

As for the limitations of our study we must first mention from the unawareness of the vitamin B12, folic acid and homocysteine levels at the end of the treatment or in time of remission. It would be valuable to compare the levels at the time of diagnosis and in time of remission. Secondly, the number of patients in control group could be much higher.

Lower vitamin B12 and folic acid levels can be used

as supportive markers at the time of diagnosis of malignant diseases. Additionally, appropriate replacement of vitamin deficiencies may contribute to succesfull cure of the malignant diseases. This relationship needs to be proved in future studies with larger series.

REFERENCES

1. Friso S, Choi SW. Gene-nutrient interactions and DNA methylation. J Nutr. 2002;132:2382-7.
2. Stern LL, Mason JB, Selhub J, Choi SW. Genomic DNA hypomethylation, a characteristic of C677T polymorphism in the methylenetetrahydrofolate reductase gene. Cancer Epidemiol Biomark Prev. 2000;9:849-53.
3. Selhub J, Jacques PF, Wilson PW, Rush D, Rosenberg IH. Vitamin status and intake as primary determinants of homocysteinemia in an elderly population. JAMA 1993;270:2693-8.
4. Önal H., Adal E, Öner T, Önal Z, Aydın A. An important problem in developing countries: maternal and neonatal vitamin B12 deficiency. Turk Arch Ped. 2010;45:242-5.
5. Sadananda Adiga MN, Chandy S, Ramaswamy G, Appaji L, Krishnamoorthy L. Homocysteine, vitamin B12 and folate status in pediatric acute lymphoblastic leukemia. Indian J Pediatr. 2008;75:235-8.
6. Thompson JR, Gerald PF, Willoughby ML, Armstrong BK. Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukemia in childhood:a case-control study. Lancet. 2001;358:1935–40.
7. Greenop KR, Miller M, Bailey HD, de Klerk NH, Attia J, Kellie SJ et al. Childhood folate, B6, B12, and food group intake and the risk of childhood brain tumors: results from an Australian case-control study. Cancer Causes Control. 2015;26:871-9.
8. Milne E, Greenop KR, Bower C, Miller M, van Bockxmeer FM, Scott RJ et al. Maternal use of folic acid and other supplements and risk of childhood brain tumors. Cancer Epidemiol Biomarkers Prev. 2012;21:1933-41.
9. Bailey HD, Miller M, Langridge A, de Klerk NH, van Bockxmeer FM, Attia J et al. Maternal dietary intake of folate and vitamins B6 and B12 during pregnancy and the risk of childhood acute lymphoblastic leukemia. Nutr Cancer. 2012;64:1122-30.
10. Greenop KR, Miller M, de Klerk NH, Scott RJ, Attia J, Ashton LJ et al. Maternal dietary intake of folate and vitamins B6 and B12 during pregnancy and risk of childhood brain tumors. Nutr Cancer. 2014;66:800-9.
11. Singer AW, Selvin S, Block G, Golden C, Carmichael SL, Metayer C. Maternal prenatal intake of one-carbon metabolism nutrients and risk of childhood leukemia. Cancer Causes Control. 2016;27:929-40.
12. Bailey HD, Miller M, Greenop KR, Bower C, Attia J, Marshall GM et al. Paternal intake of folate and vitamins B6 and B12 before conception and risk of childhood acute lymphoblastic leukemia. Cancer Causes Control. 2014;25:1615-25.
13. Greenop KR, Miller M, Bailey HD, Scott RJ, Attia J, Bower C et al. Paternal dietary folate, B6 and B12 intake, and the risk of childhood brain tumors. Nutr Cancer. 2015;67:224-30.
14. Aleksic D, Djokic D, Golubicic I, Jakovljevic V, Djuric D. The importance of the blood levels of homocysteine, folic acid and vitamin B12 in children with malignant diseases. J BUON. 2013;18:1019-25.
15. Kono S, Chen K. Genetic polymorphisms of methylenetetrahydrofolate reductase and colorectal cancer and adenoma. Cancer Sci. 2005;96:535-42.