ORIGINAL ARTICLE

ESTIMATION OF SERUM FOLATE AND VITAMIN B12 LEVELS IN CHILDREN WITH HEMATOLOGIC MALIGNANCIES

Sneha Tandon[1], Kalpana Singh[2], Anand Ruban[2], Bhawna Singh[3], Abbas Ali Mahdi[2], Archana Kumar[1]
[1]Department of Pediatrics & [2]Department of Biochemistry, King George's Medical University Lucknow, U.P, and [3]Department of Biochemistry, G B Pant Hospital, New Delhi, India

ABSTRACT

Background: Patients of hematological malignancies are reported to be deficient in folate and vitamin B12. The aim of this study was to estimate serum folate and vitamin B12 levels in children with hematological malignancies to find out the frequency of deficiencies in different type of hematological malignancies.

Material & Methods: In this case control study, 102 children (24 females and 78 males, aged 1.5-16 years) presenting with different hematological malignancies were enrolled. Serum folate and vitamin B12 levels were estimated and matched with controls (30) children with non-hematological malignancies. Folic acid and vitamin B12 levels were estimated by electrochemiluminescence technique.

Result: Children with hematological malignancies were categorized into acute lymphoblastic leukemia (ALL, 61), acute myeloid leukemia (AML, 18), Hodgkin's disease (HD, 12) and non-Hodgkin lymphoma (NHL, 11). The vitamin B12 levels in these cases and controls were 482.06±480.29 pg/mL, 582.33±500.43 pg/mL, 438.2±355.09 pg/mL, 324.91±198.14 pg/mL and 864.54±733.49 pg/mL respectively. The serum folate was 9.23±8.53 ng/mL, 6.32±3.85 ng/mL, 9.66±10.16 ng/mL, 10.56±10.30 ng/mL and 12.73±15.96 ng/mL respectively. Serum vitamin B12 levels varied differently among different hematological malignancies, it was significantly lower in ALL ($p=0.003$) followed by NHL ($p=0.02$). Serum folate was lowest in AML as compared to controls but not statistically significant.

Conclusion: The frequency of vitamin B12 deficiency was higher in children with HD while folate deficiency in AML. Serum vitamin B12 levels varied differently among different hematological malignancies, it was significantly lower in ALL followed by NHL. While folate levels were lowest in AML as compared to controls but not statistically significant.

KEY WORDS: Hematologic malignancy; Anemia; Vitamin B12; Folic acid; Cobalamin.

This article may be cited as: Tandon S, Singh K, Ruban A, Singh B, Mahdi AA, Kumar A. Estimation of serum folate and vitamin B12 levels in children with hematologic malignancies. Gomal J Med Sci 2014; 12:89-92.

INTRODUCTION

The folate and vitamin B12 (cobalamin) are two vital micronutrients that play diverse roles in body metabolism. Folate mediated one-carbon metabolic pathway is central to DNA metabolism including its synthesis, repair, and methylation.[1] This strategy is utilized therapeutically for hematological and trophoblastic malignancies.[2,3] The one carbon metabolism also involves regulation of epigenetic mechanisms including CpG island and histone methylation and DNA uracil incorporation, hence, not surprisingly a number of studies have shown aberrant folate and vitamin B12 metabolism to be associated with cardiovascular risk,[4] neurodegeneration[5] and malignancies of various tissues.[6-10] There are only some studies that explore the status of folate and vitamin B12 in acute lymphoid leukemias (ALL). It is seen that maternal folate supplementation is associated with a decreased risk of childhood acute leukemia[11] suggesting low levels of these nutrients may indeed be a contributing factor for the tumor initiation, promotion as well as progression. There are a few reports of a lack of association between methylenetetrahydrofolate reductase (MTHFR) C677T polymorphisms and ALL[12] but a recent meta analysis has concluded that the C677T polymorphism, not A1298C, in MTHFR gene is associated with a decreased risk of ALL, particularly among children and Caucasians subjects.[13] There are only a few reports that have assessed the circulating levels of folate and vitamin

Corresponding Author:
Dr. Kalpana Singh
Assistant Professor
Department of Biochemistry
King George's Medical University
Lucknow, India
E-mail: kalpanasinghdr@gmail.com

B12 in hematological malignancies in particular in pediatric group, hence, this study was planned with the objective of estimating the circulating levels of vitamin B12 and folate in childhood hematologic malignancies.[14] Since the levels are also sensitive to the chemotherapy offered and vitamins supplementation the study was planned to assess the levels at the time of diagnosis prior to chemotherapy.

The aim of our study was to estimate serum folate and vitamin B12 levels in children with hematological malignancies at the time of admission in a tertiary care hospital and to find out the frequency of deficiencies in different type of hematological malignancies.

MATERIAL AND METHODS

Study was conducted in Pediatric Hemato-Oncology Clinic, Department of Pediatric, King George's Medical University, U.P Lucknow (India). Study was approved by Institutional Ethical committee and informed consent was taken from the parents. All cases of hematological malignancies between 1.5-16 years of age, both sexes who were registered during a period of one year in the clinic were enrolled in the study. In the control group (30) age and sex matched children with non hematological malignant children were selected based on history and general physical examination. Cases of childhood hematological malignancies were selected either on the basis of hematological or bone marrow or histological findings. Under aseptic conditions 2ml peripheral venous blood sample was obtained in plain vial and the serum was used for further analysis. Samples were obtained from the cases at the time of diagnosis. Both vitamin B12 and folate levels were estimated by electrochemiluminescence on Roche cobas e411 analyzer using commercial kits (Roche diagnostics, KK Tokyo, Japan). The statistical analysis was done by using SPSS version 15.0. Chi-square test was used to find out the association of type of malignancy and vitamin B12 and folate deficiency and also to compare the deficiency between cases and controls. The values were expressed as mean ± standard deviation. Unpaired student t test was used to compare mean folate and vitamin B12 levels in different hematological malignancies.

RESULTS

Thirty age and sex matched children with non hematological malignancy constituted the control group (group 1). The demographics of group 1 are as follows: N=30, mean age 7.8 ±3.96 years (range 1.2-16.1 years). Cases enrolled in the study comprised of total 102 children (group 2) with mean age being 7.16±3.62 years (24 females and 78 males) presenting with different hematological malignancies were enrolled in the study. Mean folate levels were 8.91±8.31 ng/mL in group 2 as compared to controls 12.73 ±15.96 ng/mL while mean vitamin B12 were 479.74 ±450.23 pg/mL in group 2 as compared controls 864.54 ±733.49 ng/ml. Percentage of vitamin B12 and folate deficiencies in cases were 36.3% and 62.7%, respectively as shown in table 1. Cases (group 2) were sub-categorized in acute lymphoblastic leukemia (61), acute myeloid leukemia (18), Hodgkin Disease (12) and Non-Hodgkin lymphoma (11). The vitamin B12 and folate levels in 4 sub-groups are shown in table 2 and values are expressed as mean± standard deviation and unpaired student t test was used to compare different groups with controls. Mean

Table 1: Comparison of vitamin B12 deficiency between cases and controls.

	Patients with vitamin B12 deficiency		Patients with folate deficiency	
	Number	Percentage	Number	Percentage
Controls (n=30)	3	10	19	63.3
Cases (n=102)	37	36.3	64	62.7
Significance	p = 0.006		p = 0.953	

Table 2: Comparison of mean serum vitamin B12 and serum folate levels in different hematological malignancies as compared to controls.

Study group (n)	Serum Vitamin B12 Mean±SD (pg/mL)	Significance	Serum Folate Mean±SD (ng/mL)	Significance
Controls (30)	864.54 ± 733.49		12.73±15.96	
ALL (61)	482.06 ± 480.29*	t=2.98; p=0.003	9.23±8.53	t=1.36; p=0.17
AML (18)	582.33 ± 500.43	t=1.66; p=0.10	6.32±3.85	t=1.43; p=0.15
HD (12)	438.2 ± 355.09	t=1.91; p=0.06	9.66±10.16	t=0.61; p=0.54
NHL (11)	334.91 ± 198.14*	t=2.34; p=0.02	10.56±10.30	t=0.41; p=0.67

Table 3: Association between type of malignancy and vitamin B12 and folate deficiency.

Type of malignancy	Number of patients (n=102)	Patients with vitamin B12 deficiency		Patients with folate deficiency	
		Number	Percentage	Number	Percentage
ALL	61	23	37.7	39	63.9
AML	18	5	27.8	12	66.7
HD	12	6	50	7	58.3
NHL	11	3	27.3	6	54.5
Chi-square test	$\chi 2$	1.98		0.57	
	p	0.57		0.93	

vitamin B12 levels were lowest in children with ALL (482.06 ± 480.29 pg/ml) and NHL (334.91 ±198.14 pg/ml) as compared to controls (864.54 ± 733.49 pg/mL) which was statistically significant. Mean folate levels were lowest in AML children (6.32 ± 3.85 ng/ml) as compared to controls (12.73 ±15.96 ng/ml) but it was not statistically significant. Vitamin B12 deficiency varied differently among different hematological malignancies as shown in table 3. Fifty percent of the children diagnosed as HD were vitamin B12 deficient but it was not statistically significant (p=0.57). Similarly, the folate deficiency was 66.7% in AML but it was not significant statistically (p=0.93) as shown in Table 3.

DISCUSSION

Several molecular mechanisms that underpin genomic integrity and function are sensitive to vitamin B12 status, and in particular, are responsive to the interaction between folate nutrition and folate dependent enzyme polymorphisms. Folate, a key component in one carbon metabolism is crucial in purine and pyrimidine synthesis and provides methyl group for DNA and RNA methylation. Vitamin B12 and folate deficiency results in impairment of DNA synthesis which promotes carcinogenesis.[15,16] Hanumante et al[17] in their assessment of vitamin B12 and folic acid levels among asymptomatic Indian toddlers reported a mean level of 282.3 ±140.4 pmol/L for vitamin B12 and 18.3 ±7.5 nmol/L for folate levels whereas in our study mean vitamin B12 and folic acid levels in hematological malignant children were 479.74 ±400.23 pg/mL and 8.91 ±8.31 ng/mL. In our study mean vitamin B12 and folate levels in children with hematological malignancies were lower as compared to controls.

In the present study, mean folate levels were lower though not statistically significant, in majority of children (62.7%) with hematological disease in contrast to vitamin B12 (36.3%) which was statistically significant (p value 0.006) when compared to controls as shown in table 1. In 1998 it has been reported by Nath et al approximately 33% of Indian population suffers from folate deficiency.[18] A recent report shows vitamin B12 and folate deficiency in 26% and 6% in toddlers.[17] However, in the present study B12 and folate deficiency was observed to be 36.3% and 62.7% in hematological malignant children.

Comparison of vitamin B12 and folate levels in different sub categories of group 2 revealed statistically significant difference among groups for mean vitamin B12 levels, however the difference was not significant for folate levels. Comparison of mean vitamin B12 levels from control group was significantly lower in ALL patients (p value 0.003) followed by NHL (p=0.02) which was also statistically significant as shown in table 2. Mean vitamin B12 levels were also low in children with HD though it was not statistically significant. Mean folate levels in our study were lower in cases as compared to controls, however levels in NHL were higher than other hematological malignancies. Lowest folate levels were observed in AML which was closely followed by ALL, however no significant statistical association was seen (p=0.35). These findings are contrary to Adiga et al[14] who reported a significant difference between healthy controls and ALL for mean folate levels, however they had not observed the difference between two groups for vitamin B12 levels.

The incidence of vitamin B12 deficiency in present study varied differently among different hematological malignancies. It was maximum among children's with HD (50%) and minimum in NHL (27.3%) however, the difference among the hematological malignancies were not statistically significant (p=0.57). The incidence of folate deficiency was maximum in AML (66.7%) and minimum in NHL (54.5%) but no significant difference was observed among different type of malignancies (p=0.93).

Our study shows that the incidence of vitamin B12 deficiency was maximum in HD though it was not significant statistically, which could be due to small sample size. Studies are available according to which vitamin B12 deficiency was found in ALL

children at the time of diagnosis. But no study is available in which vitamin B12 and folate status have been evaluated in different type of childhood hematological malignancies.

CONCLUSION

In our study we found that vitamin B12 deficiency is more common in hematological diseases when compared to non-hematological diseases. The incidence of vitamin B12 deficiency was highest in children with Hodgkin's disease while the incidence of folate deficiency was highest in AML as compared to other hematological malignancies though not statistically significant. Mean vitamin B12 levels were significantly lower in ALL and NHL while folate levels were lowest in AML which was not statistically significant. From current study we can conclude that vitamin B12 deficiency is more commonly associated with ALL and NHL type of hematological malignancies. More studies on large sample size are required to dive into the depths of vitamin B12 and folate status in different type of hematological malignancies in children.

REFERENCES

1. Choi SW, Mason JB. Folate and carcinogenesis: an integrated scheme. J Nutr 2000;130: 129-32.
2. DeVita VT, Chu E. A history of cancer chemotherapy. Cancer Res 2008; 68:8643–53.
3. Chabner BA, Roberts TG Jr. Timeline: Chemotherapy and the war on cancer. Nat Rev Cancer 2005; 5: 65–72.
4. Loria CM, Ingram DD, Feldman JJ, Wright JD, Madans JH. Serum folate and cardiovascular disease mortality among US men and women. Arch Intern Med 2000; 160:3258-62.
5. Kifle L, Ortiz D, Shea TB. Deprivation of folate and B12 increases neurodegeneration beyond that accompanying deprivation of either vitamin alone. J Alzheimers Dis 2009;16: 533-40.
6. Dong LM, Potter JD, White E, Ulrich CM, Cardon LR, Peters U. Genetic susceptibility to cancer: the role of polymorphisms in candidate genes. JAMA 2008; 299: 2423-36.
7. Harris HR, Cramer DW, Vitonis AF, DePari M, Terry KL. Folate, vitamin B6, vitamin B12, methionine and alcohol intake in relation to ovarian cancer risk. Int J Cancer 2012; 131: E518-29.
8. Hou XH, Huang YM, Mi YY. Methylenetetrahydrofolate Reductase Gene C677T Polymorphism and Lung Cancer: an Updated Meta-analysis. Asian Pac J Cancer Prevv 2012; 13:2025-9.
9. Jackson MD, Tulloch-Reid MK, McFarlane-Anderson N, Watson A, Seers V, Bennett FI, et al. Complex interaction between serum folate levels and genetic polymorphisms in folate pathway genes: biomarkers of prostate cancer aggressiveness. Genes Nutr 2013; 8:199–207.
10. Vidal, AC, Grant DJ, Williams CD, Masko E, Allott EH, Shuler K, et al. Associations between intake of folate, methionine, and vitamins B-12, B-6 and prostate cancer risk in American veterans. J Cancer Epidemiol 2012; 2012: 957467. Doi:10.1155/2012/957467.
11. Amigou A, Rudant J, Orsi L, Goujon-Bellec S, Leverger G, Baruchel A, et al. Folic acid supplementation, MTHFR and MTRR polymorphisms, and the risk of childhood leukemia: the ESCALE study (SFCE). Cancer Causes Control 2012; 23:1265-77.
12. Azhar MR, Rahimi Z, Vaisi-Raygani A, Akramipour R, Madani H, Parsian A. Lack of association between MTHFR C677T and A1298C polymorphisms and risk of childhood acute lymphoblastic leukemia in the Kurdish population from Western Iran. Genet Test Mol Biomarkers 2012; 16:198-202.
13. Wang H, Wang J, Zhao L, Liu X, Mi W. Methylenetetrahydrofolate Reductase Polymorphisms and Risk of Acute Lymphoblastic Leukemia-Evidence from an updated meta-analysis including 35 studies. BMC Med Genet 2012; 13:77.
14. Adiga S, Chandy MN, Ramaswamy S, Appaji G, Krishnamoorthy L. Homocysteine, vitamin B12 and folate status in pediatric acute lymphoblastic leukemia. Indian J Pediatr 2008; 75: 235-8.
15. Blount BC, Mack MM, Wehr CM, MacGregor JT, Hiatt RA, Wang G, et al. Folate deficiency causes uracil misincorporation into human DNA and chromosome breakage: implications for cancer and neuronal damage. Proc Natl Acad Sci USA 1997; 94:3290–5.
16. Ulrich. Folate and cancer prevention: a closer look at a complex picture. Am J Clin Nutr 2007; 86:271-3.
17. Hanumante NM, Wadia RS, Deshpande SS, Sanwalka NJ, Vaidya MV, Khadilkar AV. Vitamin B12 and homocysteine status in asymptomatic Indian toddlers. Indian J Pediatr 2008; 75:751–3.
18. Nath I, Reddy KS, Dinshaw KA, Bhisey AN, Krishnaswami K, Bhan MK. Country profile: India. Lancet 1998; 351:1265–75.

CONFLICT OF INTEREST
Authors declare no conflict of interest.
GRANT SUPPORT AND FINANCIAL DISCLOSURE
None declared.