Original Article
Middle East Journal of Cancer; July 2018; 9(3): 202-207

Serum Folate and Vitamin B12 Levels in Survivors of Childhood Malignancy in Southern Iran

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Abstract

Background: Folate and vitamin B12 have a number of biologic roles that make them important in hematological disorders and malignancy. In the present study, we have assessed serum folate and vitamin B12 levels and their associated variables in patients with leukemia, lymphoma, and solid tumors.

Methods: This cross-section study investigated 98 patients (57 with leukemia, 16 lymphoma, and 25 solid tumors) between April 2015 and March 2016 in Southern Iran. Complete blood counts and serum levels of folate, vitamin B12, lactate dehydrogenase, and homocysteine were measured. Clinical characteristics of the patients were also gathered from their medical records.

Results: Patients had the following mean serum levels: serum folate (12.27±6.69 ng/ml), vitamin B12 (331.81±183.22 pg/ml), and homocysteine (11.74±26.67 µmole/L). Vitamin B12 showed a significant negative correlation with homocysteine levels (r= -0.223, *P*=0.043). Overall, there were 21(21.9%) vitamin B12 and 9 (9.8%) folate deficient patients. Vitamin B12 patients had a longer remission time (time from off therapy until study entry) of 18±16.97 months compared to those with normal vitamin B12 levels (8.81±8.08 months, *P*=025). However, logistic regression analysis showed that only mean corpuscular volume had a significant correlation with vitamin B12 deficiency (B= -0.105, odds ratio=0.9, 95% CI: 0.819-0.990, *P*=0.03). None of the variables showed significant correlation with folate deficiency (*P*>0.05).

Conclusion: Vitamin B12 and folate deficiency are frequently seen in survivors of childhood malignancy, mainly due to the effects of chemotherapy. These vitamins have important roles in hematopoiesis, as well as development and maintenance of the nervous system; therefore, timely detection of their deficiencies is of utmost importance. It is highly recommended to check the serum levels of these vitamins in children who successfully survive their cancer treatments.

Keywords: Malignancy, Pediatric cancer, Folate, Vitamin B12

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Middle East Journal of Cancer

Received: August 14, 2017; Accepted: October 28, 2017

Introduction

The biological mechanisms that lead to hematologic malignancies and solid tumors are not completely clear.[1] No single gene defects appear to be involved in hematological malignancies. Interaction between genetic variations, and environmental and dietary factors may play an important role in the genesis of hematological malignancies. One of the important dietary factors essential for normal cell growth and impact on general health is the sufficient intake of folate.[2,3] Folate and related B vitamins have a number of biological roles that may contribute to the development of cancer. Deficiencies of both vitamins and mineral elements have been frequently observed in patients with leukemia and lymphoma.[2,4,5]

Folate is a cofactor which plays a critical role in the synthesis, repair, and expression of DNA and functions as a regulatory molecule.[6]

Folate and vitamin B12 play an essential role in DNA methylation. Folate provides methyl groups for DNA methylation. Methionine synthase is a vitamin B12-dependent enzyme, which catalyzes the transfer of methyl groups for the conversion of homocysteine to methionine. Consequently, methionine becomes S-adenosyl-methionine, which is the universal methyl donor for methylation reactions.[7, 8] Deficiencies in folate and vitamin B12 are associated with chromosomal breakage, expression of chromosomal fragile sites, disruption of DNA repair, excessive uracil in DNA, hypomethylation of DNA, and high concentrations of homocysteine in the blood, which is considered to be a significant risk factor for cardiovascular diseases. The availability of S-adenosylmethionine for DNA methylation decreases. The same defects may also associate with increased cancer risk in humans and play an important role in neurological abnormalities.[7-11]

Survivors of childhood cancer are at increased risk for several complications that include obesity, diabetes, cognitive dysfunction, and osteoporosis.[12-15] The negative consequences of obesity in survivors of childhood cancer consist of metabolic abnormalities or cardiac diseases.[16]

Table 1. Demographic variables and laboratory data in the studied population.

Variables	Mean ± SD
Age (years)	8.6±4.31
Sex	
Male (%)	59 (60.2)
Female (%)	39 (39.8)
Duration of illness (months)	22.80±14.45
Remission time (months)	10.96±11.40
Serum Folate (ng/ml)	12.27±6.69
Serum Vitamin B12 (pg/ml)	331.81±183.22
WBC count ($\times 10^3$/ml)	6136.25±2020.93
Platelets ($\times 10^3$/ml)	
255.89±74.26	
RBC count ($\times 10^6$/ml)	4.98±0.86
Hb (g/dl)	12.90±1.52
MCV(fL)	79.50±7.09
RDW (%)	13.47±2.44
Homocysteine serum (µmole/L)	11.74±26.67
LDH (U/L)	365.70±109.03

On the other hand, low serum folate and vitamin B12 are associated with an increased risk of cardiovascular mortality, osteoporosis, Alzheimer's disease, and cognitive dysfunction.[15,17,18]

Several mutations as well as DNA translocations, inversions or deletions in genes involved in the regulation of blood cell development or homeostasis increase the risk of leukemia and solid tumors.[19] Deficiencies of folate and B12 are linked to different morbidities such as osteoporosis and cognitive dysfunction, which are the major treatment-related complications in survivors of childhood cancer.[20,21] There is a growing body of evidence that folate and B12 deficiencies are associated with increased risk of several solid tumors.[22-26] The aim of this study is to investigate serum folate and vitamin B12 status, and their possible associated factors in survivors of childhood cancer.

Materials and Methods

We conducted this cross-sectional study on 98 children, 18 years or younger, who had leukemia (n=57), lymphoma (n=16), and solid tumors (n=25). Patients were off therapy due to cure or disease remission. They were registered in the Pediatric Oncology Department affiliated with Shiraz University of Medical Sciences from

Table 2. Correlation of serum folate and vitamin B12 levels with LDH, homocysteine, remission time, and duration of illness in patients with hematologic malignancies and solid tumors.

	LDH		Homocysteine		Remission time		Duration of illness	
	r	*P*-value	r	*P*-value	r	*P*-value	r	*P*-value
Vitamin B12	0.157	0.190	-0.223	0.043*	-0.298	0.004*	-0.073	0.491
Folate	0.129	0.296	0.084	0.461	-0.075	0.494	0.038	0.723

*P<0.05 was considered statistically significant.

January 2015 until December 2016 in Southern Iran. The Ethics Committee of Shiraz University of Medical Sciences approved the study protocol. All patients or their parents provided written informed consent for study participation.

Folate and vitamin B12 serum levels were measured by the Hitachi Cobas E 411(Roche System). The normal values in our laboratories range between 200-900 pg/ml for vitamin B12 and 4.6-18.7 ng/ml for folate. Values less than these normal ranges were considered to be deficient. Total serum homocysteine was measured by the Hitachi 917 autoanalyzer (Roche System) with a normal range between 5-15 µmol/L; higher levels were considered to be hyperhomocysteinemia.

Statistical analysis

Data analysis was performed by SPSS software version 21 (SPSS Inc., Chicago IL, USA). Descriptive data were presented as mean, standard deviation, and percentages. The chi-square test was used to compare qualitative variables between two or more groups. The student's t-test and Mann-Whitney test were used to compare quantitative variables between two groups. Logistic regression was used to determine the independent factors that influenced folate and vitamin B 12 deficiencies. The Pearson Correlation test was used to determine the correlation between quantitative variables. A two-sided *P*-value <0.05 was considered statistically significant.

Results

In total, 98 patients (59 boys and 39 girls) enrolled in the study. Mean age of the patients was 8.67 ±4.31 years (range: 1-18 years).

Clinical and laboratory findings of patients are shown in table 1. Overall, patients had the following mean serum levels: folate (12.27±6.69 ng/ml), vitamin B12 (331±183 pg/ml), red blood distribution width (13.4±2.4%), and homocysteine (11.74±26.67 µmol/L).

Table 2 shows the correlations of folate and vitamin B12 serum levels with different variables. Only vitamin B12 showed a significant negative correlation with homocysteine levels (r= -0.223, *P*=0.043). A total of 21.9% of patients were deficient in folate, 9.8% had vitamin B12 deficiency, and 7.1% had hyperhomocysteinemia.

Table 3 summarizes demographic, clinical and laboratory variables between folate and vitamin B12 deficient patients and their normal counterparts. All clinical and laboratory variables were comparable in vitamin B12 deficient and non-deficient patients except for remission time (time from off therapy to study entry) which was longer in vitamin B12 deficient patients (18±16.97 months) compared to non-deficient patients (8.81±8.08 months, *P*=0.025). Consequently, variables with *P*<0.2 were entered into the logistic regression model. These variables included age, post-chemotherapy duration, mean corpuscular volume (MCV), and diagnosis with entering method. Only MCV showed a significant correlation with vitamin B12 deficiency in this model (B= -0.105, odds ratio=0.9, 95% CI: 0.819-0.990, *P*=0.03). There was no association between hyperhomocysteinemia and folate or vitamin B12 deficiencies (*P*>0.05).

None of the clinical and laboratory parameters showed a significant relationship with folate deficiency in univariate analysis (*P*>0.05). Due to a low number of patients in the folate-deficient group, logistic regression was not possible in this group.

Table 3. Comparison of clinical and laboratory characteristics between folate and vitamin B12 deficient and non-deficient patients.

	Vitamin B12			Folate		
	Deficient N=21	Non-deficient N=75	*P*- value	Deficient N=9	Non-deficient N=83	*P*- value
*Age (years)	10.14±5.121	8.28±4.05	0.08	9.11±4.3	8.53±4.3	0.7
*Sex (number)						
Male (%)	13 (61.9%)	45 (60%)	1.00	5 (55.6%)	50 (60.2%)	1.00
Female (%)	8 (38.1%)	30 (40%)		4 (44.4%)	33 (39.8%)	
*Diagnosis						
Leukemia	12 (57.1%)	43 (57.3%)	0.15	6 (66.7%)	45 (54.2%)	0.72
Lymphoma	6 (28.6%)	10 (13.3%)		1 (11.1%)	15 (18.1%)	
Solid tumors	3 (14.3%)	22 (29.3%)		2 (22.2%)	23 (27.7%)	
*Duration of illness (months)	22.95±13.844	22.58±14.74	0.91	29±12.25	21.16±14.3	0.17
* Remission time (months)	18±16.976	8.81±8.08	0.02*	12.75±14.44	10.74±11.3	0.77
*WBC count ($\times10^3$/ml)	5754.76±1705.153	6263.42±2123.34	0.31	6027.78±1062.16	6203.83±2151	0.96
*Platelets ($\times10^3$/ml)	249.05±59.238	259.36±78.43	0.57	282.00±70.63	256.37±75.3	0.23
*RBC count ($\times10^6$/ml)	4.91±0.588	5.02±0.94	0.63	4.83±0.49	5.00±0.91	0.56
* Hb (g/dl)	12.75±1.22	12.96±1.61	0.57	12.65±1.13	12.94±1.6	0.30
*MCV(fL)	76.085±9.95	80.51±5.86	0.06	74.50±12.77	80.13±6.13	0.144
RDW(%)	14.07±1.97	13.55±1.71	0.331	13.14±1.29	13.77±1.86	0.464
*Homocysteine (µmole/L)	23.25±58.56	8.77±4.52	0.32	10.98±5.08	12.03±28.7	0.20
*LDH (U/L)	380.06±140.87	362.84±98.08	0.57	402.00±93.16	366.72±110.227	0.31

P<0.05 was considered statistically significant.; *All parameters are written as mean ± SD, the exception of age and diagnosis, which is written as a number (%).

Discussion

Vitamin B12 and folate have key roles in nucleic acid synthesis. Deficiencies in folic acid and vitamin B12 may contribute to the development of cancer by increasing DNA damage rate, excessive accumulation of uracil in DNA, micronucleus formation and DNA hypomethylation.[27,28]

Recently, it has been postulated that nutrition may play a significant role in cancer prevention.[29,30] Since folate supplementation plays an important role in the prevention of macrocytic anemia as well as cardiovascular disease and neural tube defects, further research is needed to clearly elucidate the effect of folate on carcinogenesis.[31]

Previous investigators attempted to determine the biologic and fundamental roles of folic acid and vitamin B12 in patients with leukemia and other cancers. Hoogstraten et al. and Hellman et al. reported low folic acid activity in some cases of leukemia and solid tumors.[32] Another study showed a strong association between maternal use of folate supplements and acute lymphoblastic leukemia (ALL) in children. They reported that folate supplements in pregnancy could reduce the risk of childhood ALL.[33] Skibola et al. stated that lymphoid cells might have a higher folate requirement in comparison with myeloid cells.[26]

Some investigators evaluated the relationship between folate intake and risk of breast cancer, but reported contradictory results.[34-36] A study suggested that higher plasma levels of folate and vitamin B6 might have a protective role against breast cancer,[37] whereas another study failed to prove any association.[38]

In this study, nearly 22% of the study population had folate deficiencies, whereas 10% were deficient in vitamin B12. Jain et al. reported different statistics regarding folate (11.3%) and B12 (31.3%) deficiencies in survivors of childhood leukemia.[39] One reason might be the

heterogeneity of the current study patients in terms of primary diagnosis. The dietary habits of our patients might differ from the Indian population, so that fewer patients encountered vitamin B12 deficiency post-chemotherapy.

Another finding of our study was that the remission time was longer in vitamin B12 deficient patients compared to individuals with normal vitamin B12 levels. It seems that vitamin B12 deficiency in pediatric cancer patients is not a self-limited process and may even increase in frequency or severity as time passes unless treated appropriately. From all variables, only MCV has shown a significant correlation with vitamin B12 deficiency. While it is expected that vitamin B12 deficiency should be accompanied with macrocytosis, the deficient groups showed lower MCV values. A possible explanation is that survivors of childhood malignancies may suffer from concurrent multiple mineral and micronutrient deficiencies which can alter the effects of a vitamin B12 deficiency on RBC sizes. We did not measure other minerals or vitamins in these patients and cannot draw any robust conclusion in this regard.

This study is among the few studies in the pediatric age group that assessed folate and vitamin B12 status in children who survived their malignancies. We showed a more prevalent folate deficiency compared to vitamin B12 deficiency in the post-chemotherapy period. We demonstrated that the vitamin B12 deficiency did not resolve spontaneously over time and needed to be treated. Despite several strengths, this study faced some limitations. Besides the small sample size and short follow up period, the mentioned vitamins were not measured initially before starting chemotherapy. This could help us to more accurately investigate the effect of chemotherapy on the nutritional status of children.

Conclusion

Folate and vitamin B12 deficiencies are uncommon in survivors of childhood malignancies. Since they may implicate long-term morbidities that include second malignant neoplasms, further multicenter studies with larger sample sizes and longer follow up periods are required to elucidate the late consequences of chemotherapy in growing children. It is important to periodically check the level of vitamins and minerals in survivors of childhood malignancies in order to guarantee a healthy life for the rest of their lives.

Acknowledgment

The authors express their appreciation to Ms. Parand for assistance with manuscript preparation.

Conflict of interest

No conflict of interest is declared.

References

1. Greaves MF. Aetiology of acute leukaemia. *Lancet*. 1997;349(9048):344-9.
2. Kim YI. Folate and DNA methylation: a mechanistic link between folate deficiency and colorectal cancer? *Cancer Epidemiol Biomarkers Prev.* 2004;13(4):511-9.
3. Vanasse GJ, Concannon P, Willerford DM. Regulated genomic instability and neoplasia in the lymphoid lineage. *Blood.* 1999;94(12):3997-4010.
4. Davis CD, Uthus EO. DNA methylation, cancer susceptibility, and nutrient interactions. *Exp Biol Med* (*Maywood*). 2004;229(10):988-95.
5. Stabler SP. Clinical practice. Vitamin B12 deficiency. *N Engl J Med.* 2013;368(2):149-60.
6. Friso S, Choi SW. Gene-nutrient interactions and DNA methylation. *J Nutr.* 2002;132(8 Suppl):2382S-2387S.
7. Hultdin J, Van Guelpen B, Bergh A, Hallmans G, Stattin P. Plasma folate, vitamin B12, and homocysteine and prostate cancer risk: a prospective study. *Int J Cancer.* 2005;113(5):819-24.
8. Crott JW, Mashiyama ST, Ames BN, Fenech M. The effect of folic acid deficiency and MTHFR C677T polymorphism on chromosome damage in human lymphocytes in vitro. *Cancer Epidemiol Biomarkers Prev.* 2001;10(10):1089-96.
9. Dwivedi MK, Tripathi AK, Shukla S, Khan S, Chauhan UK. Homocysteine and cardiovascular disease. *Biotechnol Mol Biol Rev.* 2011; 6(5):101-7.
10. Mikeska T, Craig JM. DNA methylation biomarkers: cancer and beyond. *Genes (Basel)*. 2014;5(3):821-64.
11. Fenech M. Folate (vitamin B9) and vitamin B12 and their function in the maintenance of nuclear and mitochondrial genome integrity. *Mutat Res*. 2012;733(1-2):21-33.
12. Robien K, Ness KK, Klesges LM, Baker KS, Gurney JG. Poor adherence to dietary guidelines among adult

survivors of childhood acute lymphoblastic leukemia. *J Pediatr Hematol Oncol.* 2008;30(11):815-22.
13. Oeffinger KC, Adams-Huet B, Victor RG, Church TS, Snell PG, Dunn AL, et al. Insulin resistance and risk factors for cardiovascular disease in young adult survivors of childhood acute lymphoblastic leukemia. *J Clin Oncol.* 2009;27(22):3698-704.
14. Zareifar S, Shorafa S, Haghpanah S, Karamizadeh Z, Adelian R. Association of serum leptin level with obesity in children with acute lymphoblastic leukemia. *Iran J Ped Hematol Oncol.* 2015;5(3):116-24.
15. Bordbar MR, Haghpanah S, Dabbaghmanesh MH, Omrani GR, Saki F. Bone mineral density in children with acute leukemia and its associated factors in Iran: a case-control study. *Arch Osteoporos*. 2016;11(1):36.
16. Lipshultz SE, Colan SD. Cardiovascular trials in long-term survivors of childhood cancer. *J Clin Oncol*. 2004;22(5):769-73.
17. Verma M. Cancer control and prevention: nutrition and epigenetics. *Curr Opin Clin Nutr Metab Care*. 2013;16(4):376-84.
18. Kim YI. Folate and cancer prevention: a new medical application of folate beyond hyperhomocysteinemia and neural tube defects. *Nutr Rev.* 1999;57(10):314-21.
19. Robien K, Ulrich CM. 5,10-Methylenetetrahydrofolate reductase polymorphisms and leukemia risk: a HuGE minireview. *Am J Epidemiol.* 2003;157(7):571-82.
20. Bozkurt N, Erdem M, Yilmaz E, Erdem A, Biri A, Kubatova A, et al. The relationship of homocyteine, B12 and folic acid with the bone mineral density of the femur and lumbar spine in Turkish postmenopausal women. *Arch Gynecol Obstet.* 2009;280(3):381-7.
21. Koike T, Kuzuya M, Kanda S, Okada K, Izawa S, Enoki H, et al. Raised homocysteine and low folate and vitamin B-12 concentrations predict cognitive decline in community-dwelling older Japanese adults. *Clin Nutr.* 2008;27(6):865-71.
22. Slattery ML, Potter JD, Samowitz W, Schaffer D, Leppert M. Methylenetetrahydrofolate reductase, diet, and risk of colon cancer. *Cancer Epidemiol Biomarkers Prev.* 1999;8(6):513-8.
23. Stolzenberg-Solomon RZ, Pietinen P, Barrett MJ, Taylor PR, Virtamo J, Albanes D. Dietary and other methyl-group availability factors and pancreatic cancer risk in a cohort of male smokers. *Am J Epidemiol.* 2001;153(7):680-7.
24. Zhang S, Hunter DJ, Hankinson SE, Giovannucci EL, Rosner BA, Colditz GA, et al. A prospective study of folate intake and the risk of breast cancer. *JAMA*. 1999;281(17):1632-7.
25. Butterworth CE Jr. Folate status, women's health, pregnancy outcome, and cancer. *J Am Coll Nutr.* 1993;12(4):438-41.
26. Skibola CF, Smith MT, Kane E, Roman E, Rollinson S, Cartwright RA, et al. Polymorphisms in the methylenetetrahydrofolate reductase gene are associated with susceptibility to acute leukemia in adults. *Proc Natl Acad Sci U S A.* 1999;96(22):12810-5.
27. Mattson MP, Kruman II, Duan W. Folic acid and homocysteine in age-related disease. *Ageing Res Rev.* 2002;1(1):95-111.
28. Duthie SJ. Folate and cancer: how DNA damage, repair and methylation impact on colon carcinogenesis. *J Inherit Metab Dis.* 2011;34(1):101-9.
29. Key TJ, Schatzkin A, Willett WC, Allen NE, Spencer EA, Travis RC. Diet, nutrition and the prevention of cancer. *Public Health Nutr.* 2004;7(1A):187-200.
30. Supic G, Jagodic M, Magic Z. Epigenetics: a new link between nutrition and cancer. *Nutr Cancer*. 2013;65(6):781-92.
31. Ulrich CM, Potter JD. Folate and cancer--timing is everything. *JAMA.* 2007;297(21):2408-9.
32. Hellman S, Iannotti AT, Bertino JR. Determinations of the levels of serum folate in patients with carcinoma of the head and neck treated with methotrexate. *Cancer Res.* 1964;24:105-13.
33. Thompson JR, Gerald PF, Willoughby ML, Armstrong BK. Maternal folate supplementation in pregnancy and protection against acute lymphoblastic leukaemia in childhood: a case-control study. *Lancet*. 2001;358(9297):1935-40.
34. Rohan TE, Jain MG, Howe GR, Miller AB. Dietary folate consumption and breast cancer risk. *J Natl Cancer Inst.* 2000;92(3):266-9.
35. Sellers TA, Kushi LH, Cerhan JR, Vierkant RA, Gapstur SM, Vachon CM, et al. Dietary folate intake, alcohol, and risk of breast cancer in a prospective study of postmenopausal women. *Epidemiology.* 2001;12(4):420-8.
36. Negri E, La Vecchia C, Franceschi S. Re: dietary folate consumption and breast cancer risk. *J Natl Cancer Inst.* 2000;92(15):1270-1.
37. Zhang SM, Willett WC, Selhub J, Hunter DJ, Giovannucci EL, Holmes MD, et al. Plasma folate, vitamin B6, vitamin B12, homocysteine, and risk of breast cancer. *J Natl Cancer Inst*. 2003;95(5):373-80.
38. Potischman N, Swanson CA, Coates RJ, Gammon MD, Brogan DR, Curtin J, et al. Intake of food groups and associated micronutrients in relation to risk of early-stage breast cancer. *Int J Cancer.* 1999;82(3):315-21.
39. Jain P, Gulati S, Toteja GS, Bakhshi S, Seth R, Pandey RM. Serum alpha tocopherol, vitamin B12, and folate levels in childhood acute lymphoblastic leukemia survivors with and without neuropathy. *J Child Neurol*. 2015;30(6):786-8.