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The Association of Vitamin B12 and Folic Acid Levels with the Effects of Induction Chemotherapy in Acute Leukemia Patients

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ABSTRACT

Introduction: It is essential to achieve remission with induction chemotherapy and then to provide normal hematopoiesis as fast as possible in acute leukemia (AL) patients in order to minimize treatment-related problems. Both vitamin B12 (vitB12) and folic acid (FA), which are both essential vitamins in the process of cell proliferation, affect the process of reestablishing normal hematopoiesis. In patients who have AL, the purpose of this study is to explore the connection between vitB12 and FA and the accomplishment of hematological remission and bone marrow recovery following induction chemotherapy, as well as infection during induction.

Methods: A retrospective study was conducted on the collected information of 71 AL patients who were diagnosed and monitored at the department of hematology between February 2012 and May 2017. The patients' ages ranged from 21 to 67 years, with 47 being the median age. There were 37 male and 34 female patients.

Results: The median level of vitB12 was 386 pg/mL, with a range of 71-2000, while the median level of FA was 5.57 ng/mL, with a range of 2-19. A total of 57 patients (80.3%) reacted favorably to the induction chemotherapy, whereas 14 patients or 19.7%, did not. There were 67 individuals who had febrile neutropenia (94.4%) and 20 patients who developed a fungal infection (28.6%). The correlation between vitB12 and FA levels with remission, bone marrow recovery, duration of febrile neutropenia, and fungal infection was investigated, and the results showed that there was no statistical significance ($p>0.05$).

Conclusion: It was not possible to establish a connection between vitB12 and FA and the accomplishment of hematological remission and bone marrow recovery, as well as infections during induction chemotherapy in AL patients.

Keywords: Vitamin B12, folic acid, acute leukemia

Introduction

Acute leukemias (ALs), both acute myeloid leukemia (AML) and acute lymphocytic leukemia (ALL), are hematological malignancies characterized by the accumulation of blasts in the bone marrow, leading to the impairment of normal hematopoiesis (1-4). The treatment of ALs comprises induction and consolidation phases, and morbidity and mortality related to the treatment are the major concerns (2-4). During the induction phase, patients are at a fairly high-risk of complications such as infections due to neutropenia and bleeding due to thrombocytopenia. The risk of complications decreases once hematological remission and subsequently bone marrow recovery are achieved after induction chemotherapy (2,4-6).

Both vitamin B12 (vitB12) (7,8) and folic acid (FA) (9-11) are the cofactors of reactions involved in DNA biosynthesis. Thus, both vitamins play an important role in the proliferation of cells, especially highly proliferating cells such as those in the hematopoietic system. Therefore, their deficiencies cause reduced cell proliferation in the hematopoietic system, leading to mostly anemia and less frequently leukopenia and thrombocytopenia (7,10,11).

Regarding the role of vitB12 and FA in the cell proliferation of the hematopoietic system, the level of both vitamins could be important in the achievement of bone marrow remission and recovery after induction chemotherapy, and furthermore in the prevention of complications with the improvement of cytopenias. The objective of this study was to examine the correlation between vitB12 and FA levels and the achievement of hematological remission and bone marrow recovery following induction chemotherapy, as well as the incidence of infection during the induction phase, in patients with AL.

Methods

A retrospective analysis was conducted on the data of 71 patients with AL who received diagnosis and treatment at the Department of Hematology, University of Health Sciences Turkey, İstanbul Training and Research Hospital, from February 2012 to May 2017. The study was approved by

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the University of Health Sciences Turkey, İstanbul Training and Research Hospital Clinical Research Ethics Committee (approval number: 1086, date: 22/09/2017). The data collected covered several parameters, including vitB12, FA, lactate dehydrogenase, transferrin saturation, and ferritin levels at the time of diagnosis, as well as diagnostic and follow-up hemograms. As exclusion criteria, the use of drugs affecting vitb12 and FA absorption, diagnosis of malnutrition, and diagnosis of malabsorption were accepted. In addition, the study documented the patients' remission status following chemotherapy, and the occurrence of febrile neutropenia and fungal infection. The duration of febrile neutropenia and the number of days required for bone marrow recovery were also recorded. Hematological remission was assessed between the 21st and 28th days of treatment and defined as a bone marrow blast percentage <5% by morphological examination. The bone marrow recovery day was evaluated according to the peripheral blood counts at two values; days 1) neutrophil count >500x10^6/L and platelet count was >20000x10^6/L and 2) neutrophil count >1000x10^6/L and platelet count >50000x10^6/L.

The induction chemotherapies utilized for the patients were 3+7 (idarubicin 12 mg/m^2/day iv for 3 days and cytarabin 100 mg/m^2/day iv continous infusion for 7 days), 3+5 (idarubicin 12 mg/m^2/day iv for 3 days and cytarabin 100 mg/m^2/day iv continous infusion for 5 days), all trans retinoic acid (ATRA) + idarubicin (tretinoin 45 mg/m^2/day po till hematological remission was obtained, idarubicin 12 mg/m^2/day iv for 4 days at 2, 4, 6, 8 days), and hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone (HCVAD) (cyclophosphamide 300 mg/m^2 bid iv for 3 days, dexamethasone 40 mg/day iv for 4 days and on days 11-14, vincristine 2 mg/day iv on day 4 and 11, doxorubicin 50 mg/m^2/day iv on day 4).

Statistical Analysis

The data was analyzed with SPSS 24 statistical program, and was presented as numbers and percentages or median and ranges when appropriate. For evaluation of categorical values, the chi-square test and for continuous values Mann-Whitney U test were used. Spearman correlation test was used for the evaluation of correlation between vitamin levels and bone marrow recovery days, duration of febrile neutropenia. All p-values 2-sided with statistical significance of at the 0.05 alpha level.

Results

A total of 71 AL patients (60 AML, 11 ALL patients) were included in the study. The study population had a median age of 47 years, ranging from 21 to 67. Of the whole sample, 37 individuals were male, accounting for 52% of the population, whereas 34 individuals were female, representing 48% of the population. Twenty patients (28%) had lymphadenopathy, 14 (20%) had splenomegaly, and 28 (40%) had hepatomegaly. The study observed a median white blood cell count (WBC) of 16.4x10^9/L. The median hemoglobin level was found to be 8.9 g/dL. The median platelet count was measured at 41.5x10^9/L. The median mean corpuscular volume level was determined to be 91.5 fL. The median lactate dehydrogenase level was recorded as 437 U/L. The median transferrin saturation was calculated to be 39%, while the median ferritin level was measured at 470 ng/dL. The study found that the median vitB12 level was 386 pg/mL, with a range of 71-2000 pg/mL. Additionally, it was observed that 7 out of the total patients, accounting for 10% of the sample, had a vitB12 level below 126 pg/mL, indicating a low level. The study observed a median FA level of 5.57 ng/mL, with a range of 2-19. A total of 10 patients, accounting for 14% of the sample, had a low FA level of 3.1 ng/mL. Patient characteristics are presented in Table 1.

The induction chemotherapy regimens used for the patients were 3+7 in 53 (74.6%) patients, 3+5 in 3 (4.2%) patients, ATRA + idarubicin in 4 (5.6%) patients, and HCVAD in 11 (11.6%) patients. Hematological

Table 1. Patient characteristics

Characteristic	**(n=71)**
Median age, years, (range)	47 (21-67)
Gender, n (%)	
Male	37 (52%)
Female	34 (48%)
Subtype of acute leukemia, n (%)	
AML	60 (85%)
ALL	11 (15%)
Lymphadenopathy, n (%)	
Present	20 (28%)
Absent	51 (72%)
Splenomegaly, n (%)	
Present	14 (20%)
Absent	57 (80%)
Hepatomegaly, n (%)	
Present	28 (40%)
Absent	43 (60%)
Median WBC, x10^9/L, (range)	16.45 (0.81-279.84)
Median hemoglobin level, g/dL, (range)	8.9 (4.6-13.1)
Median platelet count, x10^9/L, (range)	41.5 (2-238)
Median MCV level, fL, (range)	91.5 (75.1-147.1)
Median LDH level, U/L, (range)	437 (114-3859)
Median vitamin B12 level, pg/mL, (range)	386 (71-2000)
Vitamin B12, n, (%)	
Low (<126 pg/mL)	7 (10%)
Normal (>126 pg/mL)	62 (90%)
Median folic acid level, ng/mL, (range)	5.57 (2-19)
Folic acid, n, (%)	
Low (<3.1 ng/mL)	10 (14%)
Normal (>3.1 ng/mL)	60 (86%)
Median tranferrin saturation, (%) (range)	39 (5-95)
Median ferritin level, ng/dL, (range)	470 (8-1702)
Types of chemotherapies, n (%)	
3+7	53 (74.6%)
3+5	3 (4.2%)
ATRA + idarubicin	4 (5.6%)
HCVAD	11 (11.6%)

Table 1. Continued

Characteristic	(n=71)
Response to induction chemotherapy, n (%)	
Present	57 (80.3%)
Absent	14 (19.7%)
Neutrophil count >500x10^6/L and platelet count >20000x10^6/L, day, median (range)	23 (11-40)
Neutrophil count >1000x10^6/L and platelet count >50000x10^6/L, day, median (range)	25 (13-40)
Febrile neutropenia, n, (%)	
Present	67 (94.4%)
Absent	4 (5.6%)
Duration of febrile neutropenia, days, median (range)	4 (1-16)
Fungal infection, n, (%)	
Present	20 (28.6%)
Absent	50 (71.4%)

ALL: Acute lymphocytic leukemia, AML: Acute myeloid leukemia, LDH: Lactate dehydrogenase, MCV: Mean corpuscular volume, WBC: White blood cell count, ATRA: All trans retinoic acid, HCVAD (hyper-CVAD): Hyperfractionated cyclophosphamide, vincristine, doxorubicin, and dexamethasone

remission was achieved in 57 (80.3%) patients. In those patients, regarding bone marrow recovery, the median day of neutrophil count >500x10^6/L and platelet count >20000x10^6/L was 23 days (range, 11-40) and the median day of neutrophil count >1000x10^6/L and platelet count >50000x10^6/L was 25 days (range, 13-40). Febrile neutropenia occurred in 67 (94.4%) patients, and the median duration of febrile neutropenia was median 4 days (range, 1-16). Twenty (28.6%) patients had fungal infections (Table 1).

The median vitB12 level was 438 pg/mL (range, 71-2000) in responders and 257 pg/mL (range, 111-2000) in non-responders (p=0.151). The median FA level was 5.57 ng/mL (range, 2-18.99) in responders and 5.71 ng/mL (range, 3.07-10.42) in non-responders (p=0.765). The median vitB12 level was 306 pg/mL (range, 111-927) in patients with a fungal infection and 416 pg/mL (range, 71-2000) in patients without a fungal infection (p=0.425). The median FA level was 5.73 pg/mL (range, 2.55-14.35) in patients with a fungal infection and 5.41 pg/mL (range, 2-18.99) in patients without a fungal infection (p=0.732).

vitB12 level was not correlated with neutrophil count >500x10^6/L and platelet count >20000x10^6/L (p=0.142, r=0.200) and with neutrophil count >1000x10^6/L and platelet count >50000x10^6/L (p=0.092, r=0.231). FA level was not correlated with days of neutrophil count >500x10^6/L and platelet count >20000x10^6/L (p=0.523, r=-0.087) and with days of neutrophil count >1000x10^6/L and platelet count >50000x10^6/L (p=0.677, r=-0.058). vitB12 level was not correlated with the duration of febrile neutropenia (p=0.104, r=-0.210) and also FA level was not correlated with the duration of febrile neutropenia (p=0.138, r=-0.190) (Table 2).

All analysis for vitB12 was done after exclusion of patients with WBC of >1x10^9/L, and results were not different regarding statistical significance.

Table 2. Correlation of vitamin B12 and folic acid levels with bone marrow recovery and duration of febrile neutropenia

(n=71)	Vitamin B12		Folic acid	
	r-value	p-value	r-value	p-value
Neutrophil count >500x10^6/L and Platelet count >20000x10^6/L, day	0.200	0.142	-0.087	0.523
Neutrophil count >1000x10^6/L and platelet count >50000x10^6/L, day	0.231	0.092	-0.058	0.677
Duration of febrile neutropenia (day)	-0.210	0.104	-0.190	0.138

Discussion

vitB12 ve FA are two important vitamins whose deficiencies cause megaloblastic anemia, leukopenia and thrombocytopenia in normal subjects (7,10,11). From this point of view, their levels might have an impact on the achievement of hematological remission and bone marrow recovery after induction chemotherapy, as well as the occurrence of complications such as infections in AL patients. In this study, the levels of both vitB12 and FA were similar in responders and non-responders; in patients with and without a fungal infection during induction phase. In addition, there was no correlation between both vitamins and the bone marrow recovery day and duration of febrile neutropenia.

In recent years, age, performance status of patients, and genetic risk factors have formed the basis for evaluating prognosis in AL patients (2,12). Therefore, there are inadequate data about the effects of vitB12 and FA on attainment of hematological remission after induction chemotherapy. On the other hand, the influence of vitB12 and FA on bone marrow recovery has been investigated in children with ALL. Tandon et al. (13) demonstrated that FA deficiency was associated with late bone marrow recovery in 58 children with ALL. In the same study, vitB12 deficiency was associated with only toxic deaths during induction, but not with bone marrow recovery. Roy Moulik et al. (14) showed that the incidence of thrombocytopenia and neutropenia was higher during induction chemotherapy in FA-deficient patients, in a slightly higher number of children with ALL (n=150). In fact, we have insufficient knowledge about the factors affecting bone marrow recovery after induction chemotherapy. However, Gerbing et al. (15) recently investigated the effect of telomere length on bone marrow recovery in 97 AL patients. They indicated that patients with decreased telomere content had delayed recovery of neutrophils. In contrast to previous studies, we could not exhibit an association of vitB12 and FA with bone marrow recovery.

Another important issue is the development of infections during treatment in patients with AL (2,4,5,6). vitB12 and FA deficiencies have been associated with immune system dysfunction, leading to opportunistic infections in normal people (16,17). Besides the normal population, the association of FA with infections has been studied in AL patients by Roy Moulik et al. (14). They found that FA-deficient children with ALL had an increased incidence of febrile neutropenia during induction. However, we could not demonstrate an association between

vitB12 and FA levels and fungal infection and duration of febrile neutropenia. Because most of the patients had febrile neutropenia attacks, we did not perform any analysis regarding the presence of febrile neutropenia. Our results were not surprising, considering that vitB12 and FA levels were not associated with bone marrow recovery.

Study Limitations

The retrospective nature of the study and the small number of vitamin-deficient patients is the limitations of this study. Although invalid for FA, vitB12 levels have been found to be higher in some hematological diseases such as chronic myeloid leukemia, polycythemia vera, and myelofibrosis (18). In addition, nearly 30 % of AML patients could have elevated levels of vitB12 (18). However, how vitB12 levels change in ALL patients is conflicting (19,20). Another issue regarding vitB12 is that to determine the true vit B12 deficiency, homosistein or methyl malonic acid levels are required. However, our patients did not have, which could have masked the true incidence of vitB12 deficiency. Furthermore, as this was a retrospective study, post-treatment vitB12 and FA data of the patients could not be evaluated because they could not be accessed.

Conclusion

The association of vitB12 and FA with the attainment of hematological remission and bone marrow recovery and infections during induction chemotherapy could not be established in AL patients. However, further research with extensive cohorts of patients is necessary to examine the impact of vitamin inadequacies on treatment results and consequences in individuals with AL.

Ethics Committee Approval: The study was approved by the University of Health Sciences Turkey, İstanbul Training and Research Hospital Clinical Research Ethics Committee (approval number: 1086, date: 22/09/2017).

Informed Consent: Retrospective study.

Authorship Contributions: Concept - S.E.; Design - S.E., E.S.; Data Collection or Processing - S.E.; Analysis or Interpretation - S.E., E.S.; Literature Search - S.E.; Writing - S.E., E.S.

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