

Impact of vitamin supplements on HAART related hematological abnormalities in HIV-infected patients

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Abstract

Background: The human immunodeficiency virus (HIV) is one of the most life-threatening human infections. The advent of highly active antiretroviral therapy (HAART) has dramatically changed the course of HIV infection and patients' quality of life. In addition to the benefits, HAART can have numerous side effects and toxicities. Therefore, we aimed to assess the impact of short-term vitamins treatment on hematological parameters of HIV infected patients receiving HAART.

Methods: This cross-sectional study was conducted on 100 confirmed HIV positive patients who referred to Shiraz HIV/AIDS research center in southwest of Iran. The first-line of HAART regimen contained Zidovudine, Lamivudine, and Efavirenz. The studied population received vitamin B12 weekly and folic acid daily for at least one month.

Results: After receiving HAART for at least 6 months with adherence above 90%, significant differences ($p < 0.05$) were observed in MCV, MCH, HCT, TLC and RBC status compared to the baseline parameters. After one month of treatment, vitamins in four hematological parameters including TLC, MCV, RBC, and WBC showed significant differences compared to HAART parameters.

Conclusion: Combined administration of B12 and folate supplements is a beneficial adjuster on hematologic status of HIV infected persons receiving HAART. However, future research with larger studied population and longer follow-up periods is required. Moreover, especial attention should be given to gender because the effect of vitamins was significantly different on some hematologic parameters between different genders.

Keywords: HIV, HAART, Vitamin B12, Folic acid, Hematology.

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Introduction

The human immunodeficiency virus (HIV) is one of the most life threatening infections of this century (1,2). Acquired immune deficiency syndrome (AIDS) is a systemic consequence of HIV infection, and it is characterized by severe disorders

and progressive damage of immune responses.¹ Estimates by the World Health Organization (WHO) indicate that more than 2 million people have become newly infected with HIV and nearly 2 million AIDS-related deaths occur per year (3). Moreover, estimates from UNAIDS have

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suggested that more than 90,000 HIV infected individuals are living in Iran (4).

Antiretroviral therapy (ART) is the use of drugs to treat infection by retroviruses (5). Typically, the combination of three or four antiretroviral drugs is known as highly active antiretroviral therapy (HAART) and is offered as a standard treatment to manage HIV infection (6,7). The advent of HAART has dramatically changed the course of HIV infection and led to an improvement in HIV patients' quality of life (8). In addition to all the benefits, HAART may have numerous side effects and toxicities (6). Hematological abnormalities are common complications in HIV infected patients (9). The side effects of antiretroviral drugs are common causes of the abnormalities associated with the increased risk of mortality rate (6,10).

Micronutrient deficiencies such as those of vitamin B12 and folic acid are common among HIV infected individuals and are associated with prolonged treatment (10,11). Individuals with micronutrient deficiencies are commonly at higher risk of AIDS disease progression and mortality (12). Moreover, these deficiencies may intensify the antiretroviral drugs related hematological abnormalities (13,14). Vitamins can be a useful modulator to fix hematologic adverse changes caused by antiretroviral drugs (11,12,14).

Anemia is a frequently observed disorder among HIV infected patients, which worsens the consequence of HIV disease and decreases CD4 cells counts (10). Few studies aimed to determinate the impact of micronutrient on hematological abnormalities of HIV infected patients after antiretroviral therapy. Therefore, we aimed to assess the impact of short-term treatment of vitamin B12 and folic acid combination on hematological parameters of HIV infected patients receiving HAART in Shiraz, southwest of Iran.

Methods

Study Design, Setting and Population

This was a cross-sectional study conducted on 100 confirmed HIV positive patients

who referred to Shiraz HIV/AIDS research center; this center is the second HIV/AIDS research center in Iran and is affiliated to Shiraz University of Medical Sciences. All included patients were at AIDS stage with CD4+ lymphocyte counts $\leq 200 \mu\text{l Cells/mm}^3$ (3). The study population consisted of 100 volunteer HIV patients who were selected based on convenience sampling. All participants initiated ART treatment at least three months before the study and whose rate of adherence was higher than 90%. The first-line of HAART regimen contained a fixed-dose of Zidovudine (300mg), Lamivudine (950mg), and Efavirenz (600mg). The studied population received one capsule containing 100 μg vitamin B12 weekly and 5mg folic acid, administered daily.

This study was in accordance with the declaration of Helsinki, and an informed written consent was taken from all the participants.

Assessing HIV and Hematological Parameters

An HIV seropositive patient was primarily diagnosed by the enzyme linked immunosorbent assay (ELISA) (Dia. Pro Diagnostic Bioprobes, Italy) and subsequently primary positive ELISA results were confirmed by a Western Blot test. Two blood samples, once before the initiation of vitamin treatment and once more after vitamins treatment was performed for the participants. In addition, baseline hematological parameters were obtained from the patients' recorded data from Shiraz HIV/AIDS research center database. Hematological parameters, e.g., hemoglobin (Hb), mean corpuscular volume (MCV), white blood cells (WBCs) were determined using BC-3000 MINDRAY automated hematology analyzer (Mindray, China). Moreover, demographic and clinical information including age, gender and transmission route were collected using a pretested structured questionnaire through interviews and reviewing the medical records.

Statistical analysis was performed using SPSSTM software, Version 21.0 (IBM

Corp., Armonk, NY, USA) and paired t-tests was used to analyze the results. $p < 0.05$ was considered as statistically significant.

Results

Demographic Data

Of the 100 participants, 54% and 46% were female and male, respectively. The overall mean \pm SD age was 37.8 ± 11.7 years, within the age range of 4-64 years. The common HIV transmission route in infected patients was infected husband (37%), followed by intravenous drug use (27%).

HAART Effect on Hematological Parameters

Overall, after at least six months of receiving antiretroviral therapy with adher-

ence above 90%, the patients showed an increase in the mean of Hb, hematocrit (HCT), MCV, mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), platelets (PLT), and total leukocyte count (TLC) compared to the baseline parameters. However, the observed differences were statistically significant only in MCV, MCH, HCT, and TLC ($p < 0.05$). While there was a decrease in the status of red cell distribution width (RDWCV), red blood cells (RBCs) and WBC compared to baseline, the differences were significant only in RBC (4.6 ± 3.1 to $3.9 \pm 0.8 \times 10^6/\mu\text{L}$, $p < 0.03$). There were different patterns of hematological effects of HAART on MCHC, RDWCV and WBC status between female and male patients, but the difference did not reach statistical

Table 1. The hematological characteristics of the study population in baseline, on HAART and after vitamins treatment

Studied factors	Male status	p	Female status	p	Overall status	p
Hb (g/dL)						
Baseline Mean \pm SD	12.2 \pm 2.5		11.6 \pm 2.7		11.9 \pm 2.6	
On HAART Mean \pm SD	12.6 \pm 2.7	$p = 0.2783^a$	12.1 \pm 1.7	$p = 0.1187$	12.3 \pm 2.6	$p = 0.2780$
Vitamins Mean \pm SD	12.9 \pm 2.1	$p = 0.3815^b$	12.2 \pm 1.5	$p = 0.6596$	12.5 \pm 1.8	$p = 0.5278$
HCT (%)						
Baseline Mean \pm SD	36.4 \pm 7.1		34 \pm 4.8		35.1 \pm 6.1	
On HAART Mean \pm SD	39.3 \pm 5.8	$p = 0.0018$	36.4 \pm 5	$p = 0.0007$	37.8 \pm 5.6	$p = 0.0013$
Vitamins Mean \pm SD	39.3 \pm 5.5	$p = 1.0000$	37.9 \pm 4	$p = 0.0201$	38.6 \pm 4.8	$p = 0.2794$
MCV (fL)						
Baseline Mean \pm SD	81 \pm 16.2		77.5 \pm 8.6		79.1 \pm 12.7	
On HAART Mean \pm SD	104.2 \pm 20.5	$p < 0.0001$	99.6 \pm 14	$p < 0.0001$	101.7 \pm 17.4	$p < 0.0001$
Vitamins Mean \pm SD	98.4 \pm 14.7	$p = 0.0225$	95.6 \pm 13.3	$p = 0.0396$	96.9 \pm 13.9	$p = 0.0323$
MCH (Pg)						
Baseline Mean \pm SD	29 \pm 5.5		26.3 \pm 4.2		27.6 \pm 5	
On HAART Mean \pm SD	33.2 \pm 5.4	$p < 0.0001$	31.7 \pm 5.2	$p < 0.0001$	32.4 \pm 5.3	$p < 0.0001$
Vitamins Mean \pm SD	32.3 \pm 5.8	$p = 0.2575$	31.2 \pm 5.2	$p = 0.4974$	31.7 \pm 5.5	$p = 0.3605$
MCHC (g/dL)						
Baseline Mean \pm SD	33 \pm 3.8		32.1 \pm 3.8		32.5 \pm 3.9	
On HAART Mean \pm SD	32.2 \pm 5.4	$p = 0.2271$	33 \pm 2.8	$p = 0.0580$	32.6 \pm 4.2	$p = 0.8617$
Vitamins Mean \pm SD	33.2 \pm 7.4	$p = 0.2763$	31.8 \pm 4	$p = 0.0148$	32.4 \pm 5.8	$p = 0.7803$
RDWCV (%)						
Baseline Mean \pm SD	13.6 \pm 1.4		15.3 \pm 8.3		14.5 \pm 6.2	
On HAART Mean \pm SD	14.1 \pm 2.2	$p = 0.0566$	13.7 \pm 1.8	$p = 0.0610$	13.9 \pm 2	$p = 0.3582$
Vitamins Mean \pm SD	13.9 \pm 2.5	$p = 0.5488$	13.5 \pm 1.3	$p = 0.3688$	13.7 \pm 2	$p = 0.4803$
PLT ($\times 10^3/\mu\text{L}$)						
Baseline Mean \pm SD	185.9 \pm 93.8		253.2 \pm 130.1		222.2 \pm 119.2	
On HAART Mean \pm SD	194.9 \pm 78.8	$p = 0.4634$	257.8 \pm 87.4	$p = 0.7695$	228.6 \pm 88.9	$p = 0.6674$
Vitamins Mean \pm SD	205.6 \pm 84.6	$p = 0.3558$	291.9 \pm 103.6	$p = 0.0127$	252.2 \pm 104.2	$p = 0.0864$
RBC ($\times 10^6/\mu\text{L}$)						
Baseline Mean \pm SD	5 \pm 4.5		4.3 \pm 0.9		4.6 \pm 3.1	
On HAART Mean \pm SD	4 \pm 0.9	$p = 0.0305$	3.7 \pm 0.7	$p < 0.0001$	3.9 \pm 0.8	$p = 0.0300$
Vitamins Mean \pm SD	6.4 \pm 3.8	$p < 0.0001$	5.6 \pm 5.2	$p = 0.0004$	6 \pm 4.6	$p < 0.0001$
WBC ($\times 10^3/\mu\text{L}$)						
Baseline Mean \pm SD	5 \pm 2.2		4.8 \pm 2.1		4.9 \pm 2.1	
On HAART Mean \pm SD	5.2 \pm 2	$p = 0.5019$	4.6 \pm 1.7	$p = 0.4600$	4.9 \pm 1.9	$p = 1.0000$
Vitamins Mean \pm SD	4 \pm 2.3	$p = 0.0001$	4.7 \pm 1.8	$p = 0.6867$	4.3 \pm 2.1	$p = 0.0354$
TLC ($\times 10^3/\mu\text{L}$)						
Baseline Mean \pm SD	1.7 \pm 1.1		1.5 \pm 0.7		1.6 \pm 0.9	
On HAART Mean \pm SD	2.1 \pm 1	$p = 0.0077$	1.7 \pm 0.8	$p = 0.0614$	1.9 \pm 0.9	$p = 0.0194$
Vitamins Mean \pm SD	2.4 \pm 1.4	$p = 0.0828$	2 \pm 1	$p = 0.0201$	2.2 \pm 1.2	$p = 0.0469$

a: Baseline status compared with On HAART status, b: On HAART status compared with vitamins status

significance (Table 1).

Vitamins Treatment Effect on Hematological Parameters

After one month of treatment, vitamins in four hematological parameters included Hb, HCT, PLT and TLC, enhancing the effect of HAART, but significant mean changes were observed in TLC from 1.9 ± 0.9 to $2.2 \pm 1.2 \times 10^3 / \mu\text{L}$. The mean of MCV, MCH and MCHC decreased after vitamins treatment compared to HAART status; this decreasing effect was statistically significant in MCV (101.7 ± 17.4 to $96.9 \pm 13.9 \text{ fL}$, $p < 0.03$). Following the decreasing trend of HAART, RDWCV and WBC mean declined after vitamins treatment, and the differences were statistically significant in WBC (4.9 ± 1.9 to $4.3 \pm 2.1 \times 10^3 / \mu\text{L}$, $p < 0.04$). Meanwhile, vitamins significantly adjust decreasing effect of HAART on RBC status (3.9 ± 0.8 to $6 \pm 4.6 \times 10^6 / \mu\text{L}$, $p < 0.001$).

Vitamins Treatment Effect vs. Gender

Effect of vitamins in some hematological parameters was significantly different between males and females. Among females, HCT mean significantly increased after vitamins treatment compared to HAART status (36.4 ± 5 to $37.9 \pm 4\%$, $p < 0.02$), while this change was not observed among the male patients. In MCHC, the effect of vitamins on female parameter was opposite of the male, as the MCHC mean significantly decreased in females, but the mean of MCHC increased in males. Vitamins significantly decreased the WBC mean among males (5.2 ± 2 to $4 \pm 2.3 \times 10^3 / \mu\text{L}$, $p < 0.001$), while no significant change was observed in females.

Discussion

Previously, several benefits of micronutrient supplementation in HIV positive patients have been introduced; namely, increased body weight, improving immune function, reducing inflammation and better hematological status (11,12). Hematologic toxicity is the common side effect of HIV

medications (11,15). Moreover, HIV infected persons receiving HAART have usually shown lower micronutrient concentrations, and vitamins administration could be a useful adjuvant to reduce associated complications of HAART (11). We compared hematologic parameters among 100 HIV infected patients who had experienced HAART treatment for the first time with Zidovudine, Lamivudine, and Efavirenz before and after vitamin treatment.

The elevation of MCV and depletion of RBC in HAART experienced patients are usually attributed to the adverse effect of Zidovudine, which could explain our findings (16-18). In our findings, patients receiving HAART showed a significant increase in TLC and HCT compared to the results at the baseline. In accordance to our findings, Idowu et al. showed a significant increase in TLC and HCT among HIV patients after three months of receiving HAART (19). Moreover, Florence et al. showed successful treatment with HAART increase TLC status compared to failed treatment patients and stated that the increase in both Hb and TLC compared to baseline could be a good predictor of successful treatment (20). Another significant hematologic increasing effect of HAART in our results was on MCH; this is consistent with the findings of Gedefaw et al. who showed significant differences of MCH level between HAART experienced and HAART naive participants (21).

In our study, vitamin B12 and folate treatment during observation period significantly enhanced TLC and RBC parameters compared to HAART status. Previously, in a randomized trial, Haiden et al. showed that combined vitamin B12 and folate administration significantly increased RBC counts in premature infants (22). Moreover, in two separated studies, Tamura et al. and Kim YI et al. indicated the role of vitamin B12 and folate on lymphocyte counts, respectively (23,24). Linnebank et al. showed that patients with vitamin B12 and folate normal serum levels had lower MCV level compared to those with serum levels below

the normal range (25). Findings of Linnebank et al. are consistent with ours (25), because after one month of combined vitamins treatment, we observed a significant decrease in MCV mean compared to HAART status.

This study had some limitations. First, because we administered a combination of B12 and folate, we could not discuss their individual effects. Second, more background factors such as age, digestive disorders and simultaneous infections should have been considered because of their probable effect on vitamins absorption. The final limitation was the lack of assessment of vitamins serum level after administration.

In summary, despite the limitations, combined administration of B12 and folate supplements was a beneficial adjuvant on hematologic status of HIV infected patients receiving HAART and can be recommended as a promising therapeutic option against HAART related hematological abnormalities. However, Iranian HIV/AIDS patients with different genetic and lifestyle contexts may have different responses, and to reach a comprehensive conclusion conducting further researches in other parts of the world is highly recommended. Moreover, especial attention should be paid to gender as the effect of vitamins was significantly different on some hematologic parameters between different genders.

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Conflict of interest

The authors declared none.

References

1. Basavaraj KH, Navya MA, Rashmi R. Quality of life in HIV/AIDS. *Indian J Sex Transm Dis* 2010; 31:75-80.

2. Motamedifar M, Ebrahim-Saraie HS, Abadi AR, Moghadam MN. First Outcome of MDR-TB among Co-Infected HIV/TB Patients from South-West Iran. *Tuberc Respir Dis* 2015;78(3):253-7.

3. Pawlowski A, Jansson M, Sköld M, Rottenberg ME, Källenius G. Tuberculosis and HIV Co-Infection. *PLoS Pathog* 2012;8:e1002464.

4. Zadeh AO, SeyedAlinaghi S, Hassanzad FF, Hajizadeh M, Mohamadi S, Emamzadeh-Fard S, et al. Prevalence of HIV infection and the correlates among homeless in Tehran, Iran. *Asian Pac J Trop Biomed* 2014;4:65-8.

5. Azu OO. Highly active antiretroviral therapy (HAART) and testicular morphology: current status and a case for a stereologic approach. *J Androl* 2012;33:1130-42.

6. Nubila T, Ukaejiofo EO, Nubila NI, Okorie GI. Examination of Haematotoxicity of Fixed-Dose Highly Active Antiretroviral Drug in Albino Wistar Rats. *ISRN Pharmacology* 2012;2012:6.

7. Ibeh BO, Omodamiro OD, Ibeh U, Habu JB. Biochemical and haematological changes in HIV subjects receiving zidovudine antiretroviral drug in Nigeria. *J Biomed Sci* 2013;20:1423-0127.

8. Shevitz AH, Knox TA. Nutrition in the era of highly active antiretroviral therapy. *Clin Infect Dis* 2001;32:1769-75.

9. Munyazesa E, Emile I, Mutimura E, Hoover DR, Shi Q, McGinn AP, et al. Assessment of haematological parameters in HIV-infected and uninfected Rwandan women: a cross-sectional study. *BMJ Open* 2012;2:2012-001600.

10. De Santis GC, Brunetta DM, Vilar FC, Brandão RA, de Albernaz Muniz RZ, de Lima GMN, et al. Hematological abnormalities in HIV-infected patients. *Int J Infect Dis* 2011;15:e808-e11.

11. Drain PK, Kupka R, Mugusi F, Fawzi WW. Micronutrients in HIV-positive persons receiving highly active antiretroviral therapy. *Am J Clin Nutr* 2007;85:333-45.

12. Evans D, McNamara L, Maskew M, Selibas K, van Amsterdam D, Baines N, et al. Impact of nutritional supplementation on immune response, body mass index and bioelectrical impedance in HIV-positive patients starting antiretroviral therapy. *Nutr J* 2013;12:1475-2891.

13. Volberding PA, Levine AM, Dieterich D, Mildvan D, Mitsuyasu R, Saag M. Anemia in HIV infection: clinical impact and evidence-based management strategies. *Clin Infect Dis* 2004;38:1454-63.

14. Liu E, Duggan C, Manji KP, Kupka R, Aboud S, Bosch RJ, et al. Multivitamin supplementation improves haematologic status in children born to HIV-positive women in Tanzania. *J Int AIDS Soc* 2013;16:18022.

15. Enawgaw B, Alem M, Addis Z, Melku M. Determination of hematological and immunological parameters among HIV positive patients taking highly active antiretroviral treatment and treatment

naive in the antiretroviral therapy clinic of Gondar University Hospital, Gondar, Northwest Ethiopia: a comparative cross-sectional study. *BMC Hematol* 2014;14:2052-1839.

16. Max B, Sherer R. Management of the adverse effects of antiretroviral therapy and medication adherence. *Clin Infect Dis* 2000;30:S96-116.

17. Sharma SK. Zidovudine-induced anaemia in HIV/AIDS. *Indian J Med Res* 2010;132:359-61.

18. Rivas P, Gorgolas M, Fernandez-Guerrero ML. Zidovudine and red-cell distribution width: *N Engl J Med*. 2005;352:2141-2.

19. Idowu CO, Oke OT, Afolayan DO, Olaniran O, Akinloye OA, Awodumila OO. Effect of HAART on some haematological parameters, correlations between total lymphocyte and CD4 counts of HIV clients attending ssh, ikole-ekiti. *Int J Biol Med Res* 2013; 4:3265- 3270

20. Florence E, Dreezen C, Schrooten W, Van Esbroeck M, Kestens L, Fransen K, et al. The role of non-viral load surrogate markers in HIV-positive patient monitoring during antiviral treatment. *Int J STD AIDS* 2004;15:538-42.

21. Gedefaw L, Yemane T, Sahlemariam Z, Yilma

D. Anemia and risk factors in HAART naive and HAART experienced HIV positive persons in south west Ethiopia: a comparative study. *PLoS One* 2013;8:e72202.

22. Haiden N, Klebermass K, Cardona F, Schwindt J, Berger A, Kohlhauser-Vollmuth C, et al. A randomized, controlled trial of the effects of adding vitamin B12 and folate to erythropoietin for the treatment of anemia of prematurity. *Pediatrics* 2006;118:180-8.

23. Tamura J, Kubota K, Murakami H, Sawamura M, Matsushima T, Tamura T, et al. Immunomodulation by vitamin B12: augmentation of CD8+ T lymphocytes and natural killer (NK) cell activity in vitamin B12-deficient patients by methyl-B12 treatment. *Clin Exp Immunol* 1999;116:28-32.

24. Kim YI, Hayek M, Mason JB, Meydani SN. Severe folate deficiency impairs natural killer cell-mediated cytotoxicity in rats. *J Nutr* 2002;132:1361-7.

25. Linnebank M, Moskau S, Semmler A, Widman G, Stoffel-Wagner B, Weller M, et al. Antiepileptic drugs interact with folate and vitamin B12 serum levels. *Ann Neurol* 2011;69:352-9.