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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 ≤200µl Cells/mm (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 100µg 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 SPSS™ 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±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 adherence 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±0.8x10^6/µL, $p<0.03$). 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 ± SD	12.2±2.5		11.6±2.7		11.9±2.6	
On HAART Mean ± SD	12.6±2.7	p= 0.2783[a]	12.1±1.7	p= 0.1187	12.3±2.6	p= 0.2780
Vitamins Mean ± SD	12.9±2.1	p= 0.3815 [b]	12.2±1.5	p= 0.6596	12.5±1.8	p= 0.5278
HCT (%)						
Baseline Mean ± SD	36.4±7.1		34±4.8		35.1±6.1	
On HAART Mean ± SD	39.3±5.8	p= 0.0018	36.4±5	p= 0.0007	37.8±5.6	p= 0.0013
Vitamins Mean ± SD	39.3±5.5	p= 1.0000	37.9±4	p= 0.0201	38.6±4.8	p= 0.2794
MCV (fL)						
Baseline Mean ± SD	81±16.2		77.5±8.6		79.1±12.7	
On HAART Mean ± SD	104.2± 20.5	p< 0.0001	99.6± 14	p< 0.0001	101.7± 17.4	p< 0.0001
Vitamins Mean ± SD	98.4±14.7	p= 0.0225	95.6±13.3	p= 0.0396	96.9±13.9	p= 0.0323
MCH (Pg)						
Baseline Mean ± SD	29±5.5		26.3±4.2		27.6±5	
On HAART Mean ± SD	33.2± 5.4	p< 0.0001	31.7± 5.2	p< 0.0001	32.4± 5.3	p< 0.0001
Vitamins Mean ± SD	32.3±5.8	p= 0.2575	31.2±5.2	p= 0.4974	31.7±5.5	p= 0.3605
MCHC (g/dL)						
Baseline Mean ± SD	33±3.8		32.1±3.8		32.5±3.9	
On HAART Mean ± SD	32.2±5.4	p= 0.2271	33±2.8	p= 0.0580	32.6±4.2	p= 0.8617
Vitamins Mean ± SD	33.2±7.4	p= 0.2763	31.8±4	p= 0.0148	32.4±5.8	p= 0.7803
RDWCV (%)						
Baseline Mean ± SD	13.6±1.4		15.3±8.3		14.5±6.2	
On HAART Mean ± SD	14.1 ±2.2	p= 0.0566	13.7 ±1.8	p= 0.0610	13.9 ±2	p= 0.3582
Vitamins Mean ± SD	13.9±2.5	p= 0.5488	13.5±1.3	p= 0.3688	13.7±2	p= 0.4803
PLT (x10^3/µL)						
Baseline Mean ± SD	185.9±93.8		253.2±130.1		222.2±119.2	
On HAART Mean ± SD	194.9±78.8	p= 0.4634	257.8±87.4	p= 0.7695	228.6±88.9	p= 0.6674
Vitamins Mean ± SD	205.6±84.6	p= 0.3558	291.9±103.6	p= 0.0127	252.2±104.2	p= 0.0864
RBC (x10^6/µL)						
Baseline Mean ± SD	5±4.5		4.3±0.9		4.6±3.1	
On HAART Mean ± SD	4±0.9	p= 0.0305	3.7±0.7	p< 0.0001	3.9±0.8	p= 0.0300
Vitamins Mean ± SD	6.4±3.8	p< 0.0001	5.6±5.2	p= 0.0004	6±4.6	p< 0.0001
WBC (x10^3/µL)						
Baseline Mean ± SD	5±2.2		4.8±2.1		4.9±2.1	
On HAART Mean ± SD	5.2±2	p= 0.5019	4.6±1.7	p= 0.4600	4.9±1.9	p= 1.0000
Vitamins Mean ± SD	4±2.3	p= 0.0001	4.7±1.8	p= 0.6867	4.3±2.1	p= 0.0354
TLC (x10^3/µL)						
Baseline Mean ± SD	1.7±1.1		1.5±0.7		1.6±0.9	
On HAART Mean ± SD	2.1±1	p= 0.0077	1.7±0.8	p= 0.0614	1.9±0.9	p= 0.0194
Vitamins Mean ± SD	2.4±1.4	p= 0.0828	2±1	p= 0.0201	2.2±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±1.2x10^3/µL. 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±13.9fL, $p<0.03$). 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±2.1x10^3/µL, $p<0.04$). Meanwhile, vitamins significantly adjust decreasing effect of HAART on RBC status (3.9±0.8 to 6±4.6x10^6/µL, $p<0.001$).

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±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±2.3x10^3/µL, $p<0.001$), 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 adjuster 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 prematureinfants (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). Linnebanket 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 Linnebanket 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 administrated 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 adjuster 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, UkaejiofoEO, 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 winniecure 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, BrunettaDM, 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, FawziWW. 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, AwodumilaOO. 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.