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

Esmaeil Rezaei1, Hadi Sedigh Ebrahim-Saraie2, Hamid Heidari3, Parichehr Ghane4
Khadijeh Rezaei5, Jamal Manochehr6, Mohsen Moghadami7, Parvin Afzar-Kazerooni8
Ali Reza Hassan Abadi9, Mohammad Motamedifar10

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.


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. 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
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 determine 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
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 significance.

<table>
<thead>
<tr>
<th>Studied factors</th>
<th>Male status</th>
<th>p</th>
<th>Female status</th>
<th>p</th>
<th>Overall status</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hb (g/dL)</td>
<td>12.2±2.5</td>
<td></td>
<td>11.6±2.7</td>
<td></td>
<td>11.9±2.6</td>
<td></td>
</tr>
<tr>
<td>On HAART Mean ± SD</td>
<td>12.6±2.7</td>
<td>p= 0.2783 a</td>
<td>12.1±1.7</td>
<td>p= 0.1187</td>
<td>12.3±2.6</td>
<td>p= 0.2780</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>12.9±2.1</td>
<td>p= 0.3815 b</td>
<td>12.2±1.5</td>
<td>p= 0.6596</td>
<td>12.5±1.8</td>
<td>p= 0.5278</td>
</tr>
<tr>
<td>HCT (%)</td>
<td>36.4±7.1</td>
<td></td>
<td>34±4.8</td>
<td></td>
<td>35±6.1</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>39.3±5.8</td>
<td>p= 0.0018</td>
<td>36.4±5.5</td>
<td>p= 0.0007</td>
<td>37.8±5.6</td>
<td>p= 0.0013</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>39.3±5.5</td>
<td>p= 1.0000</td>
<td>37.9±4</td>
<td>p= 0.0201</td>
<td>38.6±4.8</td>
<td>p= 0.2794</td>
</tr>
<tr>
<td>MCV (fL)</td>
<td>81±16.2</td>
<td></td>
<td>77.5±8.6</td>
<td></td>
<td>79±12.7</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>104.2±20.5</td>
<td>p= 0.0001</td>
<td>99.6±14</td>
<td>p= 0.0001</td>
<td>101.7±17.4</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>98.4±14.7</td>
<td>p= 0.0225</td>
<td>95.6±13.3</td>
<td>p= 0.0396</td>
<td>96.9±13.9</td>
<td>p= 0.0323</td>
</tr>
<tr>
<td>MCH (Pg)</td>
<td>29±5.5</td>
<td></td>
<td>26.3±4.2</td>
<td></td>
<td>27.6±5</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>33.2±5.4</td>
<td>p&lt; 0.0001</td>
<td>31.7±5.2</td>
<td>p&lt; 0.0001</td>
<td>32.4±5.3</td>
<td>p&lt; 0.0001</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>32.3±5.8</td>
<td>p= 0.2575</td>
<td>31.2±5.2</td>
<td>p= 0.4974</td>
<td>31.7±5.5</td>
<td>p= 0.3605</td>
</tr>
<tr>
<td>MCHC (g/dL)</td>
<td>33±3.8</td>
<td></td>
<td>32.1±3.8</td>
<td></td>
<td>32.5±3.9</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>32.2±5.4</td>
<td>p= 0.2271</td>
<td>33±2.8</td>
<td>p= 0.0580</td>
<td>32.6±4.2</td>
<td>p= 0.8617</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>33.2±7.4</td>
<td>p= 0.2763</td>
<td>31.8±4</td>
<td>p= 0.0148</td>
<td>32.4±5.8</td>
<td>p= 0.7803</td>
</tr>
<tr>
<td>RDWCV (%)</td>
<td>13±1.4</td>
<td></td>
<td>15.3±8.3</td>
<td></td>
<td>14.5±6.2</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>14.1±2.2</td>
<td>p= 0.0566</td>
<td>13.7±1.8</td>
<td>p= 0.0610</td>
<td>13.9±2</td>
<td>p= 0.3582</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>13.9±2.5</td>
<td>p= 0.5488</td>
<td>13.5±1.3</td>
<td>p= 0.3688</td>
<td>13.7±2</td>
<td>p= 0.4803</td>
</tr>
<tr>
<td>PLT (x10^3/µL)</td>
<td>185.9±93.8</td>
<td></td>
<td>253.2±130.1</td>
<td></td>
<td>222.8±119.2</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>194.9±78.8</td>
<td>p= 0.4634</td>
<td>257.8±87.4</td>
<td>p= 0.7695</td>
<td>228.6±88.9</td>
<td>p= 0.6674</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>205.6±84.6</td>
<td>p= 0.3558</td>
<td>291.9±103.6</td>
<td>p= 0.0127</td>
<td>252.2±104.2</td>
<td>p= 0.0864</td>
</tr>
<tr>
<td>RBC (x10^6/µL)</td>
<td>5±4.5</td>
<td></td>
<td>4.3±0.9</td>
<td></td>
<td>4.6±3.1</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>4±0.9</td>
<td>p= 0.0305</td>
<td>3.7±0.7</td>
<td>p&lt; 0.0001</td>
<td>3.9±0.8</td>
<td>p= 0.0300</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>6.4±3.8</td>
<td>p= 0.0001</td>
<td>5.6±5.2</td>
<td>p= 0.0004</td>
<td>6±6.6</td>
<td>p&lt; 0.0001</td>
</tr>
<tr>
<td>WBC (x10^3/µL)</td>
<td>5±2.2</td>
<td></td>
<td>4.8±2.1</td>
<td></td>
<td>4.9±2.1</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>5±2.2</td>
<td>p= 0.5019</td>
<td>4.6±1.7</td>
<td>p= 0.4600</td>
<td>4.9±1.9</td>
<td>p= 1.0000</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>4±2.3</td>
<td>p= 0.0001</td>
<td>4.7±1.8</td>
<td>p= 0.6867</td>
<td>4.3±2.1</td>
<td>p= 0.0354</td>
</tr>
<tr>
<td>TLC (x10^3/µL)</td>
<td>1.7±1.1</td>
<td></td>
<td>1.5±0.7</td>
<td></td>
<td>1.6±0.9</td>
<td></td>
</tr>
<tr>
<td>Baseline Mean ± SD</td>
<td>2.1±1</td>
<td>p= 0.0077</td>
<td>1.7±0.8</td>
<td>p= 0.0614</td>
<td>1.9±0.9</td>
<td>p= 0.0194</td>
</tr>
<tr>
<td>Vitamins Mean ± SD</td>
<td>2.4±1.4</td>
<td>p= 0.0828</td>
<td>2±</td>
<td>p= 0.0201</td>
<td>2.2±1.2</td>
<td>p= 0.0469</td>
</tr>
</tbody>
</table>

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

a: Baseline status compared with On HAART status, b: On HAART status compared with vitamins status
Impact of vitamin supplements on hematological parameters

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 premature infants (22). Moreover, in two separated studies, Tamura et al. and Kim YI et al. indicated the role of vitamin B12 and folate, 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 Linne-
banket al. are consistent with ours (25),
because after one month of combined vita-
mins 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 back-
ground factors such as age, digestive disor-
ders and simultaneous infections should
have been considered because of their
probable effect on vitamins absorption.
The final limitation was the lack of assess-
ment of vitamins serum level after administra-
tion.

In summary, despite the limitations, com-
bined administration of B12 and folate sup-
plements was a beneficial adjuster on he-
matologic status of HIV infected patients
receiving HAART and can be recommend-
ed 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.

Acknowledgment
We thank all the participants for their
friendly cooperation in this study. This
work was supported by Shiraz HIV/AIDS
Research Center, Shiraz University of Me-
dical sciences, Iran.

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-
3. Pawlowski A, Jansson M, Sköld M, Rottenberg
ME, Källenius G. Tuberculosis and HIV Co-
4. Zadeh AO, SeyediAlinagh S, Hassanzad FF,
Hajizadeh M, Mohamadi S, Emanzadeh-Fard S, et
al. Prevalence of HIV infection and the correlates
among homeless in Tehran, Iran. Asian Pac J Trop
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
7. Ibhe BO, Omodamiro OD, Ibhe U, Habu JB.
Biochemical and haematological changes in HIV
subjects receiving winniecure antiretroviral drug in
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 haem-
atological parameters in HIV-infected and uninf-
fected Rwandan women: a cross-sectional study.
10. De Santis GC, BrunettaDM, Vilar FC,
Brandão RA, de Albernaz Muniz RZ, de Lima
GMN, et al. Hematological abnormalities in HIV-
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 nutri-
tional supplementation on immune response, body
mass index and bioelectrical impedance in HIV-
positive patients starting antiretroviral therapy. Nutr
13. Volberding PA, Levine AM, Dieterich D,
Mildvan D, Mitsuyasu R, Saag M. Anemia in HIV
infection: clinical impact and evidence-based man-
geriment strategies. Clin Infect Dis 2004;38:1454-
63.
S, Bosch RJ, et al. Multivitamin supplementation
improves haematologic status in children born to
15. Enawgaw B, Alem M, Addis Z, Melku M. De-
termination of hematological and immunological
parameters among HIV positive patients taking
highly active antiretroviral treatment and treatment
Impact of vitamin supplements on hematological parameters

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

http://mjiri.iums.ac.ir