

Association between serum B₁₂ and folate levels and manifestations of oral lesions in HIV adult patients

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ABSTRACT

Introduction: Micronutrient deficiencies are common in Human Immunodeficiency Virus (HIV) infection. The long-term side effects of antiretroviral therapy (ART), specifically Azidothymine (AZT), include low serum levels of vitamin B₁₂ and folate, which in turn, lead to megaloblastic anaemia and oral lesions. **Methods:** A cross-sectional study was conducted to determine the associations between manifestation of oral lesions and serum vitamin B₁₂ and folate levels in HIV-adult patients with or without receiving ART therapy. Oral lesions were determined based on the EC-Clearinghouse diagnostic criteria. Serum vitamin B₁₂ and folate were assessed by electrochemiluminescence immunoassay (ECLIA). Sixty participants (48 males and 12 females) aged 20 to 51 years were recruited from a private hospital in Bandung, Indonesia. **Results:** Subnormal levels of serum vitamin B₁₂ and folate were found in 16.6% and 6.7% HIV patients, respectively. Significantly lower serum levels of vitamin B₁₂ and folate were shown in HIV patients receiving ART than those without ART. Oral lesions were found in all the participants with subnormal levels of serum vitamin B₁₂ and folate. Presence of oral lesions was significantly associated with low levels of serum vitamin B₁₂ in HIV patients with ART, but not with low folate levels. **Conclusion:** Low levels of serum vitamin B₁₂ and folate were shown in HIV patients, indicating the need for early nutritional intervention to ensure optimal nutritional status and prevention of oral lesions in HIV patients.

Keywords: Serum folate and B₁₂, HIV patients, oral lesions

INTRODUCTION

Micronutrient deficiencies are common in Human Immunodeficiency Virus (HIV) infection and occur at all stages of immunodeficiency, including asymptomatic infection. Reduced serum levels of micronutrients are associated with higher transmission of opportunistic infections, immunodeficiency, rapid disease progression and mortality (Carter *et al.*, 2015). HIV-associated anaemia was reported common (49.6%) among HIV-infected patients in Indonesia

(Wisaksana *et al.*, 2011) at higher levels (18-37%) than found in other countries (Hoffmann *et al.*, 2008; Mata-Marin *et al.*, 2010; Takuva *et al.*, 2013). Several observational studies have reported low levels of serum vitamin B₁₂ and folate in HIV patients (Remacha *et al.*, 2003; Semeere *et al.*, 2012). A deficiency of either vitamin can occur due to HIV infection itself or as a side effect of antiretroviral therapy (ART), specifically zidovudine (AZT), which clinically manifest as megaloblastic anaemia or

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neutropenia (Volberding *et al.*, 2004). Haematinic deficiency (vitamin B₁₂, folate, and iron), either as predisposing or etiological factor, can produce oral mucosal diseases including glossitis, recurrent aphthous stomatitis (RAS), angular cheilitis, or oral candidiasis (OC) (Adeyemo *et al.*, 2011).

Oral manifestations of HIV infection vary depending on the populations studied, geographical locations, clinical diversity, as well as the impact of ART (Leão *et al.*, 2009; Sharma *et al.*, 2015). Oral candidiasis, oral hairy leukoplakia (OHL), and oral hypermelanosis were predominantly found in oral lesions of HIV/Acquired Immune Deficiency Syndrome (AIDS) in Asia (Sharma *et al.*, 2015). Oral lesions play an important role as an early clinical indicator and progression predictor of HIV infection. Their occurrences, mainly OC and OHL, are strongly associated with a low cluster of differentiation 4 (CD4) count and a higher plasma viral load (Shiboski *et al.*, 2015). There have been conflicting results regarding reduction in prevalence of specific oral lesions even though expanded access to ART has significantly contributed to reducing new HIV cases, mortality, and morbidity of HIV-positive patients (Leão *et al.*, 2009; Sharma *et al.*, 2015; Shiboski *et al.*, 2015).

Long-term side effects of ART, specifically AZT, include low levels of serum vitamin B₁₂ and folate, which lead to megaloblastic anaemia and oral manifestations. Several observational studies in Indonesia have focused on the occurrence of OC and its variants (Sumintarti & Rasdiana, 2014), or OHL associated with a low CD4 count (Parmadiati *et al.*, 2017). Researchers have addressed the issue of oral manifestations and HIV-associated anaemia without receiving ART in adults (Hidayat *et al.*, 2017), and children (Lugito *et al.*, 2016). Little is known on the relationship between onset of oral lesions, vitamin B₁₂ and folate deficiency,

and HIV-patients with or without ART.

The aim of our research was to determine the correlations between serum levels of vitamin B₁₂ and folate among HIV-adult patients with or without receiving either ART or AZT. This study also aimed to determine the association between the manifestation of oral lesions in HIV-adult patients with or without receiving ART. To the best of our knowledge, this observational study is the first of its type to be conducted in Indonesia.

MATERIALS AND METHODS

Study design and population

In preparing this report, we adhered to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) statement guidelines for reporting observational studies. This cross-sectional study was conducted from November 2016 to January 2017 at Teratai outpatient clinic of Dr. Hasan Sadikin Hospital, Bandung, Indonesia.

This study was conducted in accordance with the Declaration of Helsinki and independently reviewed and approved by the Human Research Ethics Committee of the Faculty of Medicine Universitas Padjadjaran, Dr. Hasan Sadikin Hospital, number LB.02.01/C02/L4702/Z/2016.

The sample size of the participants was calculated based on the formula for comparing two independent groups. Assuming that 5% of Indonesian adults are vitamin B₁₂ and folate deficient, a sample size of 30.2 per group was calculated based on a confidence level of 80% with the confidence limits as 5%. Accounting for dropouts, an additional 30 per group was computed, giving a required total of 60 patients for the trial.

The participants were recruited based on the consecutive sampling method in which every patient who meets the inclusion criteria is selected. The inclusion criteria, include the patient's agreement to sign the informed consent,

aged ≥ 19 years, CD4 count ≤ 200 cells/mm³, and receiving ART duration for ≥ 6 months. We excluded HIV-positive patients who did not adhere to ART therapy. The patients were divided into two groups, namely patients receiving ART, and those not on ART. Informed consent was taken from all patients. Intraoral examinations were performed to collect the data on oral lesions based on the EC-Clearinghouse diagnostic criteria.

Biochemistry analysis

Five ml of participant's blood samples were collected from the median cubital vein with aseptic precautions at the Clinical Pathology Department. Serum levels of vitamin B₁₂ and folate were measured using electrochemiluminescence immunoassay (ECLIA). The normal range for serum B₁₂ is 100-400 pg/ml and levels <100 pg/ml were considered as subnormal for this study. As for serum folate, its normal range is 3-15

ng/ml and subnormal level was taken as <3 ng/ml.

Statistical analyses

Descriptive statistics were used to summarise the categorical data of demographic characteristics. Shapiro-wilk test was used to analyse numeric data which were not normally distributed and presented as median. Bivariate analysis was used to test the hypothesis, including Mann-Whitney test, for analysing differences in serum levels of vitamin B₁₂ and folate in HIV-patients with or without ART, and in HIV-patients with or without AZT. Fisher's exact test was used to analyse the correlation between oral lesions and serum levels of vitamin B₁₂ and folate. *P*-value of ≤ 0.05 was used to determine the statistical significance in the bivariate analysis.

RESULTS

The study included 60 HIV-adult patients with an equal number with or without

Table 1. Characteristics of HIV-adult patients with or without receiving ART therapy

Characteristics	ART		Non-ART		Total	
	<i>n</i>	%	<i>n</i>	%	<i>n</i>	%
Gender						
Male	25	41.7	23	38.3	48	80.0
Female	5	8.3	7	11.7	12	20.0
Age (years)						
20-29	17	28.3	15	25.0	32	53.3
30-39	12	20.0	7	11.7	19	31.7
40-49	3	5.0	4	6.6	7	11.6
≥ 50	1	1.7	1	1.7	2	3.4
CD4 count (cells/mm ³)						
≤ 50	5	8.3	19	31.7	24	40.0
51-100	7	11.7	3	5.0	10	16.7
101-200	18	30.0	8	13.3	26	43.3
Serum vitamin B-12 level (pg/ml) [†]						
Subnormal	8	13.3	2	3.3	10	16.6
Normal	22	36.7	28	46.7	50	83.4
Serum Folate (ng/ml) [‡]						
Subnormal	4	6.7	0	0.0	4	6.7
Normal	26	43.3	30	50.0	56	93.3

[†]Normal range serum vitamin B₁₂ is 100-400 pg/ml and subnormal <100 pg/ml

[‡]Normal range serum folate is 3-15 ng/ml and subnormal <3 ng/ml

Table 2. Serum levels of vitamin B₁₂ and folate in HIV-adult patients with or without receiving ART and AZT therapy

Serum level	ART (n=30)	Non-ART (n=30)	p-value
	Median (Range)	Median (Range)	
Vitamin B ₁₂ (pg/ml)	176.5 (30.0 – 844.2)	296.5 (77.3 – 1211.0)	0.006
Folate (ng/ml)	6.39 (2.22 – 11.92)	9.22 (3.80 – 20.00)	0.006
	AZT (n=17)	Non-AZT (n=13)	
	Median (Range)	Median (Range)	
Vitamin B ₁₂ (pg/ml)	147.6 (30.0 - 407.7)	258.5(114.6 - 844.2)	0.035
Folate (ng/ml)	6.07 (2.22 - 10.95)	6.50 (5.27 - 11.92)	0.174

receiving ART. There were 48 (80.0%) male and 12 (20.0%) female patients. The distribution of characteristics, such as age and sex, were largely similar for both ART and non-ART categories. In terms of CD4 count, the proportion of patients was highest among those in the 101-200 cells/mm³ group (30.0%) receiving ART, while the lowest proportion was among those in the ≤50 cells/mm³ group (31.7%) without receiving ART. None of the HIV-adult patients without ART had subnormal serum folate levels (Table 1).

Statistically significant differences were observed between serum levels of vitamin B₁₂ and folate in HIV-adult

patients with or without receiving ART (p=0.006) (Table 2). As for HIV-adult patients with or without receiving AZT, only serum B₁₂, and not serum folate, showed a significant difference between these two groups (p=0.035).

A statistically significant association was only found between serum levels of vitamin B₁₂ and manifestation of oral lesions in HIV-adult patients receiving ART therapy (p=0.014) (Table 3).

In terms of oral lesions related to either low serum levels of vitamin B₁₂, the most prevalent oral lesion was exfoliative cheilitis (38.3%) and the least prevalent was angular cheilitis (5.0%) (Table 4).

Table 3. Correlations between serum levels of vitamin B₁₂ and folic acid and oral lesions in HIV-adult patients with or without receiving ART therapy

HIV patients	Serum level	With oral lesions		Without oral lesions		p-value
		n	%	n	%	
Receiving ART	Vitamin B ₁₂ [†]					
	Subnormal	8	100.0	0	0.0	0.014
	Normal	11	50.0	11	50.0	
	Folate [‡]					
Subnormal	4	100.0	0	0.0	0.268	
Normal	15	57.7	11	42.3		
Non-ART	Vitamin B ₁₂ [†]					
	Subnormal	2	100.0	0	0.0	1.000
	Normal	20	71.4	8	28.6	
	Folate [‡]					
Subnormal	-	-	-	-		
Normal	22	73.3	8	26.7		

[†]Normal range serum vitamin B₁₂ is 100-400 pg/ml and subnormal <100 pg/ml

[‡]Normal range serum folate is 3-15 ng/ml and subnormal <3 ng/ml

Table 4. Oral lesions related to low serum levels of either vitamin B₁₂ and folate

Oral lesions	n	%
Exfoliative cheilitis	23	38.3
Oral candidiasis	15	25.0
Recurrent aphthous stomatitis	12	20.0
Glossitis	4	6.7
Median rhomboid glossitis	3	5.0
Angular cheilitis	3	5.0

DISCUSSION

A higher prevalence of male (80.0%) than females, and also a higher percentage of those aged 20-29 years were observed in this study. The 2016 data from Ministry of Health of the Republic of Indonesia for the cumulative cases of all reported HIV/AIDS in Indonesia showed that the 20-29 years age group was the most highly affected. It was also reported the cases included 55% of male, 31% of female, and 14% of those who did not report their sex. HIV primarily affects those in their most productive years; about a third of new infections are among young people (ages 15-24). Globally, an estimated 35.3 million people were living with HIV at the end of 2012; of these, 2.1 million were adolescents aged 10-19 years, of which the majority was girls (56%) (Idele *et al.*, 2014).

The degree of immunosuppression by the CD4 cell count testing in this study was found somewhat higher among patients in the 101-200 cells/mm³ group (43.3%) compared to those in the ≤50 cells/mm³ group (40.0%), irrespective of receiving ART or not. Low CD4 cell count (<200 cells/mL) is an independent risk factor for megaloblastic anaemia due to acquired deficiency in vitamin B₁₂ and folate, as it appears to be the most common haematological complication in HIV-infected adults. Although there were differences in the cut-off points for defining anaemia, its prevalence was greater in HIV patients and those with lower CD4 cell counts. The pathogenesis

of anaemia in HIV-infected adults, although multifactorial, relates primarily to a reduced production of erythrocytes. This reduction is influenced by several aetiological factors, including infection and neoplasms, use of drugs such as AZT, a direct effect of HIV on erythropoiesis, and a blunted response to erythropoietin and nutritional deficiencies (Calis *et al.*, 2008). Micronutrient deficiencies that have been associated with HIV infection leading to anaemia are iron, folate, vitamin B₁₂, vitamin A, and zinc. Folate and vitamin B₁₂ are essential for cellular proliferation and erythropoiesis, and their deficiency can depress cell-mediated immunity (Volberding *et al.*, 2004). Hidayat *et al.* (2017) in a study of 40 HIV-adult patients without receiving ART in Teratai outpatient clinic RSHS Bandung reported a correlation between oral manifestations of anaemia and CD4 count ≤200 cells/mm³. A cohort study in Uganda by Semere *et al.* (2012) reported that CD4 count ≤350 cells/mm³ was found in low serum levels of vitamin B₁₂, which was suspected to be due to the increase in HIV replication.

The study of Remacha *et al.* (2003) reported reduced serum levels of vitamin B₁₂ (10.3%) and folate (11.1%) in patients after receiving ART. This reduction was attributed to HIV infection resulting in changes in plasma protein binding capability with lymphocytes and neutrophils (Semeere *et al.*, 2012; Tang *et al.*, 1997).

Kaiser *et al.* (2006) showed that micronutrient supplementation, which included vitamin B₁₂ and folic acid twice daily for 12 weeks, significantly increased absolute CD4 cell count and the mean change in CD4 cell count from baseline in HIV-infected patients on ART therapy. Vitamin B₁₂ supplementation in HIV-infected patients on ART might be of clinical importance, since vitamin B₁₂ can inhibit nitric oxide synthase and modulate cellular immunity, particularly T lymphocytes (Kaiser *et al.*, 2006; Tang *et al.*, 1997). Nevertheless, larger trials

are needed to conclude this clinically important effect (Visser *et al.*, 2017).

In this study, serum vitamin B₁₂ and folate levels in HIV-AIDS patients with ART were found lower than those without ART. The distribution of ART, specifically AZT, can lead to mitochondria deoxyribonucleic acid (DNA) synthesis interference, which leads to haematology interference in the form of reduction of blood vitamin B₁₂ and folate levels (Shiboski *et al.*, 2015).

This study shows that various types of oral lesions were associated with vitamin B₁₂ deficiency and folic acid in HIV-AIDS patients. Manifestation of exfoliative cheilitis was highest (38.3%), followed by oral candidiasis (25.0%), recurrent aphthous stomatitis (RAS) (20.0%), glossitis (6.7%), median rhomboid glossitis and angular cheilitis (5.0%). Deficiencies of vitamin B₁₂ and folate are known to lead to the oral manifestations of glossitis, angular cheilitis, recurrent oral ulcer, oral candidiasis, and diffuse erythematous mucositis (Reynolds, 2006; Pontes *et al.*, 2009).

Reichart *et al.* (1997) showed that exfoliative cheilitis occurred in 28.5% of their study HIV-AIDS patients. Hidayat *et al.* (2017) reported at the Teratai outpatient clinic that there were 94.8% HIV-AIDS patients with anaemia had oral candidiasis with CD4 \leq 200 cells/mm³. HIV-AIDS patients with CD4 \leq 200 cells/mm³ are known to be susceptible to opportunistic infections, such as candida, thus the anaemia condition will worsen the oral candidiasis state.

While the deficiency mechanisms of both vitamin B₁₂ and folate leading to oral lesions have not yet been established, both vitamins serve as coenzymes in hematopoiesis and erythropoiesis (Kozlak *et al.*, 2010). Macrocytosis, as a result of vitamin B₁₂ and folate deficiency, is a direct effect of ineffective erythropoiesis. This process leads to changes of erythrocytes into erythroblasts with poikilocytosis, which is characteristic of megaloblastic anaemia with such clinical manifestations oral lesions (stomatitis,

cheilitis, and glossitis), malabsorption and gastrointestinal lesions, as well as diarrhoea.

CONCLUSION

The current study shows significantly lower levels of serum vitamin B₁₂ and folate in HIV-adult patients with ART therapy, compared to those without ART. Oral lesions were found significantly associated with low levels of vitamin B₁₂, but not with folate in HIV-adult patients with ART. Further large-scale studies are needed to confirm these findings and to establish whether low level of serum vitamin B₁₂ and folate in HIV patients are long-term side effects of ART therapy, before recommending routine supplementation of vitamin B₁₂ and folate among HIV patients under antiretroviral therapy.

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Authors' contributions:

IS, principal investigator, conceptualised and designed the study, prepared the draft of the manuscript and reviewed the manuscript; DP, conducted the study, data analysis and interpretation, prepared the draft of the manuscript; DMDH, data analysis and interpretation, prepared the draft of the manuscript and reviewed the manuscript.

Conflict of interest

The authors declared no conflict of interest.

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