Original Article

Prevalence of Vitamin B_{12} Deficiency in Antiretroviral Therapy Naïve Adults with Human Immunodeficiency Virus Infection in a Human Immunodeficiency Virus Treatment Center in Lagos, Nigeria

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Abstract

Background: Vitamin B_{12} deficiency is reportedly higher in human immunodeficiency virus (HIV) infection, according to reports from developed countries and is associated with worsening anemia, progressing immunodeficiency (reduced CD4 count), and reduced survival rates. The status in Nigerians with HIV has not been extensively studied. The objective of the study was to determine the frequency and correlates of Vitamin B_{12} deficiency in HIV-positive antiretroviral therapy (ART) naive patients attending an outpatient HIV clinic in Lagos, Nigeria. Specifically, the study compared HIV-positive ART-naïve patients to age- and gender-matched HIV-negative controls and determined the relationship between B_{12} status and HIV disease severity (CD4 count) and hemoglobin (Hb). **Methodology:** The study was a descriptive study of the prevalence of Vitamin B12 deficiency and its correlation with disease severity in HAART-naïve newly diagnosed HIV infection. Seventy-five ART naïve, HIV-positive patients and 75 controls fulfilling the study criteria were included. Baseline hematologic (Hb, white blood cell, platelets, and CD4 count) and Vitamin B_{12} levels were measured. Vitamin B_{12} levels were measured using urine methylmalonic acid (MMA) on spot urine normalized for urine creatinine. B_{12} deficiency was defined as urine MMA >3.6 mmol/mol urinary creatinine. CD4 count (cells/μL) was categorized as <200, 200–499, and >500. **Results:** The frequency of B_{12} deficiency was 29.3% (22/75) in HIV-positive cases and 0% (0/75) in controls ($P < 0.001$). There was no difference in the frequency of anemia in HIV cases with or without B_{12} deficiency (54.5% vs. 58.5%; $P = 0.75$). There was no significant difference in the proportions of HIV cases with or without B_{12} deficiency in the CD4 categories 1 (>500), 2 (200-499) and 3 (<200), (1: 31.8% vs. 24.5%; 2: 40.9% vs. 50.9%; 3: 27.5% vs. 24.5%, respectively; $P = 0.71$). Neither severity of HIV infection nor Hb levels was found to be associated with B_{12} status ($P > 0.05$). **Conclusion:** Vitamin B_{12} deficiency was more prevalent in HIV-positive ART naïve cases compared to age- and gender-matched HIV-negative controls. However, the presence of B_{12} deficiency was not associated with anemia or the severity of HIV infection in this study.

Keywords: Human immunodeficiency virus, Vitamin B12 deficiency, Urine methyl malonic acid

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Introduction

Since the initial descriptions of the human immunodeficiency virus Type 1 (HIV-1) in 1983 and HIV Type 2 (HIV-2) in 1986, these two viruses have been identified for over 20 years as the primary cause of the acquired immunodeficiency

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syndrome (AIDS) and approximately 37.9 million people were living with HIV at the end of 2018.[1] The hallmark of HIV disease is a profound immunodeficiency resulting from a progressive quantitative and qualitative deficiency of the Th1 and Th2 helper T-cell subpopulation. This subset of T-cells is identified phenotypically by the expression in the cell surface of the CD4 molecule, which serves as the primary receptor for HIV. Although the CD4-positive T-lymphocyte and the CD4 + monocyte lineage are the principal cellular targets of HIV, virtually any cell that expresses CD4 along with one of the co-receptors can potentially be infected with HIV.[2]

Micronutrient deficiencies such as Vitamins B, C, E, and selenium, which are required for the maintenance of a responsive immune system, have been reported in HIV and are associated with HIV disease progression. Furthermore, studies have suggested that normalization of these deficiencies might increase the interval of symptom-free survival.[3]

Vitamin B12 deficiency, like other micronutrients, has been found to occur more frequently in HIV than in the general population.[4-6] Vitamin B_{12} deficiency has been found in 10%–35% of patients with HIV and has been associated with worsening immunologic status, HIV progression, and cognitive dysfunction.[7-9]

Vitamin B_{12} deficiency is also one of the mechanisms for HIV-associated anemia and has been associated with lower hemoglobin (Hb), leukocytes, lymphocytes, CD4+ lymphocytes, and CD4/CD8 lymphocyte ratio than in HIV infected individuals with physiological serum Vitamin B_{12} levels.[10,11] The etiology and pathogenesis of B_{12} deficiency is uncertain but appears to be a defective intestinal absorption in many cases as well as evidence of an alteration of cobalamin binding proteins.[7,12-14]

Vitamin B_{12} deficiency specifically in persons with HIV infection has been infrequently studied in sub-Saharan Africa, including Nigeria. Vitamin B12 assay is not a routine pre-antiretroviral therapy (ART) test in resource-limited settings. The study is important because the frequency of B_{12} deficiency is reportedly 10%–35% in HIV infection, and some studies have found an association with worsening anemia, progressing immunodeficiency (reduced CD4 count), and reduced survival rates.[7,8,15] Furthermore, normalization of B_{12} levels has been associated with improvement in CD4 counts and this suggests that correction of B_{12} deficiency may improve care and treatment outcomes of HIV infection.[8] There is a paucity of data regarding B_{12} deficiency in Nigerians with HIV, and this study was designed to contribute to our understanding of the frequency, magnitude, and effects, and provide a basis for recommendations regarding the need or otherwise for screening in persons with HIV. The important clinical consequences of this vitamin deficiency in the clinical course of HIV and its correction (which can be easily achieved) might increase the interval of symptom-free survival thus potentially improving care and quality of life of people living with HIV/AIDS has not been fully explored in our population.

The study was a descriptive study of the prevalence of Vitamin B12 deficiency and its correlation with disease severity in HAART-naïve newly diagnosed HIV infection. The study aimed to determine the prevalence of B_{12} deficiency in ART naïve HIV infected patients, the relationship between B_{12} levels (using spot urine methylmalonic acid [MMA]) and Hb levels in HIV infection, the relationship between HIV disease severity (measured by CD4 count) and Vitamin B_{12} levels (using spot urine MMA) and to compare B_{12} levels (using spot urine MMA) in HIV-positive individuals with levels in age- and gender-matched HIV-negative individuals (controls).

The diagnosis of Vitamin B_{12} deficiency is conventionally based on the measurement of serum B_{12} levels, usually <200 pg/ml (150 pmol/L), along with clinical evidence of the disease.[16,17] However, studies have shown that about 50% of patients with the subclinical disease have normal Vitamin B_{12} levels.[18,19] Vitamin B_{12} deficiency leads to an increase in serum methylmalonyl-CoA and its metabolic product, MMA. Urine concentrations of MMA are 40-fold higher than in serum concentrations; therefore, urine MMA excretion is considered the most accurate screen for B_{12} deficiency.[20]

Methodology

This study employed a comparative case (HIV positive) and control (HIV negative) study design and was carried out at the adult HIV clinic of the Nigerian Institute of Medical Research, Yaba, Lagos, between March and June 2013. The Voluntary counseling and testing (VCT) center in practice uses two different rapid kits at a time for screening and diagnosis for HIV. Where there is a disparity between the two kits, samples are sent for confirmatory tests by Enzyme-linked immunosorbent assay.

A total of 150 participants, comprising 75 HIV-positive cases and 75 HIV-negative controls, were recruited. A simple random sampling technique was used to select one newly diagnosed HIV-positive patient at the NIMR VCT center per day. A simple random sampling technique was also used to select one HIV-negative client among those that visited the NIMR VCT per day. Where the controls were not age and gender matched, a reselection was done, ensuring that the age difference did not exceed 3 years. The specific demographic and socioeconomic parameters were gender distribution, age (and age categories), and household monthly income.

Inclusion criteria for participants were adults aged between 18 and 65 years who screened positive for HIV based on double rapid tests (Determine HIV1/2 and Unigold™), at the NIMR VCT and were antiretroviral drug naïve.

Patients who were pregnant, vegetarians, or who had a history of diabetes mellitus (random plasma glucose >200 mg/dL plus clinical features of diabetes mellitus) or drug therapy known to cause B_{12} deficiency (e.g., phenytoin, neomycin,

metformin, omeprazole), chronic kidney disease (defined using creatinine clearance <60 ml/min), and significant alcohol ingestion (>35 g/week for men, 14–21 g/week for women for >10 years), were excluded from the study.

Controls were voluntary adults aged 18 years and above who screened negative HIV using two rapid screening kits Determine HIV1/2 and Unigold™ at NIMR VCT. They were otherwise healthy and nonvegeterian.

The laboratory assessments comprised baseline hematological parameters (full blood count, red cell indices, CD4 count), baseline chemistry (random plasma glucose, serum creatinine, and determination of creatinine clearance). Urine MMA was determined on both patients and control from spot urine samples. Universally accepted laboratory reference values rather than control values were to be used to define normative values of urine MMA in the Nigerian adult.

Vitamin B_{12} deficiency was defined as urine MMA>3.6 mmol/mol urinary creatinine. CD4 count (cells/μL) was categorized as <200, 200–499 and >500.

The Statistical Package Software for the Social Sciences (SPSSR) version 17.0 (IBM Corp, Armonk, NY, USA) was used for data entry, validation, and analysis. Frequency distribution tables and charts were generated for the categorical variables. Relevant summary statistics were generated for the discrete variables. Student's *t*-test and Chi-square test were used to test the significance of differences between groups. Specifically, intergroup (case–ART naïve HIV infected versus control-HIV negative) differences in urine MMA excretion were determined using appropriate tests. Univariate linear regression analysis of urine MMA excretion with Hb and CD4 count was determined. Statistical significance was considered at $P < 0.05$

Results

The specific demographic and socioeconomic parameters were gender distribution, age (and age categories), and household monthly income. As shown in Table 1, the cases and controls were matched for age, gender, and mean monthly income.

The study participants' distribution by gender was similar, with 37 males (49.3%) and 38 females (50.7%) (male-to-female ratio 1:1.02). The age of the participants ranged from 17 to 55 years with a mean of 34.9 ± 10.3 years. The majority of the participants (37.3%) were aged between 21 and 30 years. Nearly half of the participants (40%) had a monthly income of 20,000 naira and below.

The mean Hb and CD4 counts were lower in HIV-positive cases than in controls was statistically significant [$P < 0.0001$; Table 2].

The mean urine MMA value (mmol/L) in HIV-positive cases was 51.6 ± 71.6. The HIV-negative controls had a mean value of 16.9 ± 8.4. The difference was statistically significant ($P < 0.0001$). When normalized for creatinine, the mean urine MMA/Cr (mmol/mol creatinine) was 3.20 ± 2.7 in HIV-positive cases. The HIV-negative controls had a mean value of 1.7 ± 0.7. The difference was also statistically significant [$P < 0.001$; Figure 1].

Vitamin B_{12} deficiency (defined by universally accepted laboratory cut-off values of >3.6 mmol/mol creatinine) was found in 22/75 (29.3%) of HIV-positive cases. None of the controls was found to have B_{12} deficiency. However, B_{12} deficiency in the 22 HIV-positive cases was mild based on the categorization of urine MMA/Cr excretion by universally accepted laboratory cut-off values.

Using the World Health Organization (WHO) definition of anemia as <13 g/dL in men and <12 g/dL in women, 43 of 75 (57.3%) of HIV-positive cases were anemic in contrast to 3 (4.0%) of controls. The severity of anemia was further categorized using the WHO criteria for men and women. As such, 18 (24.0%) had mild anemia (reference range 11.0–11.9 g/dL females; 11.0–12.9 g/dL males), 20 (26.7%) had moderate anemia (Hb 8.0–10.9 g/dL for both sexes), while 5 (6.7%) had severe anemia (Hb <8.0 g/dL for both sexes).

The frequency of anemia in HIV cases with and without B_{12} deficiency was also explored. The analysis showed that the frequency of anemia was similar in both categories [$P > 0.05$; Table 3]. Twelve (12) of 22 HIV-positive cases with B_{12} deficiency had anemia (54.5%), while 31 of 53 cases without B_{12} deficiency had anemia (58.5%).

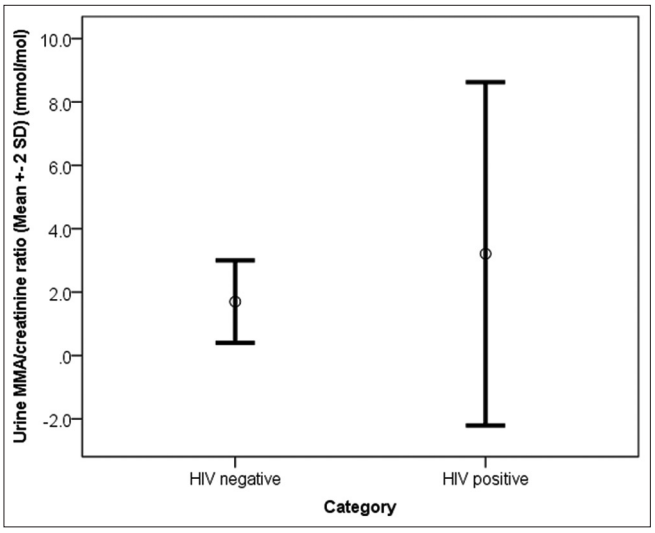

Figure 1: Comparison of mean Urinary methylmalonic acid/creatinine levels in human immunodeficiency virus positive and human immunodeficiency virus-negative controls (Error bar comparing the mean value [central circle] and standard deviation [whiskers] in human immunodeficiency virus-positive cases [3.2 ± 2.7] and human immunodeficiency virus-negative controls (1.7 ± 0.7). Mean urine methylmalonic acid/Cr [mmol/mol] was significantly higher in human immunodeficiency virus-positive cases [$P < 0.0001$])

Table 1: Baseline demographic and socioeconomic characteristics of the study participants

Parameter	Total (*n*=150), *n* (%)	HIV positive (*n*=75), *n* (%)	HIV negative (*n*=75), *n* (%)	Statistics
Gender distribution				
Number of males	74	37 (49.3)	37 (49.3)	1.00
Number of females	76	38 (50.7)	38 (50.7)	
Male-to-female ratio		1:1.02	1:1.02	
Age range (years)	17-55	17-55	18-55	
Age group (years)				
<20	8	4 (5.3)	4 (5.3)	
21-30	56	28 (37.3)	28 (37.3)	
31-40	46	23 (30.7)	23 (30.7)	
41-50	22	11 (14.7)	11 (14.7)	
51-60	18	9 (12.0)	9 (12.0)	
Mean age±SD (years)	34.9±10.3	34.8±10.5	34.9±10.3	0.96
Females	33.7±10.4	33.7±10.4	33.7±10.6	1.00
Males	36.1±10.2	36.1±10.2	36.1±10.3	1.00
Mean monthly income (₦)*	36767.3±43650.4	36580.0±43325.8	36946.7±44263.6	0.90
<20,000	60 (40)	29 (38.7)	31 (41.3)	0.99
20-50,000	68 (45.3)	35 (46.7)	33 (44.0)	
>50-100,000	14 (9.3)	7 (9.3)	7 (9.3)	
>100,000	8 (5.3)	4 (5.3)	4 (5.3)	
BMI (kg/m^2)	24.7±4.2	24.5±4.6	24.9±3.8	0.98

Pearson Chi-square test was used for comparison of categorical variables in HIV cases and controls. Student's *t*-test was used for comparison of mean values. BMI: Body mass index, HIV: Human immunodeficiency virus, SD: Standard deviation

Table 2: Comparison of the hematological parameters in human immunodeficiency virus-positive cases and matched controls

Parameter	Normal values	HIV positive (*n*=75)	HIV negative (*n*=75)	Statistics (*P*)
Packed cell volume (%)	40-52 (male) 36-47 (female)	35.7±5.9	39.4±4.0	<0.001
Hb (g/dL)	14-18 (male) 12.0-16.0 (female)	12.0±2.4	13.5±1.3	<0.001
Mean corpuscular volume (fl)	80-100	82.7±4.8	85.6±7.3	0.01
MCH (pg)	27-32	27.6±2.1	29.9±2.9	0.04
MCH concentration (g/dL)	32-36	33.3±2.7	34.1±1.5	0.02
Total WBC count (x10^9/L)	4-11	5.0±1.1	5.1±1.8	0.87
Neutrophil (%)	40-75	49.9±7.6	50.6±8.0	0.59
Lymphocytes (%)	20-45	43.0±7.0	44.4±8.6	0.29
Basophils (%)	0-1	0.2±0.10	0.2±0.1	0.2
Monocytes (%)	2-10	3.8±3.1	1.0±0.9	<0.001
Eosinophil (%)	1-6	1.4±1.3	0.5±0.4	<0.001
Reticulocyte (%)	0.5-2.5	1.1±0.3	1.5±0.3	<0.001
Platelet count (x10^9/L)	150-400	235.7±81.4	232.37±59.8	0.71
CD4 count (cells/μL)	500-1,500	410.8±278.8	947.3±154.8	<0.001

MCH: Mean corpuscular hemoglobin, WBC: White blood cell, Hb: Hemoglobin

Table 3: Relationship between B_{12} deficiency and anemia in human immunodeficiency virus cases

Hb status	B_{12} deficiency MMA/Cr >3.6 mmol/mol (*n*=22), *n* (%)	No B_{12} deficiency MMA/Cr <3.6 mmol/mol (*n*=53), *n* (%)	Total (*n*=75)	*P*
Anemia	12 (54.5)	31 (58.5)	43	0.75
No anemia	10 (45.5)	22 (41.5)	32	

Hb: Hemoglobin

Correlation analysis (Pearson's) was conducted to explore the association between Hb levels and urine MMA/creatinine ratio (representing B_{12} levels). There was no correlation between the parameters (Pearson correlation 0.125; $P = 0.28$).

The HIV cases were categorized based on the CD4 count levels (cells/μL) as follows: <200 (19 i.e., 25.3%); 200–499 (36 or 48.0%) and >500 (20 i.e., 26.7%). The mean CD4 counts of the HIV cases with (436.3 ± 308.4) and without (400.2 ± 267.9) B_{12} deficiency did not differ significantly ($P = 0.61$).

Of the HIV-positive cases, 6 (27.5%) of those with B_{12} deficiency had CD4 count was <200 cells/μL in 6 (27.5%), between 200 and 499 cells/μL in 9 (40.9%), while 7 (31.8%) had CD4 counts >500 cells/μL. There was thus no significant association between severity of HIV disease (measured by CD4 count) and B_{12} status [$P = 0.7$; Table 4].

Discussion

This study assessed the frequency and correlates of Vitamin B_{12} deficiency using urine MMA (which is a metabolite of Vitamin B_{12} excreted in the urine) in HIV-positive adults in an outpatient clinic setting. Urine excretion of MMA has however been documented to be the most sensitive assay for B_{12} deficiency.[20] Besides its sensitivity, the test is noninvasive and not cumbersome, requires a very small urine sample specimen (1 ml), is relatively inexpensive and does not show significant individual daily variation or sample instability in processing.[20]

The mean age (years) of the cases was 34.9 ± 10.3, and the majority (40%) were aged between 21 and 30 years. This is consistent with global demographic statistics for HIV, including those from the by the Centers for Disease Control, USA and the Joint United Nations Programme on HIV/AIDS on the age range of HIV diagnosis of between 20 and 24.[21,22] This has been attributed to the fact that this age group is the most sexually active, and hence the group is at the highest risk for HIV infection.

Sociodemographic factors are important determinants of the risk of HIV infection. In this study, monthly income earnings (classified by the Department of International Development 2004)[23] showed that over a third of the cases (38.7%) earned <20,000 naira monthly-a figure close to the country's official minimum wage of 18,000 naira per month which has only in the year 2019 been increased to N30,000 per month. This is consistent with the fact that HIV infection is very common in the low socioeconomic group, who constitute most of those infected in Africa.[21,22] Sub-Saharan Africa has the lowest gross domestic product in the world, with more than 60% of the population spending less than the US $1 a day.[24]

The mean Hb and packed cell volumes levels were significantly lower in HIV-positive cases than in controls. This study reported anemia in 57.5% of HIV-positive cases using the WHO references in males and females.[25] This finding corroborates previous documentation of anemia being the most frequent complication of HIV occurring in 30%–80% of HIV-infected persons. And confirms other reports in Nigeria and Ghana which also documented significantly lower mean Hb levels in HIV-positive persons.[25-27] The lower Hb levels in HIV cases may be due to the effect of the virus itself on erythropoiesis, and disease progression.[15]

The mean CD4 cell counts were also significantly lower in HIV-positive cases than in controls as expected. This finding was consistent with that observed in Kano, Nigeria, which reported a two-fold or greater reduction in CD4 counts of HIV-positive adults compared to HIV seronegative controls.[28] The CD4 positive lymphocyte (and monocyte) lineage are the principal cellular targets for HIV and infection results in a progressive quantitative (and qualitative) deficiency of these cells.[2] In this study, only 19 (25.3%) of the HIV cases were in late-stage disease (CD4 counts of <200 cells/μL), whereas 56 (74.7%) were in middle and early-stage disease. Specifically, 48.0% were in middle-stage disease (CD4 counts between 200 and 499 cells/μL), and 26.7% were in early-stage disease (CD4 counts >500 cells/μL).

The prevalence of vitamin B_{12} deficiency in HIV-positive individuals in this study was 29.3% which concurred with earlier reported with findings of Vitamin B_{12} deficiency in 10%–35% in early asymptomatic HIV disease.[8,12] Using standard laboratory reference values, the severity of B_{12} deficiency was mild based on the finding of mild to moderate elevations of urine MMA/Cr (3.9–39.930 mmol/molcr).[29] The prevalence of B_{12} deficiency was significantly higher than in controls, none of whom were found to be B_{12} deficient. The finding of significantly lower B_{12} levels among HIV-positive cases is in consonance with the findings by Masaisa *et al*. In their cross-sectional study conducted in Rwanda, 200 HIV-infected and 50 uninfected women were evaluated to determine the prevalence and risk factors for anemia among HIV-infected women in Rwanda. The study found that mean serum Vitamin B_{12} concentration was significantly lower among HIV-positive than among HIV-negative women.[30] Although there is a paucity of published data on B_{12} deficiency among the

Table 4: Comparison of B_{12} levels (and frequency of B_{12} deficiency) in relation to CD4 count category in human immunodeficiency virus

CD4 count category	B_{12} deficiency MMA/Cr >3.6, *n* (%)	No B_{12} deficiency MMA/Cr <3.6, *n* (%)	Total (*n*=75), *n* (%)	*P*
<200	6 (27.5)	13 (24.5)	19 (25.3)	0.71
200-499	9 (40.9)	27 (50.9)	36 (48.0)	
>500	7 (31.8)	13 (24.5)	20 (26.7)	
Total	22	53	75	

MMA: Methylmalonic acid

ART-naïve population in sub-Saharan Africa, a cross-sectional study with a retrospective chart review over a period of 1 year among 218 HIV-positive, ART-naïve adults (≥18 years) attending two HIV treatment centers in Uganda reported sub-optimal serum B_{12} levels (defined as <300 pg/ml in 75 (36.8%) patients while 21 (10.8%) were defined as B_{12} deficient using a cut-off value of 200 pg/ml.[31] In the Medical Research Council Concorde trial conducted over a 2.5 year period in London, England, Rule *et al*. measured serum B_{12} levels in 218 asymptomatic HIV seropositive patients using radioimmune assay techniques, and found that 22 (10.1%) had low concentrations of B_{12} (serum B_{12} level of <210 ng/L).[12] In a prospective study by Remacha *et al*. in Santa Creu, Barcelona, aimed at determining the cause of the low serum Vitamin B_{12} and its clinical effects, 60 consecutive HIV-positive adult patients admitted over a 6 month period were studied. Low serum B_{12} ≤ 150pmol/l (determined by radioassay) was found in 10 (16.7%) patients.[8] A study of 150 HIV-infected patients in south India to evaluate the prevalence of folate and Vitamin B_{12} deficiency in HIV-positive patients with or without tuberculosis and its association with neuropsychiatric symptoms and immunological response revealed that 30% of the HIV patients' study had a folic acid deficiency and about 10% of the HIV patients had Vitamin B_{12} deficiency. The prevalence of Vitamin B_{12} deficiency found in all these studies was lower than that in the present in the study. This is likely due to the methodological difference. Different screening assays for B_{12} deficiency were used, and that employed in this study (urine MMA) measured as an indicator of B_{12} deficiency has been found to be a more sensitive and earlier marker of tissue B_{12} deficiency than serum B_{12}.[20] However, ethnic and cultural dietary differences may also have contributed to the variation.

In this study, there was no demonstrable correlation between Hb and B_{12} levels (Pearson correlation 0.125; $P = 0.28$). The frequency of anemia was high in HIV cases with and without B_{12} deficiency, with a similar magnitude (54.5% and 58.5%). This finding suggests that B_{12} deficiency is not the only cause of anemia in HIV. The causes of anemia in HIV-infected individuals are multifactorial and involve various pathogenetic mechanisms such as decreased red blood cell (RBC) production, increased RBC destruction, and ineffective RBC production, and blood loss.[25] Decreased RBC production may be a consequence of chronic disease, which is commonly associated with anemia due to low erythropoietin levels, decreased production of endogenous erythropoietin, as well as a blunted response to erythropoietin.[32] In addition, cytokines such as interleukin-1, tumor necrosis factor, and interferons play an important role in impairing erythropoietin by reducing the concentration of the marrow progenitors and erythroid colonies.[33] Furthermore, opportunistic diseases including neoplasms (especially lymphomas), bone marrow infections (e.g., parvovirus B19, cytomegalovirus, Mycobacterium avium, and Cryptococcus neoformans), use of myelosuppressive medications, myelofibrosis, and even the direct effect of HIV itself lead to marrow suppression and resultant decreased RBC production.[25] A range of medications used in the setting of HIV also causes myelosupression. Zidovudine (AZT) is known to cause anemia by various pathogenic mechanisms, which include inhibition of Hb synthesis and globin gene transcription[34] and toxicity to the bone marrow cells (particularly erythroid cells) by the metabolite of AZT (3' amino-3-deoxythymidine) (AMT).[35] Other myelosuppressive drugs used in HIV include anti-infectives (dapsone, pyrimethamine, trimethoprim), antivirals (ganciclovir, foscarnet,) and antineoplastics (doxorubicin, epotoside, cyclophosphamide), and so on.[25] Other cytokines such as tumour necrosis factor alpha and transforming growth factor may be upregulated in HIV infection, contributing to ineffective red cell production.[33]

Ineffective RBC production can also result from deficiencies of iron, folic acid and vitamin $B_{12.}$ In patients with HIV disease, folic acid deficiency is generally caused by either dietary deficiency or jejunal pathology, while Vitamin B_{12} deficiency may result from malabsorption as a result of gastric and intestinal pathologies as a consequence of HIV infection or opportunistic infections.[25] Iron deficiency may result from blood loss which may be associated with conditions such as neoplastic disease (e.g., Kaposi sarcoma in the gastrointestinal tract) or gastrointestinal lesions that accompany opportunistic cytomegalovirus infection.[32] Increased RBC destruction in HIV may result from red cell autoantibodies and hemophagocytic syndrome.[32]

In this study however, the HIV cases with B_{12} deficiency had mild B_{12} deficiency. This may also have contributed to the lack of demonstrable difference in frequency of anemia in B_{12} deficient compared to nondeficient cases**.** From this study, one can therefore suggest that mild B_{12} deficiency may not necessarily translate to anemia. This position is supported by a case report from Delva in Canada, of a 50 year old woman who was found to have mild B_{12} deficiency defined by serum levels 147 pmol/L (reference values165–140 pmol/L) but had normal Hb levels (14.3 g/dl) even though she presented with vague neuropsychiatric symptoms.[36] Another study by Lindenbaum *et al*. found that 40 of 141 consecutive HIV-positive patients with neuropsychiatry symptoms from cobalamin deficiency did not have anemia or macrocytosis. From this series, it appeared apparent that at least one-quarter of cobalamin deficient patients may present without anemia.[37] Anaemia and megaloblastosis are typically present when B_{12} deficiency is severe (serum level <74 pmol/L (100 ng/L).[38]

This study showed no significant relationship between severity of disease and B_{12} deficiency and suggests that B_{12} deficiency can occur at any stage of HIV infection and may not be used as a marker for disease severity. Findings from this study agree with those by Baum *et al.* in California, USA who, in a prospective study of 50 patients with HIV infection (in which 10 of them had AIDS), found that serum cobalamin levels appeared to be as frequent in the AIDS and the non-AIDS group and implied that low cobalamin levels are not related to the

disease states in question.[3] Furthermore, Rule *et al*. in London, England, observed serum B_{12} levels of 218 asymptomatic HIV patients and found that 22 (10%) had B_{12} deficiency but serum B_{12} levels showed no correlation with CD4 lymphocyte counts. Follow-up studies over a 2.5 year period conducted on 59 of the HIV patients in which repeated serum B_{12} and CD4 assays were performed found that serum B_{12} fell in 38 (64%) of the 59 patients. Although CD4 counts fell during this period, these drops did not correlate with those in serum B_{12}, further obscuring this connection between Vitamin B_{12} and HIV disease severity.[12] Findings from this study, however differed from those by Remacha *et al*. in Barcelona in a study of 60 HIV-positive patients who found lower CD4 cell counts in those with B_{12} deficiency compared to those with normal serum B_{12} levels.[11] It is worth pointing out that 9 of 10 low Vitamin $B_{12\ patient}$s had AIDS, compared to 33 of 50 in the group with physiological Vitamin B_{12} values, although in their study, the difference was not statistically significant. It was concluded that larger studies would be required to evaluate the significance of low vitamin B_{12} levels as a prognostic factor in the different stages of HIV infection.

Conclusion

This study demonstrated a significantly higher frequency of vitamin B_{12} deficiency in HIV positive treatment-naïve cases compared to HIV-negative age-, gender-, and socioeconomically-matched controls. The severity of B_{12} was mild in all the cases and did not correlate with disease severity or Hb level. This study is important in that it highlights the relatively high frequency of Vitamin B_{12} deficiency early in the course of HIV disease, highlighting a metabolic derangement that can potentially be aggravated in the course of treatment or with the occurrence of comorbid opportunistic diseases or other nonopportunistic conditions, and thus needs to be identified early.

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Conflicts of interest

There are no conflicts of interest.

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