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ORIGINAL RESEARCH

The serum concentration of vitamin B_{12} as a biomarker of therapeutic response in tuberculosis patients with and without human immunodeficiency virus (HIV) infection

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Background: Prior to clinical trials of new tuberculosis (TB) drugs or therapeutic vaccines, it is necessary to develop monitoring tools to predict treatment outcomes in TB patients.

Methods: Micronutrients concentration level was determined from a total of 262 study participants with five clinical groups: 57 TB patients coinfected with HIV (HIV+TB+), 87 active TB Patients (TB cases), 71 HIV infected without active and latent TB infection (HIV +TST-), 22 latent TB infection (TST+) and 25 healthy controls (TST-). Vitamin A concentration was measured using high-performance liquid chromatography (HPLC), whereas iron and vitamin B_{12} concentrations were measured using Cobas® 6000 analyzer.

Result: The serum concentration levels of iron, vitamin A and vitamin B_{12} had a significant difference between active TB and latent (LTBI) or healthy controls. Six months after treatment, the serum concentration levels of vitamin A, vitamin B_{12} and iron in tuberculosis became indistinguishable from the levels of LTBIs and healthy control individuals. The concentration levels of iron and vitamin B_{12} in HIV+TB+patients at the end of TB treatment were normalized to the levels observed in healthy controls (TST–) regardless of HAART treatment. However, the concentration level of vitamin A in HIV+TB+patients HAART untreated at the end of TB treatment was not normalized to the levels observed in healthy controls (TST–) or HAART untreated HIV+TST–.

Conclusion: Detecting serum concentration levels of vitamin B_{12} and vitamin A might be used as a biomarker of the diagnostic method of active TB regardless of HIV-infected individuals. Moreover, detecting serum concentration of vitamin B_{12} might also be used for TB treatment responses monitoring biomarker in TB-HIV-co-infected individuals regardless of HAART (in)eligibility and therapy.

Keywords: biomarker of diagnostic, vitamin A, Vitamin B_{12}, anti-TB treatment, retinol

Introduction

Most immunocompetent individuals maintain latent tuberculosis (TB) infection with only 5–10% lifelong risk of developing the clinical disease,[1] while the risk rises to 10% per year in immunocompromised individuals.[2] Most of the new TB cases in 2015 has occurred in Asia (61%) and the African Region (26%).[3] The available diagnostic tools of TB (smear microscopy, solid and liquid sputum culture, Genexpert) have several limitations to detect latent and active TB[4–7] and for monitoring TB treatment response,[8] and therefore, it is necessary to develop diagnostic

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and monitoring tools to predict treatment outcomes in TB patients. Nutritional status is one of the most important determinants of immune response to resist infection.[9] Micronutrients are key contributors to the development of the immune system and cytokine kinetics of the body. In addition, micronutrients have important roles in cellular function and immune competence[10] and increase the ability to produce immunoglobulin and activating lymphocyte transformation for immune protection.[11] Micronutrient malnutrition has been described in pulmonary TB patients previously.[12] Detecting serum concentration levels of vitamin A, vitamin B_{12} and iron might be used for a diagnostic method of detecting active TB and monitoring TB treatment response. Therefore, the aim of this study was to assess whether the three micronutrient elements (vitamin A, vitamin B_{12}, and iron) concentration could be used for a biomarker of active TB with and without HIV infection and these were also used for monitoring TB treatment during anti-TB treatment therapy.

Methods

Study Design And Study Population

A total of 262 study participants and adults with age 15–65 year range were enroled from the previous cohort study.[13] Study participants included 5 clinical groups: 57 TB patients coinfected with HIV (HIV+TB+), 87 active TB Patients (TB cases), 71 HIV infected without active and latent TB infection (HIV+TST-), 22 latent TB infection (TST+) and 25 healthy controls (TST-). All these study participants had not received any vitamin supplements during their treatment. Demographic data were collected from the previous study. A control group of 47 (TST+and TST-) subjects without a prior diagnosis of TB was recruited without any clinical symptoms or signs of illness due to active TB and HIV/AIDS. Exclusion criteria for enrollment were the refusal of HIV testing, pregnancy, co-morbidity with diabetes mellitus or chronic bronchitis, receiving steroid therapy, receiving TB and/or HAART treatment (at recruitment or previously), and alcohol or drug abuse that could compromise the previous study.[13] The initiation of HAART treatment at baseline or during follow-up visits was determined by the physician at the health center, using the national guidelines for ART[14] based on immunological criteria (CD4 count<200 cells/μL) and clinical criteria. HAART was provided free of charge to eligible patients. All active TB cases confirmed at enrollment were treated according to the national guideline.[15] All active TB with and without HIV infection participants were followed for 6 months of ATT. The outcomes of all TB cases were assessed clinically based and/or laboratory-based at the end of TB treatment, and they were cured. A 0.1 mL tuberculin solution (RT23, State Serum Institute, Copenhagen) was injected intradermally into the dorsal surface of the forearm: TST positivity was classified as skin induration diameter ≥5 mm in HIV-infected individuals while ≥10 mm in HIV-negative individuals.

The HIV testing was determined using the Determine HIV-½ (Abbott Laboratories, Japan) as the screening test, the Capilus HIV-½ (Trinity Biotec, Ireland) as the confirmatory test and Unigold HIV-½ recombinant (Trinity Biotec, Ireland) as a tie breaker test.[15] The CD4 cell count was determined by flow cytometry using a FACS Calibur (Becton Dickinson, San Jose, USA).

Micronutrient Concentration Testing

Vitamin A concentration level of study participants was measured from stored plasma sample using high-performance liquid chromatography (HPLC) (Shimadzu Corporation, Japan) according to the standard operating procedure with the normal reference range 0.70–1.4 μmol/L.[16] Plasma was diluted with retinyl acetate solution in ethanol. The retinyl acetate acts as internal standard and the ethanol precipitate release the retinol then extract with hexane. Retinol is separated by HPLC using reversed-phase C18 column in methanol as mobile phase with ultraviolet detector at 325 nm. Its concentration was determined from the ratio of its peak area to that of retinyl acetate.

Serum iron and vitamin B_{12} concentration levels were measured from stored plasma sample using a solid-phase, competitive chemiluminescent enzyme immunoassay analyses on cobas® 6000 analyzer (Roche Diagnostics Corporation, USA) automated immunochemistry analyser (e601 module for B_{12} and c501 module for Iron), which was operated according to manufacturer's instructions.[17,18] Two levels of quality control materials for iron (PCCC1 and PCCC2, Roche) and vitamin B_{12} (PV1 and PV2, Roche) plasma concentration were run prior to sample testing and analysed each day to set instrument sensitivity.

Statistical Analysis

To compare the concentration level of micronutrients in the clinical-stage groups of TB, Kruskal–Wallis test was used to compare the concentration level of micronutrients among the study groups, while a two-tailed Wilcoxon rank-sum (Mann–Whitney) test was used to compare two unpaired

micronutrient concentration levels of study groups or a Wilcoxon signed-rank test for paired two group concentration level data after assessment of normality of the data using the Kolmogorov–Smirnov test. All data analysis was done using STATA version 12.0 (College Station, Texas, USA). *P*-value of 5% was taken as a cut-off to determine statistical significance.

Results

Characteristics Of The Study Population At Baseline

Malnutrition (BMI<18.5 kg/m^2) was detected in 49.1% of HIV-positive and 50.6% of HIV-negative TB patients. Severe malnutrition (BMI<16 kg/m^2) was more pronounced in TB patients with and without HIV coinfection (Supplementary Table 1).

Micronutrient Levels Of Active And Latent TB in HIV-Negative Individuals

The plasma iron and vitamin concentrations are summarized in Table 1. TB patients had low median concentrations of vitamin A (0.7 µmol/L) and iron (0.25 µg/mL) and high concentration of vitamin B_{12} (626.7 pg/mL) compared to TST+ (1.5 µmol/L, 0.86 µg/mL and 354.5 pg/mL, respectively) and to TST– (1.5 µmol/L, 0.90 µg/mL and 360.4 pg/mL, respectively) (Table 1), suggesting that this difference might be strongly associated with TB disease. For better evaluation, the study participants have been divided into two groups on the basis of age (greater and less than 30 years) and genders. The levels of serum iron, retinol and vitamin B_{12} in TB patients have significant difference compared to healthy controls with no difference by age category and gender (data not shown).

Non-parametric receiver operator characteristic (ROC) curves was done to determine the accuracy of diagnostic of TB using plasma concentration level of iron, retinol, and vitamin B_{12} compared to controls. Area under the curves (AUCs) of iron, retinol, and vitamin B_{12} were 0.04, 0.124 and 0.82, respectively (Figure 1). This indicates that there are 96%, 87% and 82% chances that low serum concentrations of iron, retinol, and high concentration of vitamin B_{12} respectively could be able to distinguish TB cases from TST+. The low AUC values of iron and retinol indicated that 4% and 12.4% had the chance that high serum concentrations of iron and retinol could be able to distinguish TB cases from TST+. Those micronutrients might be the most powerful diagnostic method for classifying potential to discriminate between TB cases and TST+. The concentration levels of iron, retinol and vitamin B_{12} could also best classify TB patients and TST– with AUCs of 0.022, 0.17 and 0.76, respectively (Figure 1). This also indicates that 98%, 83% and 76% of TB cases could be able to distinguish from TST– using low serum concentrations of iron and retinol, and high concentration of vitamin B_{12}, respectively. The concentration level of these micronutrients could not discriminate TST+ from TST–.

TB patients with HIV infection (HIV+TB+) had no statistically significant difference in concentrations of plasma iron (0.23 µg/mL), vitamin B_{12} (655 pg/mL), and vitamin A (0.6 µmol/L) compared to TB patients (Table 2). Moreover, except iron, HIV+TST– patients had no statistically significant difference in concentrations of plasma vitamin B_{12} (402 pg/mL) and vitamin A (1.4 µmol/L) compared to TST– controls. This suggested that HIV may not have any impact on the concentration level of vitamin B_{12} and vitamin A.

Impact of Anti-TB treatment (ATT) On Micronutrient Concentration Level In Active TB+ Patients

In this study, we assessed also the effect of ATT treatment on the level of micronutrient. Thus, the concentrations of iron, vitamin B_{12} and vitamin A in TB patients were measured at six months (M6) of ATT and compared to the baseline value (M0) of the same patients (TB cases) and with that of apparently healthy control (TST+and TST– groups). The

Table 1 Plasma Concentration Levels Of Micronutrients In The HIV-Negative Study Groups

Micronutrient Name	TB (n=87)	TST+(n=22)	TST– (n=25)	*P*-Value		
				TB vs TST+	TB vs TST–	TST+vs TST–
Iron (µg/mL)	0.25(0.21–0.37)	0.86(0.64–1.07)	0.90(0.76–1.03)	**<0.0001**	**<0.0001**	0.9699
Vitamin B_{12} (pg/mL)	626.7(479.4–829.4)	354.5(283.6–482.7)	360.4(256.7–565.5)	**<0.0001**	**<0.0001**	0.8559
Vitamin A (µmol/L)	0.7(0.5–1.3)	1.5(1.4–1.8)	1.5(1.1–1.6)	**<0.0001**	**<0.0001**	0.1413

Notes: Median (interquartile range) of micronutrient level values are shown at baseline and significant differences between study groups were determined using Kruskal–Wallis H and Wilcoxon Mann–Whitney test. *P*-values ≤ 0.05 for the comparison between the groups are indicated in bold.

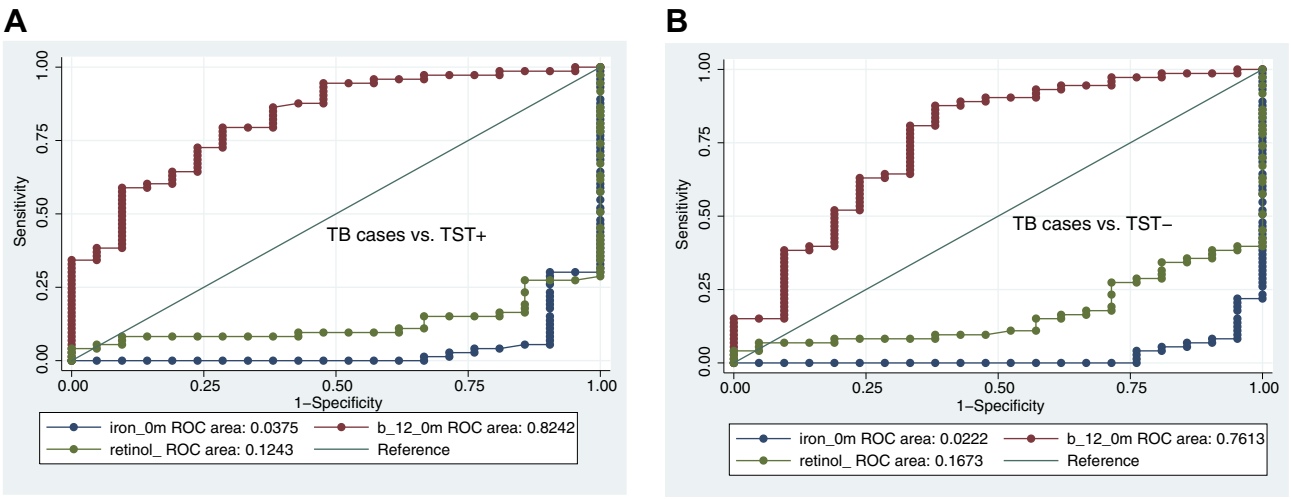

Figure 1 Diagnostic power of TB using plasma concentration level of single micronutrient. Receiver operator characteristics (ROC) curves showing the accuracies of diagnostic of TB using plasma concentration level of single micronutrients. (**A**) Area under the curve (AUCs) of iron, retinol, and vitamin B_{12} was 0.04, 0.124 and 0.82, respectively, to distinguish between TB cases and TST+. The low AUC value (4%) of iron and (12.4%) of retinol indicated that low concentration of these micronutrients could predict TB cases than TST+. This shows that serum concentrations of iron, retinol, and vitamin B_{12} could distinguish 96%, 87% and 82% of TB cases accurately from TST+. (**B**) Area under the curves (AUCs) of iron, retinol, and vitamin B_{12} were 0.022, 0.17 and 0.76, respectively, to distinguish between TB cases and TST–. This also indicates that 98%, 83% and 76% of TB cases could be able to distinguish from TST– using low serum concentrations of iron and retinol and high concentration of vitamin B_{12} respectively.

Table 2 The Effect Of HIV On The Serum Concentration Level Of Micronutrient To Discriminate Active TB From Healthy Control Study Participants (M0)

Micronutrient Name	HIV+TB+ (n=57)	TB (n=87)	HIV+TST– (n=71)	TST– (n=25)	*P*-Value	
					HIV+TB+vs TB	HIV+TST– vs TST–
Iron (µg/mL)	0.23(0.19–0.36)	0.25(0.21–0.37)	0.58(0.36–0.84)	0.90(0.76–1.03)	0.3212	**0.0005**
Vitamin B_{12} (pg/mL)	655(491–869)	627(479–829)	402(329–512)	360(257–566)	0.6350	0.3942
Vitamin A (µmol/L)	0.6(0.4–0.9)	0.7(0.5–1.3)	1.4(1.1–1.7)	1.5(1.1–1.6)	0.0983	0.8588

Notes: Median (interquartile range) micronutrient level values are shown at baseline and significant differences between active TB with and without HIV, HIV infection and controls were determined using the Wilcoxon Mann–Whitney test. *P*-values for the comparison between the groups are shown.

concentrations of plasma iron and vitamin A in TB were higher at M6 of ATT compared to the baseline concentration, while the concentration of vitamin B_{12} in TB patients was lower at M6 of ATT compared to baseline concentration (Table 3). The concentrations of iron, vitamin $B_{12,}$ and vitamin A in TB patients at M6 of ATT were not significantly

Table 3 Anti-TB Treatment (ATT) Response On Micronutrient Concentration Level In TB Patients After Treatment

Micronutrient Name	TB (M0)	TB (M6)	TST+(M0)	TST– (M0)	*P*-Values		
					TB (M6) vs TB (M0)	TB (M6) vs TST+(M0)	TB (M6) vs TST– (M0)
Iron(µg/mL)	0.25(0.21–0.37)	0.85(0.51–1.22)	0.86(0.64–1.07)	0.90(0.76–1.03)	**<0.0001**	0.5507	0.4807
Vitamin B_{12} (pg/mL)	627(479–829)	389(279–611)	354 (284–483)	360 (257–566)	**0.0001**	0.4173	0.4797
Vitamin A (µmol/L)	0.7(0.5–1.3)	1.5(1.1–1.9)	1.5(1.4–1.8)	1.5(1.1–1.6)	**<0.0001**	0.9401	0.3459
CD4 T-cell	440(286–632)	643(501–795)	714(582–956)	805(657–870)	**0.0011**	0.0514	**0.0306**

Notes: Median (interquartile range) micronutrient level values are shown. Significant differences between M0 and M6 of TB patients were determined using Wilcoxon signed-rank test. Significant differences between TB patients at M6 and TST+or TST– at M0 were determined using the Wilcoxon Mann–Whitney test. *P*-values for the comparison between the groups are shown.

different compared to the level of TST+and TST–. The CD4 T-cell was also significantly increased in the TB after treatment and normalized with the CD4- T cell of TST+, but still lower with compared with the CD4- T cell of TST– (Table 3).

Impact of HAART Therapy On Serum Concentration Of Micronutrients In HIV-Coinfected TB Patients

To determine if (in)eligibility for HAART might be a confounding parameter in the concentration level of micronutrients during the analysis of TB treatment responses, study groups (HIV+TB+and HIV+TST–) were classified at baseline as either eligible or ineligible for HAART based on the physician during the sample collection. Clearly, only iron concentration was higher in ineligible for HAART than eligible for HAART (Table 4). These data indicate that in (eligibility) for HAART stratification based on in(eligibility) for HAART at baseline may not have any impact on the concentration level of vitamin B_{12} and vitamin A.

Similarly, at the end of TB treatment, the concentration levels of iron, vitamin B_{12} and vitamin A in ATT plus HAART treated HIV+TB+ patients had no significant difference with the level of ATT only treated patients. Moreover, the concentrations of iron, vitamin B_{12} and vitamin A in HAART treated HIV+TST– patients had no significant difference with the level of HAART untreated HIV+TST– patients (Table 5). These data indicate that HAART treatment in HIV+TB+ patients merely affects the concentration level of micronutrients.

Importantly, the concentration levels of iron, vitamin B_{12} and vitamin A in HAART plus ATT treated HIV+TB+ patients at the end of TB treatment were comparable with the level of HAART treated HIV+TST– patients at six months followup and TST– at baseline. However, the concentration level of vitamin A in ATT only treated HIV+TB+ patients at the end of TB treatment was lower than HAART untreated HIV+TST– patients at six months followup and TST– at baseline (Tables 6 and 7). While at baseline the concentration levels of iron and vitamin B_{12} had significant difference between active TB cases versus TST+and TST– controls (Table 1), longitudinal follow-up analysis showed that the level of these micronutrients in TB treated patients with and without HIV infection at the end of 6 months ATT therapy became indistinguishable from those of TST+and TST– controls. HAART (in)eligibility and HAART treatment has not had any impact on this outcome (Tables 6 and 7).

Discussion

Assessing candidate biomarkers diagnosing TB disease and monitoring treatment responses using concentration

Table 4 Impact Of (In)eligibility For HAART At Baseline (Month 0) On the Concentration Level Of Micronutrients In TB-HIV Co-infected

Micronutrient Name	HIV+TB +HAART–	HIV+TB +HAART+	HIV+TB+HAART– vs HIV+TB+HAART+	HIV+TST– HAART–	HIV+TST– HAART+	HIV+TST–HAART- vs HIV+TST– HAART+
Iron(μg/mL)	0.32(0.25–0.46)	0.2(0.15–0.28)	**0.0128**	0.74(0.59–0.94)	0.55(0.36–0.70)	**0.0082**
Vitamin B_{12} (pg/mL)	686 (500–984)	679 (484–785)	0.8433	402 (349–453)	488 (332–614)	0.1347
Vitamin A (μmol/L)	0.59(0.42–1.08)	0.94(0.41–1.15)	0.4464	1.55(1.40–1.95)	1.33(1.08–1.66)	0.0715

Notes: Median (interquartile range) micronutrient values are shown. Significant differences between HAART eligible and HAART ineligible HIV+TB+ patients at baseline (Month 0) and between HAART eligible and HAART ineligible HIV+TST–- atients at baseline (Month 0) were determined using the Wilcoxon signed-rank test. *P*-values ≤ 0.05 for the comparison between the groups are indicated in bold.

Table 5 Effect of HAART During TB treatment Response On The Concentration Of Micronutrients (Month 6)

Micronutrient Name	HIV+TB +HAART–	HIV+TB +HAART+	HIV+TB+HAART- vs HIV+TB+HAART+	HIV+TST– HAART–	HIV+TST– HAART+	HIV+TST–HAART– vs HIV+TST–HAART+
Iron(μg/mL)	0.75(0.44–1.00)	0.82(0.54–1.48)	0.4875	0.81(0.71–0.88)	0.66(0.51–1.02)	0.3111
Vitamin B_{12} (pg/mL)	367(264–532)	381(294–532)	0.8170	400(378–411)	446(355–587)	0.4211
Vitamin A (μmol/L)	1.08(0.59–1.36)	1.38(1.06–2.02)	0.1649	1.78(1.64–1.85)	1.78(1.15–1.99)	0.7655

Notes: Median (interquartile range) micronutrient values are shown. Significant differences between HAART treated and HAART untreated HIV+TB+ patients and between HAART treated and HAART untreated HIV+TST– patients at completion of treatment (Month 6) were determined using the Wilcoxon signed-rank test. *P*-values ≤ 0.05 for the comparison between the groups are indicated in bold.

Table 6 Impact Of ATT Plus HAART On The Level Of Micronutrients In HIV+TB+Patients Compared To HAART Treated HIV+TST– and TST– Control Subjects

Micronutrient Name	HIV+TB +(M0)	HIV+TB +(M6)	HIV+TST– (M6)	TST– (M0)	HIV+TB +(M6) vs HIV +TB+(M0)	HIV+TB +(M6) vs HIV +TST– (M6)	HIV+TB +(M6) vs TST– (M0)
Iron(μg/mL)	0.2(0.15–0.28)	0.82(0.54–1.48)	0.66(0.51–1.02)	0.90(0.76–1.03)	**0.0117**	0.4649	0.8073
Vitamin B_{12} (pg/mL)	679 (484–785)	381(294–532)	446(355–587)	360 (257–566)	0.0687	0.4901	0.9281
Vitamin A (μmol/L)	0.94(0.41–1.15)	1.38(1.06–2.02)	1.78(1.15–1.99)	1.5(1.1–1.6)	0.0793	0.5027	0.8830

Notes: Median (interquartile range micronutrient values are shown. Significant differences between HAART eligible HIV+TB+patients at baseline (Month 0) and 6 months following HAART and ATT treatment initiation (Month 6) were determined using Wilcoxon signed-rank test. Significant differences between the different HAART treated study groups at the 6-month time point were determined using the Wilcoxon Mann–Whitney test.

Table 7 Micronutrient Level Of The ATT Treatment Response In HAART Untreated HIV+TB+ Patients Compared To HAART Ineligible HIV+TB+, HAART Untreated HIV+TST– and TST– Control Subjects

Micronutrient Name	HIV+TB +(M0)	HIV+TB +(M6)	HIV+TST– (M6)	TST– (M0)	HIV+TB +(M6) vs HIV +TB+(M0)	HIV+TB +(M6) vs HIV +TST– (M6)	HIV+TB +(M6) vs TST– (M0)
Iron(μg/mL)	0.32(0.25–0.46)	0.75(0.44–1.00)	0.81(0.71–0.88)	0.90(0.76–1.03)	**0.0180**	0.8120	0.2028
Vitamin B_{12} (pg/mL)	686 (500–984)	367(264–532)	400(378–411)	360 (257–566)	0.3980	0.2845	0.8637
Vitamin A (μmol/L)	0.59(0.42–1.08)	1.08(0.59–1.36)	1.78(1.64–1.85)	1.5(1.1–1.6)	0.0500	**0.0011**	**0.0424**

Notes: Median (interquartile) range micronutrient values are shown. Significant differences between HAART ineligible HIV+TB+ patients at baseline (Month 0) and 6 months following HAART untreated and ATT treatment initiation (Month 6) were determined using the Wilcoxon signed-rank test. Significant differences between the different HAART untreated study groups at the 6-month time point were determined using Wilcoxon Mann–Whitney test. *P*-values ≤ 0.05 for the comparison between the groups are indicated in bold.

level of micronutrients will be important for the future direction of TB disease control. Here, we used three micronutrients which could discriminate clinical stages of TB and monitoring treatment responses. The concentration levels of iron, vitamin B_{12} and vitamin A in TB patients with and without HIV infection had a significant difference compared to TST+and TST– control individuals, and this difference was strongly associated with TB disease, and these micronutrients indeed play critical roles in the response against TB.

In this study, TB patients had low plasma concentrations of vitamin A and iron and high levels of vitamin B_{12} compared to TST+and TST– individuals. The lower concentration of vitamin A in TB patients was in line with previous reports from Rwanda,[19] India.[20] Vitamin A is known to play a vital role for normal functioning and proliferation of T and B lymphocytes, for macrophage activity and for generation of antibody response.[21] The lower concentration level of plasma vitamin A in TB patients might be due to increased urinary excretion of vitamin A, appetite loss and anorexia, reduced absorption of fat, and increased utilization of vitamin A by tissues.[20,22]

Low iron concentration is known in patients with TB and HIV.[23,24] Low iron concentration in TB and TB-HIV-coinfected patients was reported in a previous study in the north-west of Ethiopia,[25] and in Iran,[26] which was similar to our finding. Iron has a significant role in the myeloperoxidase-dependent generation of hypochlorous acid which is a microbicidal factor.[27] This low iron concentration circulation in the plasma might be due to increasing degree of anemia, increased blood loss from hemoptysis, decreased proliferation of red blood cell, poor appetite and food intake, inflammation or deficiency of iron by the shift of iron from a transferrin-bound available state to a ferritin incorporated storage state.[24]

Unlike vitamin A and iron, a significantly high concentration level of plasma vitamin B_{12} was observed in adult TB patients in this study. A study in India showed that the serum concentration level of vitamin B_{12} in the TB patients was lower than controls among HIV-infected individuals, which is in contrast to our finding.[28] In contrast to

the above finding, a higher vitamin B_{12} plasma concentration is related to the dysfunction of the cell due to a reduced intracellular concentration as a result of cell damage.[29] This higher vitamin B_{12} plasma concentration might be a pseudo elevation which might be associated with liver dysfunction.[30]

We assessed also the levels of iron, vitamin $B_{12,}$ and vitamin A concentrations in response to ATT. We showed that the concentration levels of vitamin A, vitamin $B_{12,}$ and iron had significant difference between TB patients at baseline and at 6 months of ATT treated individuals. The concentration levels of those micronutrient parameters in TB patients at the end of ATT treatment were normalized to levels observed in latent TB and healthy controls (TST+and TST–). The finding that TB patients treated with ATT had significantly increased the vitamin A and iron concentrations compared to baseline, and this was consistent with the previous findings.[31,32] The increment of vitamin A and iron and decrement vitamin B_{12} concentrations after taking ATT treatment in TB patients might be due to the body reclaiming normal physiological function of the affected organs[33] and immune function improves by cleaning or a rapid drop in the bacterial load, which had been driving and circulating their production in the blood.[34] Our results also showed that ATT induced high CD4 cells restored in TB patients at the end of ATT compared to that at the baseline (P<0.0011) which support the above statement. Interestingly, HIV and HAART (in)eligibility of TB patients at baseline had no effect on the concentration levels of vitamin B_{12} and vitamin A. Moreover, HAART treatment had no effect on the concentration levels of iron, vitamin B_{12} and vitamin A in ATT treated HIV+TB+ patients. The concentration levels of iron and vitamin B_{12} in HIV+TB+ patients at the end of ATT treatment were normalized to the levels observed in healthy controls (TST–) regardless of HAART treatment and this could be associated with TB treatment response on the concentration of these micronutrients. However, the concentration level of vitamin A in HAART untreated HIV+TB+ patients at the end of ATT treatment was not normalized to the level observed in healthy controls (TST–). This indicates that treatment response on the concentration level of vitamin A might be dependent on HAART treatment to normalize to the level of healthy controls. Larger multicentre studies would be required to confirm whether the results are generally applicable to TB patients in a variety of settings. The change difference of micronutrients might be specific to the population studied and may not be specific to infection with *M. tuberculosis*.

Conclusion

Detecting serum concentration levels of vitamin B_{12} and vitamin A might be used as a biomarker of the diagnostic method of active TB with and without HIV infection. Detecting the concentration level of these micronutrients might also be used for TB treatment response monitoring biomarker in HIV-uninfected individuals. Moreover, detecting serum concentration of vitamin B_{12} might also be used for TB treatment response monitoring biomarker in TB-HIV-co-infected individuals regardless of HAART (in)eligibility and therapy.

Ethical Statement

This study was conducted in accordance with the Declaration of Helsinki. All study participants provided written, informed consent and assent for those under the age of 18 from a parent or legal guardian on their behalf at enrollment. The previous study obtained ethical clearance from the Scientific and Ethics Research Office of Ethiopian Public Health Institute (Ref. EPHI 6.13/268), and the Health Research Ethics Review Committee of the ministry of Ethiopian Science and Technology (Ref. RDHE/54-75/2002), and this study obtained ethical clearance from the Ethiopian Public Health Institute Sciences Ethics Review Committee (Ref.EPHI 6.13/268) to use the stored sample. The research article is original. This article is not published in any other journal previously or not under consideration for publication currently by another journal.

Availability Of Data and Material

All raw data generated or analysed during this study are included in this published article as supplementary data files.

Abbreviations

AFB, Acid Fast Bacilli; AIDS, Acquired Immune Deficiency Syndrome; ART, Anti-retroviral Treatment; ATT, Anti-tuberculosis Treatment; BMI, Body Mass Index; CD, Cluster Differentiation; HPLC, high performance liquid chromatography; HIV, Human Immune deficiency Virus; HIV+TB+, Active TB HIV positive patients; HIV+TST+, HIV positive with Latent TB infection; TB, Active TB and HIV negative patients; TST-, HIV negative and Latent TB negative; TST+, HIV negative and Latent TB positive; M0, Month 0; M6, Month 6; Mtb, *Mycobacterium tuberculosis*; SOP, Standard operation procedure; TB, Tuberculosis; TST, tuberculin skin test; TST-, negative TST tests; TST+, positive TST tests.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

Disclosure

The authors report no conflicts of interest in this work.

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