Research Article

Consumption of Sun-Exposed Oyster Mushrooms Help Patients Fight Tuberculosis

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ABSTRACT

Introduction: Tuberculosis (TB) is an airborne infectious disease that usually affects the lungs leading to severe coughing, fever, and chest pains.

Objective: This study aimed to assess the effects of consuming sun-exposed mushrooms on the treatment outcomes of TB

Methods: Participants were TB patients and categorized into block-1 (32) and block-2 (32) based on their willingness to consume sandwich bread containing sun-exposed oyster mushrooms. Blood and sputum samples were taken at the beginning (Day 0) and end of the study (4th month). Assays of 25-hydroxy (OH) D, cytokines, LL-37, and CRP were performed using Enzyme Linked-Immunosorbent Assay (ELISA) technique, and mycobacterial cultures were performed using Löwenstein Jensen media. A p-value less than 0.05 was considered significant.

Results: Consumption of the sandwich bread induced a 27.8% increase in the mean serum 25(OH)D level with 35.5% and 32.3% reduction in the proportion of vitamin D deficiency (VDD) and insufficiency (VDI), respectively. There were progressive changes in TB score (mean ± SD of 2.6 ± 1.8; 95% CI of 1.95 to 3.17; $p<0.001$) and Karnofsky performance status scale (80.3 ± 6.9%, $p < 0.001$) with significant improvements in IFN-γ and LL-37 levels ($p<0.05$).

Conclusion: Consumption of sun-exposed oyster mushrooms effectively improved the deficiencies of vitamin D in TB patients. The accelerated improvements on the clinical and immunological outcomes give us a clue that sun-exposed oyster mushrooms could serve as a potential, safe, easily available, and affordable adjunctive treatment and help patients fight TB.

Keywords: Sun-exposure; Mushrooms; Vitamin D; Tuberculosis; Treatment outcomes

Abbreviations: 25(OH)D = 25-hydroxy vitamin D; AFB = Acid Fast Bacilli; ELISA = Enzyme Linked Immunosorbent Assay; TB= Tuberculosis; UVB = Ultra Violet B; VDD = Vitamin D Deficiency.

INTRODUCTION

Tuberculosis (TB) is an airborne infectious disease caused by *Mycobacterium tuberculosis* that usually affects the lungs leading to severe coughing, fever, and chest pains [1-3]. Studies done until the recent time provided valuable insight into TB transmission, diagnosis, and treatment. However, much remains to be discovered to effectively reduce the incidence and eventually eradicate TB [4,5]. TB has a relationship with vitamin D. A meta-analysis found that low serum vitamin D status was associated ith an increased risk of TB [6]. In the pre-antibiotic era, vitamin D was used to treat TB. The use of Vitamin D for TB treatment started in 1849, with the observation that oil from fish liver improved appetite and strength [7]. The major circulating metabolite of vitamin D, 1,25-dihydroxyvitamin D ($1,25[OH]_2D$), supports innate antimicrobial immune responses, suggesting a potential mechanism by which adjunctive vitamin D might enhance response to anti-TB therapy [8].

Mushrooms are the only non-animal food source that contains vitamin D [9]. Ergosterol is the principal sterol and precursor for vitamin D_2 in mushrooms. It is converted via absorption of ultraviolet B (UVB) light energy (290–320 nm). The absorption of light causes the isomerization of the molecule and bond cleavage between carbons 9 and 10, resulting in unstable intermediate "pre-vitamin D_2". The conversion of pre-vitamin D_2 to vitamin D_2 then follows via a thermally catalyzed process [10, 11].

Commercially cultivated mushrooms contain low amounts of vitamin D_2 [12, 13]. Some commercial producers include UVB radiation steps to increase the content of vitamin D_2 in their products [14, 15]. Sun-exposure produced a sufficient amount of vitamin D_2 in mushrooms. Our previous study revealed that sun-exposed oyster mushroom is an excellent resource of vitamin D which is comparable to the content of vitamin D_3 in cod liver oil [16]. Having this, we hypothesized that sun-exposed mushroom has an impact on TB. Therefore, the present study aimed to assess the effects of consuming sun-exposed oyster mushrooms on the treatment outcomes of TB.

METHODS

Study site and design

The study was performed in the central part of Ethiopia from December 2014 to June 2015. Participants were TB patients and categorized into two blocks based on their willingness to consume sandwich bread containing sun-exposed oyster mushrooms.

Sun-exposure of oyster mushroom

Sun-exposure improves the content of vitamin D_2 in oyster mushrooms (*Pleurotus ostreatus*) [16]. Fresh oyster mushrooms were procured from Bio-Enguday Production and Sale Micro-enterprise (Debre Birhan, Ethiopia) with a moisture content of 92.5% as determined by the oven drying method. To facilitate the production of vitamin D_2, mushrooms were chopped down to the volume of $9cm^3$ (3 cm x 3 cm x 1cm) and exposed to the sunlight for 3 hours. Immediately after sun-exposure, mushrooms were put into a plastic bag and kept at - 20°C. The detailed process of Sun-exposure was described in our previous work [16].

Vitamin D_2 analysis

Samples were taken, packed into insulated plastic bags together with dry ice, and transported to the University of Hohenheim (Stuttgart, Germany) where further analysis of vitamin D_2 was done. The extraction of vitamin D_2 was performed based on the method indicated in Keflie et al. [16]. A system of High-Performance Liquid Chromatography (HPLC) (Shimadzu technologies) equipped with a DGU-20A3R degassing Unit, two LC-20AT pumps, a SIL-20ACHT autosampler, and a CBM-20A communication bus module (Shimadzu GmbH, Duisburg, Germany) as well as Reprosil 80 ODS-2 analytical column, 4.6 × 250 mm, 3 µm particle size (Dr. Maisch GmbH, Ammerbuch, Germany) was used to measure vitamin D_2 at Institute of Biological Chemistry and Nutrition. Our pre-test experiment on the effect of cooking temperature indicated that vitamin D_2 was almost stable during the process of cooking (Data not shown).

Sandwich bread preparation

Sandwich bread was prepared everyday morning using ingredients of sun-exposed oyster mushroom, wheat bread, olive oil, onion, and salt. We used 27g of sun-exposed oyster mushroom (containing 146 µg of VD_2) to prepare one sandwich bread.

Inclusion and exclusion criteria

Eligibility assessment was performed at health facilities. The major inclusion criteria were: newly diagnosed, smear-positive, and active pulmonary TB. Patients with extrapulmonary TB, multi-drug resistant TB, HIV infection, chronic diseases, or taking immunosuppressive drugs, or vitamin D supplementation were excluded.

Follow-up and data collection

All patients were attending a directly observed treatment short-course (DOTS) programme and received anti-TB drugs. Follow-up continued for 4 months. Patients who showed their willingness to consume the sandwich bread (Block-1) were provided with the sandwich for 5 days in a week for 16 weeks. Adherence to the DOTS programme and consumption of mushroom sandwich bread were supervised by the clinical nurses. Patients were checked every week for any kind of complaints and undergone physical examination.

Socio-demographic data were collected using a pre-tested and structured questionnaire which is translated to Amharic (local language). Medical records were also used to collect clinical characteristics. Vitamin D intake was assessed using a modified food frequency questionnaire (FFQ). The questionnaire included the availability of vitamin D-rich foods, monthly income, money spent for foods, duration of sun-exposure, working hours in the sun, clothing style, and use of sun protection. The types of all market and traditional foods were completely listed and, self-administered supplementary vitamin D intake was also considered in the assessment of dietary intake.

Samples collection

Blood and sputum samples were taken at the beginning (Day 0) and end of the study (4^{th} month). After overnight fasting, 10

mL of venous blood was withdrawn from the antecubital fossa vein into a non-heparinized vacutainer tube between 8:00 and 10:00 am at all health facilities. Blood samples were allowed to clot for about 1 h in the dark and subjected to centrifugation at 2504xg for 10 min at room temperature.

Samples with visible haemolysis were discarded. The sera were separated immediately into aliquots of sterile Eppendorf tubes using sterile Pasteur pipettes and stored at -20 °C. Later, the sera were transported in the icebox to Armauer Hansen Research Institute (AHRI) in Addis Abeba, Ethiopia, where they were stored at −80 °C until the time of analysis. We used the sera for the analyses of 25(OH)D, cytokines, cathelicidin (LL-37), and C-Reactive Protein (CRP). Similarly, early morning sputum specimens were collected in a sterile plastic cap for AFB (Acid Fast Bacilli) smear examination and mycobacterial culture. Sputum specimens were stored at -20 °C and later transported in the icebox to AHRI.

Laboratory analyses

25 hydroxy (OH) vitamin D: Serum 25(OH)D (vitamin D_2 plus D_3) levels were assayed in duplicate using an Enzyme-Linked Immunosorbent Assay (ELISA) kit purchased from Enzo Life Sciences, GmbH (Lörrach, Germany). The assay was done as per the manufacturer‟s instructions. The kit had 1.98 ng/mL detection limits with a 0.5 to 1010 ng/mL assay range. Measurements were categorized as severe vitamin D deficiency (sVDD) (≤ 10 ng/mL), deficiency (VDD) (≤ 20 ng/mL), insufficiency (VDI) (≤ 30 ng/mL) and sufficiency (adequate level) (VDS) (> 30 ng/mL) [17, 18].

Cytokines, Cathelicidin (LL-37), and C-Reactive Protein (CRP): Levels of IFN-γ, Interleukin-4 (IL-4), IL-10, LL-37, and CRP in the serum were measured using ELISA kits. The kits for IFN-γ and IL-4 were purchased from Sigma-Aldrich (Saint Louis, MO63103 USA) with detection limits of 15 pg/mL and 5 pg/mL, respectively. The kit for IL-10 was purchased from Enzo Life Sciences, GmbH (Lörrach, Germany). This kit had a detection limit of <7.81 pg/mL. Likewise, the kits of LL-37 and CRP were purchased from Hycult GmbH (Beutelsbach, Germany) and their detection limits were 0.1 ng/mL and <5 ng/mL, respectively. The Mean value of CRP ≥10 µg/mL was considered as a positive indicator of infection or inflammation. The assays were carried out as per the manufacturer‟s instructions.

Acid Fast Bacilli smear examination and Culture: Duplicated sputum specimens were collected from each patient. AFB smears were prepared using the Ziehl-Neelsen staining technique as previously described in Keflie and Ameni [19]. AFB smears were denoted as +1, +2, +3, and +4 whenever 1-9 AFB in 100 high-powered fields, 1-9 AFB in 10 high-powered fields, 1-9 AFB in 1 high powered field, or > 9 AFB in 1 high powered field were observed, respectively [20].

Sputum specimens were decontaminated with 4% sterile NaOH solutions and culture was performed using Löwenstein-Jensen solid media as previously indicated in Keflie and Ameni [19]. Bacterial cultures were monitored for 6 to 8 weeks until colonies were detected.

Anthropometry

Anthropometric measurements such as body weight, height, and mid-upper arm circumference (MUAC) were obtained using standardized procedures. All patients were weighed while wearing light clothes using an electronic platform weighing scale to the nearest 0.1 kg. Height was measured to the nearest 0.1 cm using a Seca stadiometer. Body mass index (BMI) was calculated as body weight (kg) divided by height (m) squared (kg/m^2). BMI values of 18.5, 17.0, and 16.0 kg/m^2 were used as the cut-off values below which patients were classified as having mild, moderate, or severe malnutrition, respectively [21]. MUAC was measured halfway between the olecranon and acromion processes of the left arm using a flexible non-stretch measuring tape to the nearest 0.1 cm while the arm is hanging relaxed, without compressing the tissues. MUAC less than 23 cm for males and 22 cm for females are used to define undernutrition as per FANTA III [22].

Outcomes

The primary outcomes were changes in vitamin D status, clinical improvements, and immunologic responses. Clinical outcomes were assessed using TB score and Karnofsky performance status scale. TB score measures change in the clinical status of TB patients and its components include self-reported symptoms (cough, dyspnoea, night sweat, chest pain, haemoptysis), clinical signs (tachycardia, pallor, fever, auscultatory findings), BMI (Low BMI: ≤ 18.5 kg/m^2, <16 kg/m^2) and MUAC (<23 cm for male or < 22 cm for female, < 20 cm). Each variable contributed 1 point and the total score varies from 0 to 13 points. The score was grouped as mild (Severity Classes (SC)-I: 0-5 points), moderate (SC-II: 6-7 points), or severe (SC-III: 8 points and more). A low TB score correlates with favourable outcomes, cure, and completed treatment [23]. Likewise, Karnofsky performance status scale correlates purely to physical ability and covers 11 points, each scored as a percentage from normal health to death (100 to 0%) [24]. Sputum smear conversion and culture negativity were evaluated as the secondary outcome.

Adverse effects

Patients were interviewed for the occurrence of adverse events related to hypercalcemia such as nausea, vomiting, excessive

thirst, anorexia, symptoms of kidney stones, and confusion. In addition, the occurrence of itching, arthralgia, jaundice, headache, malaise, dyspepsia, and others were asked.

Ethics

This study was undertaken in accordance with Helsinki declaration and approved by the Ethics Review Committee of AHRI-ALERT (All Africa Leprosy Rehabilitation and Training) as part of the project entitled „" Effect of micronutrients on the treatment outcomes of TB"" (Project Reg. No. P057/14). Written informed consent was obtained from the patients after explaining the aim and purpose of the study. All concerned health bureau of North Shewa Zone of Amhara Regional State gave supports and facilitated the processes of the present study.

Statistical analysis

Sample size estimation was done based on the previous findings of TB Score reduction after 2 months of anti-TB treatment with the mean ± SD of 3.2 ± 2.3 [23]. With the assumption of a 36% more reduction in the primary clinical TB score after vitamin D_2 intervention at a 5% level of significance, 80% power, and 10% dropout rate, the sample size was calculated to be 34 for each group with a total of 68 patients. The study was analyzed using IBM SPSS version 23 statistical program and data were summarized as mean± SD or median with IQR (Inter Quartile Range) for continuous variables and frequencies with percentages for categorical variables. Means were compared using a two-tailed paired t-test or Wilcoxon signed-rank sum test depending on the results of the Shapiro-Wilk test for normality of distribution.

The differences in the proportions were analyzed using Chi-square (X^2) or Fisher"s exact test (when more than 20% of the cells have expected count less than 5). Kruskal-Wallis test was applied to assess the changes in the serum 25(OH) D levels across different categorical variables. Pearson"s and Spearman correlation tests were used to identify the associations between parametric and non-parametric variables, respectively. Variables with a p-value less than 0.25 in bivariate analysis were entered into a multiple linear regression model to evaluate the relationship between the changes in the serum 25(OH)D levels and the clinical outcomes by adjusting independent factors. A p-value less than 0.05 was considered statistically significant.

RESULTS

A total of 64 patients (32 patients assigned in block-1 and 32 patients assigned in block-2) completed the study. Basic characteristics were compared between block-1 and 2. As it is indicated in Table 1, the two blocks had almost similar baseline characteristics. The median age with IQR was 28 (14) years in block-1 and 26 (8.5) years in block-2.

Sun-exposed oyster mushroom

The content of vitamin D_2 in fresh oyster mushroom was almost nil. However, a large amount of vitamin D_2 was produced after Sun-exposure and we obtained concentrations of 540.9 µg/ 100 g fresh weight.

Vitamin D

More than 65% of the study participants spent 30 to 50% of their monthly income on food. But, less than 26% of them tried to include vitamin D-rich foods like oily fish in their diet. The correlation between the serum 25(OH)D level and vitamin D-rich food intake, working in the sun for 1 hour per day, use of sun protection, and clothing style were not significant. Although more than 40% of TB patients were working in the sun for about 1 hour per day, their skin did not directly expose to the sunlight as most of them (>68%) cover their bodies with clothes and using sun protection like an umbrella (Table 1).

The mean ± SD of serum 25(OH)D level at baseline was 29.1 ± 12.3 ng/mL versus 30.6 ± 16.8 ng/mL in block-1 and 2, respectively. The proportions of VDD and VDI at baseline were nearly 42% and 58% in block-1 and about 41% and 59% in block-2 (Table 2). After 4 months, the mean serum 25(OH) D level was higher in block-1 than block-2. Consumption of the sandwich bread induced a 27.8% increase in the mean serum 25(OH)D level in block-1 (for the difference: mean ± SD of 8.1 ± 6.2 ng/mL; 95% CI of 5.9 to 10.3 ng/ mL, $p < 0.001$) (Figure 1). The proportions of VDD and VDI were significantly reduced in block-1 by 35.5% and 32.3%, respectively. Although the reduction was not statistically significant, the corresponding values were 17.3% and 6.9% in block-2 (Table 2). The changes in the serum 25(OH)D level were not significantly different across sex and age categories.

Clinical outcomes

At baseline, block-1 and 2 had the mean ± SD of TB score of 6.1 ± 3.2 and 6.9 ± 2.1 points, espectively. About 34% of TB patients in block-1 and 38% in block-2 were found in the TB score SC-III. After 4 months, progressive change was observed in TB score of block-1 (mean ± SD of 2.6 ± 1.8; 95% CI of 1.95 to 3.17; $p<0.001$) as compared to block-2 (mean ± SD of 6.7 ± 1.8; 95% CI of 6.13 to 7.37; $p= 0.21$). The number of TB patients with TB score SC-I was significantly improved by 56.3% in block-1, but this improvement was very small in block-2 (only 3.1%) (Table 3). Holding location, occupation, and family size constant, there was an inverse relationship between TB score and serum 25(OH)D levels in block-1 (β=-0.630, $p <0.001$). About 33% of the variability of TB score in block-1 was accounted for by the change in the serum 25(OH) D level. However, the contribution of the change in 25(OH)D level for such variability in block-2 was 22%.

Table 1: Basic Characteristics of TB patients assigned in block-1 and -2

Variables		Block-1	Block-2	p-value
Age (years) median (IQR)	Age	28 (14)	26 (8.5)	0.90
Sex n (%)	Male	18 (56.3)	16 (50.0)	0.62
Location n (%)	Urban	21 (65.6)	20 (62.5)	0.79
Educational Status n (%)	Illiterate	2 (6.3)	3 (9.4)	0.43
	Primary Education	11 (34.4)	15 (46.9)	
	Secondary Education	14 (43.7)	13 (40.6)	
	Tertiary Education	3 (9.4)	1 (3.1)	
	Religious Education	2 (6.2)	0 (0.0)	
Occupation n (%)	Student	7 (21.9)	6 (18.7)	0.79
	Farmer	6 (18.7)	6 (18.7)	
	Working for the government	6 (18.8)	10 (31.3)	
	Working for NGO*	9 (28.1)	6 (18.8)	
	House Wife	4 (12.5)	4 (12.5)	
Marital Status n (%)	Single	17 (53.1)	11(34.4)	0.13
	Married	15 (46.9)	21 (65.6)	
Family Size n (%)	1 to 4	12 (37.5)	5 (15.6)	0.12
	5 to 8	15 (46.9)	22 (68.8)	
	> 8	5 (15.6)	5 (15.6)	
Monthly Income n (%)	≤ 1000 ETB*	3 (9.4)	10 (31.3)	0.11
	1001 to 3000 ETB*	20 (62.5)	12 (37.5)	
	3001 to 5000 ETB*	6 (18.7)	6 (18.7)	
	> 5000 ETB*	3 (9.4)	4 (12.5)	
Money spent for food n (%)	30 to 40% of monthly income	16 (50.0)	7 (21.9)	0.072
	40.1 to 50% of monthly income	7 (21.9)	14 (43.7)	
	50.1 to 60% of monthly income	5 (15.6)	4 (12.5)	
	> 60% of monthly income	4 (12.5)	7 (21.9)	
Working in the Sun for 1hour/day n (%)	No	12 (37.5)	18 (56.3)	0.13
	Yes	20 (62.5)	14 (43.7)	
Clothing style (covering the body) n (%)	No	5 (15.6)	10 (31.3)	0.14
	Yes	27 (84.4)	22 (68.7)	
Use of sun protection n (%)	No	7 (21.9)	10 (31.3)	0.39
	Yes	25 (78.1)	22 (68.7)	
Vitamin D-rich food consumption (E.g. Oily Fish) n (%)	No	24 (75.0)	25 (78.1)	0.77
	Yes	8 (25.0)	7 (21.9)	
AFB Smear Examination n (%)	AFB Smear ≥ 3+	24 (75.0)	31 (96.9)	0.012
BMI mean (SD)	BMI	17.5 (2.4)	18.7 (3.8)	0.13
MUAC median (IQR)	MUAC	21 (5)	20 (3)	0.93
*NGO-Non-Governmental Organization; 1USD ≅ 38.37 ETB (Ethiopian Birr)				

Table 2: Serum 25(OH) vitamin D status of TB patients in block-1 and -2 before and after consuming the sandwich bread

Categories of 25(OH)Vitamin D Status	Block-1			Block-2		
	Beforen (%)	Aftern (%)	p - value	Beforen (%)	Aftern (%)	p - value
sVDD‡	-	-	**0.0012**	1 (3.4)	1 (3.4)	0.73
VDD§	13 (41.9)	2 (6.4)		12 (41.4)	7(24.1)	
VDI†	18 (58.1)	8 (25.8)		17 (58.6)	15 (51.7)	
Sufficient VD¡	13 (41.9)	23 (74.2)		12 (41.4)	14 (48.3)	
‡sVDD - Severe Vitamin D Deficiency (≤10ng/mL); §VDD - Vitamin D Deficiency (≤20ng/mL), †VDI - VitaminD Insufficiency (≤30ng/mL), and ¡Sufficient VD - Sufficient Vitamin D (>30ng/mL)						

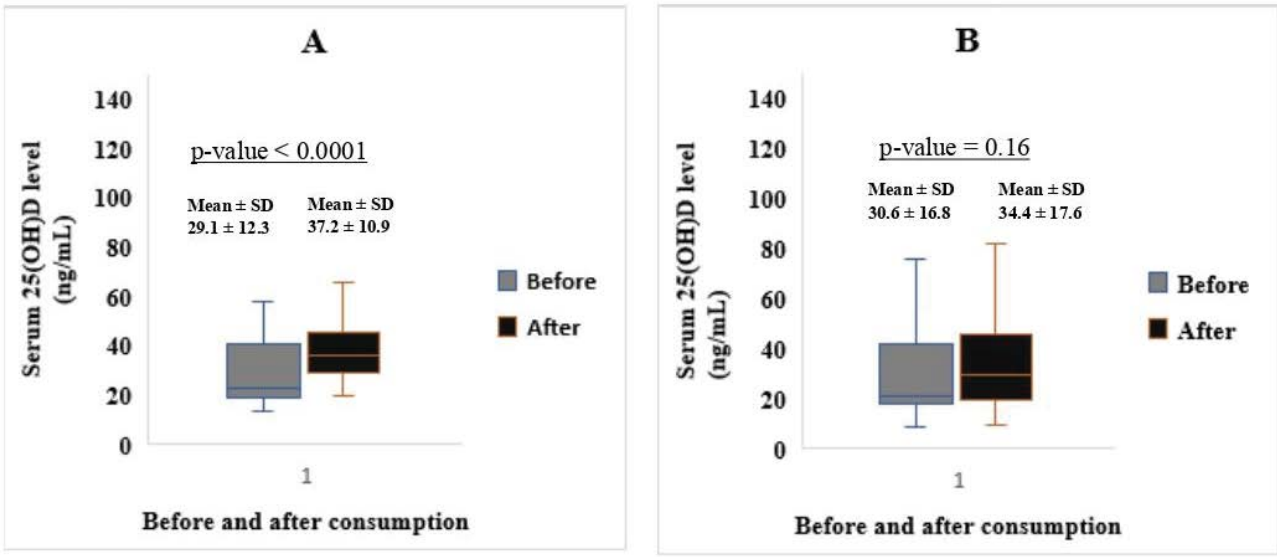

Figure 1: Changes in serum 23(OH)Dlevel of TB patients before and after consuming the sandwich bread. Significant change was observed in block-1 **(A)** but not in block-2 **(B).**

Table 3: Clinical and laboratory findings in block-1 and -2

Variables	Block-1			Block-2		
	Before n (%)	After n (%)	p-value	Before n (%)	After n (%)	p-value
TB Score Severity Class (SC)						
SC-I (0 to 5 Points)	13 (40.6)	31 (96.9)	<0.001	8 (25.0)	7 (21.9)	0.88
SC-II (6 to 7 Points)	8 (25.0)	1 (3.1)		12 (37.5)	14 (43.7)	
SC- III (8 Points)	11 (34.4)	0 (0.0)		12 (37.5)	11 (34.4)	
Karnofsky performance status scale						
50 Points	5 (15.6)	0 (0)	<0.001	1 (3.1)	0 (0.0)	0.52
60 Points	16 (50.0)	0 (0)		15 (46.9)	13 (40.6)	
70 Points	11(34.4)	7 (21.9)		16 (50.0)	18 (56.3)	
≥ 80 Points	0(0.0)	25 (78.1)		0 (0.0)	1 (3.1)	
Acid Fast Bacilli (AFB)†						
Smear +1, n (%)	0 (0)	10 (31.3)	<0.001	0 (0.0)	14 (43.7)	<0.001
Smear +2, n (%)	8 (25.0)	14 (43.7)		1 (3.1)	15 (46.9)	
Smear +3, n (%)	16 (50.0)	5 (15.6)		21 (65.6)	1 (3.1)	
Smear +4, n (%)	8 (25.0)	3 (9.4)		10 (31.3)	2 (6.3)	

† **AFB Smear +1**: 1 - 9 AFB Observed/ 100 high powered fields; **+2**: 1 - 9 AFB Observed/ 10 high powered fields; **+3**: 1 - 9 AFB Observed/ high powered fields); and **+4**: > 9 AFB Observed/ 1 high powered field.

TB score had statistically significant inverse correlation with Karnofsky performance status scale (r=-0.554, p=<0.001), body weight (r=-0.393, p=0.001), BMI (r=-0.498, p<0.001), and MUAC (r=-0.515, p<0.001). The mean ± SD of Karnofsky performance status scale before and after consumption were 61.9 ± 6.9% and 80.3 ± 6.9% in block-1, and 64.7 ±5.7% and 66.2 ± 5.5% in block-2, respectively. The change in the Karnofsky performance status scale was statistically significant in block-1 (p < 0.001), but not in block-2 (p=0.52) (Table 3). There was a strong correlation between BMI and MUAC (r=0.71, p<0.001). After 4 months, block-1 had significant improvements in BMI (by 0.91 kg/m^2) and MUAC (by 1.16 cm). However, these improvements were not statistically significant in block-2 (BMI by -0.01 kg/m^2 and MUAC by -0.1 cm).

Laboratory outcomes

AFB smear examinations and bacterial culture were performed to analyze the changes in the bacterial load. After 4 months, both blocks had significant changes in AFB load ($p<0.001$), in which more than 30% of TB patients had AFB smear examination level +1 (1 to 9 AFB observed/ 100 high powered fields). Despite the changes in the bacterial load, total AFB smear negativity could not be achieved and most of the patients were culture positive. In both blocks, the changes in the culture conversion were not statistically significant (Table 3).

Table 4 illustrates the changes in cytokines and cathelicidin (LL-37) levels before and after consumption. There were significant changes in the means of IFN-γ and LL-37 ($p<0.05$) levels in block-1, but not in block-2. IFN-γ had significant positive correlation with 25(OH)D ($r=0.426$, $p= 0.017$). The levels of IFN-γ ($\beta= 0.349$, $p=0.039$) and LL-37 ($\beta=0.366$, $p=0.0.028$) had significant relationship with 25(OH)D level holding location, occupation and family size constant. In both blocks, the difference in the level of CRP was not significant.

DISCUSSION

We demonstrated for the first time the role of consuming sun-exposed oyster mushroom on the treatment outcomes of TB. A high proportion (≥ 40%) of VDD was found in this study and it was directly related to factors such as lack of Sun-exposure and inadequate intake of vitamin D-rich diets. These two factors were identified together with others in our previous systematic review as the main predictor variables of vitamin D status among TB patients in Africa [18].

Consuming vitamin D_2 enriched oyster mushrooms brought a significant difference of 8.1 ng/mL in the mean 25(OH)D level and corrected the deficiency in more than 35% of TB patients without showing any adverse effects. Urbain et al. [25] demonstrated that intervention with vitamin D_2-enhanced button mushrooms via UVB irradiation was effective in improving vitamin D status. Comparable to our study, Yesudian et al. [26] found a 9.16 ng/mL increase in the mean 25(OH)D level from a baseline of 11.23 ng/mL in 3 consecutive days of UVB exposure to Asian immigrants in the UK. Tukvadze et al. [20] in Georgia also showed that adjunctive high-dose oral vitamin D_3 was safe and led to a substantial increase in plasma 25(OH)D concentrations over 16 weeks.

A previous study revealed that oral vitamin D supplementation was associated with significant suppression of the concentrations of circulating inflammatory markers, like CRP [27]. In this study, there was no significant change in the level of CRP. Similarly, Wu et al. [28] confirmed that there was no evidence of the improvement of CRP after the intervention. Our previous work underscored that CRP was not the best option to control the change in the acute phase response of micronutrient levels in the serum of TB patients [29].

Intriguingly, consumption of the sandwich bread ameliorated the clinical outcomes and immune responses of TB patients, but not sputum culture conversion. Clinical outcomes were assessed by TB score and Karnofsky performance status scale. Wejse et al. [23] found that TB score declined for 96% of the surviving patients from initiation to end of treatment. At the end of standard chemotherapy at 6 months, most patients had a TB score below 1 [30].

In this study, a progressive change was observed during 4 months of consumption in block-1. Most patients (96.9%) found in TB score SC-I, having more than 55% improvement from baseline. During the same duration of treatment, about 22% of patients in block-2 were found in TB score SC-I with less than 5% improvement. There was a significant inverse relationship between TB score and serum 25(OH)D level. The variability of TB score in more than one-third of TB patients was attributed to the change in the serum 25(OH)D level. In line with this, a study done in Iran indicated a reduction of TB score in patients who took a single oral dose of 450,000 IU cholecalciferol after 2 and 3 months of treatment [31]. Another study done in Egypt showed better healing after 1000 IUs of oral vitamin D supplementation [32]. More recently, Bekele and his colleagues [30] reported that an additional 25% reduction in the TB score in the intervention group was considered as a significant effect.

Table 4: Changes in the serum cytokines and cathelicidin LL-37 levels in block-1 and -2.

Cytokines (pg/mL)	Block-1 (Mean ± SD)		p-value	Block-2 (Mean ± SD)		p-value
	Before	After		Before	After	
IFN-g	14.9 ± 12.2	59.5 ± 39.8	**0.01***	13.8 ± 7.0	14.8 ± 8.7	0.69
IL-4	4.6 ± 3.4	6.5 ± 5.0	0.73	9.8 ± 10.4	9.0 ± 8.2	0.34
IL-10	15.2 ± 5.8	17.8 ± 5.9	0.44	20.3 ± 8.8	14.6 ± 5.6	0.26
Anti-microbial peptide (ng/mL)						
Cathelicidin LL-37	192.7 ± 65.2	245.3 ± 99.3	**0.02***	165.9 ± 92.7	174.8 ± 90.1	0.21
NB: * indicated statistically significant at $p<0.05$.						

TB score was inversely associated with the Karnofsky performance status scale. In this study, there was a significant change in the Karnofsky performance status scale in block-1 having a mean of 80.3% as compared to 66.2% in block-2. Similarly, there was a significant change in nutritional status as assessed by BMI and MUAC. BMI had a strong relationship with MUAC. We observed the improvements of BMI (by 0.91 kg/m^2) and MUAC (by 1.16 cm) solely in block-1. In agreement with this, Salahuddin et al. [33] showed that 2 doses of 600,000 IU vitamin D administered intramuscularly resulted in a greater weight gain by 1.14 kg and improvement in BMI. Two small randomized studies [32, 34] have also suggested the beneficial effects of vitamin D on weight gain. These implied that the improvement in the level of vitamin D has a contribution to the improvement of the nutritional status of TB patients.

Regarding bacterial load, we could not find a whole AFB smear negativity and sputum culture conversion. Comparable to this, several studies done elsewhere [30, 33, 35, 36] demonstrated that vitamin D supplementation did not affect the time to sputum smear and culture conversion. However, there were some contrasting reports. Studies done by Nursyam et al. [34] and Coussens et al. [37] indicated that vitamin D supplementation accelerated sputum smear conversion. Martineau et al. [38] also showed that administration of four doses of 2.5 mg vitamin D_3 had a faster effect on sputum smear conversion in patients with the tt genotype of the TaqI VDR polymorphism. Therefore, the reasons for such inconsistencies could be the presence of the variants of VDR polymorphisms, variability in vitamin D dosages, or different phases of baseline serum 25(OH)D level as indicated in Farazi et al. [31].

Vitamin D is implicated in the induction of IFN-γ mediated activity in macrophages [39]. In this study, a significant increase in IFN-γ level but not IL-4 and IL-10 levels were found in block-1. In agreement with our study, Salahuddin et al. [33] showed that vitamin D administration brought significant change in IFN-γ production in TB patients with VDD at baseline. In vitro study also indicated that supplementation of vitamin D deficient serum with 25(OH)D_3 restored IFN-γ [39]. However, some studies reported that vitamin D intervention inhibits the level of IFN-γ, but enhances the production of IL-4, and IL-10 [40-42]. These recapped that the difference in the effects of vitamin D intervention on the levels of cytokines was mainly dependent on VDD at baseline.

In the present study, there was a significant change in the level of cathelicidin LL-37 in block-1. This was supported by several studies done elsewhere [20, 40, 41, 43, 44]. We found a direct relationship between LL-37, IFN-γ, and 25(OH)D levels in block-1. This indicated that better status of vitamin D increases the level of IFN-γ mediated cathelicidin LL-37. According to different studies, vitamin D promotes mycobacterial killing [44] or reduced intracellular viability of *M. tuberculosis* [27] in macrophages through the production of cathelicidin LL-37, after activation of macrophages via toll-like receptor [45] or IFN-γ pathways [39].

LIMITATION

We did not analyze the nutrient contents of oyster mushrooms except vitamin D. Data on the genotypes of VDR polymorphisms were not included as we could not have access to measure the genes of VDR polymorphisms at the time of the study. VDR polymorphisms are, however, expected to influence the effects of vitamin D intervention.

CONCLUSION

In conclusion, consumption of sun-exposed oyster mushrooms effectively corrected VDD in TB patients without showing any adverse reaction. The accelerated improvements on the clinical and immunological outcomes, although not on sputum smear conversion, give us a clue that sun-exposed oyster mushrooms could serve as potential, safe, easily available, and affordable adjunctive treatment and helps patients fight TB. As this is the first study, further investigations on the interactions of vitaminD_2, sputum smear conversion, and immunological responses on the large size and diverse groups of TB patients are warranted.

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STATEMENT OF AUTHORSHIP

TSK and HKB conceived and designed the experiments; TSK performed the experiments, analyzed the samples, collected, analysed, and interpreted the data, and wrote the manuscript; and AS, AZW, CL, DN, and HKB critically reviewed and approved the manuscript.

CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

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