ORIGINAL ARTICLE

Nutritional Status and Lung Cavity in Pulmonary Tuberculosis Patient

Christi Giovani Anggasta Hanafi[1*], Fariz Nurwidya[2], Wiji Lestari[1], Heidy Agustin[2], Shaogi Syam[3]
[1]Department of Nutrition, Faculty of Medicine, Universitas Indonesia/Dr. Cipto Mangunkusumo General Hospital, Jakarta, Indonesia.
[2]Department of Pulmonology and Respiratory Medicine, Faculty of Medicine, Universitas Indonesia/Persahabatan National Respiratory Referral Hospital Jakarta, Indonesia.
[3]Respiratory Medicine, Division of Medicine, University College London, United Kingdom.

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ABSTRACT

Introduction: As one of the leading causes of death worldwide, pulmonary tuberculosis (PTB) is an infectious disease that continues to pose a serious threat to public health. The presence of cavities in radiological imaging of patients with PTB is associated with malnutrition, age, gender, and other comorbidities, including diabetes mellitus. This study aimed to find the association between nutritional status and lung cavity in PTB patients.
Methods: This was an analytical observational study with a cross-sectional design that involved 134 adult patients who were diagnosed with PTB at Persahabatan National Respiratory Referral Hospital, Jakarta. All patients were interviewed using a questionnaire for sociodemographic and anthropometric data, the nutritional status was assessed using the subjective global assessment (SGA), and the lung cavity was determined using a chest X-ray interpreted by radiologists. The Chi-square test was performed using the Statistical Package for the Social Sciences (SPSS) version 25 for Windows.
Results: Of 134 PTB patients, 61.9% were males, and 92.5% were from the 18-59 years old age group. Based on the SGA score, 77 (57.5%) were grouped as mild-moderate malnutrition/SGA B and 22 (16.4%) as severe malnutrition/SGA C. Lung cavity was found in 42 (31.3%) patients. The analysis showed that malnutrition was statistically significantly associated with lung cavity with OR=6.933 (95%CI 1.986-24.205; p=0.002) and the adjusted OR were 7.303 (95%CI 2.060-25.890; p=0.002) after controlling for age, sex, smoking, education, and comorbidities.
Conclusion: This study found that malnutrition was associated with lung cavities in PTB patients. These findings might indicate how malnutrition impaired the immune function in PTB patients.

INTRODUCTION

Tuberculosis (TB) is an infectious disease caused by *Mycobacterium tuberculosis* (MTB), which transmits through the air. Tuberculosis can impact many organs but primarily affects the lung, referred to as pulmonary TB (PTB). It is a major health concern and a leading cause of death in the world. Before the coronavirus disease (COVID-19) pandemic, TB was the biggest cause of death and resulted in nearly double the number of deaths as human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS).[1] In 2022, the Ministry of Health of the Republic of Indonesia reported around 459,789 cases of PTB and an estimated 107,000 deaths, equating to a rate of 40 deaths per 100,000 people.[2] This situation challenges achieving the World Health Organization (WHO)'s Sustainable Development Goal (SDG) of eradicating the TB epidemic by 2030.[1]

Radiological examination (chest X-ray) is a mandatory examination for PTB patients, and the lung cavity is found to be the frequent clinical sign, with more than 40% of patients with PTB presenting these at the time of diagnosis.[3] These lung cavities are crucial in defining the severity of TB and contribute to a less favorable prognosis. Patients with lung cavities tend to have a slower rate of sputum conversion, poorer clinical results, including a higher likelihood of treatment failure

*Corresponding author: christi.giovani41@ui.ac.id

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and relapse, and increased rates of infection spread compared to those without lung cavities. These conditions are due to a higher bacterial load and the persistence of lung cavities, even after completing anti-TB therapy, caused by incomplete recovery and the development of fibrotic scar tissue.[3,4] Several factors are related to the presence of lung cavities in PTB patients, including older age, sex, and comorbidities such as diabetes and malnutrition.[3,5–8] The correlation between TB and malnutrition is well-documented, as malnutrition can worsen TB, and TB can aggravate malnutrition.[9]

On the other hand, malnutrition refers to a state caused by a lack of intake or uptake of nutrition, leading to altered body composition (reduced fat-free mass) and body cell mass. This results in weakened physical and also mental capabilities and impaired clinical outcomes from disease.[10] Among PTB patients, malnutrition is estimated to affect more than 50% of individuals, and it is found to be associated with an increased risk of all-cause death.[11,12] It has been suggested that malnutrition reduces the expression of interferon-gamma (IFN-γ), tumor necrosis factor (TNF)-α, and other mycobactericidal agents and also selectively impairs cell-mediated responses that are important for stemming and limiting the pathogenesis of TB.[13] This study aimed to measure the association between nutritional status and lung cavity and assess the frequency distribution of nutritional status and the prevalence of lung cavity in PTB patients.

METHODS

Study Population

This cross-sectional design was conducted at Persahabatan National Respiratory Referral Hospital, Jakarta, between January and February 2023, among eligible adult PTB patients who came as out-patients as well as in-patients, had confirmed PTB diagnosis from a pulmonologist that involved clinical examination and sputum microscopy with the support of chest X-ray, aged >18 years old, and willing to participate in this study by signing the informed consent. The exclusion criteria were pregnancy or breastfeeding, having not had a chest X-ray in the last six weeks, and being unable to measure body weight and height according to anthropometric measurements. This study acquired 134 patients from the screening criteria and added them as participants to the study. Before enrolment, patients were thoroughly informed about the study, and each participant's written consent was obtained. Interviews were used to gather data using a pre-tested collection sheet. The patients' sociodemographic information was gathered and documented.

Ethical Approval

Ethical approval was granted by the Ethics Commission of the Department of Research and Development at Persahabatan National Respiratory Referral Hospital, Jakarta, under registration number 02/KEPK-RSUPP/01/2023. This study complied with the principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all participants.

Anthropometric Measurement

All anthropometric measurements were performed. Patients were asked to wear light clothing when measuring their height and body weight using standard instruments. A height measurement board (ShorrBoard, USA) was used to determine the height, and body weight was measured using SECA 876 calibrated by a laboratory unit of the Southeast Asian Ministers of Education Organization Regional Center for Food and Nutrition (SEAMEO RECFON). The measurement was taken twice to give a more reliable estimate of the true measurement than a single measurement, and the average value was computed.[14] Body mass index (BMI) was computed by dividing body weight and height square, then classified using the WHO Asia-Pacific category for BMI as underweight (<18.5 kg/m^2), normal (18.5-22.9 kg/m^2), overweight (23.0-24.9 kg/m^2), obese I (≥25 kg/m^2), and obese II (≥30 kg/m^2).

Nutritional Status and Intake

The SGA score was used to evaluate the nutritional status. Five elements of a patient’s medical history (weight changes, dietary intake, gastrointestinal symptoms, functional capacity, metabolic demands related to underlying disease) and two components of a nutritional-related physical examination (signs of fat and muscle wasting, nutrition-associated alterations in fluid balance) were measured. The patients were assigned a rating of SGA A (well nourished), SGA B (mild-moderate malnourished), or SGA C (severely malnourished). An enumerator interviewed all participants to collect food recall (1x24-hour) data for daily energy intake (in kcal and kcal/kgBW).

Chest Radiograph

Patients with chest X-ray data from diagnostic examinations in the last six weeks that the radiologists had interpreted were included in this study. Current guidelines recommend that patients who have started anti-TB medication should be re-evaluated after 2 to 3 months of therapy.[15] However, Lee, *et al.* (2020) found that chest X-ray re-evaluation of one month’s findings would be beneficial for judging whether re-evaluation would be necessary earlier than re-evaluation in two months.[16] This study used six weeks as the average interval, suggesting clearer and more accurate imaging.

Data Analysis

Statistical analyses using computer-based methods were performed using suitable techniques and systems. Qualitative data were expressed as frequency distribution and percentage, and quantitative data were tested for normality using the Kolmogorov-Smirnov test. If the data followed a normal distribution, they were represented by the mean and standard deviation. On the other hand, they were described using the median and the range of minimum to maximum values if they did not follow a normal distribution. Normally distributed data were then continued to be tested using a bivariate Chi-square test, and the rest of the data that were not distributed were tested by Fisher's exact test for calculating the odds ratio (OR) of malnutrition to lung cavity. All the statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS) version 25 for Windows.

RESULTS

Study Population Characteristics

Table 1. Sociodemographic characteristics of the study population[a]

Variable	Lung Cavity Tuberculosis n=42 (31.3%)	Non-Lung Cavity Tuberculosis n=92 (68.7%)	Total n=134 (100%)
Sex			
Male	26 (19.40)	57 (42.54)	83 (61.9)
Female	16 (11.94)	35 (26.12)	51 (38.1)
Age (years old)[b]	40.29 ± 13.77	40.50 (18-69)	
18-59	39 (29.10)	85 (63.5)	124 (92.5)
>59	3 (2.20)	7 (5.20)	10 (7.5)
Smoking			
Yes	17 (12.69)	43 (32.09)	60 (44.8)
No	25 (18.65)	49 (36.57)	74 (55.2)
Comorbidities			
Diabetes mellitus	10 (7.46)	19 (14.18)	29 (21.6)
Hypertension	5 (3.73)	16 (11.94)	21 (15.7)
Cancer	0	5 (3.73)	5 (3.7)
Chronic kidney disease	0	3 (2.20)	3 (2.2)
Liver disease	2 (1.49)	4 (2.99)	6 (4.5)
Education			
Elementary school/no education	4 (2.99)	11(8.21)	15 (11.2)
Middle school	8 (5.97)	10 (7.46)	18 (13.4)
High school/universities	30 (22.38)	71 (52.99)	101 (75.4)
Income			
Low income	30 (22.38)	64 (47.76)	94 (70.1)
Enough income	12 (8.96)	28 (20.90)	40 (29.9)
Body mass index	18.9 ± 4.45	19.5 ± 3.87	
Underweight	22 (16.42)	39 (29.10)	61 (45.5)
Normal	16 (11.94)	39 (29.10)	55 (41.0)
Overweight	0	7 (5.20)	7 (5.2)
Obese I	2 (1.49)	6 (4.48)	8 (6.0)
Obese II	2 (1.49)	1 (0.75)	3 (2.2)
Treatment failure			
Yes	11(8.21)	17 (12.69)	28 (20.9)
No	31 (23.13)	75 (55.97)	106 (79.1)
Treatment phase			
Intensive	23 (17.16)	39 (29.10)	62 (46.3)
Continuation	19 (14.18)	53 (39.55)	72 (53.7)
Mycobacterium test			
Positive	34 (25.38)	55 (41.04)	89 (66.4)
Negative	8 (5.97)	37 (27.61)	45 (33.6)
Tuberculosis classification			
Drug-resistant	22 (16.42)	12 (8.96)	34 (25.4)
Drug-sensitive	20 (14.93)	80 (59.69)	100 (74.6)
Daily energy intake (kcal)	1,525.32±450.45	1,495.55±487.16	
Energy (kcal/kgBW)	33.21±12.35	29.76 (11.87-103.76)	

Data were presented as mean±standard deviation if the distribution was normal and as median (minimum-maximum) if the data were not normally distributed; [a]Descriptive statistic; [b]Based on Daily Requirement Intake (AKG) Indonesia

Table 2. Characteristics of the study population based on SGA[a]

Nutritional Status Based on SGA	N	%
Well-nourished (SGA A)	35	26.1
Malnourished		
Mild-moderate (SGA B)	77	57.5
Severe (SGA C)	22	16.4
Total	134	100

SGA: subjective global assessment; [a]Descriptive statistic

Table 3. Characteristics of the study population based on lung cavity[a]

Lung Cavity	N	%
Yes	42	31.3
No	92	68.7
Total	134	100

[a]Descriptive statistics

Table 4. Distribution of nutritional status based on the presence of lung cavity[a]

Nutritional Status Based on SGA	Lung Cavity		Total n (%)
	Yes n (%)	No n (%)	
Malnourished (SGA B-C)	39 (29.1)	60 (44.8)	99 (73.9)
Well-nourished (SGA A)	3 (2.2)	32 (23.9)	35 (26.1)
Total, n (%)	42 (31.3)	92 (68.7)	134 (100)

SGA: subjective global assessment; [a]Descriptive statistics

Table 5. Odds ratios of association between nutritional status and lung cavity

	Unadjusted OR[a] (95% CI)	p	Adjusted OR[b] (95% CI)	p
Nutritional status based on SGA	6.933 (1.986-24.205)	0.002*	7.303 (2.060-25.890)	0.002*

OR: odds ratios; 95% CI: 95% confidence interval; [a]Chi-square test; [b]Logistic regression (adjusted by sex, age, smoking, education, comorbidities); *p-value<0.05

DISCUSSION

In this study, most cases were males, accounting for 61.9%, consistent with previous studies showing a higher proportion of male patients with PTB.[5,8] The WHO Global TB Report and national surveys indicate that TB disproportionately affects adult males, likely due to gaps in detection and reporting, with males contributing to over 50% of all TB cases.[1] The majority of subjects in this study were non-smokers (55.2%), which aligns with previous studies showing high rates of non-smoking among PTB patients.[17,18] Although the relationship between smoking and the incidence of PTB has been established, and a previous study showed that MTB can induce lung cavity by promoting the secretion of epithelial matrix metalloproteinase (MMP)-1 while suppressing tissue inhibitor of metalloproteinase (TIMP)-1, the relationship between the two remains unclear.[19] This study only collected data from current smokers at the time of the study, not estimating former smokers and passive smokers. Therefore, further research should be conducted to address this issue.

Almost half of the study population (45.5%) had comorbidities, which were dominated by diabetes mellitus (21.6%) and hypertension (15.7%). Studies in India and Korea found that diabetes mellitus is a common comorbidity in TB patients, with rates ranging from 18% to 26.58%.[20,21] The exact mechanisms behind this are not fully understood, but changes in immune function, including reduced cellular immunity and cytokine levels, have been proposed as possible explanations.[22] The study participants were predominantly high school or university graduates (75.4%). This study contrasts with previous studies, which generally show a higher prevalence of PTB among individuals with lower education levels.[23,24] People with lower levels of education may face limited access to healthcare, leading to delays in TB diagnosis and treatment, as well as limited access to healthy housing and insufficient nutrition.[24] Nonetheless, data from the Central Bureau of Statistics in Indonesia showed that most Indonesians, with a total of 40.12% of the population, graduated from high school or its equivalent, consisting of 42.06% males and 36.95% females in 2022.[25] Furthermore, the sociodemographic factors of the area where the study was conducted, Jakarta, the capital city, may influence these results.

However, the participants in this study had a lower income (70.1%), similar to results obtained from a study in Bandar Lampung, which showed that, based on the income indicator, the majority of TB patients had a very low per capita income, i.e., less than IDR 8,046,000 per year.[26] Several reasonable explanations exist for the connection between socioeconomic factors and TB. First, these factors may increase exposure to TB, such as in overcrowded and poorly ventilated workplaces and living conditions. Second, malnutrition contributes to greater vulnerability. Third, limited access to healthcare leads to delays in diagnosis and treatment.[27] According to the BMI classification, 45.5% of the study population was underweight. A previous study found that 27 (29.03%) PTB patients had a BMI <18.5 kg/m².[28] A higher proportion of malnutrition was found in Banjarmasin, where 33 out of 42 TB patients (78.6%) had a BMI <18.5 kg/m².[29] A strong link between active TB and low BMI has been observed in TB incidences across various countries and among all BMI levels in PTB patients.[9–11]

Most patients had no history of TB treatment failure (79.1%) and were drug sensitive-TB (74.6%), with some participants in the continuation phase (53.7%) and bacteriologically positive (66.4%). For the history of TB treatment failure, this study aligns with a study from Birhane, *et al*. (2023) that found 503 (85.4%)

participants to have no history of TB treatment failure.[30] These findings can be associated with the high number of subjects (74.6%) in this study who had drug-sensitive TB, which may also be related to lower case detection of drug-resistant TB (33.5%). This number was below the national target of 70%, and the treatment coverage was only 20.6%, which was against a target of 60%, according to Indonesia's 2021 TB management report.[31] No studies were found linking patients' treatment phase to the incidence of PTB. However, when associated with the presence of lung cavities, a study suggested a tendency for an extended treatment duration.[21] This study found more patients in the continuation phase. Additionally, 66.4% of subjects in this study had positive acid-fast bacilli (AFB)/molecular rapid test/culture results. This aligns with global data showing that 63% of the 6.2 million people diagnosed with PTB worldwide in 2022 were bacteriologically confirmed.[1] A previous study found similar results, with 57.5% of patients testing positive for AFB upon admission, and the bacteriologically confirmed prevalence of PTB was 852 cases per 100,000 people.[32]

From the food recall 1x24-hour data, the study population took approximately 1,553.53 kcal per day for males and 1,406.18 kcal per day for females. The findings align with the study by Ren, *et al.* (2019), which reported an average daily energy intake of 1,655 kcal for males and 1,360.3 kcal for females.[33] This study is lower than other studies, one found that TB patients had a daily intake of 2,153.5 kcal, and another found averages of 2,001.2±345.3 kcal/day for males and 1,860±478 kcal/day for females.[34,35] There is no guideline available currently, but guidelines by the Ministry of Health and Family Welfare, Government of India, suggested an average energy requirement of 37 kcal/kg for adults with low activity and 40 kcal/kg for TB patients due to the additional energy needed for the disease.[36] Meanwhile, other studies recommend 35-40 kcal/kg for TB patients.[37] It means the energy intake found in this study, 31.55±12.60 kcal per kg BW per day, was insufficient to meet the daily needs of TB patients. This factor should be associated with the high number of malnourished patients found.

Table 2 shows that most of the study subjects fell into the SGA B category (mild-to-moderate malnutrition) with 77 subjects (57.5%), followed by SGA A (well nourished) with 35 subjects (26.1%), and SGA C (severe malnutrition) with 22 subjects (16.4%). A study by Miyata, *et al.* (2011) in Japan found that, out of 39 TB patients, 12 (30.1%) were categorized as SGA A, 14 (35.9%) as SGA B, and 13 (33.3%) as SGA C.[38] In a study of 128 patients, Lin, *et al.* (2021) found that 65 (50.78%) patients were categorized as well-nourished (SGA A), while 63 (49.22%) patients were categorized as malnourished (SGA B and SGA C).[39] Another study by Nguyen, *et al.* (2023) on 221 patients found that 57.9% of patients were at risk of undernutrition (SGA A), 40.7% were at moderate risk of undernutrition (SGA B), and 17.2% were at risk of severe undernutrition (SGA C).[40]

The correlation between malnutrition and the incidence of PTB is well-known as a bidirectional relationship, in which malnutrition can reduce the expression of IFN-γ, TNF-α, and other mycobactericidal agents while also selectively impairing cell-mediated responses that are crucial for controlling the progression of TB.[9,13] As a new insight, this study proposed using the SGA as a malnutrition assessment tool. So far, no such study in Indonesia uses this method to assess malnutrition in PTB patients. The European Society for Clinical Nutrition and Metabolism (ESPEN) conceptually defines malnutrition as consisting of three domains: domain A (nutritional intake or absorption), domain B (body composition), and domain C (physical and cognitive function). The use of the SGA covers all three domains. Meanwhile, BMI, used in many previous studies, only fulfills one of the three domains, namely domain B.[10,21,26,28] For that reason, relying solely on BMI to evaluate malnutrition in patients with PTB is questionable because the most common form of malnutrition observed is disease-related malnutrition, which is characterized as a sub-acute condition where reductions in body weight and muscle or fat-free mass do not necessarily correlate with a lower BMI. While decreases in body weight and fat-free mass are directly linked to worse prognosis, including higher rates of morbidity and mortality, BMI may not fully capture these changes.[9,41] This study used the SGA instrument because it is efficient, inexpensive, non-invasive, easy to learn, can be performed bedside, and has also been proven to have good sensitivity and specificity for detecting malnutrition in hospital patients.[42,43]

This study also found lung cavities in 42 (31.3%) patients (Table 3). The result is lower than previous studies, with Kim, *et al.* (2021) reporting that, out of 410 patients, 166 (40.5%) had lung cavities on chest X-ray.[21] A higher proportion of lung cavities was reported by Balogun, *et al*. (2021), who found that 534 (55.7%) of 958 TB patients with positive cultures had lung cavities.[5] A higher proportion was also observed in a study by Hoyt, *et al*. (2019), where 132 (76%) of 273 TB cases with chest X-ray data had lung cavities.[8] The differences in the results of this study may be attributed to the inclusion criteria in previous studies, where subjects were patients with positive cultures or bacteriological examinations, which likely increased the likelihood of developing lung cavities.[44] The use of computed tomography, which detects lung cavities more effectively than chest X-rays, could also be a contributing factor.[4]

This study found an association between nutritional status and lung cavity (Table 5). Malnutrition was significantly associated with lung cavities found in chest X-rays compared to well-nourished patients, controlling for confounders. Previous studies have found several factors to be related to the presence of lung cavities in PTB patients, including older age, sex, and comorbidities such as diabetes and malnutrition.[3,5–8] Therefore, this study adjusted for age, sex, smoking, education, and comorbidities to understand the true association between malnutrition and lung cavities. This study is consistent with a previous study, which found severely malnourished patients were more likely to have lung cavities with an OR of 3.4 (95%CI: 1.2-9.8; p=0.06) and adjusted OR of 4.6 (95%CI: 1.5-14.1; p=0.03).[8] A study by Kim, *et al.* (2021) also showed that malnutrition was significantly associated with lung cavity TB (OR=0.90; 95%CI: 0.84-0.96; p=0.002/aOR=0.88; 95%CI: 0.81-0.97; p=0.002).[21] Abilbayeva, *et al*. (2022) also found malnutrition is associated with lung cavity in TB patients (OR=6.3271; 95%CI: 2.6746-14.9673, p<0.0001/aOR=5.719; 95%CI: 2.049–15.965; p=0.001).[45] However, this study could not find any studies in Indonesia related to this.

Severe malnutrition was significantly associated with multiple lung cavity lesions compared with those with well-nourished status after controlling for confounders. This finding likely reflects an immune dysfunction in the malnutrition state. Malnutrition is a major factor of acquired immune deficiency and has been referred to as acquired immune deficiency syndrome.[46] *Mycobacterium tuberculosis* containment necessitates a strong innate and adaptive immune response marked by a robust T-helper 1 (Th1) response and the formation of granulomas.[47] Human research has demonstrated that malnourished patients exhibit reduced levels of Th1 cytokines (such as IL-2 and IFN-γ) and proinflammatory cytokines (including TNF, IL-6, IL-1α, and IL-1β). Meanwhile, their Th2 cytokines (such as IL-4, IL-5, and IL-13) are elevated. Severe protein-energy malnutrition also leads to the atrophy of the thymus and peripheral lymphoid organs, resulting in leukopenia, a decreased CD4/CD8 ratio, an increase in CD4 and CD8 double-negative T cells, and a higher presence of immature T cells in the peripheral blood.[13,48] Other studies indicate lung cavity formation in patients with PTB is linked to a predominance of Th2 $CD4^+$ cells in the alveoli and increased activity of free neutrophil elastase in the lungs.[49] These combined effects on the immune response may alter the pathogenesis of PTB in malnourished patients and lead to more extensive lung cavities and lesions.[8,13] Many underlying conditions, such as chronic energy deficiency, lung inflammation, elevated oxidative stress, and changes in the body's composition, can contribute to the development of the lung cavity in PTB.[21]

Malnutrition is a modifiable factor affecting many aspects, including prognosis and treatment outcome. Therefore, promptly assessing and addressing malnutrition in PTB patients, with a better point of view and approach, is necessary to overcome and find the best-modified solution for each patient.[50] The latest WHO recommendation (2022) emphasizes the importance of nutritional care and support for TB patients, highlighting that nutrition screening, assessment, and management are integral components of TB treatment and care.[51] The guidelines stress the importance of individualized nutritional assessments and management for all active TB patients, including dietary counseling and nutritional interventions, to enhance the nutritional status and potentially prevent treatment failure.[51]

CONCLUSION

Most of the study population was in mild-moderate malnutrition, and one-third had lung cavities shown in their chest X-ray. This study found a statistically significant relationship between nutritional status based on SGA and lung cavity, which shows that malnourished PTB patients are at risk for the formation of lung cavities. Therefore, clinicians must be alerted concerning malnourished patients, as they are susceptible to having lung cavities, which may affect transmission to others and treatment outcomes. The findings of this study also highlight the need for and importance of nutritional management in optimizing daily energy intake in PTB patients. The use of the SGA assessment to detect malnutrition is more effective than BMI, suggesting that the use of SGA by trained healthcare personnel should be considered.

LIMITATIONS OF THE STUDY

Despite the association found in this study, it has several limitations. First, it was a cross-sectional study, meaning it could not show the cause and effect of two variables. Second, the chest X-ray for assessing the lung cavity was not performed at the same time as nutritional data were taken. Thus, it could not show patients' current lung condition. Third, the confidence intervals obtained for the OR and adjusted OR were wide, suggesting that more data or a larger sample size would be needed in future studies. Lastly, the sample was not homogeneous, as it included patients with comorbidities and varying statuses of drug-sensitive and drug-resistant TB, which could affect the outcomes. Further research on drug-resistant TB patients is needed.

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Conflict of Interest
The authors declared there is no conflict of interest.

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Authors' Contributions
Conceived and designed the study, collected, analyzed, interpreted the data, and wrote the manuscript: CGAH. Offered guidance, contributing to substantial intellectual content during the drafting process, and revising the manuscript: FN, WL, HA, SS. All authors contributed and approved the final version of the manuscript.

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