ORIGINAL PAPER

NUTRITIONAL STATUS AND ASSOCIATED FACTORS AMONG ADULT PATIENTS WITH TUBERCULOSIS

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ABSTRACT

Introduction. Malnutrition increases the risk of tuberculosis (TB), death, and relapse. The association between TB and malnutrition is bi-directional. TB leads patients to malnutrition, and malnutrition increases the risk of developing active TB by 6 to 10 times. This causes economic burden and affects the patient's quality of life.

The objective of the study was to assess the nutritional status of hospitalized patients with pulmonary TB in Thua Thien Hue province and to determine its associated factors.

Material and methods. A cross-sectional descriptive study was conducted on 250 adult pulmonary TB patients admitted at Thua Thien Hue Lung Hospital from May 2021 to May 2022. Baseline demographic information, anthropometry, and nutritional indicators were measured.

Results. The mean age of the patients was 55.4 ± 17.6 years (range 18 – 92 years), the male: female ratio was 3:1. The prevalence of undernutrition was 60.4%, of this 34.3% had mild, 16.8% moderate and 9.2% severe undernutrition based on body mass index. On multivariate logistic regression analysis, the predictors of undernutrition were wealth status (OR = 2.64; 95%

Résumé

État nutritionnel et facteurs associés chez les patients adultes atteints de tuberculose

Introduction. La malnutrition augmente le risque de tuberculose (TB), de décès et de rechute. L'association entre tuberculose et malnutrition est bidirectionnelle. La tuberculose conduit les patients à la malnutrition, et la malnutrition augmente de 6 à 10 fois le risque de développer une tuberculose active. Cela entraîne un fardeau économique et affecte la qualité de vie du patient.

L'objectif de l'étude était d'évaluer l'état nutritionnel des patients hospitalisés atteints de tuberculose pulmonaire dans la province de Thua Thien Hue et de déterminer les facteurs associés.

Matériel et méthodes. Une étude descriptive transversale a été menée sur 250 patients adultes atteints de tuberculose pulmonaire admis à l'hôpital pulmonaire de Thua Thien Hue de mai 2021 à mai 2022. Les informations démographiques de base, l'anthropométrie et les indicateurs nutritionnels ont été mesurés.

Résultats. L'âge moyen des patients était de 55,4 ± 17,6 ans (extrêmes 18 – 92 ans), le ratio hommes/

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CI: 1.19 – 5.85; p = 0.017), physical activity (OR = 7.14; 95% CI: 1.87 – 27.27; p = 0.004), and total energy intake of the 24-hour diet (OR = 7.35; 95% CI: 2.86 – 18, 87; p < 0.0001).

Conclusions. Undernutrition remains prevalent among adult patients with pulmonary TB. Routine nutritional screening and assessment upon admission and during treatment, as well as nutritional counselling and communication, are crucial to improve patients' healthcare.

Keywords: undernutrition, pulmonary tuberculosis, diet.

List of abbreviations:

TB – tuberculosis BMI – body mass index CI – confidence interval GLP-1 – glucagon-like peptide 1 HIV – human immunodeficiency virus OR – odd ratio SD – standard deviation

INTRODUCTION

Tuberculosis (TB) is a contagious disease caused by Mycobacterium tuberculosis. Globally, the incidence of TB in 2019 was approximately 10 million, with an estimation of 1.4 million deaths¹. Vietnam is ranked 11th among the 30 countries with the highest TB burden, with the annual rate of TB infection of 1.7%².

Nutritional status is one of the most important determinants of resistance to infection, including TB. The interaction between TB and malnutrition has been well-established in the literature³⁻⁶. Undernutrition causes approximately 1/4 of all new TB cases worldwide and TB is one of the most frequent underlying causes of wasting⁷. Individuals with TB frequently experience loss of appetite, poor nutrient absorption, and metabolic changes that can lead to malnutrition. Meanwhile, malnutrition itself increases the risk of TB infection and relapse, aggravates its severity and sequelae, and increases mortality^{4,8}. A study in Ethiopia found that patients who weights <35 kg had a 3.9 times higher rate of death than patients who weights \geq 35 kg⁹. Malnourished pulmonary TB patients had an increased risk of all-cause death during long-term follow-up¹⁰. In extensively drug-resistant TB patients, odds of survival decreased by 7.5% (p <0.01) for every kg decrease in weight at the start of treatment¹¹. In addition, coexisting malnutrition may worsen TB treatment outcomes, contribute to growing TB drug-resistance as well as prolong hospital stay, and eventually affect femmes était de 3/1. La prévalence de la dénutrition était de 60,4 %, dont 34,3 % souffraient de dénutrition légère, 16,8 % modérée et 9,2 % sévère, sur la base de l'indice de masse corporelle. Dans l'analyse de régression logistique multivariée, les prédicteurs de la dénutrition étaient le statut de richesse (OR = 2,64 ; IC à 95 % : 1,19 – 5,85 ; p = 0,017), l'activité physique (OR = 7,14 ; IC à 95 % : 1,87 – 27,27 ; p = 0,004). et l'apport énergétique total du régime de 24 heures (OR = 7,35 ; IC à 95 % : 2,86 – 18, 87 ; p < 0,0001).

Conclusions. La dénutrition reste répandue chez les patients adultes atteints de tuberculose pulmonaire. Le dépistage et l'évaluation nutritionnelle de routine à l'admission et pendant le traitement, ainsi que les conseils et la communication en matière de nutrition, sont essentiels pour améliorer les soins de santé des patients.

Mots-clés: dénutrition, tuberculose pulmonaire, alimentation

patients' life quality and impose an economic burden on family and society⁸.

Addressing undernutrition alongside with TB treatment has been shown to improve treatment outcomes, and reduce the risk of recurrence. Regular nutritional supplement counselling helps ensure adequate energy intake for significant improvement in body weight, total lean mass, and physical functioning¹². Therefore, a comprehensive evaluation of the nutritional status of TB patients upon admission, to provide proper nutritional support during hospitalization is crucial.

THE OBJECTIVE OF THE STUDY was to assess the nutritional status of hospitalized patients with pulmonary TB in Thua Thien Hue province and to determine its associated factors.

MATERIALS AND METHODS

Ethics

This study received institutional review board approval (approval number H2021/349) of the Hue University of Medicine and Pharmacy (Vietnam). The Declaration of Helsinki was followed when conducting the study. Written informed consent was provided by each participant.

Study design and population

A cross-sectional study was conducted on 250 patients with active pulmonary TB treated at Thua

Thien Hue Lung Hospital, Thua Thien Hue Province, Vietnam, from May 1, 2021 to May 31, 2022. Thua Thien Hue is a southernmost coastal province in the North Central Coast region, the Central of Vietnam, with a 5.033 km² surface. According to the central statistical agency of Vietnam in 2022, the province had a population of 1,160,224 inhabitants, including 578,223 men and 582,001 women. The urban population was 612,827 inhabitants and the rural population was 547,397 inhabitants. The provincial population density was 234.5 inhabitants/km².

The inclusion criteria were: (i) age \geq 18 years, (ii) active pulmonary TB requiring hospitalization, (iii) under standard anti-TB treatment, and (iv) voluntarily sign the informed consent form and cooperate with the relevant questionnaire. The exclusion criteria were patients with: (i) extrapulmonary TB, (ii) non-tuberculous mycobacteria infection, (iii) severe comorbidities such as malignant tumours, gastrointestinal, cardio-vascular, and respiratory diseases, (iv) severe mental illness and cognitive dysfunction, (v) during lactation or pregnancy, and (vi) missing baseline data. The diagnostic criteria of TB were in line with the National Tuberculosis Prevention and Control Guideline 2020.

Procedure

An interviewer-administered questionnaire was used to collect the socio-demographic, health, and nutritional characteristics of all TB patients upon admission. The demographic characteristics of participants (age, sex, marital status, educational status, occupational status and monthly income per capita), health status (history of TB treatment, comorbidities, HIV co-infection), and habits (alcohol consumption, cigarette smoking, and physical activity) were documented.

The monthly income per capita was classified according to the decree of the Vietnamese Ministry of Labour-Invalids and social affairs in 2021. All charts were reviewed to record comorbidities and to ascertain HIV co-infection. A participant was considered to have alcohol consumption if he consumes at least one glass of beer (340ml) or liquor (45ml) or wine (150ml) daily. An occasional alcohol consumption person was deemed non-consumer. Physical activity was defined as regular exercise with moderate intensity at least 30 minutes/day for 5 days/week. Regarding cigarette smoking, the patients were categorized as current, former and never smokers. The current smokers were those who have been smoking a regardless number of cigarettes within the last 12 months. Former smokers were those who quitted and did not smoke any cigarette within the last 12 months, otherwise considered never smokers.

The outcome variable was undernutrition. The nutritional status of TB patients was determined

based on their body mass index (BMI), which was calculated using height and body weight (kg/m²). In order to measure weight, the patients were requested to remove shoes, in minimal clothing, standing erect on the center of the balance, face the recorders, and put their hands at their side while looking straight ahead. Weight was recorded to the nearest 0.1 kg. Height was measured in the similar fashion with the patient being barefoot, wearing no head gear, knees were fully straight and both hands were held down to the side and record the height to the nearest 0.1 cm.

Patients with a BMI <18.5 kg/m² are considered undernourished. The patients with a BMI <16.0 kg/ m², between 16.0 and 16.99 kg/m², and between 17.0 and 18.49 kg/m² were defined as having severe, moderate and mild undernutrition, respectively. A BMI between 18.5-23 was considered normal, and a BMI \geq 23 was classified as overweight or obesity. The 24-hour recall method was used to record participants' diet in the previous 24 hours preceding the date of survey. The diet was then analysed using a designated software (Eiyokun, National Institute of Nutrition, Vietnam) based on the Vietnamese food composition table to calculate the total energy and macronutrients intake. An individual was finally dichotomized as either meet or do not meet the recommended nutritional need for Vietnamese people.

Data analysis

Continuous data are presented as mean ± standard deviation (SD) if the variables were normally distributed or as median and range if the variables were not normally distributed. Categorical data are given as count and percentage. Pearson's Chi-squared test was used to compare percentages of categorical variables. A multivariate logistic regression was run to predict associated factors of malnutrition. All analyses were performed using SPSS software version 20.0 (IBM®, USA). P values <0.05 indicated statistical significance for all comparisons.

RESULTS

Socio-demographic characteristics

Among 250 patients with pulmonary TB, the mean age was 55.4 ± 17.6 years (range 18 - 92 years), the male: female ratio was 3:1. Most participants were married and living with spouse (82.8%) and had a personal monthly income >58 USD (76%). Nearly half of the study population completed high school (47.6%) and were unemployed (49.6%) (Table 1).

Health status and lifestyle habits

In this sample, 26% of TB patients had a history of TB diagnosis and treatment. Approximately

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Characteristics	<u>Male (n = 189)</u> N (%)	$\frac{\text{Female } (n = 61)}{N (\%)}$	Total
	N (%)	N (%)	(n - 250)
			(n - 250)
Marital status			
• Married	159 (63.6)	48 (19.2)	207 (82.8)
• Unmarried	16 (6.4)	7 (2.8)	23 (9.2)
 Divorced/Separated 	12 (4.8)	0 (0.0)	12 (4.8)
• Widowed	2 (0.8)	6 (2.4)	8 (3.2)
Educational status			
• Illiterate	17 (6.8)	8 (3.2)	25 (10.0)
 Primary/Secondary school 	110 (44.0)	25 (10.0)	135 (54.0)
 High school 	33 (13.2)	17 (6.8)	50 (20.0)
Tertiary education	29 (11.6)	11 (4.4)	40 (16.0)
Occupational status			
• Employed	14 (5.6)	3 (1.2)	17 (6.8)
 Merchant/ self-employed 	3 (1.2)	6 (2.4)	9 (3.6)
 Daily laborers/ house made 	81 (32.4)	19 (7.6)	100 (40.0)
• Unemployed	91 (36.4)	33 (13.2)	124 (49.6)
Monthly income per capita			
• <40 USD	31 (12.4)	7 (2.8)	38 (15.2)
• ≥40 - 58 USD	13 (5.2)	9 (3.6)	22 (8.8)
• >58 USD	145 (58.0)	45 (18.0)	190 (76.0)
History of TB treatment	53 (21.2)	12 (4.8)	65 (26.0)
Cigarette smoking			
Current smoker	35 (14.0)	2 (0.8)	37 (14.8)
 Former smoker 	8 (3.2)	0 (0.0)	8 (3.2)
Never smoker	146 (58.4)	59 (23.6)	205 (82.0)
Alcohol consumption	51 (20.4)	1 (0.4)	52 (20.8)
Physical activity	18 (7.2)	9 (3.6)	27 (10.8)
Chronic comorbidity			100 (40.0)
• 1 comorbidity	83 (33.2)	17 (6.8)	83 (33.2)
• ≥2 comorbidities		· · ·	17 (7.8)
HIV co-infection	2 (0.8)	0 (0.0)	2 (0.8)
Nutritional status (BMI)	< /	- \/	
Severe undernutrition	36 (14 4)	15 (6.0)	51 (2.0.4)
 Moderate undernutrition 	25 (10.0)	9 (3 6)	34 (13.6)
Mild undernutrition	52 (20.8)	14 (5.6)	66 (2.6.4)
Normal	69 (27.6)	22 (8.8)	91 (36.4)
Overweight	7 (2.8)	1 (0.4)	8 (3.2)

Table 2. Summary of 24-hour energy intake				
Energy intake	Male $(n = 189)$	Female $(n = 61)$	Total ($n = 250$)	
	N (%)	N (%)	N (%)	
Meet Energy need x ± SD (Kcal/day)	46 (18.4) 1763.5 ± 538.2	17 (7.8) 1397.6 ± 372.6	63 (25.2)	
Meet Protein need x ± SD (g/day)	34 (13.6) 55.3 ± 10.1	18 (7.2) 40.8 ± 7.9	52 (20.8)	
Meet Lipid need x ± SD (g/day)	86 (34.4) 64.5 ± 26.6	24 (9.6) 52.4 ± 21.2	110 (44.0)	
Meet Carbohydrate need $\bar{x} \pm SD (g/day)$	22 (8.8) 226.4 ± 114.7	16 (6.4) 182.6 ± 76.1	38 (15.2)	

one-fifth of the participants were smokers (18%) and alcohol consumers (20.8%), 10.8% reported regular physical activity. Chart review found that 40% of TB patients had co-existing chronic illnesses such as diabetes mellitus, hypertension, chronic obstructive pulmonary disease; most of them had more than one comorbidity. Two male patients had HIV co-infection.

Table 3. Associated factors of nutritional status				
Characteristics	BMI < 18.5 (n = 151)	$BMI \ge 18.5$ $(n = 99)$	Total	Þ
	N (%)	N (%)	- (n = 250)	
Gender				
• Male	113 (45.2)	76 (30.4)	189 (75.6)	0.73
• Female	38 (15.2)	23 (9.2)	61 (24.4)	
Age group				
• <60 years	71 (28.4)	38 (15.2)	109 (43.6)	0.18
• ≥60 years	80 (32.0)	61 (24.4)	141 (56.4)	
Marital status				
 Married 	123 (49.2)	84 (33.6)	207 (82.8)	0.49
• Others	28 (11.2)	15 (6.0)	43 (17.2)	
Educational status				
 Under high school 	102 (40.8)	58 (23.2)	160 (64.0)	0.15
From high school	49 (19.6)	41 (16.4)	90 (36.0)	
Occupational status				
 Employed 	81 (32.4)	43 (17.2)	124 (49.6)	0.11
• Unemployed	70 (28.0)	56 (22.4)	126 (50.4)	
Monthly income "per capita				
• ≤58 USD	43 (17.2)	17 (6.8)	60 (24.0)	
• >58 USD	108 (43.2)	82 (32.8)	190 (76.0)	0.04
History of TB				
• Yes	40 (16.0)	25 (10.0)	65 (26.0)	0.83
• No	111 (44.4)	74 (29.6)	185 (74.0)	
Cigarette smoking				
• Yes	123 (49.2)	82 (32.8)	205 (82.0)	0.78
• No	28 (11.2)	17 (6.8)	45 (18.0)	
Alcohol consumption				
• Yes	30 (12.0)	22 (8.8)	52 (20.8)	0.65
• No	121 (48.4)	77 (30.8)	198 (79.2)	
Physical activity				
• Yes	10 (4.0)	17 (6.8)	27 (10.8)	<0.01
• No	141 (56.4)	82 (32.8)	223 (89.2)	
Chronic comorbidity				
• Yes	60 (24.0)	40 (16.0)	100 (40.0)	0.92
• No	91 (36.4)	59 (23.6)	150 (60.0)	

Table 4. Predictors of undernutrition among TB patients.

Characteristics	Adjusted OR	95% CI	Þ		
Income per capita	2.64	1.19 - 5.85	0.017		
Physical activity	7.14	1.87 - 27.27	0.004		
Meet recommended energy intake	7.35	2.86 - 18.87	<0.001		
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OR: Odd ratio, CI: confident interval

Nutritional status

The prevalence of undernutrition was 60.4%, of this 34.3% had mild, 16.8% moderate and 9.2% severe undernutrition based on BMI classification. Table 2 summarized the 24-hour energy intake of the participants. The average energy intake of TB patients was 1763.5±538.2 kcal/24 hours for males and 1397.6±372.6 kcal/24 hours for females. One-fourth of the sample (25.2%) met the recommended daily allowance, of which 20.8%, 44.0%, and 15.2% met the recommended protein, lipid, and carbohydrate intake, respectively.

The monthly income per capita and physical activity were associated with nutritional status (Table 3). A multivariate logistic regression was run to ascertain the effects of socio-demographic characteristics, health status and nutritional status on the likelihood that participants had undernutrition (Table 4). TB patients with low monthly income per capita were 2.64 times more likely to have undernutrition than others. The odds of undernutrition were >7 times higher among individuals who did not have regular physical activity and who did not meet the commended energy intake than their counterparts.

DISCUSSION

Vietnam has a high burden of TB and has a population attributable fraction that exceeds 40%¹³. In the present study, we found that 60.4% of patients with pulmonary TB had undernutrition, in which one-fifth of the sample (20.4%) were severely malnourished. The prevalence of underweight among TB patients varies across studies and continents, mostly reported over 40%¹⁴⁻¹⁶. A meta-analysis involving 48,598 participants reports a prevalence of malnutrition of 48.0% (95% CI, 40.9-55.2%), in which the prevalences of mild, moderate, and severe malnutrition are 21.4%, 14.0%, and 29.4%, respectively¹⁶. Another comparative cross-section study reveals the prevalence of underweight among TB patients of 57.17% (95% CI: 54.80 - 59.54%) compared to 23.37% (95% CI: 21.93-24.80) of TB-free residents⁸. In India, the median BMI for TB patients reported in a large cohort study is 16 kg/m² for men and 15 kg/m^2 for women, substantially lower than that of non-TB ones $(21.6 \text{ kg/m}^2)^{13}$.

Nearly three-fourth (74.8%) of TB patients in our sample were deficient in calories. This finding mirrors the data from high TB burden countries such as India and China^{13,17}. In the United States of America, adults who have low BMI, reduced subcutaneous fat or reduced muscular mass, are at increased risk of developing TB compared to those with a normal nutritional status¹⁸. Moderate-to-severe malnutrition is a risk factor for mortality (8%) during the first 4 weeks of treatment for TB¹⁹.

The complex interaction between undernutrition and TB has been observed across countries with different income levels. A poor nutrition induces proteinenergy malnutrition, micronutrients deficiencies and eventually immunodeficiency. This secondary immunodeficiency increases the host's susceptibility to infection and thus increases the risk for developing TB. TB in turn causes reduction in appetite, macro- and micronutrient malabsorption, and altered metabolism, leading to wasting and a poor nutritional status^{12,15}.

Undernourished individuals are also more likely to a greater severity of TB. Severely undernourished patients are 4.6 times (95% CI, 1.5–14.1) more likely to have lung cavitation and 11% more lung affected (95% CI: 4.0–13.3) on chest X-ray compared to those with a normal BMI²⁰. Another research observed that underweight participants are significantly more likely to be both smear and culture positive compared to those with a normal BMI²¹.

Cell-mediated immunity is the primary host defence against TB and the host protective immune mechanism of infection with *Mycobacterium tuberculosis* depends critically on the interaction and cooperation between monocyte-macrophages and T-lymphocytes and their cytokines. Previous studies also demonstrated significant alterations of appetite-regulatory hormones in TB patients, including elevations in peptide YY2, ghrelin, resistin, and reductions in plasma leptin²². Generalized malnutrition can cause significant impairment of several important mechanisms of immune protection, including phagocytic function, cell-mediated immunity, antibody concentration, and cytokine production¹². Therefore, TB patients usually experience decreased appetite, nutrient intake and micronutrient malabsorption.

In active TB, catabolic processes leading to wasting usually occur before symptom onset. During this time, the basal metabolic rate or resting energy expenditure is increased, resulting in increased energy needs to meet the basic demands for body function. Simultaneously, energy intakes are likely to decline as a result of illness-associated anorexia. Amino acids and protein synthesis utilization may be inhibited due to the presence of pro-inflammatory cytokines. Several nutritional parameters are worse among newly diagnosed TB patients compared to healthy controls. The combination of these conditions induces weight loss with ultimate wasting²³. Consequently, undernourished TB patients have delayed recovery and increased death rates in comparison to patients with TB with normal nutrition¹⁵. As hormones normalize during effective antituberculosis treatment, appetite is restored and nutritional status consequently improves¹⁵.

Nutritional status may have an effect on relapse of active TB. Reactivation of previously sub-clinical TB infection is often related to worsening nutritional status. The relapse risk was increased amongst those who were $\leq 90\%$ of ideal body weight or had a BMI \leq 18.5 Kg/m² at the time of diagnosis. In patients who were underweight at diagnosis, a weight gain of less than 5% between diagnosis and completion of the initiation phase of therapy was significantly associated with relapse¹².

The likelihood of undernutrition was increased by 2.64 times among TB patients with low monthly income per capita. This observation is congruent with that of various studies¹⁴. Patients with poor socioeconomic status usually have a cereal-based monotonous diet instead of nutrient-dense and protein-rich food, which diminishes their body ability to counteract excessive TB-associated fat and fat-free loss overtime¹⁴. Moreover, these individuals tend to reside in high density places with low hygienic condition which favours the transmission of *Mycobacteria*. Poverty also limits access to health care, leading to delayed diagnosis, prolonged exposure within the community and inadequate treatment of TB.

We observed that the odds of undernutrition were more than 7 times higher among individuals who neither have regular physical activity nor meet the recommended daily allowance. The interaction between energy intake, physical activity, malnutrition and TB is a vicious circle. TB patients often experience loss of appetite, which reduces micro-and macronutrients intake. Nausea and diarrhoea also contribute to the loss of nutrients²². Conversely, physically active individuals appear to have an improved appetite sensitivity, which may facilitate long-term energy balance. Several research found that in the active group, the concentrations of fasting acylated ghrelin and GLP-1 are elevated, whereas plasma levels of insulin are lower compared with inactive group²⁴. The detailed underlying mechanism of this interaction is discussed elsewhere.

Nevertheless, the awareness of risk factors of malnutrition remains controversial in patients with pulmonary TB. TB types, bacterial positivity, wealth status, residence in rural areas, alcohol consumption, working ability, occupation, food frequency, calorie intake, nutritional status at the time of registration and bedridden are among the common reported risk factors^{14-16,25}.

Current TB treatment guidelines provide insufficient emphasis on the role of nutritional supplementation, probably due to evidence scarcity⁸. Moreover, the prevalence, risk factors and potential impact of nutritional support on improved treatment outcomes and prognosis are not homogeneous due to the differences in burden settings, study population, design and endpoints, treatment protocols and durations. Therefore, interventional programs should be nation- or region-specific. The findings from this study aim to provide additional evidence to the national database.

This study has several limitations. The cross-sectional and single institutional nature hinders the generalization of the findings to the wider population and those who did not admit to the hospital. The study is not free from recall bias, as the measurement of dietary diversity was relied on memory. Micronutrients were not included in the analysis. Details of TB treatment, such as duration of current and previous anti-TB treatment, were not documented.

CONCLUSIONS

Undernutrition remains prevalent among adult patients with pulmonary TB. Routine nutritional screening and assessment upon admission and during treatment should be given to all TB patients. Awareness of nutrition, balanced diet and regular physical activity should be improved by counselling and communication.

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Author Contributions:

Conceptualization, T.T.N.N; methodology, T.T.N.N; software, T.T.N.N, T.B.Y.H; validation, T.T.N.N, T.B.Y.H; formal analysis, T.T.N.N, T.B.Y.H; investigation, T.T.N.N, T.B.Y.H; resources, T.T.N.N, T.B.Y.H; data curation, T.T.N.N, T.B.Y.H; writing – original draft preparation, T.T.N.N; writing – review and editing, T.T.N.N, T.B.Y.H; visualization, T.T.N.N, T.B.Y.H; supervision, T.T.N.N, T.B.Y.H; project administration, T.T.N.N. All the authors have read and agreed with the final version of the article.

Compliance with Ethics Requirements:

"The authors declare no conflict of interest regarding this article"

"The authors declare that all the procedures and experiments of this study respect the ethical standards in the Helsinki Declaration of 1975, as revised in 2008(5), as well as the national law. Informed consent was obtained from all the patients included in the study".

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