ORIGINAL ARTICLE

The Role of Omega-3/Omega-6 Ratio on Appetite in Pulmonary Multidrug-Resistant Tuberculosis Patients

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ABSTRACT

Introduction: Tumour necrosis factor-alpha (TNF-α) levels, pro-inflammatory cytokines that suppress appetite, were discovered to be much higher in multidrug-resistant tuberculosis (MDR-TB) than in drug-sensitive TB. Research on the omega-3/omega-6 (ω-3/ω-6) polyunsaturated fatty acids (PUFAs) intake, which can support appetite in patients with MDR-TB, has never been performed. This study aimed to examine the relationship between ω-3/ω-6 PUFAs intake ratio and appetite mediated by TNF-α in pulmonary MDR-TB patients.

Methods: This was a cross-sectional study conducted on 46 male and female adults with MDR-TB undergoing intensive phase therapy. Data were collected through questionnaires, 1x24 hours food recall, anthropometric measurements, 100 mm visual analogue appetite scale, and venous blood collection.

Results: Correlation analysis used the Pearson and Spearman tests. The findings revealed the ratio of ω-3/ω-6 PUFAs intake of 0.11 ± 0.05, the median value of TNF-α 7.49(1.66-447.62) pg/mL, and an average appetite of 58.72 ± 26.7. There was no relationship between ω-3/ω-6 PUFAs intake ratio and TNF-α ($r = 0.016$; $p = 0.91$), likewise between ω-3/ω-6 PUFAs intake ratio with appetite ($r = -0.1$; $p = 0.54$), but there was a relationship between TNF-α and appetite ($r = 0.031$; $p = 0.04$).

Conclusion: There was no relationship between ω-3/ω-6 PUFAs intake ratio and TNF-α and appetite in pulmonary MDR-TB patients. However, there was a relationship between TNF-α and appetite. This study is the first to determine the correlation between ω-3/ω-6 PUFAs intake ratio and appetite in pulmonary MDR-TB patients.

INTRODUCTION

One of the world's top ten causes of death is tuberculosis (TB). The World Health Organization (WHO) has determined TB as a global emergency.[1] Around 20% of TB strains are resistant to at least one anti-TB drug. There were 465,000 cases of rifampicin-resistant TB (RR-TB) in 2019, of which 78% had developed into multidrug-resistant TB (MDR-TB).[2] Indonesia is one of the 20 countries with the highest MDR-TB cases. WHO estimated 24,000 cases per year in Indonesia, where West Java was the province with the most MDR-TB cases, with 1,566 new cases in 2018 and 2,073 cases in 2019.[3]

TB can cause malnutrition due to metabolic increase and decreased appetite.[4] TB patients usually show malnutrition.[5] Previous studies stated that primary factors associated with poor treatment outcomes for MDR-TB patients were age (> 60 years old), body weight (≤ 50 kg), cavitary disease or hemoptysis symptoms, and treatment irregularity.[6] The inflammatory process in TB leads to the increased production of interleukin (IL)-l, IL-6, tumour necrosis factor-alpha (TNF-α), and malondialdehyde (MDA). The increase in

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TNF-α is associated with anorexia.[7] A study reported that levels of interferon-gamma (IFN-γ) and TNF-α were discovered to be higher in MDR-TB patients compared to drug-sensitive TB patients.[8]

In a randomized control trial (RCT), the administration of eicosapentaenoic acid (EPA), which is a group of omega-3 (ω-3) polyunsaturated fatty acids (PUFAs), was found to be associated with an increase in appetite in healthy adults. In anorexia-cachexia syndrome, it reduces the production of C-reactive protein (CRP), IL-6, and TNF-α. In addition, it inhibits the ubiquitin-proteasome pathway in muscle loss. Hence, insulin sensitivity increases and improves protein and calorie intake. In another study, EPA supplementation improved and cured anorexia nervosa in young patients. Oral EPA supplementation in non-small cell lung cancer can increase eating intake, and restore appetite and lean body mass compared to control with a 1g dose of EPA twice daily.[9] Pizzini, *et al*. (2018) reported that ω-3 PUFAs could limit and improve the inflammatory process in people with chronic obstructive pulmonary disease (COPD), including lowering TNF-α levels.[10] The properties of ω-3 and omega-6 (ω-6) PUFAs have opposite ways of working. EPA and docosahexaenoic acid (DHA), which are the ω-3 PUFAs group, produce specialized pro-resolving lipid mediators (SPMs), such as resolvins, that can reduce inflammation, while linoleic acid (LA) and arachidonic acid (ARA), which are groups of ω-6 PUFAs, increase inflammation and proliferation of fat cells.[11]

Research on the effect of ω-3 PUFAs on reducing inflammation and appetite has never been performed in people with MDR-TB. Therefore, this study aimed to examine the relationship between PUFAs consumption ratios of ω-3/ω-6 to appetite, which TNF-α mediates in people with MDR-TB at Dr. M. Goenawan Partowidigdo Lung Hospital (RSPG) Bogor, Indonesia.

METHODS

Study Participants

From December 2019 to March 2020, a cross-sectional study with a consecutive sampling method was performed at RSPG Bogor, Indonesia. Adults, male and female aged 19-49 years old, diagnosed with MDR-TB, and undergoing intensive phase therapy of MDR-TB, were included. Subjects with a history of autoimmune and immunocompromised diseases, taking ω-3 and ω-6 PUFAs supplements, and pregnant or breastfeeding were not allowed to participate. Before recruitment, a written consent form was received from participants. Subjects completed a socio-demographic questionnaire and a 100 mm visual analogue appetite scale. The Ethical Committee Faculty of Medicine, Universitas Indonesia, Jakarta, approved the study procedure (No. KET-1317/UN2.F1/ETIK/PPM.00.02/2019).

Dietary Intake

For the study, a semi-quantitative Food Frequency Questionnaire (FFQ) was used to collect dietary consumption of ω-3 and ω-6 PUFAs during the previous month. For total energy, fat, and protein intake, 1x24 hours of food recall was used. Dietary consumption was determined using food composition data from local and Asian countries.

Anthropometric Measurement

The height measurement was performed with a height measuring board (ShorrBoard, Olney, USA). Weight and body composition measurements were performed using a Tanita type SC-330 body composition analyzer (Tanita, Tokyo, Japan) for body weight.

Laboratory Analysis

Venous blood samples were drawn and centrifuged at 3000 rpm for 10 minutes, the serum was then refrigerated at -20°C until it was tested at the Department of Biochemistry and Molecular Biology Laboratory, Faculty of Medicine, Universitas Indonesia, Jakarta. Enzyme-Linked Immunosorbent Assay (ELISA) kit (Elabscience, USA) measured TNF-α level.

Data Analysis

The Statistical Package for the Social Sciences version 20 (SPSS Ins, Chicago, IL, USA) was used to analyze the data. Dietary intake was evaluated using NutriSurvey 2007 (Germany). The normality data of subjects' characteristics, dietary intake, TNF-α serum levels, and appetite were assessed using the Kolmogorov-Smirnov test. The Spearman correlation test was applied to evaluate the relationship between the ω-3/ω-6 PUFAs ratio with TNF-α. The Pearson correlation test was applied to determine the relationship between TNF-α and appetite, and the relationship between the ω-3/ω-6 PUFAs ratio and appetite. If $p < 0.05$, the association was considered significant.

RESULTS

Forty-six adults diagnosed with MDR-TB and undergoing an intensive therapy phase participated. The mean age of participants was 34.26 ± 11.64 years old, with a female majority. Most subjects had low to middle educational backgrounds and a low-income level. The fat-free mass (FFM) average was 37.04 ± 7.2 kg. Most subjects had a low body weight, and most were former active smokers, as seen in Table 1.

Table 1. Subject characteristics

Parameter (n=46)	n (%)	Average/median
Age (years old)		34.26 ± 11.64
Gender		
Male	21(45.7%)	
Female	25(54.3%)	
Education level		
Low	26(56.5%)	
Middle	16 (34.8%)	
High	4 (8.7%)	
Income level		
Low	40(87%)	
Enough	6(13%)	
FFM (kg)		37.04 ± 7.2
BMI (kg/m^2)		17.96 ± 3.75
Nutritional status		
Low	31(67.4)	
Normal	9(19.6)	
Overweight	4(8.7)	
Obese I	2(4.3)	
Smoking history		
Former active smoker	24(52.2)	
Not active smoker	1(2.2)	
Passive smoker	13(28.3)	
Not passive smoker	8(17.4)	

Table 2 shows that the majority of the individuals did not consume enough total energy and total protein to meet the Institute of Medicine Dietary Reference Intake (DRI). On the other hand, most participants had enough total fat intake. The median intake of ω-3 PUFAs was 0.13(0.00-1.38) g/day and ω-6 PUFAs was 1.09(0.04-10.38) g/day. This study found that the average ω-3/ω-6 PUFAs ratio was 0.11 ± 0.05. The median TNF-α serum level was 7.49(1.66-447.62) pg/mL. The average 100 mm VAS score of appetite was 58.72 ± 26.7. Most of the subjects experienced anorexia.

Table 2. Distribution of dietary intakes

Dietary intake (n=46)	n (%)	Average/median
Total energy (kcal/day)		1499.88 ± 549.32
Level of energy		
Low	24(52.2)	
High	22(47.8)	
Level of fat		
Low	22(47.8)	
High	24(52.2)	
Total protein (g/day)		51.63 ± 26.68
Level of protein		
Low	32(69.6)	
High	14(30.4)	
ω-3 (g/day)		0.13(0.00-1.38)
Level of ω-3		
Low	45(97.8)	
High	1(2.2)	
ω-6 (g/day)		1.09(0.04-10.38)
Level of ω-6		
Low	46(100)	
High	0(0)	
ω-3/ω-6		0.11 ± 0.05
TNF-α (pg/ml)		7.49(1.66-447.62)
Appetite		58.72 ± 26.7
Anorexia	29(63)	
Not anorexia	17(37)	

The Spearman test was performed to see the correlation between ω-3/ω-6 PUFAs intake ratio with TNF-α level and between TNF-α level and ω-3/ω-6 PUFAs ratio with appetite. Table 3 shows no correlation between ω-3/ω-6 PUFAs intake ratio with TNF-α level and between ω-3/ω-6 PUFAs intake ratio with appetite. TNF-α level and appetite had a significant correlation.

Table 3. Correlation between variables

Variables	R coefficient*	p-value**
ω-3/ω-6 PUFAs and TNF-α	0.016	0.91
TNF-α and appetite	0.031	0.04
ω-3/ω-6 PUFAs and appetite	-0.1	0.54

Notes: *R > 0.7 = very strong correlation; 0.4-0.69 = strong relationship; 0.3-0.39 = moderate relationship; 0.2-0.29 = weak relationship; 0.01-0.19 = negligible relationship. **Statistically significant ($p < 0.05$)

In this study, an additional analysis was performed using tests on the relationship between age, body mass index (BMI), FFM, total fat intake, and total energy with appetite. Spearman correlation test was conducted on gender, education level, income level, and smoking history. The correlation test results indicated a link between FFM and appetite ($r = 0.33$; $p = 0.025$). Total energy also showed a correlation with appetite ($r = 0.37$; $p = 0.01$), intake of ω-3 PUFAs and appetite ($r = 0.33$; $p = 0.03$), and intake of ω-6 PUFAs and appetite ($r = 0.34$; $p = 0.02$). Although the analysis found several variables with a significant p-value, the R-value (correlation coefficient) was weak.

DISCUSSION

This study found that most MDR-TB patients at RSPG consumed ω-3 and ω-6 PUFAs under DRI (97.8% and 100%), although most of the total fat intake was sufficient (52.2%). The adequacy of the total fat intake was likely because most of the food consumed was fried and sauteed food, while the lack of intake amounts of ω-3 and ω-6 PUFAs was likely since most of the subjects experienced anorexia (63%), as well as having low income and education (56.5% and 87%). Van Der Meij, *et al.* studied a healthy population in the United States and reported that decreased appetite had a significant relationship with females, black skin, smokers, and low-educated and low-income individuals ($p < 0.05$).[12]

This study also found no correlation between ω-3/ω-6 PUFAs ratio and serum TNF-α level in pulmonary MDR-TB patients at RSPG. From previous studies, the administration of EPA and DHA decreases the production of pro-inflammatory cytokines TNF-α, IL-1, and IL-6, and increases the concentration of IL-10, known as anti-inflammatory cytokines.[13] EPA and DHA

have been shown to inhibit TNF-α, a cytokine mostly produced in the infection process by M. tb. This mechanism allows to reduce anorexia and decrease body weight. Conversely, the study by Hu, *et al*. stated no significant difference in TNF-α levels between subjects who consumed ω-3 PUFAs and controls.[14] The study reported that ω-3 PUFAs supplementation had no significant effect on serum IL-6, TNF-α, and CRP levels reduction in chronic kidney disease (CKD) patients who were or were not on dialysis. Therefore, research on the effect of ω-3 PUFAs on TNF-α levels in MDR-TB patients still requires further research.

This study found no correlation between serum TNF-α levels and appetite in pulmonary MDR-TB patients because cytokine testing was only performed on TNF-α, whereas several other cytokines that promote inflammation were not checked, such as IL-1, IL-2, IL-6, IL-8, and IFN-γ which are known to suppress appetite. In addition, the TNF-α measurement was only performed once in the intensive treatment phase and not in the advanced treatment phase. This process allows for differences in results. TNF-α levels will decrease with treatment progression, hence there is no comparison in the follow-up phase. IL-1 and TNF-α induce insulin resistance and sensation of fullness in the hypothalamic anorexia centers.[15]

In this study, there was no correlation between the ω-3/ω-6 PUFA consumption ratio and appetite in pulmonary MDR-TB patients. This study is the first to establish the correlation between the ω-3/ω-6 PUFA consumption ratio and appetite in patients with MDR-TB. Sari, *et al*. reported that the ω-3/ω-6 PUFAs consumption ratio in 54 healthy women who worked in Semarang government offices was 1:16.[16] Although in this study the results did not show a relationship between the ω-3/ω-6 ratio, several studies showed that the ω-3/ω-6 ratio could affect appetite. A clinical study reported that administering EPA and DHA of 600 mg/d to 3.6 g/d can maintain and increase body weight, and improve and minimize lean body mass loss.[17] This is in contrast to Simopoulus, *et al*., who conducted research on ω-3 as a weight-lowering agent and found a high intake of ω-6 PUFAs and a high ratio consumption of ω-6/ω-3 PUFAs were linked to western gain, while ω-3 PUFAs reduced the risk of gaining weight.[18] Sedighiyan, *et al*. reported the results of a single-blind controlled trial research in Amir-Alam Hospital in Tehran, which showed that COVID-19 patients given ω-3 (EPA) showed an improvement in appetite ($p = 0.03$).[19] The central nervous system produces endocannabinoids (eCBs), which have a role in neurobiology. eCBs metabolites come from ARA, EPA, and DHA. eCBs bonds with cannabinoids (CB)-1 and CB-2 receptors can affect appetite stimulation, metabolism, energy, the immune system, and the neuroplasticity of the brain.[20]

LIMITATIONS

This study only measured TNF-α, whereas other cytokines could affect appetite. Data collection of ω-3 and ω-6 PUFAs intake was only performed by the 1x24 hours recall method. No standard gold instrument exists to assess appetite in people with pulmonary MDR-TB.

CONCLUSION

There was no relationship between ω-3/ω-6 PUFAs intake ratio and TNF-α and appetite in pulmonary MDR-TB patients. However, there was a relationship between TNF-α and appetite. A prospective cohort study is recommended to understand better the relationship between ω-3/ω-6 PUFAs intake ratio and appetite. Further studies could enrich the ω-3 and ω-6 PUFAs content in the Indonesian food database and studies on a specific gender. Studies measuring ω-3 and ω-6 PUFAs concentrations in erythrocytes membrane to see the ω-3/ω-6 PUFAs ratio more objectively are also warranted. The authors suggest a study of cytokines and other factors that can affect appetite in MDR-TB patients for future research.

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Conflict of Interest

The authors declared there is no conflict of interest.

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Authors' Contributions

Conceiving the study, designing the experiment, gathering, analyzing, and interpreting the data, making tables and figures, and writing the manuscript: FHJ. Providing guidance, contributing significant intellectual content during drafting, and revising the manuscript: FN, NRMM, IP, NS, BR, and AW. All authors reviewed and approved the final version.

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