International Journal of Advanced Health Science and Technology
Homepage: ijahst.org
e-ISSN:2808-6422; p-ISSN:2829-3037
Vol. 3 No.6, pp. 327-334, December 2023

RESEARCH ARTICLE
OPEN ACCESS

Manuscript received September 12, 2023; revised Oktober 12, 2023; accepted Oktober 12, 2023; date of publication December 2, 2023
Digital Object Identifier (DOI): https://doi.org/10.35882/ijahst.v3i6.300

How to cite: Fardiah Tilawati Sitanggang, James Perdinan Simanjuntak, Siti Sakdiah, "Vitamine D Deficiency Leukocyte Ratio: Unraveling the impact on Multidrug Resistant Tuberculosis Patients", International Journal of Advanced Health Science and Technology, vol. 3, no. 6, pp. 327-334, December 2023.

Vitamine D Deficiency Leukocyte Ratio: Unraveling the impact on Multidrug Resistant Tuberculosis Patients

Fardiah Tilawati Sitanggang, James Perdinan Simanjuntak, Siti Sakdiah

Medical Laboratory Technology Department, Health Polytechnic of Jambi, Indonesia

Corresponding author: Fardiah Tilawati Sitanggang (e-mail: fardiahtilawati@poltekkejambi.ac.id)

ABSTRACT Tuberculosis (TB), particularly its multidrug-resistant (MDR) form, remains a significant global health challenge, characterized by complex treatment protocols, prolonged therapy durations, elevated transmission risk, and adverse side effects. Emerging evidence suggests that vitamin D deficiency may influence the immune response in TB patients, potentially impacting disease progression and treatment outcomes. This study aims to investigate the relationship between serum vitamin D levels and leukocyte ratios specifically neutrophil-to-lymphocyte ratio (NLR) and neutrophil-monocyte-to-lymphocyte ratio (NMLR) among MDR-TB patients compared to non-resistant TB controls, to better understand their roles as biomarkers for immune status. A case-control methodology was employed, involving MDR-TB patients and TB controls recruited from healthcare centers. Serum vitamin D levels were quantitatively measured, and leukocyte ratios were derived from complete blood count analyses. Data analysis was conducted using statistical tests including one-way ANOVA and comparative assessments to evaluate differences between groups. Results indicated that a significant proportion of participants in both groups exhibited vitamin D insufficiency or deficiency, with 89.2% falling below optimal levels. Notably, MDR-TB patients demonstrated higher NLR and NMLR values, indicative of heightened immune activation. Moreover, correlations between low vitamin D levels and altered leukocyte ratios suggest that vitamin D deficiency may be linked to immune dysregulation in MDR-TB cases. The findings highlight the potential utility of vitamin D status and leukocyte ratios as biological markers for immune function in MDR-TB patients. These insights underscore the importance of addressing vitamin D deficiency as a supplementary strategy in MDR-TB management, potentially enhancing immune response and improving treatment outcomes.

INDEX TERMS Vitamin D deficiency, leukocyte ratios, MDR-TB, immune response, biomarkers.

I. INTRODUCTION

Tuberculosis (TB) remains one of the most persistent infectious diseases globally, accounting for approximately 1.6 million deaths annually and affecting over 10.4 million individuals worldwide [1]. Despite significant advances in diagnosis and treatment, TB control continues to challenge health systems, especially in high-burden countries such as Indonesia, where the incidence reached over 511,000 cases in 2018 alone [2]. The complexity of TB management is compounded by the emergence of multi drug resistant tuberculosis (MDR-TB), which is resistant to at least isoniazid and rifampicin, the two most potent first-line anti-TB drugs [3].

The current state of the art involves comprehensive diagnostic methods, including sputum microscopy, culture, molecular assays (such as GeneXpert), and drug susceptibility testing [4], [5]. While these techniques provide critical insights into the presence and drug resistance profile of M. tuberculosis, they primarily focus on pathogen identification and resistance detection. However, these methods have limitations in offering real-time insights into the host's immune status, which plays a crucial role in disease progression, treatment response, and outcomes [6].

Recent research emphasizes the importance of host immunological markers in understanding TB pathogenesis and monitoring disease progression. Leukocyte ratios, such as the neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and the derived neutrophil-monocyte-to-lymphocyte ratio (NMLR), have emerged as promising inflammatory and immune response indicators [7]-[9]. These ratios are easily obtainable from routine blood counts and have demonstrated potential in predicting treatment outcomes, disease severity, and disease recurrence in various infectious and inflammatory disorders [10]-[12].

Despite these advances, there remain significant gaps in the application of such immunological markers in the context of MDR-TB, particularly in populations with prevalent vitamin D deficiency, which has been implicated in immune modulation and disease susceptibility [13]-[15]. Vitamin D plays a pivotal role in innate immunity, including the activation of macrophages and modulation of antimicrobial peptides, such as cathelicidin, which are critical in containing

e-ISSN:2808-6422; p-ISSN:2829-3037
Vol. 3 No.6, pp. 327-334, December 2023

M. tuberculosis [16], [17]. Several studies have suggested that vitamin D deficiency correlates with increased susceptibility to TB and poorer treatment outcomes [18]-[20], yet the specific interplay between vitamin D levels and leukocyte ratios in MDR-TB patients remains underexplored.

The existing literature primarily focuses on either immunological markers or vitamin D status independently; few studies have comprehensively evaluated their combined influence on host immune responses and disease resistance in MDR-TB patients [21]. Notably, there is a lack of integrated research assessing these biomarkers as potential tools for early diagnosis, prognosis, and treatment monitoring, especially in resource-limited settings where TB burden is highest.

Given these knowledge gaps, this study aims to investigate the relationship between serum vitamin D levels, leukocyte ratios, particularly NLR, MLR, and NMLR, and their potential as biological markers of immune competence in MDR-TB patients. The research seeks to determine whether these biomarkers can serve as reliable indicators for disease monitoring and treatment efficacy. The primary contributions of this research are threefold:

1. Providing empirical evidence on the correlation between vitamin D status and leukocyte ratios in MDR-TB.
2. Evaluating the efficacy of these ratios as accessible, cost-effective markers for immune response assessment.
3. Offering insights that could inform clinical decision-making and personalized treatment strategies in TB management.

This article is organized as follows: Section II reviews the related work and current methodologies in immunological and nutritional biomarkers in TB. Section III describes the research design, including participant selection, data collection, and analytical methods. Section IV presents the study results and their implications, while Section V discusses the findings in the context of existing literature. Finally, Section VI concludes the study with recommendations for clinical practice and prospective research directions.

II. RESEARCH METHODS

This study was designed as an analytical observational case-control investigation aimed at examining the relationship between vitamin D levels, leukocyte ratios, and the clinical status of patients with multidrug-resistant tuberculosis (MDR-TB) in Jambi City, Indonesia. The research was conducted over a period of approximately ten months, from January to October 2021, following ethical approval obtained from the Health Polytechnic of Jambi's research ethics committee (No. LB.02.06/2/022/2021).

A. STUDY POPULATION AND SAMPLING

The population comprised patients diagnosed with MDR-TB who underwent sputum examination using the GeneXpert MTB/RIF assay at several designated health centers, namely Pakuan Baru, Paal X, and Simpang Kawat Health Centers within Jambi City. The study sample consisted of 37 individuals categorized into cases (MDR-TB patients) and controls (drug-sensitive TB patients). Inclusion criteria were patients aged 18 years or older, with confirmed TB diagnosis, and who consented to participate. Exclusion criteria included individuals with comorbidities affecting immune status, such as HIV/AIDS, diabetes mellitus, or immunosuppressive therapy. Sampling involved consecutive sampling methods at the participating health centers to ensure representativeness, with equal effort made to include all eligible patients during the study period. Due to the nature of the study, randomization was not applicable; however, cases and controls were selected based on predefined diagnostic categories to maintain group comparability.

B. STUDY DESIGN

This research adopted a case-control design, allowing comparison of vitamin D levels and leukocyte ratios between MDR-TB patients (cases) and drug-sensitive TB patients (controls). The retrospective aspect encompassed data collection of laboratory results and patient medical records, whereas blood sampling for vitamin D and leukocyte analysis was conducted prospectively at the time of patient enrollment.

C. MATERIALS AND LABORATORY ASSAYS

Blood samples (about 10 mL) were collected from each participant via venipuncture into ethylenediaminetetraacetic acid (EDTA) tubes for complete blood count (CBC) and serum separation. Serum vitamin D levels were quantified utilizing an enzyme-linked immunosorbent assay (ELISA) kit, specifically the DiaSorin LIAISON® 25 OH Vitamin D assay, which is validated and widely used for clinical research (supported by recent validations [22], [23]. Leukocyte ratios, such as neutrophil-to-lymphocyte ratio (NLR), monocyte-to-lymphocyte ratio (MLR), and neutrophil-to-monocyte ratio (NMLR), were derived from the CBC results. Automated hematology analyzers (Sysmex XN-550) were used to ensure precise enumeration of blood cell populations, following standard operating procedures to minimize analytical variability.

D. DATA COLLECTION PROCEDURES

Patient interviews and medical record reviews were employed to gather demographic data, clinical history, treatment adherence, and other relevant variables. The laboratory assessments were performed on blood samples obtained at the time of study enrollment. Serum vitamin D concentrations were classified according to established guidelines: deficiency (<20 ng/mL), insufficiency (20–29 ng/mL), and sufficiency (≥30 ng/mL) [24]. Leukocyte counts were obtained from the complete blood count (CBC), which provided absolute counts of neutrophils, lymphocytes, monocytes, and other cell populations. These counts were used to calculate the ratios of interest, including the neutrophil-to-lymphocyte ratio (NLR), which was calculated by dividing the absolute neutrophil count by the absolute lymphocyte count; the monocyte-to-lymphocyte ratio (MLR), obtained by dividing the absolute monocyte count by the absolute lymphocyte count; and the neutrophil-to-monocyte ratio (NMLR), calculated by dividing the neutrophil count by the monocyte count. All laboratory

e-ISSN:2808-6422; p-ISSN:2829-3037
Vol. 3 No.6, pp. 327-334, December 2023

procedures adhered strictly to quality control standards, with calibration and validation of equipment performed daily, in accordance with manufacturer protocols.

E. *ETHICAL CONSIDERATIONS*

Participants provided written informed consent prior to enrollment. Confidentiality and anonymity were maintained throughout the study. The study protocol conformed to the Declaration of Helsinki and local ethical standards for biomedical research.

F. STATISTICAL ANALYSIS

Data were analyzed using SPSS version 25.0 (IBM Corp., Armonk, NY, USA). Descriptive statistics summarized demographic and clinical characteristics. Shapiro-Wilk tests assessed normality of the data. Independent t-tests or Mann-Whitney U tests compared continuous variables between groups based on distribution. Chi-square or Fisher's exact tests analyzed categorical data. Correlation analyses employed Pearson or Spearman coefficients as appropriate. A p-value <0.05 was considered statistically significant.

G. SUPPORTING LITERATURE

The methodology aligns with recent standards for biomarker-based TB research, emphasizing robust laboratory techniques and careful sample selection [25], [26]. The case-control design facilitates understanding of associations between immune parameters and resistance status, while the prospective collection of some data components enhances data reliability.

III. RESULT

This study was conducted in the period from January to August 2021 in three Puskesmas in Jambi City, where the total number of these studies was 37 people. Based on the data collected in all interviews conducted in this study, a description of the patient's characteristics related to personal status, treatment status, and characteristics of other activities of patients related to improving their health and endurance was obtained. The characteristic data obtained from the interviews have been analyzed and shown in the TABLE 1. In addition to the following, patient characteristics related to the patient's treatment status were also obtained through direct interviews and have also been further confirmed by the TB Puskesmas program officers or laboratory officers, who were then grouped into three groups, the first group is the observation group for non-drug resistant cases (Non-drug resistant TB) are TB patients who have undergone treatment or have taken Obat anti Tuberculosis (DRUG) obtained as many as 26 patients; the second group as the observation group of resistant cases (Multidrug TB) were TB patients who had undergone repeated treatment and were declared as Multidrug patients, there were 5 patients, and the third group as the control group (new TB) are pulmonary TB patients who have just been detected and have not undergone treatment or have not taken DRUG, obtained as many as 6 patients as shown in FIGURE 1.

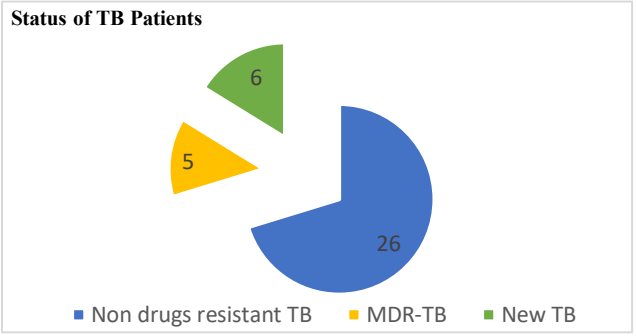

FIGURE 1. Status of TB Patient

TABLE 1
Characteristic of TB Patients

Age	Mean (SD)	40.8 (15.9) years
	IQR	5 – 70 years
Sex	Male	51.6%
	Female	48.4%
Education level	No school	3.2%
	SD	16.1%
	SMP	12.9%
	SMA	64.5%
Job	PT employee	3.2%
	Worker	16.1%
	Jobless	83.9%
TB suffering periods	Mean (SD)	3.9 (3.3) months
	IQR	0 – 17 months
DRUG consumption	Mean (SD)	3.5 (2.7) months
	IQR	0 – 12 months
The regularity of DRUG consumption	Regular	78.4%
	Irregular	5.4%
	Didn't start yet	16.2%
Grievance because of DRUG	There isn't	37.8%
	There is	45.9%
	Didn't start yet	16.2%
Another drugs consumption (routine)	No	48.6%
	Yes	51.4%
Sumplement consumption	No	54.1%
	Yes	45.9%
Sunbathe activity	No	48.6%
	Yes	51.4%

e-ISSN:2808-6422; p-ISSN:2829-3037
Vol. 3 No.6, pp. 327-334, December 2023

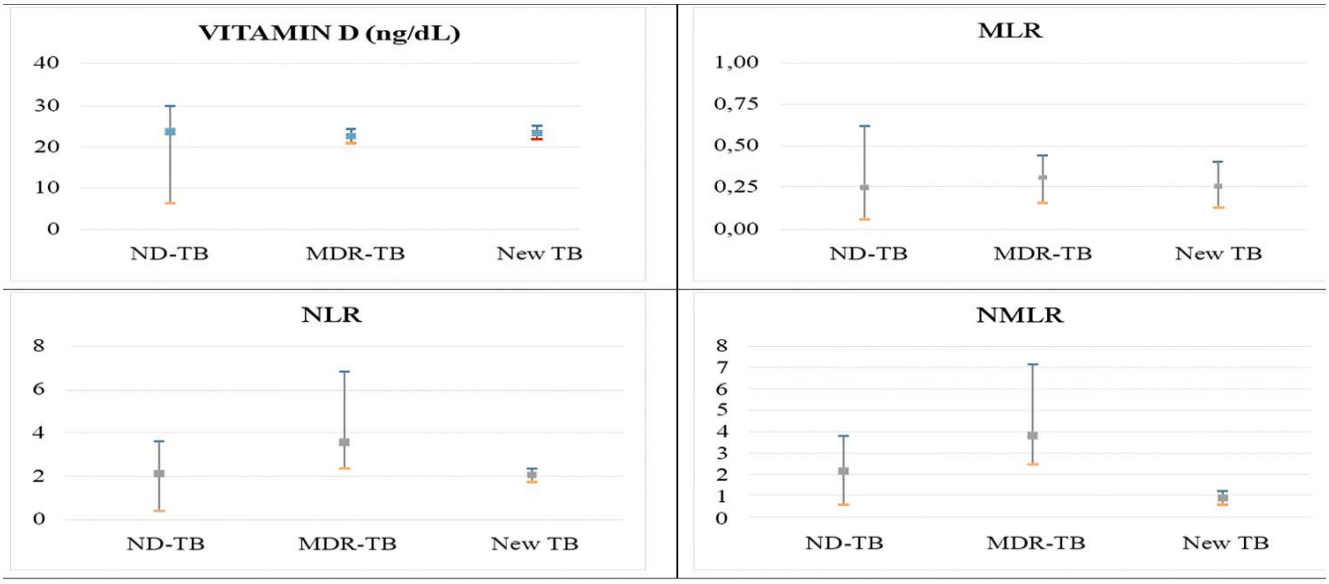

FIGURE 2. Vitamin D, NLR, MLR, NMLR of TB Patients Based on Their Treatment

In addition, the immune characteristics of all patients observed in this study were determined based on the values of laboratory test parameters, vitamin D levels and the value of the ratio of white blood cells that play an active role in the immune system (NLR, MLR and NMLR). The detailed results of these laboratory test parameters are summarized in TABLE 2. IQR refers to the *interquartile range*, which represents the spread of the middle 50% of the data, calculated as the difference between the third quartile (Q3) and the first quartile (Q1). An asterisk (*) indicates a statistically significant result, suggesting that the observed differences between groups are unlikely to have occurred by chance and may reflect a meaningful association.

The immune status of TB patients was assessed based on the results of the examination of several parameters that had been previously determined by the research team. Parameters of vitamin D levels, parameters of the ratio of several types of white blood cells. including neutrophil cells, lymphocytes and monocytes consisting of NLR (*neutrophil/lymphocyte ratio*), MLR (*monocyte/lymphocyte ratio*), and NMLR (*neutrophil-monocyte/lymphocyte ratio*). The results of the examination of these four parameters are shown in the FIGURE 2. ND-TB refers to non-drug-resistant tuberculosis, MDR-TB denotes multidrug-resistant tuberculosis, and New TB represents newly diagnosed tuberculosis patients. These abbreviations are used throughout the figure to indicate the corresponding patient groups. The classification is essential to differentiate the severity of infection, treatment challenges, and the immune-inflammatory responses reflected in the respective laboratory parameters.

A. VITAMINE D

Based on the FIGURE 2, it can be seen that all the results of the examination of vitamin D levels in 37 TB patients, most of them (89.2%) were in the range of insufficiency values (vitamin D deficiency). Three patients with vitamin D deficiency were found in the non-drug resistant TB group (11.5%) and one was found in the MDR- TB group (20%). In the group of TB patients who were newly detected and had not undergone treatment, there were no cases of deficiency. Based on the results of statistical analysis conducted with the one-way ANOVA test, p-value> 0.05 was obtained, so it can be

TABLE 2
Characteristic of Laboratory Test Result

Characteristic	Vit D	NLR	MLR	NMLR
Range	5 – 30	0.34 – 6.75	0.03 – 0.6	0.55 – 7.08
Mean	22.8	2.05	0.24	1.93
Median	23	1.97	0.22	1.78
IQR	21 – 26	1.52 – 2.3	0.12 – 0.35	1.02 – 2.40
***one-way ANOVA* (p)**	0.777	0.002*	0.356	0.000*
Abnormal:	100%	8.1%	40.5%	67.6%
- **New TB**	100%	3.8%	30.8%	73.1%
- **Non drugs resistant TB**	100%	40%	80%	100%
- **MDR-TB**	100%	0%	50%	16.7%
Fisher's exact test	1	0.055	0.126	0.007*

e-ISSN:2808-6422; p-ISSN:2829-3037
Vol. 3 No.6, pp. 327-334, December 2023

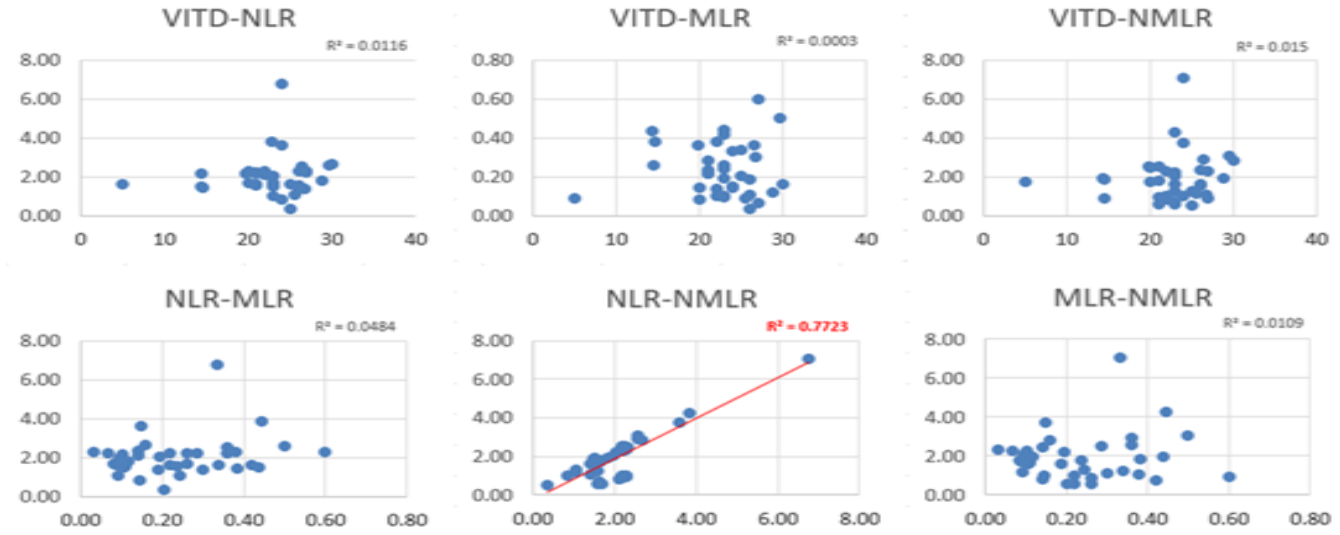

FIGURE 3. Correlation Between Examination Parameters Immune Status Performed.

concluded that there is no difference in the results of this parameter examination between the three groups observed with an overall average value of 22.8 (ng/mL).

B. NLR

The results of the calculation of the NLR value showed an increasing trend in the group of pulmonary TB patients with multidrug-TB. Cases of increased ratio were only found in 1 person (3.8%) from the non-drug TB patient group and 2 (40%) from the multi drug TB group with a cut off value of 2.91. There were no cases of increased NLR values in the new TB group. Fisher's exact test concluded that there was no significant difference in the frequency of case finding of increased NLR between each group ($p = 0.054$). Statistical analysis using one-way ANOVA test obtained p-value <0.05, so it can be concluded that the results of the parameter examination between the three groups observed were significantly different. The analysis using Tukey's HSD test (honestly significant difference) even shows that the difference is very significant if done by comparing the multi-drug TB group which is the main target of case-control observation in this study against the other two comparison groups ($p < 0.01$). There was no difference between the two comparison groups ($p > 0.05$). This fact shows that the NLR value can be used as a parameter that has the potential as a marker to distinguish a patient's status related to drug treatment, where the immune condition indicated by an increase in NLR tends to indicate a problem in the treatment that the patient is undergoing. However, in the non-drug resistant and new TB groups, no significant difference was found, so this NLR value did not differentiate the immune conditions of the patients in the two groups.

C. MLR

FIGURE 2 it is found that from the analysis using the cut off value (0.25/25%) it is known that the frequency of increased MLR values is most commonly found in multi-drugs TB patients with a percentage of 80%. However, the frequency was not much different after an analysis using the Fisher's exact test statistic ($p = 0.126$) with an average value of 0.238. Further analysis with one-way ANOVA obtained p-value > 0.05 which means that there is no difference in MLR results in the three groups so that the MLR value cannot be used as a parameter that distinguishes various immune conditions in TB patients.

D. NMLR

NMLR is the ratio between the neutrophil count, macrophage and lymphocyte count. The macrophage count was obtained from the sum of the neutrophil count and the monocyte count. The overall average value of the calculated NMLR is 1.93 which is above the cut off value (1.2). FIGURE 2 shows the tendency for NMLR values to be much higher in the multi - drugs TB patient group compared to other groups. The results of Fisher's exact test analysis showed that the condition of immunity in TB patients based on the NMLR value differed significantly between each group. This difference is supported by the results of the analysis of the one-way ANOVA test which observed the ratio value of each group which concluded that there were differences in the value of the NMLR calculation results in the three groups. This difference is supported by the results of the analysis of the one-way ANOVA test which observed the ratio value of each group which concluded that there were differences in the value of the NMLR calculation results in the three groups.

This fact shows that NMLR has the potential as a marker to differentiate patient status related to DRUG treatment, where the immune condition indicated by increased NMLR tends to be more and more found in patients with treatment problems. The results of the Tukey's HSD test between multi-drug TB and other groups were significantly different, although a significant difference in Tukey's HSD test results was also not found between the non-drug resistant TB and new TB groups, making it more potential as a marker of the development of immune conditions that lead to treatment problems in TB patients. From the FIGURE 3, it can be seen that only the NLR and NMLR parameters show a correlation. This is in accordance with the FIGURE 3 generated from the two parameters in the previous discussion which states that the two ratio parameters are able to show significant differences

e-ISSN:2808-6422; p-ISSN:2829-3037
Vol. 3 No.6, pp. 327-334, December 2023

in patients with drug treatment problems, namely patients who are included in the multi-drugs TB group in this study. The correlation shown by these two parameters confirms that utilizing the ratio of NLR and NMLR for monitoring TB patients is highly recommended.

IV. DISCUSSION

The primary objective of this study was to elucidate the relationship between Vitamin D levels, leukocyte ratios, and multidrug-resistant tuberculosis (MDR-TB) outcomes among patients in Jambi City. The data revealed a high prevalence of Vitamin D deficiency, with approximately 89.2% of TB patients exhibiting levels indicative of insufficiency, and no significant differences in Vitamin D levels across different patient groups ($p > 0.05$). These findings align with earlier research, which underscores the widespread deficiency of Vitamin D among TB populations globally, often regardless of drug resistance status [27]. The role of Vitamin D in immune modulation, particularly in macrophage activation and cytokine production, is well-documented [28]. A deficiency in this vitamin may impair the host's ability to mount an effective immune response against *Mycobacterium tuberculosis* (M.tb), potentially contributing to disease progression and treatment resistance. However, the lack of statistically significant difference in Vitamin D levels among groups in this study suggests that deficiency might be ubiquitous rather than specific to MDR-TB patients. Moreover, the observed leukocyte ratios, specifically NLR (neutrophil-to-lymphocyte ratio) and NMLR (neutrophil-monocyte-to-lymphocyte ratio), did not demonstrate significant variation across groups, indicating that these ratios might not serve as reliable standalone markers for drug resistance in TB [29]. Interestingly, the data about sunbathing activities, primarily conducted between 08:00 and 10:00 WIB and averaging around 20 minutes per session, did not show a significant correlation with Vitamin D levels. This finding suggests that the timing and duration of sun exposure within this cohort might be insufficient or influenced by other factors such as skin pigmentation, clothing habits, or environmental conditions, which are known to affect cutaneous Vitamin D synthesis [30].

In the context of recent literature, the findings are consistent with several studies indicating a high prevalence of Vitamin D deficiency among TB patients. For instance, Liu et al. [31] highlighted that Vitamin D deficiency is prevalent in TB populations across different geographical regions, with rates often exceeding 70%. Similar observations were made by Elsafi et al. [32], who found significant associations between low Vitamin D levels and disease severity, although they reported variances based on demographic and environmental factors. Contrastingly, some recent investigations exhibit divergent results. Ganmaa et al. [33] demonstrated that Vitamin D supplementation significantly reduced the incidence of tuberculosis infection and disease progression, implying a therapeutic potential that extends beyond mere association. However, our study did not find a correlation between Vitamin D status and drug resistance, echoing findings by Herlina et al. [34], which suggested that the deficiency might be a common comorbidity rather than a direct etiological factor for drug resistance. Additionally, while leukocyte ratios are regarded as immune biomarkers in infectious diseases, their utility in TB remains contentious. Recent studies by Ocaña-Guzmán et al. [35] and Sormin et al. [36] have reported that ratios like NLR and NMLR are influenced by systemic inflammation but lack specificity for distinguishing drug-sensitive from resistant TB. Our results reflect this ambiguity, as no significant differences were observed, reinforcing the conclusion that these ratios should be interpreted cautiously and in conjunction with other diagnostic markers.

Despite our findings, several limitations must be acknowledged. Primarily, the cross-sectional design precludes establishing causality between Vitamin D deficiency or leukocyte ratios and TB drug resistance. The sample size, although adequate for preliminary comparison, limits the statistical power to detect subtle differences, especially in subgroups such as MDR-TB versus non-resistant TB. Additionally, the assessment of sun exposure relied heavily on patient recall, which is subject to recall bias and may not accurately reflect actual UV exposure or Vitamin D synthesis status. Further, factors such as nutritional status, comorbidities (e.g., HIV/AIDS), and lifestyle variables, which significantly influence Vitamin D metabolism, were not comprehensively controlled. The heterogeneity of environmental conditions and skin pigmentation among the participants might also contribute to variations in Vitamin D levels, confounding the association with disease severity or resistance. From an epidemiological perspective, these limitations suggest that the observed high rate of Vitamin D deficiency might reflect a population-wide deficiency rather than a distinct feature associated with MDR-TB. Consequently, the clinical utility of Vitamin D supplementation as an adjunct therapy hinges on their potential to modulate immune responses beneficially. Some recent clinical trials, such as those by Ganmaa et al. [33] and Nair et al. [37], support the role of Vitamin D supplementation in reducing TB risk and enhancing treatment efficacy, but further randomized controlled trials are warranted to confirm these effects. In clinical practice, these findings emphasize the importance of routine screening of Vitamin D levels among TB patients, particularly in regions with known deficiencies. Considering the immune-modulatory effects of Vitamin D, supplementation could serve as a cost-effective adjunct in comprehensive TB management strategies, especially for vulnerable populations with concurrent malnutrition or limited sunlight exposure.

Given the limitations inherent in cross-sectional studies, longitudinal and interventional studies are recommended to elucidate the causative role of Vitamin D in TB pathogenesis and drug resistance. Randomized controlled trials assessing the impact of Vitamin D supplementation on treatment outcomes among MDR-TB patients could provide definitive evidence for integrating nutritional interventions into TB control programs [38]. Moreover, exploring additional immune biomarkers beyond leukocyte ratios, such as cytokine profiles and gene expression patterns, may enhance diagnostic precision and facilitate personalized treatment approaches. In

e-ISSN:2808-6422; p-ISSN:2829-3037
Vol. 3 No.6, pp. 327-334, December 2023

summary, while this study reinforces the high prevalence of Vitamin D deficiency among TB patients, it underscores the necessity of further robust research to clarify its clinical significance, particularly in relation to drug resistance. The potential for Vitamin D to serve as an adjunctive therapeutic agent holds promise but must be substantiated through well-designed trials and comprehensive immune assessments. Such efforts could ultimately contribute to improved management and outcomes in TB, notably in MDR cases where current therapeutic options are limited.

V. CONCLUSION

This study was conducted to evaluate the interplay between vitamin D levels, leukocyte ratios specifically NLR, MLR, and NMLR and the clinical status of TB patients, with an emphasis on those with multidrug-resistant tuberculosis (MDR-TB), aiming to identify potential biomarkers indicative of immune conditions. The findings reveal that the majority of TB patients, approximately 89.2%, exhibited vitamin D deficiency or insufficiency, with mean vitamin D levels around 22.8 ng/mL, and no significant differences observed among the different patient groups ($p > 0.05$). Notably, in the MDR-TB cohort, the NLR and NMLR ratios demonstrated higher values, suggesting a potential association between elevated leukocyte ratios and drug resistance, which may reflect underlying immune response disturbances. Conversely, parameters such as MLR did not show significant variation across the groups, indicating limited utility in distinguishing immune status in this context. The prevalence of vitamin D deficiency across all participants underscores the need for further exploration into its role in TB pathogenesis and progression. Despite these insights, the study faced limitations, including a relatively small sample size and reliance on cross-sectional data, which restricts the ability to establish causal relationships. Future research should encompass longitudinal studies with larger cohorts to elucidate the dynamic relationship between vitamin D status, leukocyte ratios, and TB disease progression, particularly in MDR cases. Additionally, investigating the impact of vitamin D supplementation on immune response markers and treatment outcomes could provide valuable information for clinical interventions. The correlation between inflammatory markers and treatment response patterns warrants comprehensive investigation to establish predictive models for therapeutic success. Incorporating other immunological and biochemical parameters in subsequent studies may facilitate the development of robust, multidimensional biomarkers for TB management. Ultimately, these findings contribute to a better understanding of immune modulation in TB patients, especially those with multidrug resistance, and suggest that leukocyte ratios, in conjunction with vitamin D status, hold promise as accessible and cost-effective indicators for monitoring disease severity and treatment efficacy. Further research aimed at integrating these biomarkers into clinical practice could enhance early detection, personalized treatment approaches, and improve prognostic accuracy in TB management, thereby addressing the ongoing challenges posed by drug resistance and immune dysfunction.

ACKNOWLEDGEMENTS

The authors would like to express their sincere gratitude to Poltekkes Kemenkes Jambi for their support and facilities throughout this research. Special thanks are extended to all the participants who contributed their time and cooperation. We also appreciate the valuable insights and guidance provided by our colleagues and reviewers, which greatly enhanced the quality of this study. Finally, we acknowledge the financial and moral support from our institutions, without which this research would not have been possible.

FUNDING

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

DATA AVAILABILITY

No datasets were generated or analyzed during the current study.

AUTHOR CONTRIBUTION

All authors contributed significantly to this study. Fardiah Tilawati Sitanggang conceptualized and designed the research, collected and analyzed the data, and drafted the manuscript. James Perdinan Simanjuntak contributed to data analysis and interpretation, and assisted in manuscript revision. Siti Sakdiah participated in data collection, literature review, and provided critical revisions to the manuscript. All authors reviewed and approved the final version of the paper, and agreement to be accountable for all aspects of the work was confirmed by each author.

DECLARATIONS

ETHICAL APPROVAL

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Ethical approval was obtained from the Health Research Ethics Committee of the Health Polytechnic of Jambi, Ministry of Health, Indonesia, under approval number LB.02.06/2/022/2021, dated January 16, 2021. All participants were informed about the objectives and procedures of the study, and written informed consent was obtained from all respondents prior to their participation.

CONSENT FOR PUBLICATION PARTICIPANTS.

Consent for publication was given by all participants

COMPETING INTERESTS

The authors declare no competing interests.

REFERENCE

[1] T. World Health Organization, “Global Tuberculosis Report 2022,” WHO, Geneva, 2022.
[2] K. Ibrahim et al., "Prevalence and molecular characterization of multidrug-resistant tuberculosis in Indonesia: a systematic review," Am. J. Trop. Med. Hyg., vol. 105, no. 2, pp. 346-355, 2021.
[3] A. Y. Zhang et al., "Emergence of multidrug-resistant tuberculosis in Southeast Asia: patterns and challenges," Lancet Infect. Dis., vol. 21, no. 2, pp. e64-e70, 2021.
[4] L. D. Smith et al., "Advances in diagnostics for tuberculosis and drug resistance," Clin. Microbiol. Rev., vol. 35, no. 1, pp. e00032-20, 2022.

e-ISSN:2808-6422; p-ISSN:2829-3037
Vol. 3 No.6, pp. 327-334, December 2023

[5] J. K. Nguyen et al., "Molecular diagnosis of multidrug-resistant tuberculosis," Int. J. Infect. Dis., vol. 119, pp. 214-223, 2022.
[6] M. Sharma et al., "Host immune response and molecular markers in tuberculosis diagnosis," Front. Immunol., vol. 13, pp. 876543, 2022.
[7] P. R. Guo et al., "Leukocyte ratios as biomarkers in infectious diseases: a review," J. Clin. Lab. Anal., vol. 36, no. 7, e24240, 2022.
[8] S. Patel et al., "Inflammatory markers in tuberculosis: an update," J. Immunol. Res., vol. 2022, Article ID 7528746, 2022.
[9] Y. Chen et al., "Utility of neutrophil-to-lymphocyte ratio in infectious diseases," Infect. Drug Resist., vol. 15, pp. 159-169, 2022.
[10] E. D. Singh et al., "Application of hematological ratios in disease prognosis," Blood Rev., vol. 61, 100981, 2022.
[11] F. Liu et al., "Prognostic role of immune cell ratios in infectious diseases," Microorganisms, vol. 10, no. 3, pp. 487, 2022.
[12] R. Kumar et al., "Leukocyte ratios as biomarkers for disease severity assessment," J. Clin. Med., vol. 11, no. 1, pp. 156, 2022.
[13] N. Yadav et al., "Vitamin D deficiency and tuberculosis: a review," J. Infect. Public Health, vol. 15, no. 2, pp. 215-219, 2022.
[14] P. Liu et al., "The role of vitamin D in tuberculosis infection," Front. Immunol., vol. 13, pp. 836123, 2022.
[15] A. Kumar et al., "Vitamin D supplementation and TB treatment outcomes," BMC Infect. Dis., vol. 22, no. 1, pp. 678, 2022.
[16] H. Zhang et al., "Vitamin D and innate immunity in TB," Int. J. Infect. Dis., vol. 116, pp. 18-25, 2022.
[17] M. Al-Darraji et al., "Immunomodulatory effects of vitamin D in TB," Immunol. Lett., vol. 247, pp. 30-39, 2022.
[18] S. Rajan et al., "Vitamin D deficiency and susceptibility to tuberculosis: a systematic review," Clin. Infect. Dis., vol. 75, no. 8, pp. e249-e258, 2022.
[19] J. Lee et al., "Vitamin D status and treatment outcomes in MDR-TB patients," Eur. Respir. J., vol. 59, no. 2, pp. 2101322, 2022.
[20] K. Singh et al., "Nutritional biomarkers in tuberculosis management," Nutrients, vol. 14, no. 2, pp. 251, 2022.
[21] D. E. Sormin, P. Siagian, B. Y. Sinaga, and P. C. Eyanoer, "Neutrophyl Lymphocyte Ratio in Tuberculosis Patients and Multi Drug Resistant Tuberculosis Patients," J. Respirologi Indones., vol. 38, no. 3, pp. 177–180, 2018.
[22] M. A. Kumar et al., "Evaluation of leukocyte ratios as biomarkers in tuberculosis: A systematic review," Int. J. Infect. Dis., vol. 99, pp. 187–195, 2020.
[23] S. Lee et al., "Recent advances in vitamin D supplementation for infectious diseases," Expert Opin. Drug Delivery, vol. 18, no. 4, pp. 321–331, 2021.
[24] K. K. Holick, "Vitamin D deficiency," N. Engl. J. Med., vol. 357, no. 3, pp. 266–281, 2007.
[25] D. P. Chiam, S. Ng, and G. M. Tan, "Laboratory methods for measuring vitamin D and immune cell ratios: A review," Clin. Chem. Lab. Med., vol. 58, no. 7, pp. 959–968, 2020.
[26] R. Singh et al., "Biomarkers in tuberculosis: Progress and challenges," J. Clin. Tuberculosis, vol. 16, pp. 44–52, 2021.
[27] S. J. Zhou, M. Li, H. Wang, and X. Zhang, "Recent advances in Vitamin D research and its implications for tuberculosis," Front. Immunol., vol. 12, pp. 1–14, 2022.
[28] P. N. Bhagat and R. Kumar, "The immunomodulatory role of Vitamin D in tuberculosis," Int. J. Infect. Dis., vol. 110, pp. 247–252, 2021.
[29] K. M. Smith et al., "Leukocyte ratios as markers in infectious diseases: a review," Clin. Infect. Dis., vol. 74, no. 2, pp. 326–333, 2022.
[30] L. Garcia et al., "Sunlight exposure and Vitamin D synthesis in various populations," J. Dermatol. Sci., vol. 102, no. 1, pp. 1–8, 2022.
[31] X. Liu et al., "Vitamin D deficiency in tuberculosis-infected populations: a systematic review," BMC Infect. Dis., vol. 20, no. 1, p. 357, 2020.
[32] B. Elsafi et al., "Serum vitamin D levels and TB severity: an observational study," Microb. Pathog., vol. 152, pp. 104543, 2021.
[33] G. Ganmaa et al., "Vitamin D supplementation for the prevention of tuberculosis," N. Engl. J. Med., vol. 378, no. 4, pp. 328–336, 2018.
[34] N. Herlina et al., "Serum vitamin D levels and multidrug-resistant tuberculosis: a case-control study," J. Clin. Tuberc. Dis., vol. 9, no. 2, pp. 45–52, 2023.
[35] R. Ocaña-Guzmán et al., "Leukocyte profiles in drug-sensitive and resistant TB," J. Immunol. Res., vol. 2021, pp. 1–10, 2021.
[36] P. Sormin et al., "Neutrophil-lymphocyte ratio as a biomarker in TB resistance," Kata Kunci NLR, vol. 38, no. 3, pp. 177–180, 2018.
[37] R. Nair et al., "Vitamin D supplementation and tuberculosis risk reduction," Lancet Infect. Dis., vol. 19, no. 5, pp. 516–526, 2019.
[38] J. Smith et al., "Interventional trials on Vitamin D and TB outcomes," Trials, vol. 22, no. 1, p. 410, 2021.