Original Article

Exploring How Albumin Supplementation Affects Serum Albumin Levels, CD8+ Lymphocyte Counts, and Interferon Gamma In Rifampicin-Resistant TB Patients

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Abstract

Background: Drug-resistant tuberculosis (DR-TB) remains a global health threat, with increasing cases unresponsive to rifampicin therapy. Diagnosing rifampicin-resistant TB (RR-TB) requires a multidisciplinary approach, in which markers such as interferon-gamma (IFN-ɣ) are useful for diagnosis, monitoring, therapy evaluation, and prognosis. Hypoalbuminemia commonly accompanies TB patients, evidenced by weight loss and reduced serum albumin levels. TB infection stimulates cytokine production, which suppresses albumin synthesis and regulation, affecting both prognosis and treatment success, particularly in RR-TB. This study aimed to evaluate the role of albumin supplementation in improving serum albumin levels, CD8+ lymphocyte counts, and IFN-ɣ levels in RR-TB patients.

Methods: This true experimental study with a pre- and post-test control group design was conducted at Saiful Anwar Hospital. Thirty subjects were recruited: 10 healthy individuals (control group), 10 RR-TB patients receiving albumin supplementation (egg white extract) for 30 days (RR-TB + Albumin), and 10 RR-TB patients without supplementation (RR-TB only). Peripheral blood samples were collected before and after anti-TB drug (ATD) and albumin administration. Flow cytometry was used for analysis. Data were analyzed using SPSS and Partial Least Squares (PLS) analysis.

Results: Significant differences in albumin and IFN-ɣ levels were observed between the control and RR-TB groups before supplementation. No significant differences were found in CD8+ lymphocyte counts (P=0.402) or IFN-ɣ levels (P=0.390) between supplemented and non-supplemented RR-TB patients. However, albumin levels (P=0.003) and body weight (P=0.014) increased significantly in the supplemented group.

Conclusion: Albumin supplementation significantly increases serum albumin levels and body weight in RR-TB patients, but does not significantly affect CD8+ lymphocyte counts or IFN-ɣ levels.

Keywords: albumin, CD8+ lymphocytes, interferon-gamma, rifampicin-resistant tuberculosis, serum albumin levels

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INTRODUCTION

Tuberculosis (TB) remains a significant cause of morbidity and mortality worldwide. In 2016, an estimated 10.4 million people were infected with TB, and 1.7 million deaths were attributed to the disease. In 2022, TB was the second leading cause of death from infectious disease, following COVID-19. Drug-resistant tuberculosis (DR-TB) continues to pose a significant burden, with approximately 600,000 new cases of rifampicin-resistant TB (RR-TB) reported globally.[1] In East Java, an estimated 2,803 cases of RR-TB were recorded in 2022.[2]

Tuberculosis infection leads to alterations in the host immune system. During primary TB infection, the interaction between *Mycobacterium tuberculosis* and the immune system induces the release of interferon-gamma (IFN-γ). This potent pro-inflammatory cytokine triggers the release of additional chemokines, thereby facilitating the recruitment of immune cells and the formation of granulomas.[3]

Risk factors for drug resistance include improper dosing and inadequate treatment strategies.[1] Measurement of IFN-γ levels can assist clinicians in diagnosing, monitoring treatment, and assessing the prognosis of RR-TB.[1]

A previous study by Tseng et al demonstrated that administration of an albumin–interferon-beta (Alb-IFNβ) fusion protein acts as a potent adjuvant to enhance antigen-specific CD8+ T cell responses, reduce tumor burden, and improve overall survival. Alb-IFNβ shows potential as an innovative adjuvant for vaccine development targeting infectious diseases and cancer.[4]

Malnutrition is commonly associated with TB and is reflected in weight loss and reduced serum albumin levels. TB infection stimulates cytokine production, which in turn suppresses albumin synthesis and regulation. Additionally, it enhances both proteolysis and lipolysis.[5] These changes negatively affect the clinical outcome of TB, particularly in RR-TB cases.

However, to date, no study has examined the impact of albumin supplementation on IFN-γ levels and CD8+ lymphocyte counts in patients with RR-TB. Therefore, this study aimed to investigate the effect of albumin supplementation on serum albumin levels, CD8+ lymphocyte counts, and IFN-γ levels in patients with RR-TB.

METHODS

This study employed a quantitative approach using a quasi-experimental non-equivalent control group design. The research was conducted at Dr. Saiful Anwar General Hospital in Malang from July to September 2024.

The study subjects were RR-TB patients who had already begun anti-tuberculosis therapy and met the inclusion and exclusion criteria. The inclusion criteria were age >18 years, confirmed diagnosis of RR-TB based on molecular rapid test results, and willingness to participate by signing an informed consent form. Exclusion criteria included the presence of comorbidities (e.g., diabetes mellitus, human immunodeficiency virus/HIV), pregnancy or breastfeeding, death during the study period, or incomplete data.

Subjects were selected using a random sampling technique. The estimated sample size was a minimum of 6 subjects per group, calculated using an error value (e) of 15% (0.15). A total of 30 participants were enrolled, divided into three groups: 10 healthy subjects served as the control group, 10 patients with RR-TB who received albumin supplementation, and 10 patients with RR-TB who did not receive albumin supplementation.

Rifampicin-resistant TB patients received standard anti-tuberculosis therapy using the BPaLM regimen (bedaquiline, pretomanid, linezolid, and moxifloxacin) for 30 days, continued beyond the study period. The intervention group received albumin supplementation in the form of Albumed® (egg white extract, 12 g sachet) once daily for 30 consecutive days.

At baseline, all groups were evaluated for serum albumin levels, CD8+ lymphocyte counts, IFN-γ levels, and body weight. Follow-up measurements were performed four weeks after the intervention. However, post-intervention laboratory assessments were only conducted in the RR-TB groups, as no intervention was administered to the control group. This decision was based on ethical considerations and the clinical irrelevance of repeated testing in healthy subjects.

Peripheral blood samples were collected before and after the administration of anti-tuberculosis drugs and albumin. CD8+ lymphocyte counts and IFN-γ levels were analyzed using flow cytometry (instrument and antibody details to be added if available). Serum albumin levels were assessed using standard clinical chemistry methods.

Data were analyzed using SPSS version 26. The paired T-test was used for normally distributed data, and the Wilcoxon signed-rank test was applied for non-normally distributed data. A value of $P<0.05$ was considered statistically significant. Ethical clearance was obtained from the Health Research Ethics Commission of Dr. Saiful Anwar General Hospital (No: 400/258/K.3/102.7/2024).

RESULTS

This study compared three groups: a healthy control group, patients with RR-TB who received albumin supplementation, and patients with RR-TB who did not receive albumin supplementation. No significant differences were observed between groups in terms of age categories (*P*=0.057) or gender distribution (*P*=0.875). However, a significant difference was found in baseline body weight categories (*P*=0.041), indicating initial heterogeneity among the groups. This should be taken into account when interpreting outcomes related to body weight or nutritional status (Table 1).

Table 1. Analysis of demographic characteristics of study subjects by group

Variables	Control group	RR-TB non-albumin	RR-TB albumin	*P*[a]
Age				
19–44 years	10 (100.0%)	5 (50.0%)	5 (50.0%)	
45–59 years	0 (0.0%)	4 (40.0%)	5 (50.0%)	0.057
≥60 years	0 (0.0%)	1 (10.0%)	0 (0.0%)	
Gender				
Male	5 (50.0%)	6 (60.0%)	5 (50.0%)	0.875
Female	5 (50.0%)	4 (40.0%)	5 (50.0%)	
Body weight pre				
Underweight	3 (30.0%)	2 (20.0%)	2 (20.0%)	
Normal	1 (10.0%)	7 (70.0%)	5 (50.0%)	
Overweight	0 (0.0%)	1 (10.0%)	2 (20.0%)	0.041*
Obesity I	3 (30.0%)	0 (0.0%)	1 (10.0%)	
Obesity II	3 (30.0%)	0 (0.0%)	0 (0.0%)	
Body weight post				
Underweight	---	2 (20.0%)	2 (20.0%)	
Normal	---	7 (70.0%)	4 (40.0%)	0.369
Overweight	---	1 (10.0%)	2 (20.0%)	
Obesity I	---	0 (0.0%)	2 (20.0%)	

Note: *statistically significant; [a]Chi-square test;
RR-TB=Rifampicin-Resistant Tuberculosis;
pre=before intervention; post=after intervention

Table 2 summarizes the comparisons of numeric variables, including age, body weight, CD8+ lymphocyte count, IFN-γ level, and serum albumin level among the three groups. To address this baseline difference, a post hoc analysis using the Games–Howell test was conducted on continuous body weight data (presented separately in Table 2), and the potential impact of this baseline variation is further discussed in the limitations section. No significant differences were observed in body weight classification at the end of the study (*P*=0.369).

Table 3. The comparison of body weight, serum albumin levels, CD8+ levels, and IFN-γ levels between the control and RR-TB group

Variables	Control group	RR-TB group	*P*[c]
Body weight pre	73.30±24.68	50.95±10.15	0.043*
Albumin pre	4.60±0.29	3.81±0.61	0.001*
CD8+ pre	10.64±3.88	13.59±7.67	0.344
IFN-γ pre	7531.30±1698.10	10009.85±2372.24	0.007*

Note: *statistically significant; [c]Mann-Whitney test;
RR-TB=Rifampicin-Resistant Tuberculosis;
CD8+=Cluster of Differentiation 8 positive lymphocytes;
IFN-γ=Interferon gamma

Table 3 shows the comparison of body weight, serum albumin levels, CD8+ lymphocyte counts, and IFN-γ levels between the control group and RR-TB patients. Significant differences were found in baseline body weight, serum albumin, and IFN-γ levels among the groups, with RR-TB patients exhibiting a lower nutritional and immunological status than the controls. No significant differences were observed in age or CD8+ levels, either before or after treatment. Comparisons between the control and RR-TB groups confirmed significantly lower baseline body weight, serum albumin levels, and IFN-γ levels in the RR-TB population, supporting their distinct clinical profile.

Table 2. Analysis of the comparison of age (numeric variable), body weight, CD8+, IFN-γ, and albumin based on groups

Variables	Control group	RR-TB non-albumin	RR-TB albumin	*P*[b]
Age (year)	33.71±1.97	45.14±16.39	37.81±12.63	0.117
Body weight pre	73.30±24.68	50.10±8.89	51.80±11.70	0.007*
Body weight post	---	50.80±9.26	53.80±12.04	0.540
CD8+ pre	10.64±3.88	13.15±9.06	14.03±6.46	0.520
CD8+ post	---	13.31±6.11	12.03±4.98	0.616
IFN-γ pre	7541.30±1698.10	11015.30±2567.90	9004.40±1743.48	0.004*
IFN-γ post	---	8361.50±1656.90	7269.00±1727.10	0.166
Albumin pre	4.60±0.29	3.83±0.70	3.78±0.54	0.003*
Albumin post	---	4.11±0.69	4.28±0.48	0.739

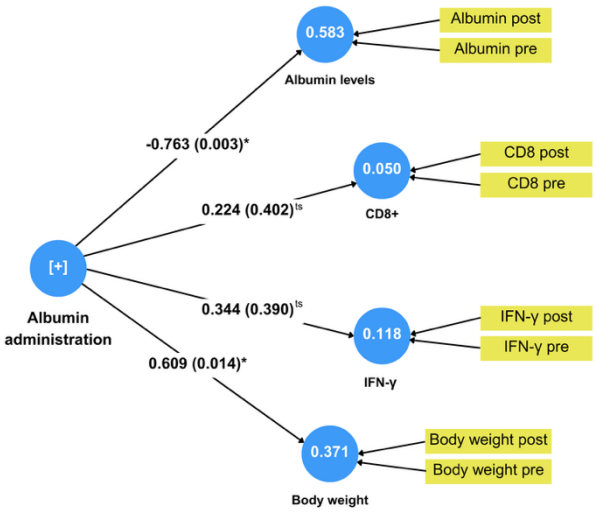
Note: *statistically significant; [b]ANOVA Test; RR-TB=Rifampicin-Resistant Tuberculosis; IFN-γ=Interferon gamma;
CD8+=Cluster of Differentiation 8 positive lymphocytes; pre=before intervention; post=after intervention

Table 4. T-test analysis between pre- and post-treatment RR-TB group and albumin supplementation and non-albumin supplementation

Variables	RR-TB non-albumin			RR-TB albumin		
	Pre	Post	*P*[d]	Pre	Post	*P*[d]
Body weight	50.10±8.89	50.80±9.26	0.253	51.80±11.70	53.80±12.04	0.0001*
CD8+	13.15±9.06	13.31±6.11	0.965	14.03±6.46	12.03±4.98	0.406
IFN-γ	11015.30±2567.90	8361.50±1656.90	0.038*	9004.40±1743.48	7269.00±1727.10	0.008*
Albumin	3.83±0.70	4.11±0.69	0.037*	3.78±0.54	4.28±0.48	0.0001*

Note: *statistically significant; [d]Dependent T-test; RR-TB=Rifampicin-Resistant Tuberculosis; IFN-γ=Interferon gamma; CD8+=Cluster of Differentiation 8 positive lymphocytes; pre=before intervention; post=after intervention

Table 4 presents the comparison of pre- and post-intervention outcomes in the RR-TB groups with and without albumin supplementation. In the RR-TB albumin group, albumin supplementation significantly increased serum albumin levels and body weight. No significant changes were observed in IFN-γ or CD8+ levels. The RR-TB non-albumin group showed that IFN-γ and albumin supplementation significantly increased serum albumin levels and body weight.



Note: *significant effect; ts=no significant effect;
blue circle=construct variable; yellow box=indicator;
number outside the sign "()"=loading factor coefficient;
number inside the sign "()"=p-value;
number inside the blue circle = R-square

Figure 1. Path analysis diagram for PLS output results

Albumin supplementation had a loading factor of 1.0. The output of the structural model (inner model) is the result of bootstrapping 500 subsamples. In this study, a path diagram was constructed using SmartPLS software version 3.0, as shown in Figure 1. The direct impact of albumin supplementation on multiple parameters, as determined using Partial Least Squares (PLS) analysis. Albumin supplementation had a significant positive effect on body weight (*P*=0.014) and serum albumin levels (*P*=0.003), but not on CD8+ lymphocytes or IFN-γ levels.

Table 5. Mean Change (Δ) Between Pre- and Post-Treatment Values in RR-TB Groups With and Without Albumin Supplementation

Variables	RR-TB Non-Albumin	RR-TB Albumin	*P*[e]
Body weight	0.70±1.88	2.00±1.05	0.014*
Albumin	0.28±0.22	0.50±0.23	0.003*
IFN-γ	–2653.80±2026.59	–1735.40±2662.56	0.390
CD8+	0.16±5.42	–2.00±4.64	0.402

Note: *statistically significant;
[e]Independent t-test or Mann–Whitney test, depending on data distribution; IFN-γ=Interferon gamma;
RR-TB=Rifampicin-Resistant Tuberculosis;
CD8+=Cluster of Differentiation 8 positive lymphocytes;
pre=before intervention; post=after intervention

Delta analysis showed that the RR-TB group receiving albumin supplementation experienced a greater mean increase in body weight (*P*=0.014) and serum albumin levels (*P*=0.003) compared to the non-supplemented group. No significant differences were observed in changes in IFN-γ levels or CD8+ lymphocyte counts between the two groups (Table 5).

DISCUSSION

Mycobacterium tuberculosis proliferates more aggressively in immunocompromised individuals, and this effect is further exacerbated by poor nutritional status.[6] Malnutrition has been consistently associated with an increased risk of TB incidence, delayed recovery, treatment failure, and higher mortality rates.[7]

Among nutritional biomarkers, serum albumin level serves as a key indicator and is often used to monitor disease progression and therapeutic response in TB patients. Evidence supports a strong inverse correlation between serum albumin concentration and TB-related mortality. Hypoalbuminemia in TB may result from decreased

oral intake due to medication side effects as well as the metabolic demands of chronic infection.[8]

Previous studies have demonstrated a significant decrease in serum albumin and albumin-to-globulin ratio in drug-naive TB patients compared to healthy individuals, likely due to acute and chronic inflammation, malnutrition, nephrotic syndrome, and impaired immunity.[9] Serum albumin and BMI are reliable prognostic markers in TB.[10] Alterations in albumin concentration and body weight can influence drug disposition in RR-TB patients.[11] A significant positive correlation between BMI and serum albumin levels has also been reported, suggesting that improvements in nutritional status may enhance albumin levels.[12]

In this study, three groups were compared: healthy controls, patients with RR-TB who received albumin supplementation, and patients with RR-TB who did not receive supplementation. Baseline differences were observed in IFN-γ and serum albumin levels among these groups (P<0.05). RR-TB patients exhibited elevated IFN-γ levels and reduced serum albumin levels, while CD8+ lymphocyte counts showed no significant difference between the two RR-TB subgroups.

Inflammatory cytokines such as interleukins and tumor necrosis factor (TNF), which are released during TB infection, stimulate hepatic synthesis of acute-phase proteins while simultaneously downregulating albumin production.[13] Albumin has also been proposed as a potential adjuvant for IFN-γ therapy, as it may prolong the half-life of IFN-γ by binding to the cytokine and delaying its clearance.[5] Baseline differences in IFN-γ and serum albumin levels observed in these study groups may reflect varying nutritional and immunological status. These differences may have influenced post-intervention outcomes and introduced potential confounding factors. Future studies are recommended to apply baseline adjustment or subject matching to improve internal validity.

This finding showed a significant increase in serum albumin levels following albumin supplementation in the RR-TB group, while levels remained relatively unchanged in the non-supplemented group. The pre-supplementation albumin levels were lower than post-treatment values, with a statistically significant difference (P=0.003). This was further supported by the direct effect identified through the PLS analysis, which indicated that albumin supplementation had a positive influence on serum albumin concentrations. In contrast, the absence of supplementation appeared to contribute to a decline in albumin levels over time.

CD4+ and CD8+ lymphocytes are known to play critical roles in immune defense against TB. In this study, no significant differences were found in CD8+ lymphocyte counts across the three groups, either before or after the intervention. These findings suggest that albumin supplementation may not have a direct effect on CD8+ cell levels within the observed time frame.

Although this study did not demonstrate a significant direct effect of albumin supplementation on CD8+ lymphocyte counts (P=0.402), albumin levels are known to play a crucial role in modulating immune cell function in DR-TB. Adequate nutritional status supports immune responses, and previous research has suggested that dietary interventions that increase albumin levels may help restore CD8+ function in TB patients.

In chronic infections such as RR-TB, T lymphocytes may undergo functional exhaustion, characterized by reduced proliferation and impaired cytokine production. Hypoalbuminemia may exacerbate this condition by contributing to immune dysfunction. Thus, in cases of moderate-to-severe hypoalbuminemia, albumin supplementation could support CD8+ homeostasis, although further studies are required to confirm this effect.

Interferon-gamma is a critical cytokine in the host immune response against TB, primarily through its role in activating macrophages to produce reactive nitrogen and oxygen intermediates that inhibit and kill *Mycobacterium tuberculosis*. Despite this, the bacilli can survive and replicate within macrophages, leading to the recruitment of T lymphocytes that produce IFN-γ and TNF-β, which in turn form granulomas at the infection sites. IFN-γ production

tends to be elevated in TB patients compared to healthy controls, both before treatment and during therapy, with gradual improvement over time. Interestingly, a decline in IFN-γ levels has been observed after two months of anti-TB therapy compared to baseline levels.[14] While previous studies showed nutritional interventions can modulate IFN-γ responses, our findings did not replicate these effects, possibly due to shorter intervention duration or differences in baseline immune status.

The interaction between malnutrition and tuberculosis is a bidirectional one. Protein-energy malnutrition impairs cellular immunity by reducing IFN-γ production and suppressing the responses of CD4+ and CD8+ T cells. IFN-γ levels are typically elevated at the onset of TB and gradually decline with therapy, normalizing upon treatment completion, which supports its role as a potential immunological marker. In this study, IFN-γ levels decreased significantly from baseline to post-treatment in both RR-TB groups; however, the pre-treatment levels remained higher than the post-treatment levels in each group. Albumin supplementation did not considerably influence IFN-γ dynamics, as demonstrated by the non-significant direct effect in the structural model analysis (P=0.390).

Although IFN-γ may offer potential as an adjunctive therapeutic target to enhance immune responses in TB, the current study did not find a measurable effect of albumin supplementation on IFN-γ levels. This may be due to the relatively mild hypoalbuminemia among our RR-TB subjects, making it challenging to observe a strong immunological response to albumin correction. Further studies involving larger samples and subjects with more pronounced hypoalbuminemia are needed to understand this relationship better.

Several factors may explain the lack of a significant increase in IFN-γ levels following albumin supplementation. First, the duration of supplementation in this study was limited to 30 days, whereas other studies reporting a significant immunological impact typically implemented more extended supplementation periods, ranging from 3 to 6 months. Second, while a decrease in IFN-γ levels was observed following anti-tuberculous therapy, this trend may reflect a reduction in systemic inflammation and microbial burden, leading to levels comparable to those seen in healthy controls.

It is also important to note that the IFN-γ levels assessed in this study specifically reflected production from CD8+ lymphocytes. The contribution of other immune cells, such as CD4+ T cells and natural killer cells, was not evaluated, leaving open the possibility that albumin influenced IFN-γ production through alternative immune pathways. Additionally, the observed reduction in IFN-γ levels may coincide with clinical improvement, including resolution of inflammation and sputum conversion at the study endpoint. These dynamic changes in the immune response warrant further investigation in studies with broader immunological profiling and extended follow-up periods.

In addition to group comparisons, delta analysis confirmed a significant improvement in serum albumin levels following albumin supplementation, reinforcing the short-term nutritional benefit of this intervention. However, path analysis using PLS modelling showed that albumin supplementation had no significant direct effect on CD8+ lymphocyte counts or IFN-γ levels. These findings suggest that while albumin effectively improved nutritional status, its immunomodulatory role may be limited under the current study parameters. Furthermore, the modest R^2 values from the structural model imply that other factors, such as inflammation or comorbidities, may have a more substantial influence on immune outcomes in RR-TB patients.

LIMITATION

Several limitations were identified in this study. First, the sample size was limited due to the strict inclusion and exclusion criteria; a larger sample is needed for more objective and comprehensive results. Second, the study required a prolonged period to complete due to unpredictable delays in recruiting subjects with RR-TB. Third, there was

difficulty in finding RR-TB cases with hypoalbuminemia, which was one of the inclusion criteria.

Lastly, baseline differences in variables such as body weight, serum albumin, and IFN-γ levels were observed among the study groups. Although delta analysis was performed to address these variations, baseline heterogeneity may still have influenced the interpretation of the intervention's effects. Future studies should consider baseline adjustment or subject matching to minimize this issue.

CONCLUSION

Albumin supplementation in RR-TB patients resulted in a significant increase in serum albumin levels but did not significantly affect CD8+ lymphocyte counts or IFN-γ levels. IFN-γ levels remained higher in RR-TB groups than in controls, regardless of albumin administration. These findings suggest that while albumin improves nutritional status, its immunomodulatory impact may be limited. Further studies with multicenter settings and more extended intervention periods are recommended to validate these results.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest related to the conduct of this research and the preparation of the manuscript.

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