Arginine Supplementation in Patients Diagnosed with Drug-sensitive Pulmonary Tuberculosis

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ABSTRACT

Objective. To determine the effects of arginine in the rates of sputum conversion in patients with drug-sensitive pulmonary tuberculosis.

Methods. Studies from PubMed, Medline, EMBASE, and Cochrane were reviewed and appropriate studies were included. Randomized controlled trials comparing arginine with placebo in adult patients with drug-sensitive pulmonary tuberculosis were included. The risk of bias was assessed using the Cochrane Risk of Bias tool. A meta-analysis of the rate of sputum conversion at 8 weeks, was conducted. Post hoc analyses of sputum conversion at 4 weeks, and cough reduction at 4 and 8 weeks were done.

Results. Three articles included in this study had a pooled population of 452 participants. This meta-analysis showed no significant difference in the sputum conversion at 4 and 8 weeks, with a relative risk of 0.96 (95% CI 0.77-1.20) and 1.07 (95% CI 0.96-1.19), respectively. However, cough was significantly reduced at 4 and 8 weeks, with subtotal relative risks of 0.91 (95% CI 0.82-1.00) and 0.43 (95% CI 0.22-0.81), and a total relative risk for cough reduction of 0.83 (95% CI 0.73-0.93).

Conclusion. While arginine may not significantly reduce sputum conversion rates, it may be used as an adjunct to decrease cough in patients with tuberculosis.

Key Words: arginine, pulmonary tuberculosis, sputum conversion

INTRODUCTION

Description of the condition

Tuberculosis is an infection caused by the bacteria Mycobacterium tuberculosis and is an important cause of mortality worldwide. In 2014, the World Health Organization (WHO) reported an estimated 9.6 million incident cases of tuberculosis (TB) around the world, equivalent to 133 cases per 100 000 population. In the Philippines, prevalence of TB is estimated at 410,000, incidence at 290,000, and mortalities at 10,000 (Global Tuberculosis Report, 2015). Currently, it is recommended that the standard regimen (comprised of isoniazid, rifampicin, pyrazinamide and ethambutol for two months and isoniazid and rifampicin for four months) against TB should be given for at least six months.

Description of the intervention

Several food supplements have already been studied in the past to determine their efficacy as adjunctive treatment for patients with tuberculosis.[1] One such supplement is L-arginine, which is the sole biological precursor of nitric oxide (NO). In macrophages, arginine is converted by nitric

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oxide synthase 2 (NOS2) to NO, concentrations of which correlate with the inhibition of TB bacilli[2,3] and the immune response related to it.[2]

NO directly affects *M tuberculosis* by damaging the cell itself, causing apoptosis of the macrophage.[4] High levels of NO are shown in an in vitro study to have bactericidal properties comparable to that of antibiotics.[5] Low levels of NO, however, are thought to contribute to induction of a non-replicating state for mycobacteria, causing them to be less susceptible to antibiotic treatment.[6] Thus, appropriate levels of NO are hypothesized to contribute to the faster treatment of tuberculosis.

In a study by Ralph and his colleagues in 2015, they measured the Fractional Exhaled NO (FENO) in patients diagnosed with tuberculosis and corresponding controls. These patients were followed up during their course of treatment and serial measurements of FENO were conducted. At the end of the study, the investigators were able to correlate lower NO levels with more severe forms of the disease and delayed mycobacterial clearance from the culture. Owing to this result, the authors recommended evaluating means which increase pulmonary NO as an adjunct to TB treatment.

Significance of the review

Several studies have already tried to determine the efficacy of adding arginine in the treatment of pulmonary tuberculosis. Findings regarding this adjunctive treatment have been conflicting thus far, with some studies showing benefit.[3,7] while others showed no net clinical benefit.[2,8] Hence, the two authors performed a systematic review and meta-analysis to estimate the effect of such micronutrient on TB treatment, mainly measured in the conversion of sputum positive patients on the 8th week of treatment.

Research question

Among adult patients diagnosed with drug-sensitive pulmonary tuberculosis, how effective is supplementation of arginine to the standard regimen as compared to standard regimen alone (or supplemented with a placebo) in the rates of sputum conversion at 8 weeks of treatment?

OBJECTIVE

To determine the effect of arginine supplementation in the rates of sputum conversion in patients diagnosed with drug-sensitive pulmonary tuberculosis.

METHODS

Search strategy

A systematic search of online/electronic databases such as PubMed, EMBASE, the Cochrane Central Register of Controlled Trials, and other collections of published studies, registries of ongoing clinical trials, and unpublished articles was done to identify all studies and publications which dealt with the effect of arginine in the treatment of tuberculosis. No language restrictions were imposed. All the articles were retrieved online. Search terms comprised of the following: the MeSH or Medical Subject Headings terms "tuberculosis", "tuberculosis, pulmonary", "Mycobacterium tuberculosis", and "arginine" as well as free text terms "tuberculosis", "pulmonary tuberculosis", "arginine", and "sputum conversion". Review of references of the retrieved articles were conducted to search for other applicable studies. All relevant articles which were retrieved through these search strategies are considered for inclusion in this study.

Selection criteria

The two authors independently assessed and scanned the titles and abstracts of the retrieved articles and identified those relevant to this systematic review. The following pre-specified inclusion criteria were used for the selection of studies: (1) randomized clinical trials, (2) adult patients diagnosed with drug-sensitive pulmonary tuberculosis, (3) comparison of supplementation of arginine to standard regimen versus standard regimen alone or standard regimen with placebo in the treatment of tuberculosis, and (4) outcomes reported as sputum conversion at 8 weeks of treatment.

Analysis and appraisal of data

Quality assessment of studies

The two authors independently assessed the validity of each included trial using Cochrane Collaboration Risk of Bias tool. Validity was determined in terms of the following characteristics: randomization, similarity of baseline characteristics in the treatment groups, allocation concealment, blinding, follow-up rate, and intention-to-treat analysis. Disputes in the assessment of validity were settled by discussion with the consultant co-author. The judgment on each category was classified as either low risk, high risk or with unclear risk for bias. The results of this quality assessment are stated in the results section, under "Assessment of validity".

Extraction of data

The two authors of this systematic review independently extracted the data using a tailored data extraction form. The following information were gathered: study design, participant characteristics (age, sex, etc), interventions (arginine supplementation: specific formulation and preparation, brand name if any, dosing, duration of treatment), comparison (placebo or none), standard regimen given (dosage and dosing, duration of treatment, etc) and outcome.

Synthesis of data

The data were analyzed and synthesized using Review Manager 5. For dichotomous data, the relative risk was calculated. Data from each trial included were combined

Table 1. Characteristics of included studies

Study (year)	Population (N)	Design	Intervention	Outcome	Study quality
Farazi (2015)	68	Randomized controlled trial	1. Isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months 2. Isoniazid, ethambutol to 6 months 3. 1000mg pure L-arginine hydrochloride OD for 30 days	1. Final treatment success, sputum conversion, weight gain, and clinical symptoms after one and two months were considered as primary outcomes 2. Secondary outcomes were ESR, CRP, and Hg	Good
Ralph (2013)	200	Randomized factorial trial	1. HRZE daily for 2 months 2. HR thrice weekly for 4 months 3. Supplementary L arginine 6g for 8 weeks, active cholecalciferol 50,000 IU at baseline and on day 28 4. L arginine 6g for 8 weeks, placebo cholecalciferol 5.	1. Primary outcome measures were the proportion of participants with negative sputum culture on liquid medium at week 4, and a composite clinical severity score at week 8. The clinical score allocated points at week 8 for %change in weight and FEV1, cough (worse or same, improved, ceased), and presence/absence of sputum and hemoptysis. 2. Secondary outcomes were safety (death, hospitalization, hypercalcemia); sputum smear conversion time (2 consecutive negative smears without a subsequent positive); change in 6-minute walk test, modified St George's Respiratory Questionnaire, chest radiograph severity score, %predicted forced expiratory volume in one second (FEV1); and primary end points stratified by HIV status and ethnicity.	Good
Schon(2003)	184	Randomized controlled trial	1. Isoniazid, pyrazinamide, rifampicin, and streptomycin or ethambutol for 2 months followed by isoniazid and ethambutol for 6 months 2. 1 g arginine for four weeks	1. Sputum conversion at Week 8 2. Cough at Weeks 2 and 8	Good

whenever it was deemed appropriate. A 95% confidence interval is presented with each result. Heterogeneity of the different trials were determined using the I^2 and the chi-square measures of heterogeneity, with a value of 50% and p-value of less than 0.10, respectively, to denote significant heterogeneity. The data gathered are presented in a Forrest plot in the results section.

Subgroup analysis

Post-hoc subgroup analysis was done to compare the following: sputum conversion rates at 4 weeks, and the presence of cough at 4 and 8 weeks.

RESULTS

Results of the search

The original search yielded five articles, and these studies were screened by the authors for inclusion in the review. Two studies were excluded, one because it did not deal with supplementation of arginine for the treatment of PTB, and the other because the intervention done was a comparison between low-dose and high-dose arginine, and not comparison of arginine with a placebo drug. A diagrammatic flow chart is shown in Appendix A. For the full details of the excluded studies, please see Appendix B.

Characteristics of included studies

All three studies included in this meta-analysis were randomized controlled trials comparing the supplementation of arginine versus placebo on top of accepted treatment regimens of pulmonary tuberculosis. For the full details of the included studies, please see Appendix D.

Participants and study sites

The three trials included a total of 452 participants. All these trials included patients 15-60 years old being treated with active tuberculosis. Farazi et al (2015) conducted the study in Iran while Ralph et al (2013) conducted the study in Imika, Indonesia. Schon et al (2003) conducted the study in Ethiopia. All patients were treated in Directly Observed Treatment Shortcourse facilities in their respective countries.

Interventions

The interventions in the three trials differed in the amount of arginine supplemented (ranging from 1g to 6g) and the duration for which arginine was being given (ranging from 4 weeks to 8 weeks). One study included supplementation of Vitamin D as an additional intervention.[4]

Table 1 shows a summary of the characteristics of the studies included, and Appendix C shows a more detailed version of this summary.

Table 2. Summary of the assessment of validity of the included studies

	Farazi 2015	Ralph 2013	Schon 2003
Method of random sequence generation (Selection bias)	A	A	A
Method of allocation concealment (Selection bias)	A	A	A
Incomplete outcome data/Loss of participant to follow up (Attrition bias)	A	C	A
Blinding of participants and personnel (Performance bias)	A	A	A
Blinding of outcome assessment (Detection bias)	A	B	B
Selective reporting/Intention to treat Analysis (Reporting bias)	B	A	A
Other bias	NA	NA	NA

Legend: A: Low risk, B: Unclear, C: High risk

Assessment of validity

The risk of bias assessment is summarized in Table 2.

Allocation

All three included studies showed an adequate method of randomization. These studies also demonstrated satisfactory methods for preservation of allocation sequence.

Blinding

All three included studies showed blinding of both the participants and the caregivers. However, only one study showed clear indication of blinding of the outcome assessor.[3] The authors both deemed that it was unclear whether the outcome assessor was blinded in the remaining two studies.

Incomplete outcome data

One study was considered at high risk of attrition bias due to the high number of drop-outs.[4]

Selective reporting

Two out of the three studies reported intention-to-treat analyses and were thus deemed to be at low risk for reporting bias. However, this was not clearly stated in the study by Farazi et al.

Effects of Interventions

Three studies[3,4,5] have compared the effect of arginine supplementation in the treatment of pulmonary tuberculosis in terms of sputum conversion. Figure 1 shows the results of the individual studies and the pooled effect of the treatment. The combined risk ratio from three studies is 1.07 (95% CI 0.96-1.19). Two studies,[3,4] on the other hand, evaluated sputum conversion at 4 weeks (Figure 2). The risk ratio for the pooled results is 0.96 (95% CI 0.77-1.20).

Several studies also evaluated cough reduction in patients being supplemented with arginine. The overall risk ratio, computed by combining the three studies, was 0.83 (95% CI 0.73-0.93). Two studies[3,4] measured cough reduction after a four-week period. The risk ratio was 0.91 (95% CI 0.82-1.00). Farazi et al. and Schon et al. also measured cough reduction at 8 weeks, with a subtotal risk ratio of 0.43 (95% CI 0.22-0.81). These results are illustrated in Figure 3.

DISCUSSION

Summary of main results

Sputum conversion

The results of pooled analysis showed no significant change in the sputum conversion between the treatment group and the control group. The relative risk of sputum conversion at 4 weeks (RR 0.96, CI 0.77-1.20) is relatively similar to that of the analysis after 8 weeks of treatment (RR 1.07, 0.96-1.19). There was no noted significant heterogeneity between the two groups.

Cough reduction

The available trials showed increased rates of cough reduction both at 4 and 8 weeks of treatment supplemented with arginine, with a more significant improvement shown for the longer time interval. Separately, the analysis for cough reduction at 4 weeks and 8 weeks did not show significant heterogeneity. When pooled together, however, the chi^2 showed significant p-value of less than 0.10 (p-value=0.003) and an I^2 of 80.7%, both measures identifying significant heterogeneity. This could be attributed to the difference in the time interval at which cough reduction was assessed.

Overall completeness and applicability of evidence

The included studies slightly differed in the interventions that they used. Most of them used isoniazid, rifampicin, pyrazinamide, and ethambutol as part of the standard regimen for the first two months of treatment; however, most used isoniazid and ethambutol for the succeeding six months of treatment, while one study used isoniazid and rifampicin thrice weekly for four months. The available trials also varied with the source, dosage, and frequency of administration of their separate arginine supplementation regimens. These differences in the characteristics of the utilized interventions could have accounted for the heterogeneity seen in some of our analyses.

L-arginine supplementation may be available through increasing intake of food rich in arginine such as chocolate, peanuts, coconut, soy beans, almond, dairy products, white flour, and wheat or through intake of L-arginine capsules which are readily available.

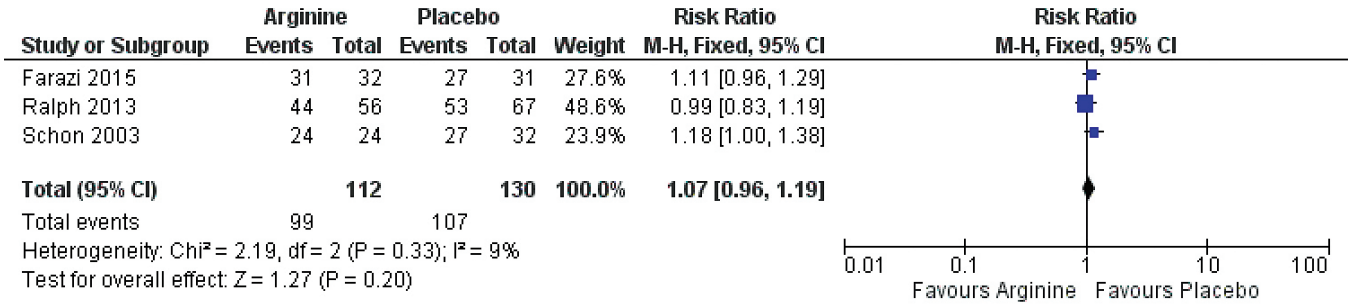

Figure 1. Comparison of arginine and placebo. Outcome 1 sputum conversion at 8 weeks.

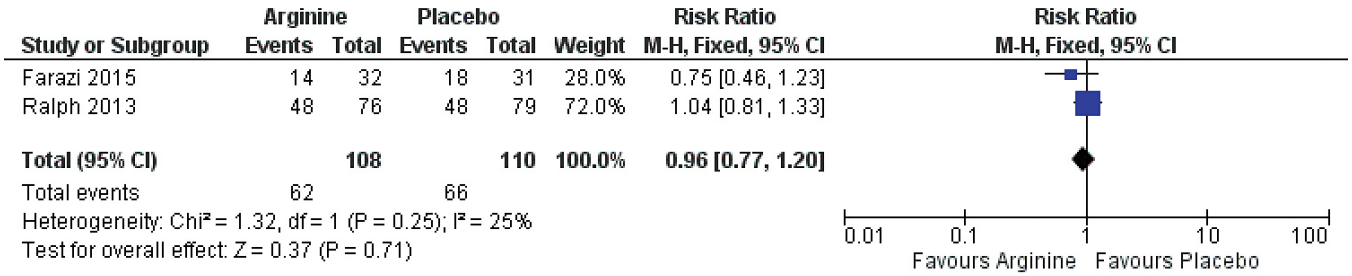

Figure 2. Comparison of arginine and placebo. Outcome 1 sputum conversion at 4 weeks.

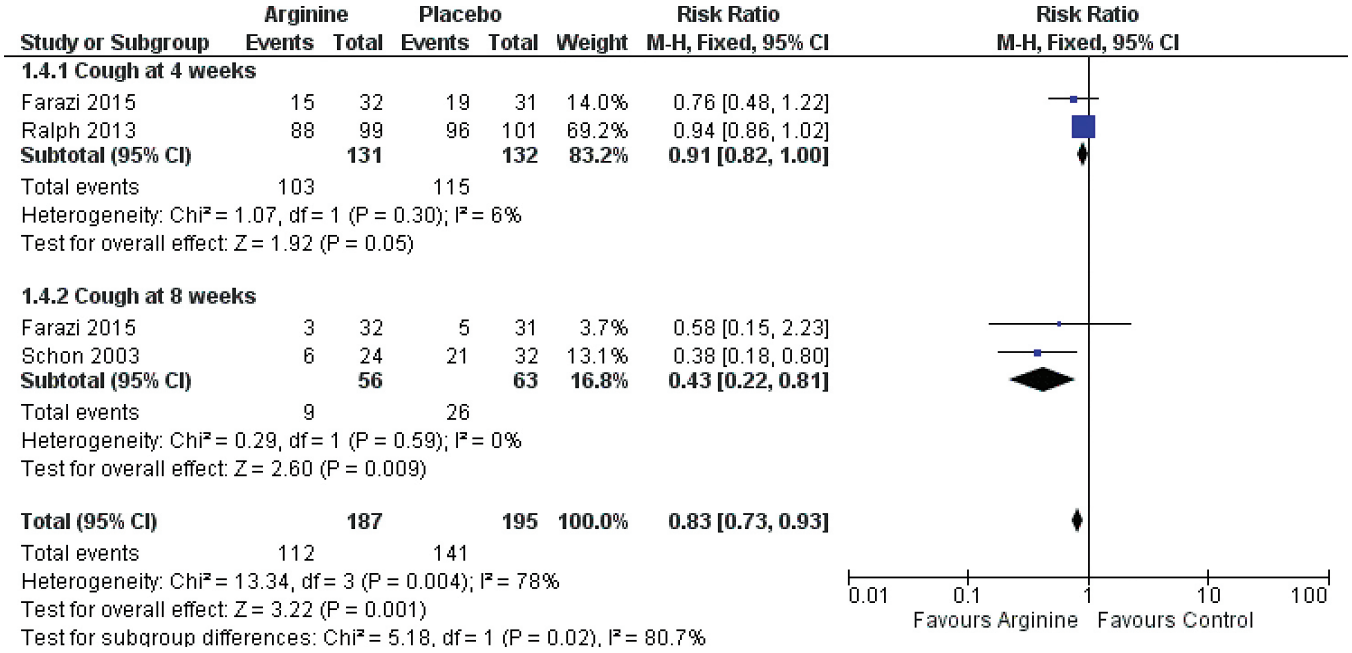

Figure 3. Comparison of arginine and placebo. Outcome 2 cough.

Quality of the evidence

The quality of evidence has been assessed using the Cochrane Risk of Bias Tool and is displayed in the characteristics of included studies and assessment of validity tables. 'Good' quality evidence implies that we could have some confidence in the result, but further research evidence might still be useful. 'Poor' quality reflects a low level of confidence in the result.

Agreements and disagreements with other studies or reviews

Arginine supplementation in adult patients with drug-sensitive pulmonary tuberculosis has also been assessed by one previous Cochrane review.[7] The study made by Sinclair in 2011 assessed one trial and found no significant increase in sputum smear conversion at eight weeks of treatment. They, however, reported a decrease in cough at second and eighth weeks of treatment.

CONCLUSION

Arginine is not recommended to increase sputum conversion rates in patients with pulmonary tuberculosis but it may be helpful in improving cough reduction.

Implications for practice

Arginine supplementation may be useful in decreasing cough in patients with pulmonary tuberculosis. This may help in decreasing the infectivity of patients with the disease, given that pulmonary tuberculosis is primarily transmitted via airborne respiratory droplets.

Implications for research

The failure to demonstrate faster rates of sputum conversion with arginine supplementation does not imply that it is not effective. Further studies might still be done involving a higher dosage of arginine and a more adequate sample size. For other possible benefits, it would have been useful if some standardization of outcome measurements were made (for example, a tuberculosis response scoring system), to more completely assess treatment response.

Limitations of the study

The sample size may be too small to see a significant effect in terms of sputum conversion. Moreover, the differences in the dosing of the interventions in each study may account for differences in their results.

Statement of Authorship

All authors have approved the final version submitted.

Author Disclosure

All the authors declared no conflict of interest.

Funding Source

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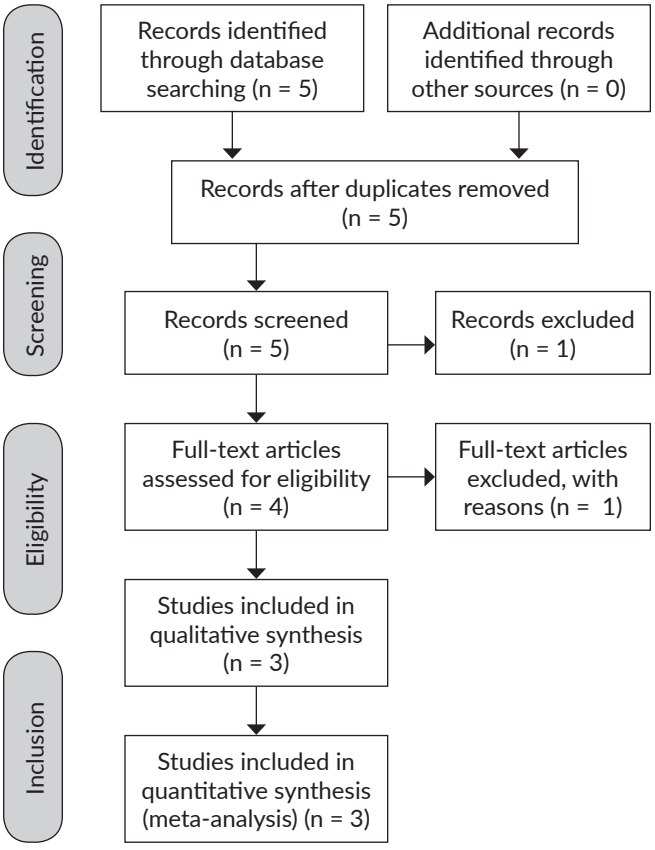
APPENDICES

Appendix A. Prisma flow chart



Appendix B. Characteristics of excluded studies

Schon 2011

Methods	Randomized controlled trial Duration: Feb 2004 - Dec 2006, with a final follow up until August 2007 Generation of allocation sequence: described as "randomized" Allocation concealment: sealed individual envelopes Blinded participants: no Blinded providers: no Blinded outcomes assessors: unclear Inclusion of randomized participants in the analysis: 173/180 (96%) of those who met the inclusion criteria
Participants	Number: 180 enrolled; outcomes presented for 173 Inclusion criteria: age above 15 years old and acid fast bacilli (AFB) smear positive tuberculosis by microscopy as recommended by the WHO for DOTS Exclusion criteria: patients requiring hospital admission, peanut allergy, pregnancy, previous treatment for TB, clinical signs or medical treatment indicating any concomitant disease such infectious diseases other than TB/HIV
Interventions	Group 1: HRZE for 2 months HE for 6 months 30g of wheat crackers (daboqolo) = 0.1 g arginine for 4 weeks Group 2: HRZE for 2 months HE for 6 months 30g of peanuts = 1 g arginine for 4 weeks
Outcomes	1. Cured, Not cured 2. Smear conversion, weight gain >10%, cough, SR (mm/h), CXR improvement, Eno (ppb), uNO (microM)
Notes	Location: Ethiopia Setting: Direct Observed Treatment Short-Course (DOTS) Clinic at Gondar University Hospital, Gondar, Ethiopia

Appendix C. Characteristics of included studies

Farazi 2015

Methods	Randomized controlled trial Duration: December 2012-May 2014 Generation of allocation sequence: described as "randomized" Allocation concealment: sealed envelopes Blinded participants: yes Blinded providers: yes Blinded outcomes assessors: unclear Inclusion of randomized participants in the analysis: 63/68 (93%) of those who met the inclusion criteria
Participants	Number: 68 enrolled; outcomes presented for 63 Inclusion criteria: age of ≥15 years and smear-positive pulmonary TB, as recommended by the World Health Organization (WHO) for DOTS Exclusion criteria: hospitalization, pregnancy, clinical signs of any comorbidity (such as diabetes mellitus, malignancy, hepatic or renal failure, HIV+, etc) according to physician's judgement and patient's medical documents, patients who received L-arginine supplement during the past month, and allergic reactions to L-arginine
Interventions	Group 1: Isoniazid, rifampicin, pyrazinamide ,and ethambutol for 2 months Isoniazid, ethambutol to 6 months 1000mg pure L-arginine hydrochloride OD for 30 days Group 2: Isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months Isoniazid, ethambutol to 6 months Placebo with 1000mg of sugar OD for 30 days
Outcomes	1. Final treatment success, sputum conversion, weight gain, and clinical symptoms after one and two months were considered as primary outcomes 2. Secondary outcomes were ESR, CRP, and Hg
Notes	Location: Iran Setting: DOTS clinics in Markazi province

Ralph 2013

Methods	Randomized factorial trial Duration: June 12, 2008 - February 22, 2010 Generation of allocation sequence: described as "randomized" Allocation concealment: opaque envelope Blinded participants: yes Blinded providers: yes Blinded outcomes assessors: unclear Inclusion of randomized participants in the analysis: 155/200 (78%) of those who met the inclusion criteria
Participants	Number: 200 enrolled; outcomes presented for 155 Inclusion criteria: sputum smear positive, >15 years, agreeing to stay in Timika for 6 months and providing written informed consent Exclusion criteria: pregnant, with hypercalcemia, not previously treated for TB
Interventions	Group 1: HRZE daily for 2 months HR thrice weekly for 4 months A: Supplementary L arginine 6g for 8 weeks, active cholecalciferol 50, 000 IU at baseline and on day 28 B: L arginine 6g for 8 weeks, placebo cholecalciferol Group 2: HRZE daily for 2 months HR thrice weekly for 4 months A: Placebo L arginine, active cholecalciferol 50, 000 IU at baseline and on day 28 B: Placebo L arginine, Placebo Vitamin D
Outcomes	1. Primary outcome measures were the proportion of participants with negative sputum culture on liquid medium at Week 4, and a composite clinical severity score at Week 8. The clinical score allocated points at Week 8 for % change in weight and FEV1, cough (worse or same, improved, ceased), and presence/absence of sputum and hemoptysis. 2. Secondary outcomes were safety (death, hospitalization, hypercalcemia); sputum smear conversion time (2 consecutive negative smears without a subsequent positive); change in 6-minute walk test, modified St George's Respiratory Questionnaire, chest radiograph severity score, %predicted forced expiratory volume in one second (FEV1); and primary end points stratified by HIV status and ethnicity.
Notes	Location: Timika, Indonesia Setting: Timika TB clinic

Schon 2003

Methods	Randomized controlled trial Duration: December 2000 - December 2001 Generation of allocation sequence: described as "randomized" Allocation concealment: sealed copy of treatment code Blinded participants: yes Blinded providers: yes Blinded outcomes assessors: unclear Inclusion of randomized participants in the analysis: 120/184 (65%) of those who met the inclusion criteria
Participants	Number: 184 enrolled; outcomes presented for 120 Inclusion criteria: age of 15–60 yrs and acid fast bacilli (AFB) smear-positive TB by microscopy, using Ziehl-Nielsen staining, as recommended by the World Health Organization (WHO) for DOTS Exclusion criteria: hospitalization, pregnancy or clinical signs of any concomitant disease, such as diabetes mellitus, acute renal failure, or infectious diseases other than TB/HIV
Interventions	Group 1: Isoniazid, pyrazinamide, rifampicin, and streptomycin or ethambutol for 2 months followed by isoniazid and ethambutol for 6 months 1 g arginine for four weeks Group 2: Isoniazid, pyrazinamide, rifampicin, and streptomycin or ethambutol for 2 months followed by isoniazid and ethambutol for 6 months 1 g placebo for four weeks
Outcomes	1. Sputum conversion at Week 8 2. Cough at Weeks 2 and 8
Notes	Location: Ethiopia Setting: Direct Observed Treatment Short-Course (DOTS) Clinic at Gondar Hospital

Appendix D. Data extraction sheets

Trial ID: Farazi 2015	Extractor: Cheng, So	Year of Publication: 2015
Title: Arginine adjunctive therapy in active tuberculosis		
Authors: Aliasghar Farazi, Omid Shafaat, Masoomeh Sofian, Manijeh Kahbazi		
Citation: Farazi A, Shafaat O, Sofian M, Kahbazi M. Arginine Adjunctive Therapy in Active Tuberculosis. Tuberculosis Research and Treatment. 2015;2015:1–5		

Participants

Inclusion criteria: 1. New cases of smear positive TB (December 2012-May 2014, DOTS clinics in Markazi province) 2. Age ≥ 15years old and smear positive TB (two or three positive morning sputum samples or one of three positive sample with a chest x-ray and clinical symptoms suggestive of pulmonary TB)
Exclusion criteria: 1. Hospitalizations 2. Pregnancy 3. Clinical signs of any co-morbidity such as DM, malignancy hepatic or renal failure, HIV, etc according to the physician's judgment and patient's medical documents 4. Patients who received L-arginine supplementation in the past month 5. Allergic reaction to L-arginine

Intervention

Experiment group: 1. Isoniazid, rifampicin, pyrazinamide and ethambutol for 2 months 2. Isoniazid, ethambutol to 6 months 3. 1000mg pure L-arginine hydrochloride OD for 30 days
Control/Comparison group: 1. Isoniazid, rifampicin, pyrazinamide, and ethambutol for 2 months 2. Isoniazid, ethambutol to 6 months 3. Placebo with 1000mg of sugar OD for 30 days

Quality/Assessment/Risk of Bias Table

Domain	Judgment Low Risk/High Risk/Unclear	Support for Judgment/ Description
Method of random sequence generation (Selection bias)	Low risk	"Patients were randomly assigned to either the arginine group or the placebo group. The randomization is 1:1 for the 2 groups and was performed by using random number tables for the two assign regimen" p.2
Method of allocation concealment (Selection bias)	Low risk	"Preparation of the sealed envelopes was performed by a member of the staff who was not directly involved in the study and a sealed copy of the treatment code was kept by the project leader until all data had been collected and analysed" p.2
Incomplete outcome data/Loss of participant to follow up (Attrition bias)	Low risk	
Blinding of participants and personnel (Performance bias)	Low risk	Placebo-controlled trial
Blinding of outcome assessment (Detection bias)	Unclear	No mention of blinding of outcome assessor, just assumed
Selective Reporting/Intention to treat analysis (Reporting bias)	Low risk	
Other bias		

Outcomes

Outcome measures (Dichotomous)	Total number of participants			
	Intervention group N=32		Control group N=31	
	Events	Total	Events	Total
Primary outcome(s)				
Sputum conversion (smear negative) at 8 weeks	31	32	27	31
Secondary outcome(s)				
Constitutional symptoms at 4 weeks	11		19	
Constitutional symptoms at 8 weeks	3		8	
Cough at 4 weeks	15		19	
Cough at 8 weeks	3		5	
BMI < 18.5 at 4 weeks	4		11	
BMI < 18.5 at 8 weeks	1		6	
Positive sputum smear at 4 weeks	14		18	
Anemia at 4 weeks	6		12	
Anemia at 8 weeks	2		7	
Increasing ESR at 4 weeks	25		26	
Increasing ESR at 8 weeks	11		15	
Increasing CRP at 4 weeks	8		16	
Increasing CRP at 8 weeks	2		6	
Final treatment success	32		31	

Trial ID: NCT00677339	Extractor: Cheng, So	Year of publication: 2013
Title: L-arginine and vitamin D adjunctive therapies in pulmonary tuberculosis: a randomised, double-blind, placebo-controlled trial		
Authors: Anna P. Ralph, Govert Waramori, Gysje J. Pontororing, Enny Kenangalem, Andri Wiguna, Emiliana Tjitra, Sandjaja, Dina B. Lolong, Tsin W. Yeo, Mark D. Chatfield, Retno K. Soemanto, Ivan Bastian, Richard Lumb, Graeme P. Maguire, John Eisman, Ric N. Price, Peter S. Morries, Paul M. Kelly, Nicholas M. Anstey		
Citation: Ralph AP, Waramori G, Pontororing GJ, Kenangalem E, Wiguna A, Tjitra E, et al. L-arginine and Vitamin D Adjunctive Therapies in Pulmonary Tuberculosis: A Randomised, Double-Blind, Placebo-Controlled Trial. PLoS ONE. 2013;8(8)		

Participants

Inclusion criteria: 1. Sputum smear positive, > 15 yo, not pregnant, without hypercalcemia (ionized calcium ≤1.32 mmol/L), not previously treated for TB (p2)
Exclusion criteria: 1. Participants with protocol violations or poor adherence were excluded (p5)

Intervention

Experiment group: Directly-observed anti-TB therapy (weight-dosed rifampicin, isoniazid, pyrazinamide, ethambutol daily x 2 months, then rifampicin, isoniazid thrice weekly x 4 months) with either of the ff: 1. Supplementary active L-arginine 6 g daily for 8 weeks + active cholecalciferol 50000 IU at baseline and on day 28 2. Active L-arginine + placebo cholecalciferol 3. Placebo L-arginine + active cholecalciferol
Control/Comparison group: Directly-observed anti-TB therapy (weight-dosed rifampicin, isoniazid, pyrazinamide, ethambutol daily x 2 months, then rifampicin, isoniazid thrice weekly x 4 months) with: 1. Placebo L-arginine + placebo cholecalciferol

Quality/Assessment/Risk of Bias Table

Domain	Judgment Low risk/High risk/Unclear	Support for judgment/ description
Method of random sequence generation (Selection bias)	Low risk	A block random allocation sequence stratified by ethnicity was generated and remained concealed from all investigations throughout the study (p3)
Method of allocation concealment (Selection bias)	Low risk	Independent assistants prepared study medication packs, labelling them with a code corresponding to the randomisation sequence. Participants were assigned the next sequential code, and dispensed an opaque envelope containing the study medications. (p3)
Incomplete outcome data/Loss of participant to follow up (Attrition bias)	High risk	
Blinding of participants and personnel (Performance bias)	Low risk	This study was a double-blind factorial 2x2 design (p3)
Blinding of outcome assessment (Detection bias)	Unclear	
Selective reporting/Intention to treat analysis (Reporting bias)	Low risk	Outcomes were analysed according to the arm to which the participant was originally designed (p5) In modified intention-to-treat analyses, participants with protocol violations or poor adherence were excluded (p5)
Other bias		

Outcomes

Outcome measures (Dichotomous)	Total number of participants = 200			
	Intervention group Arms AB (Arginine ± Vitamin D): N=99		Control group Arms CD (No Arginine ± Vitamin D): N=101	
	Events	Total	Events	Total
Primary outcomes				
Culture negative at week 4	48/76 (63.2%)		48/79 (60.8%)	
<5% weight gain	49 (57%)		49 (52%)	
5.0-9.9% weight gain	22 (26%)		26 (27%)	
≥10% weight gain	15 (17%)		20 (21%)	
≥10% fall in FEV1	4 (5%)		13 (14%)	
< 10% fall or <10% improvement	44 (55%)		50 (55%)	
≥10% FEV1 improvement	32 (40%)		28 (31%)	
Worse/same cough	6 (7%)		9 (10%)	
Improved cough	69 (80%)		77 (85%)	
Ceased cough	11 (13%)		5 (6%)	
Sputum present	63 (73%)		68 (72%)	
Hemoptysis present	2 (2%)		1 (1%)	
Secondary outcomes				
Culture negative at week 8	44/56 (79%)		53/67 (79%)	
HIV (-)	27/36 (75%)		42/55 (76%)	
HIV (+)	8/9 (89%)		5/5 (100%)	
Time to smear negativity: Median weeks (IQR)	4 (2-8)		5 (2-8)	
Death	1%		1%	
Hospitalization	3%		2%	
Mild hypercalcemia (1.33-1.39 mmol/L)	12%		14%	
Mod hypercalcemia (1.40-1.49 mmol/L)	0%		3%	
Severe hypercalcemia (≥1.33-1.39 mmol/L)	0%		0%	
Nausea	33%		34%	
Vomiting	21%		14%	
CNS symptom (headache, dizziness, delirium)	51%		56%	
Itch	43%		44%	
Rash	21%		27%	
Arthralgia	59%		59%	

Trial ID:	Extractor: Cheng, So	Year of publication: 2003
Title: Arginine as an adjuvant to chemotherapy improves clinical outcome in active tuberculosis		
Authors: T. Schon, D. Elias, F. Moges, E. Melese, T. Tessema, O. Stendahl, S. Britton, T. Sundqvist		
Citation Schon T, Elias D, Moges F, et al. Arginine as an adjuvant to chemotherapy improves clinical outcome in active tuberculosis. Eur Respir J 2003;21:483-488 doi: 10.1183/09031936.03.00090702		

Participants

Inclusion criteria: 1. Age of 15–60 yrs and acid fast bacilli (AFB) smear-positive TB by microscopy, using Ziehl-Nielsen staining, as recommended by the World Health Organization (WHO) for DOTS (p484)
Exclusion criteria: 1. Hospitalisation, pregnancy or clinical signs of any concomitant disease, such as diabetes mellitus, acute renal failure, or infectious diseases other than TB/HIV (p484)

Intervention

Experiment Group: isoniazid, pyrazinamide, rifampicin and streptomycin or ethambutol during the intensive phase of 2 months followed by isoniazid and ethambutol for 6 months. 1. Supplementation with 1 g arginine daily, administered orally for 4 weeks.
Control/Comparison group: isoniazid, pyrazinamide, rifampicin and streptomycin or ethambutol during the intensive phase of 2 months followed by isoniazid and ethambutol for 6 months. 1. Supplementation with identical capsules of 1 g placebo (State Pharmacy of Sweden) daily, administered orally for 4 weeks.

Quality/Assessment/Risk of Bias Table

Domain	Judgment Low risk/High risk/Unclear	Support for judgment/ Description
Method of random sequence generation (Selection bias)	Low risk	In a randomised double-blind study (abstract)
Method of allocation concealment (Selection bias)	Low risk	A sealed copy of the treatment codewas kept by the project leader until all data had been collected and analysed.
Incomplete outcome data/Loss of participant to follow up (Attrition bias)	Low risk	Protocol was by intention-to-treat but as the drop out rate was low (4.2%, five of 120) and equally distributed among groups, the authors did not include these patients in the statistical analysis (p484)
Blinding of participants and personnel (Performance bias)	Low risk	The study was double blinded
Blinding of outcome assessment (Detection bias)	Unclear	
Selective reporting/Intention to treat analysis (Reporting bias)	Low risk	Protocol was by intention-to-treat (p484)
Other bias		

Additional Information Requested

Notes

Outcomes

Outcome Measures (Dichotomous)	Total number of participants =			
	Intervention Group N=		Control Group N=	
	Events	Total	Events	Total
Primary outcome(s)				
Sputum conversion at week 8 (HIV-/TB+)	24	24	27	32
Secondary outcome(s)				
Cough at week 2 (HIV-/TB+)	16	24	30	32
Cough at week 8 (HIV-TB+)	6	24	21	32