Impact of L-arginine as an adjuvant drug in smear-positive TB cases

[1]Kumar Raja Madasu - PhD Scholar, Department of Pharmacology, Santosh Medical College, Santosh Deemed To be University, Ghaziabad, NCR-Delhi.
[2]Dr. Virendra Singh - Associate Professor,Department of Respiratory Medicine, KMMCH, Mathura.
[3]Dr. Jyotsna Sharma - Professor, Department of Pharmacology, Santosh Medical College, Santosh Deemed To be University, Ghaziabad, NCR-Delhi.
[4]Dr. Shaktibala Dutta - Professor & HOD, Department of Pharmacology, Santosh Medical College, Santosh Deemed To be University, Ghaziabad, NCR-Delhi.

Corresponding: Dr. Jyotsna Sharma -Professor, Department of Pharmacology, Santosh Medical College, Santosh Deemed To be University, Ghaziabad, NCR-Delhi.

Abstract

Smear-positive tuberculosis (TB) is a global public health issue. Standard treatment regimens have medication resistance and extensive treatment duration. Due to its immunomodulatory and anti-inflammatory properties, L-arginine may be an adjuvant therapy for TB. This study examined L-arginine as an adjuvant medication in smear-positive TB patients. From December 2020 to June 2021, a tertiary care hospital in Mathura, K.M Medical College & Hospital, conducted a randomised controlled experiment. 150 patients were randomly randomised to L-arginine and 150 to placebo and evaluated for clinical and biochemical data. Adding L-arginine to anti-TB medication considerably improved clinical outcomes. In conclusion, L-arginine supplementation may be an effective adjuvant therapy for smear-positive TB patients, increasing treatment outcomes, constitutional symptoms, CRP, and cough. Further research is needed to validate these findings and evaluate L-arginine as a TB treatment supplement.

DOINumber:10.48047/nq.2022.20.22.NQ10268 NeuroQuantology2022;20(22):2759-2767

Introduction

Mycobacterium tuberculosis, which causes tuberculosis (TB), is a bacterial infection that usually affects the lungs but can also damage the gut, bones, and joints (Moule et al. 2020). According to data published by the WHO, 10 million people worldwide (5.7 men, 3.2 women, and 1.1 children) were reported to have been afflicted by TB in 2018, with around 1.5 million people died as a result of the disease (WHO Global Tuberculosis Report, 2019). The growth of drug-resistant tuberculosis and the use of a combination of medicines, which are known to have a number of adverse effects, are both factors in the treatment of TB. Thus, current research has focused on using adjuvant medications to increase the effectiveness of the available TB medications (Amin et al. 2021; Fatima et al. 2021). In order to increase the effectiveness of the treatment and shorten the course of treatment, adjuvant therapy for tuberculosis (TB) refers to the use of supplementary medications or therapies in addition to the regular anti-TB therapy. Targeting the numerous factors that contribute to the onset and progression of TB symptoms, adjuvant therapy aims to improve the results of TB treatment (Young et al. 2020). One such adjuvant medicine that has been studied for

its possible effect on the treatment of tuberculosis is l-arginine.

Particularly in foods like meats and nuts, L-arginine is abundant. Endogenous L-arginine production is possible, although it may be impaired in young infants and those with specific diseases (such as illness or trauma) (Farazi et al. 2015). Many studies using animal models demonstrate that nitric oxide plays a crucial role in immunomodulatory processes, notably in the synthesis of nitric oxide (NO) and macrophage activation, which are both crucial for host resistance during the acute phase of tuberculosis (Jamaati et al. 2017; Mishra et al. 2017; Chandra et al. 2022).

L-arginine is a preferable choice for TB therapy since NO is a powerful microbicidal agent that is essential for controlling Mtb in mice macrophages and is also known to have antimycobacterial capabilities (Chan et al, 1992; MacMicking et al, 1997). In addition to its involvement in NO generation and its contribution to host defence, L-arginine supports the modulation of various immune cells such as neutrophils release the L-arginine sequestering enzyme arginase into the extracellular environment (McKell et al. 2021). L-arginine is also required for the activation and cytotoxicity of NK cells (Munder and Arginase, 2009). L-effect arginine's on the treatment of tuberculosis has been the subject of several research, with varying degrees of success (Vrieling et al. 2019; Crowther et al. 2021).

Recent studies revealed that patients with pulmonary TB required much less time to convert sputum cultures when L-arginine was added to the conventional TB medication regimen (Weiner et al. 2012; Farazi et al. 2015). Another research published in 2017 indicated that L-arginine supplementation did not enhance clinical outcomes in individuals with active TB (Ralph et al. 2017).

However, it should be recognized that these studies have drawbacks, such as small sample numbers, varied doses of L-arginine, and diverse study methodologies. The effectiveness and safety of L-arginine as an adjuvant medication in the treatment of TB must thus be determined via more study. The goal of the current study was to examine the effects of L-arginine, an adjuvant drug used in addition to first-line medicines for the treatment of tuberculosis.

Methods

Patients

To evaluate the effects of L-arginine as an adjuvant drug in active tuberculosis, we included newly diagnosed smear-positive TB cases with informed consent from December 2020 to June 2021 at the K.M Medical College & Hospital and Mathura. Patients older than 15 to 60 years, smear-positive TB by microscopy, no other chronic diseases other than TB, radiological findings, the World Health Organization's (WHO) DOTS 1 recommendation for Ziehl-Nieelsen staining, patients older than 15 to 60 years, and a willingness to participate in the study were the inclusion criteria. The exclusion criteria included hospitalisation, pregnancy, or any clinical signs of a concomitant disease, such as diabetes mellitus, acute renal failure, or infectious illnesses other than HIV and TB. patients who have recently used a L-arginine supplement, patients who have had L-arginine allergies, and patients based on the physician's evaluation and the patient's medical records. Smear positives were defined as two out of three positive morning sputum samples or one out of three positives with a chest radiograph and clinical symptoms suggestive of pulmonary TB. The overall number of patients who received all necessary care and went on to be cured is how the WHO assessed treatment success. Patient Patients were initially questioned about the duration of clinical symptoms, and then again after one and two months to determine if symptoms were present or not. Using a standard form, data were collected (cough, constitutional symptoms, and body mass index).

Using the same individuals and calibrated equipment, one person measured all clinical indications and symptoms as well as all laboratory tests at the same lab. Evaluation During the beginning of therapy and one and two months later, sputum samples were collected. During the beginning of therapy, one, two, and six months later, the body mass index was assessed. Blood samples were

obtained to assess C-reactive protein (CRP), erythrocyte sedimentation rate (ESR), and haemoglobin content at baseline, one and two months after beginning therapy (Hg). ESR was determined using the Westergren technique, C-reactive protein by latex agglutination assays, and Hg by haematology.

Treatment Regime

Treatment Planning In accordance with the instructions of the WHO, all treatment was given outside of a hospital setting. During the two-month intense phase of therapy, which included isoniazid, pyrazinamide, rifampicin, and ethambutol, isoniazid and ethambutol were also used. At the beginning of their anti-TB treatment, TB patients were given either identical L-arginine supplements from Karen Pharma & Food Supplement Co. in Iran, which contain 1000 mg of pure L-arginine hydrochloride, or a placebo that included 1000 mg of sugar twice daily for 30 days. Patients were randomly assigned to receive either the L-arginine supplements or the placebo. The final effectiveness of the therapy, the conversion of sputum, the accumulation of weight, and the resolution of clinical symptoms at one, two, and six months were the primary goals. ESR and CRP were secondary outcomes that were observed.

Statistical analysis

The findings are displayed as frequency distributions, percentages, and mean + SD. The Chi-square test was used to compare the categorical variables. To compare continuous variables, the Paired/Unpaired t-test was applied. A 0.05 p-value was deemed significant. The SPSS 16.0 version was used to conduct all of the analyses (Chicago, Inc., USA).

Results and Observation

Patients

The study was conducted between December 2020 and June 2021 at the K.M Medical College & Hospital, Mathura, with a total of 150 patients and 150 controls. 150 patients were randomly allocated to the L-arginine group and 150 patients were assigned to the placebo group out of the 300 patients who underwent therapy. More than a third of individuals who were diagnosed with the condition (44%) and those who acted as controls (46.7%) were between the ages of 41 and 50. Nearly one quarter of the cases, or 28 percent, and 30.7 percent of the controls were between the ages of 30 and 40. The average age of those who had the condition and those who served as controls was, respectively, 43.16 ± 9.54 and 42.53 ± 8.44 years. There was no statistically significant difference in age between the cases and the controls ($p > 0.05$), indicating that the groups were comparable in terms of their ages (Table 1). About half of cases (54.7%) and 45.3% of controls were males. Both the L-arginine and placebo groups did not experience any negative effects. Weight, BMI, and HB were the only baseline parameters that did not significantly change between the L-arginine group and the placebo group. Comparability of the groups for gender showed no significant ($p>0.05$) difference in gender between the patients and controls (Table 1).

Table 1. Demographics and clinical characteristics of the patients.

Characteristics			
		L-Arginine(Mean±SD)	**Placebo (Mean±SD)**
Patients (n)		150	150
Age		43.16±9.54	42.53±8.44
Sex	**Male (n)**	82	68
	Female (n)	68	82
Resident	**Rural (n)**	90	80
	Urban (n)	60	70
Presence of constitutional symptoms	**Baseline**	122	114

	1 month	82	80
	2 months	36	74
	6 months	16	62
Cough	**Baseline**	122	114
	1 month	84	82
	2 months	28	62
	6 months	16	60
Weight (kg)	**Baseline**	66.61±6.83	66.79±6.81
	1 month	67.43±6.85	67.49±6.77
	2 months	68.89±6.68	68.84±6.66
	6 months	70.74±6.43	70.81±6.44
BMI	**Baseline**	25.75±3.17	25.69±3.01
	1 month	26.08±3.26	25.97±3.11
	2 months	26.64±3.24	26.50±3.16
	6 months	27.36±3.23	27.25±3.16
Sputum Status	**Baseline**	102	80
	1 month	72	70
	2 months	20	62
	6 months	8	40
HB	**Baseline**	9.24±1.40	9.66±1.43
	1 month	9.90±1.31	10.13±1.35
	2 months	11.08±1.03	11.12±1.03
	6 months	12.23±0.99	12.25±1.01
CRP	**Baseline**	122	110
	1 month	88	80
	2 months	38	68
	6 months	18	50
ESR	**Baseline**	128	112
	1 month	96	78
	2 months	38	72
	6 months	24	58

L-arginine supplementation reduces constitutional symptoms in cases

L-arginine supplement decreases the constitutional symptoms in cases while the controls have more symptoms in 2 and 6 months of treatment. At baseline, patients (81.3%) had more constitutional symptoms than controls (76%) but not statistically significantly more symptoms (p>0.05). At one month, there were fewer constitutional symptoms in cases (54.7%) than in controls (53.3%), although the difference was statistically insignificant (p>0.05). At two months, patients (24%) had fewer constitutional symptoms than controls (49.3%), on average. At six months, patients (10.7%) had fewer constitutional symptoms than controls (41.3%) did. Constitutional symptoms were present differently in cases and controls at 2 months (p=0.001) and 6 months (p=0.000) (Figure 1).

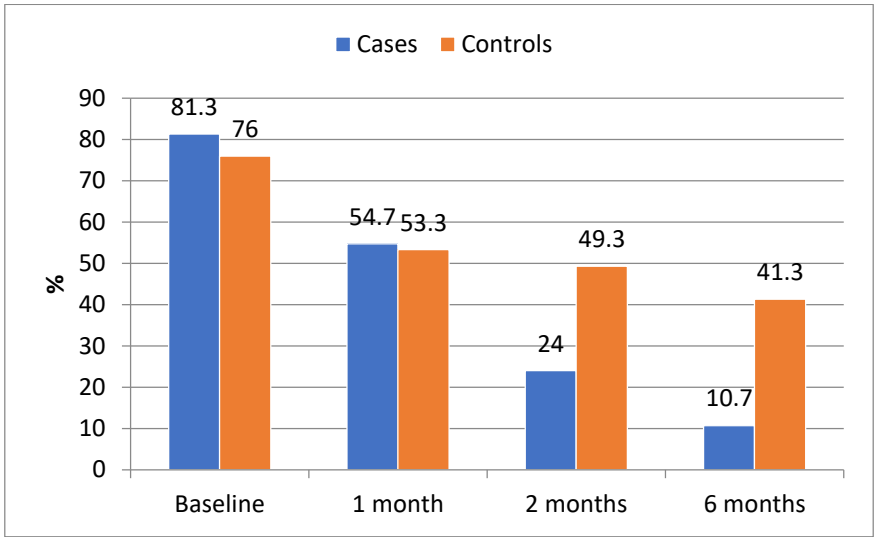

Fig. 1: Comparison of constitutional symptoms between cases and controls across the time periods

L-arginine supplementation induces increased sputum conversion and reduced cough in cases

Pertaining to the examination of sputum samples obtained from both the patients and the controls at all times. Across groups, there was no significant variance in the percentage of sputum that was positive at the start of the trial and one month later (p>0.05). At two months, the proportion of patients in the case group (13.3%) compared to the control group (41.3%) had a significantly reduced (p = 0.001) positive sputum rate. As compared to controls (26.7%), the proportion of positive sputum tests in patients was significantly (p = 0.0001) lower after 6 months (5.3% vs. 26.7%). In Figure 2.

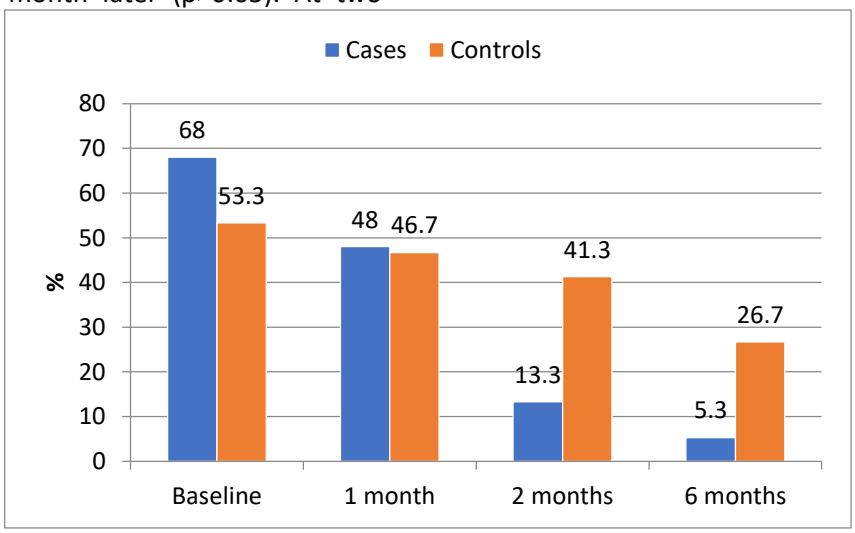

Fig. 2: Comparison of sputum between cases and controls across the time periods

It is possible to compare the coughs of the patients and the controls throughout all time periods. Coughing was not significantly more prevalent (p>0.05) among the patients (81,3%) than it was among the controls (76%) during the research period. Coughing decreased to 56 percent among patients and 54.7% among controls during the course of a month, but there was no statistically significant difference between the two groups (p>0.05). As compared to controls (41.3%), individuals with the cough had a lower likelihood of becoming cases at 2 months (18.7%). The prevalence of cough was similarly lower among patients at 6 months of age (10.7%) than it was among controls (40%). When it came to the occurrence of cough at two months and six months, there was a statistically significant difference between patients and controls (p=0.0001 and p=0.0001, respectively) (Figure 3).

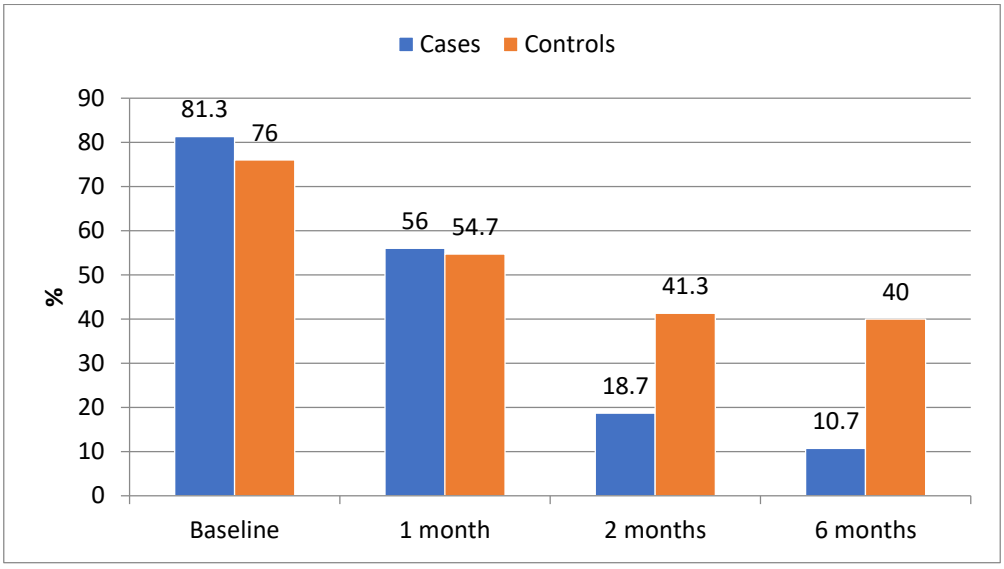

Fig. 3: Comparison of cough between cases and controls across the time periods

L-arginine supplementation induces reduction of C-reactive protein (CRP) in cases

When comparing the CRP levels of the patients to those of the controls across all time periods. Elevated CRP was found to be substantially greater among patients (81.3%), but not significantly higher among controls (73.3%). At 2 months, researchers discovered that the percentage of patients with elevated CRP was considerably (p=0.01) lower than the percentage of controls with elevated CRP. It was also discovered that the percentage of patients (12%) with elevated CRP was considerably (p=0.001) lower than the percentage of controls (33.3%) at 6 months (Figure 4).

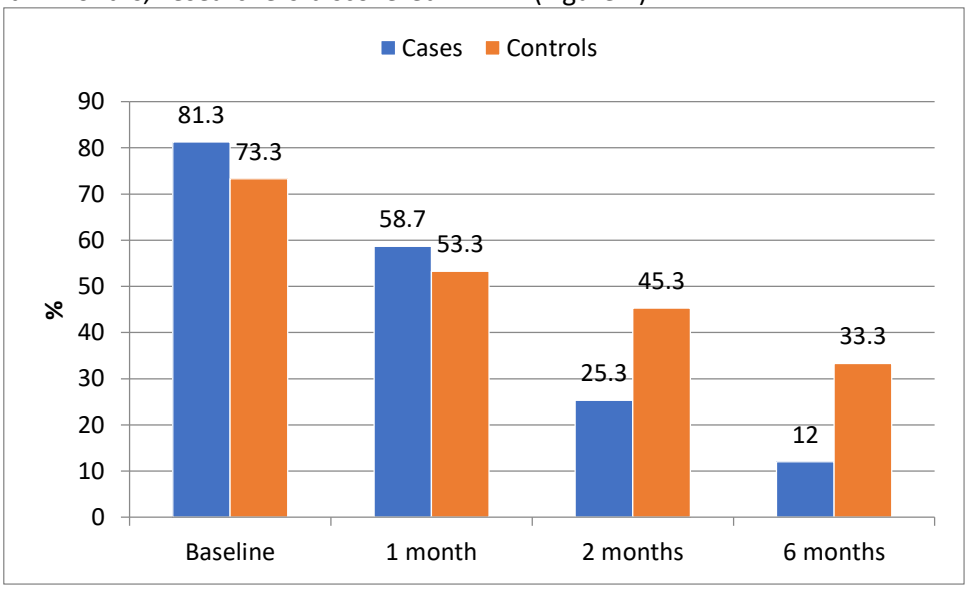

Fig. 4: Comparison of CRP between cases and controls across the time periods

L-arginine supplementation induces reduction of Erythrocyte Sedimentation Rate (ESR) in cases

When compared across all time periods, the ESR of the patients and controls were found to be significantly different. Elevated ESR was insignificantly (p>0.05) greater among patients (85.3%) compared to controls (74.7%). At 2 months, it was discovered that the percentage of patients with an elevated ESR was considerably (p=0.001) lower than the percentage of controls who had an elevated ESR. At six months, the percentage of patients with an elevated ESR was found to

be considerably (p=0.001) lower in the case population (16%) compared to the control population (38.7%). (Figure 5).

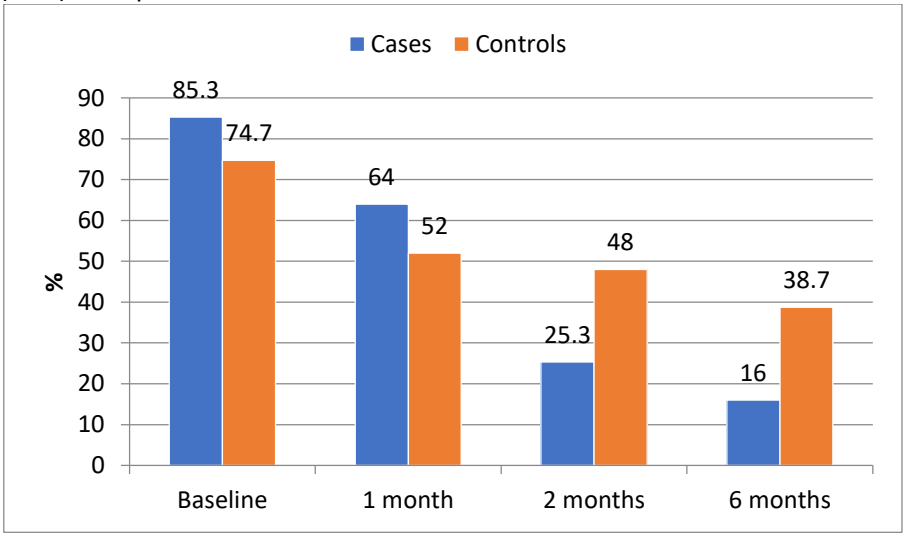

Fig. 5: Comparison of ESR between cases and controls across the time periods

Discussion

L-arginine is an amino acid that plays a key role in many physiological processes, including immune function. According to studies, taking L-arginine supplements can improve immune function by boosting the synthesis of nitric oxide, an antibacterial molecule that also helps control the immunological response (Wilson et al 2019).In addition, L-arginine supplementation has been found to reduce inflammation in TB patients by modulating the activity of immune cells called macrophages (Qualls et al. 2016; Chen et al. 2020). Macrophages play a key role in the immune response to TB, but excessive activation of these cells can lead to tissue damage and inflammation. LIt has been demonstrated that giving TB patients more -arginine can help control macrophage activity and lessen inflammation. The evidence for the effectiveness of L-arginine supplementation in TB patients is still sketchy, and further studies are required to validate its advantages. We thus undertook this study to close this gap, and the findings demonstrated that L-arginine supplementation has a substantial and positive influence on constitutional symptoms, sputum conversion, and the abatement of symptoms like cough. Moreover, case group patients' CRP and ESR readings were lower than those of the control group's patients. A significant effect on disease transmission may result from the decreased cough and enhanced mycobacterial clearance in sputum shown during the first two months of therapy in TB patients getting L-arginine supplementation. Sputum conversion and the disappearance of symptoms like the cough are crucial gauges of a patient's response to therapy (TB).There is limited research on the effect of L-arginine supplementation on sputum conversion and reduction of symptoms in TB patients. One study published in 2015 investigated the effect of L-arginine supplementation on TB patients undergoing standard anti-TB treatment. The study discovered that L-arginine supplementation significantly reduced cough frequency and intensity after four weeks of therapy and increased sputum conversion rates (Farazi et al. 2015). Another 2016 study looked into how L-arginine supplementation affected TB patients' immune systems and symptoms. The results of the study revealed that L-arginine supplementation greatly enhanced immune function and significantly decreased cough frequency and intensity in addition to other symptoms like fever and night sweats (Grober er al. 2016). We observed that the primary symptoms, such as sputum conversation and a decrease in cough, are shown to be statistically significant together with the constitutional symptoms, such as fever, night

sweats, and weight loss in our study, which is also in agreement with these findings.

Overall, these trials indicate that supplementing with L-arginine may help TB patients convert their sputum and experience less symptoms. To fully comprehend the potential advantages of L-arginine supplementation in the treatment of tuberculosis and to establish the ideal dosage and course of therapy, more study is necessary. Nevertheless, several studies found that taking 500–1000 mg twice a day relieved symptom, and we found that taking 1000 mg twice a day for at least 2–6 months produced results that were statistically significant. This result further supported the treatment regimens found in earlier research.

C-reactive protein (CRP) and erythrocyte sedimentation rate (ESR) are markers of inflammation and are commonly used to monitor the response to treatment in patients with tuberculosis (TB). L-arginine is an amino acid that has been demonstrated to have immunomodulatory effects and has the potential to enhance the immunological response in people suffering from tuberculosis. There has only been a little amount of study done on how taking L-arginine supplements affects CRP and ESR levels in TB patients. According to the findings of one investigation that was published in 2015, supplementation with L-arginine resulted in a substantial reduction in both CRP and ESR levels after eight weeks of therapy (Farazi et al. 2015). Nevertheless, a meta-analysis conducted by Nazarian et al. (2019) found that supplementation with L-arginine had no significant effect on inflammatory biomarkers such as CRP, IL-6, or TNF. Because of this, the results of our study indicated a significant shift in CRP and ESR levels, which is consistent with the findings of the preceding study and may be used as a basis for the design of additional research on TB patients. The findings of these research indicate that L-arginine supplementation may have a favourable effect on inflammation in TB patients, as demonstrated by decreases in CRP levels. This conclusion is drawn from the overall findings of the investigations. However, further study is required to fully understand the potential advantages of L-arginine supplementation in the treatment of tuberculosis (TB), as well as to discover whether or not it can also influence the levels of CRP and ESR.

Conclusion

Further L-arginine medication can assist patients being treated for active TB, with the advantages most likely mediated by increased nitric oxide production. This research suggests that supplementing the treatment of active TB with L-arginine is effective. This action probably enhanced constitutional symptoms including weight gain and a decrease in CRP, as well as cough, sputum smear conversion, and treatment success.

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