

VITAMIN C AS AN ADD ON SUPPLEMENTATION TO STANDARD ANTITUBERCULAR TREATMENT IMPROVES SPUTUM SMEAR CONVERSION IN NEWLY DIAGNOSED DRUG SENSITIVE PULMONARY TUBERCULOSIS PATIENTS

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ABSTRACT

Objective: This study was aimed to evaluate the effect of oral supplementation of Vitamin C along with standard anti-tubercular (anti-TB) drugs on the sputum smear conversion in newly diagnosed pulmonary tuberculosis (TB) patients.

Methods: This was a prospective, observational study in which 49 newly diagnosed pulmonary TB patients were given oral vitamin C supplementation along with standard anti-TB treatment during the intensive phase (2 months) of the treatment. An examination of the sputum smear at the end of the intensive phase of anti-tubercular treatment was done. At the end of the intensive phase, a comparison was done between the results of the sputum smear examination of the study population and the TB patients of the general population from the same locality who were receiving only standard anti-TB treatment and not add on Vitamin C supplementation.

Results: The comparison between the results of the study population (sputum conversion-100%) with those of the general population (sputum conversion-92.47%) shows a significant difference between both populations with $p < 0.05$ ($p = 0.041$).

Conclusion: This study showed that Vitamin C supplementation as an add-on therapy to the existing standard anti-TB treatment improves sputum smear conversion and decreases disease activity to a greater extent than routine standard anti-TB treatment alone.

Keywords: Tuberculosis, Vitamin C, Sputum smear conversion, Antitubercular treatment.

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INTRODUCTION

Tuberculosis (TB) is currently one of the most important health issues in India as well as the world. India has been fighting this disease for a long time, and for more than 50 years, the activities to control TB have been going on and are still going on. Currently, the National TB Elimination Programme (NTEP) has been going on to eliminate TB from the country. As per the Global TB Report 2023, India stands among the top 3 countries with the highest TB disease burden [1]. TB kills approximately 480,000 Indians every year, i.e., more than 1400 every day. So eliminating TB has become the mainstay of NTEP [2].

The challenges against effective TB control in India are delayed diagnosis and inadequate treatment. Due to the long duration of treatment, patients tend to be non-compliant with anti-TB treatment. These factors also contribute to the development of drug-resistant TB (DR-TB), which is even more difficult to treat. A significant proportion of the Indian population is undernourished, which leads to weakened immunity and TB reactivation. A considerable population also suffers from conditions weakening immunity, including diabetes, indoor air pollution from cook stoves, or smoking, that increase the likelihood of progression to active TB [3]. These factors make it difficult to combat TB, yet better control of TB is possible as long as enough TB is diagnosed early and treated appropriately, appropriately interrupting further transmission. Hence, for better control of TB and to prevent the emergence of resistance in TB, it is important to make an early diagnosis and more effectively treat TB as soon as it is diagnosed.

Vitamin C has been playing an important role as an anti-oxidant agent and in boosting immunity in humans [4,5]. It was seen that a deficiency of Vitamin C is associated with a higher incidence of diseases such as TB [6,7]. Vitamin C has been shown to accelerate the recovery from TB [8]. Hence, there has been an association between Vitamin C and TB. Based on this, many bacterial culture studies, as well as *in vitro* and *ex vivo* studies, have been carried out to know more about the effects of Vitamin C on *Mycobacterium tuberculosis*. These studies have proven Vitamin C to be helpful in more effective killing of *M. tuberculosis* in many ways, e.g., Fenton and Haber-Weiss reaction [9,10], induction of bacterial dormancy in TB, [11] and synergism with anti-tubercular drugs [12]. This indicated that the addition of Vitamin C supplementation to standard anti-tubercular treatment can improve the recovery of TB patients. In spite of many such evidences, there is a lack of studies showing the effects of Vitamin C supplementation in TB patients.

Hence, this study was aimed to analyze the effect of Vitamin C as an add-on supplementation with standard anti-tubercular treatment on the sputum smear conversion of drug-sensitive pulmonary TB patients.

METHODS

This was a prospective, observational study done for 1 year, from January 01st, 2019 to December 31st, 2020. The study was carried out in the respiratory medicine outpatient department (OPD) of a tertiary care hospital. The study was approved by the scientific review committee and the human research and ethics committee of the institution.

Patients attending respiratory medicine OPD with symptoms pertaining to TB were advised by the chest physician to get a sputum smear examination by Zeihl-Neelsen staining for acid-fast tuberculous bacilli. A cartridge-based - nucleic acid amplification test (CB-NAAT) was performed for confirmation of disease and rifampicin sensitivity estimation. The participants were subjected to thorough history-taking regarding clinical features and previous treatment. Patients who satisfied the inclusion criteria were included in the study. The study included newly diagnosed patients aged between 18 and 45 years with a positive sputum smear for acid-fast bacilli and rifampicin susceptible in CB-NAAT analysis. Patients with human immunovirus (HIV) seropositivity, multi DR - extensive DR TB, Extra-pulmonary TB, TB with hemoptysis, diabetes, pregnant women, immunosuppressive therapy, or those not willing to take part in the study were excluded.

All the study patients were given standard anti-tubercular treatment according to revised national TB control program (RNTCP) guidelines, along with a Vitamin C tablet at a dosage of 500 mg/day by the treating physician during the intensive phase (initial 2 months/8 weeks) of anti-tubercular treatment. A sputum smear examination was done at the end of the intensive phase. The results of the sputum smear examination at the beginning of the study and at the end of the intensive phase were recorded for comparison.

Sputum was classified as given in the Table 1 below (as per RNTCP guidelines) [13].

For the purpose of comparing the results of the sputum smear examination of the study population at the end of the intensive phase, sputum conversion data of the general population on standard anti-tubercular treatment was obtained from the local municipal corporation district TB center database. The obtained data contained patients from the same time period, a similar age group, and matched the inclusion-exclusion criteria of the study.

The data was entered and analyzed using Microsoft office Excel 2016. The results were expressed as numbers and percentages. The χ^2 value, degree of freedom d(f), critical value (CV), and p-value were calculated using the chi-square test wherever applicable. $p < 0.05$ was considered statistically significant.

RESULTS

During the study, 49 patients were diagnosed with pulmonary TB who fulfilled the inclusion criteria and were enrolled in the study. All the patients completed the study.

Comparison between the number of sputum smear conversion of study population and the general population on anti-tubercular treatment after the intensive phase:

- Study Population: Out of 49 patients enrolled in the study, all 49 of them were tested negative on sputum smear microscopy after the intensive phase of anti-tubercular treatment, as shown in Table 2. There were no positive patients left in the study population after the intensive phase.
- General (local municipal corporation) Population: Out of 1053 patients, 970 were tested negative on sputum smear microscopy after the intensive phase. 83 patients were still positive after the intensive phase (8 weeks of therapy), as shown in Table 2.
- Statistical analysis with Chi-square gave $\chi^2=4.177$ with $p=0.041$ suggesting statistical significance.

% of sputum smears conversion in the study population and the general population:

Table 3 and Graph 1 shows % of sputum smear conversion of study population and general population after the intensive phase of anti-tubercular treatment.

Table 1: AFB Sputum classification

Examination finding	Number of fields examined	Grading	Result
No AFB in 100 oil immersion fields	100	0	Negative
1-9 AFB per 100 oil immersion fields	100	Scanty*	Positive
10-99 AFB per 100 oil immersion fields	100	1+	Positive
1-10 AFB per oil immersion fields	50	2+	Positive
More than 10 AFB per oil immersion field	20	3+	Positive

*Record actual number of bacilli seen in 100 fields - e.g., "Scanty 4". AFB: Acid fast bacilli

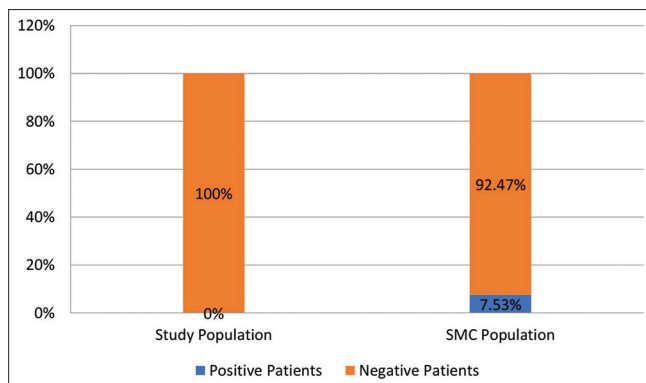
Table 2: Number of sputum smear conversion of study versus general population

Sputum Smear Result	Study population	General population
Sputum positive patients (After intensive phase)	0	83
Sputum negative patients (After intensive phase)	49	970
Total	49	1053

$\chi^2=4.177$, d (f)=1, CV=3.841. $p=0.041$ (Significant)

Table 3: % of sputum smear conversion of study versus general population

Sputum Smear Result	Study population (%)	SMC population (%)
Sputum positive patients % (After intensive phase)	0	7.53
Sputum negative patients % (After intensive phase)	100	92.47



Graph 1: % of sputum smear conversion of study versus general population

DISCUSSION

From the results of this study, it was evident that after 8 weeks of anti-tubercular therapy, the percentage of sputum smear conversion was higher (100%) in the patients in the study group who received Vitamin-C supplementation than in the general population who were receiving standard anti-tubercular treatment without add on Vitamin C supplementation.

The present study was similar to the research conducted by Susanto et al. [14], whose findings showed that the sputum conversion at the end

of the study was 100% in the patients who were given add-on Vitamin C supplementation. That study showed that Vitamin C supplementation has effects on improving the healing process in TB patients, as indicated by higher sputum conversion. These findings are similar to those of the present study.

The increase in sputum smear conversion in the study population was majorly due to anti-tubercular drugs because the chosen study population was rifampicin-sensitive. Vitamin C accelerates bacterial cell death with rifampicin [12].

Better sputum smear conversion in the study population can be explained by several aspects of Vitamin C, which causes DNA damage and eventually cell death in *M. tuberculosis*, either by synergism with first-line anti-tubercular drugs or by the Fenton reaction that induces an oxidative stress response and generates reactive oxygen species [9]. A study has shown that amongst the bacteria, *M. tuberculosis* is most sensitive to the Vitamin C-induced Fenton reaction, [9] which indicates that Vitamin C has a pleiotropic effect on biological processes such as DNA synthesis, lipid synthesis, and redox homeostasis against *M. tuberculosis*. This combination of effects might be the reason why Vitamin C can sterilize drug-susceptible TB strains.

The present study's findings also support Khameneh *et al.* [15], whose results showed that Vitamin C increased the antibacterial effects of rifampicin and isoniazid against *M. tuberculosis*. It was suggested that in pharmacologic doses, Vitamin C can act as a pro-drug for hydrogen peroxide (H₂O₂) formation, thereby helping the antibacterial agents to combat against bacteria [16].

The findings of the study favor the knowledge that Vitamin C, as an add-on supplementation along with first-line anti-tubercular drugs, has the potential to boost the efficacy of these drugs and enhance sputum smear conversion.

Limitations

In this study, due to ethical issues, keeping a control group for comparison was not approved by the human research and ethics committee. The results of the study could have been more decisive if there had been a control group.

Serum levels of Vitamin C before and after taking supplementation would provide insight on the importance of add on Vitamin C supplementation.

CONCLUSION

This study shows that Vitamin C supplementation as an add-on therapy to the existing standard anti-TB treatment improves sputum smear conversion and decreases disease activity to a greater extent than with routine standard anti-TB treatment alone.

Vitamin C, being an inexpensive and nontoxic compound, could easily be added to the standard anti-TB regimen to substantially improve treatment outcomes without much increase in the cost of the present treatment guidelines.

These findings could be of great assistance and have a significant impact on achieving the goals set to eliminate *M. tuberculosis* infection from the country and the worldwide TB community.

AUTHORS CONTRIBUTIONS

All six authors contributed equally toward the study, starting from a literature review, identifying the research gap, research proposal writing, data collection and analysis, manuscript writing, and publication.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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