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Original Article

Randomized Clinical Trial of the Combined Effects of Vitamin C and Anti-tuberculosis Drugs in Tuberculosis Patients

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ABSTRACT

Background and Objectives: Tuberculosis is one of the major health problems in Bangladesh as well as other regions of the world. The aim of this clinical trial was to investigate combined effects of vitamin C and anti-tuberculosis drugs in tuberculosis patients.

Materials and Methods: A total of 32 tuberculosis patients were enrolled in the study through random sampling and separated into intervention (vitamin C and anti-tuberculosis drugs) and control (only anti-tuberculosis drugs) groups, where the intervention group was treated with 1000 mg of vitamin C daily in addition to anti-tuberculosis drugs and the control group was treated with only anti-tuberculosis drugs for 28 days. In the second stage, the microbial count of tuberculosis patients was assessed using sputum smear test for control and intervention groups after a 7-d time interval.

Results: Smear test of the patient first week showed that the microbial loads comparatively decreased compared to the test of the second week using combined treatment of vitamin C and anti-tuberculosis drugs for the intervention group (p=0.034). In contrast, the control group included p=0.000, p=0.341 and p=0.346 for the second, third and fourth-week tests with no significance.

Conclusions: Combined effects of vitamin C and anti-tuberculosis drugs in TB patients.

Keywords: Tuberculosis, Treatment, Vitamin C, Tuberculosis drugs

Introduction

Nowadays, tuberculosis (TB) is one of the major health issues and top ten causes of the worldwide death in 2015 (1). Nearly one-third of the global population is infected with *Mycobacterium tuberculosis* (2). Every year, more than 9 million individuals contract active TB with over 2 million deaths (3). More than 90% of the global TB cases and deaths occur in developing countries, where 75% of the cases are in the most economically productive age group of 15-54 years old (4). According to world health organization (WHO Global TB report 2016), incidence and prevalence of all forms of TB in 2015 were 225 and 382 per 100,000 population, respectively (5). Basics of TB care includes active drugs, case management and direct monitoring therapy (6). Combination of drugs was suggested more than 40 years ago, allowing for a successful treatment of the vast majority of patients; however, this protocol includes major drawbacks; such as necessity for prolonged treatments, side effects, drug interactions and inadequate efficacies against latent MTB germs (7).

Vitamin C simulates a variety of intracellular stresses and includes a wide range of regulatory effects on MTB gene expression and physiology, resulting in growth inhibition (8). High concentrations of vitamin C sterilize cultures of drug-susceptible and drug-resistant of *M. tuberculosis* and combinations of vitamin C with first-line TB drugs of isoniazid and rifampin decreased bacterial

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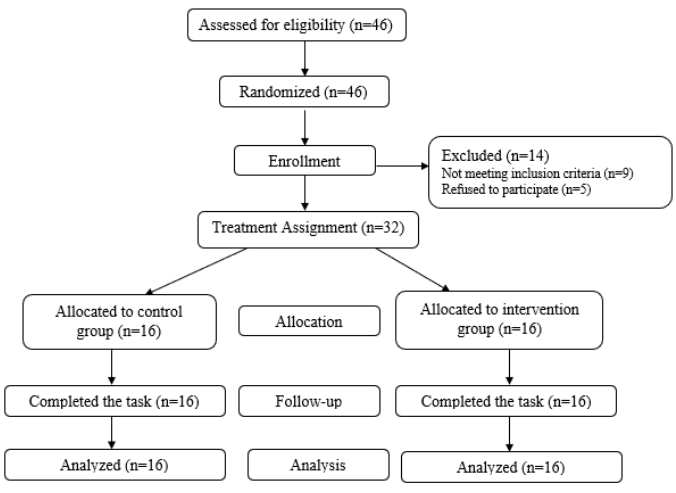
burden in the lungs of M. tuberculosis-infected patients (9). Another study detected correlations between the high vitamin C contents of medicinal plant extracts and their activities against *M. tuberculosis* (10). In Bangladesh, TB is one of the most serious health concerns, with new cases being discovered every day. Anti-TB treatments are usually used to cure the infection within long treatment courses. Vitamin C seems to include effects on *M. tuberculosis*. As a result, it is noteworthy to investigate if combinations of vitamin C with medications are more successful in treatment of TB. Various combinations of medications and medicinal plants have been used in treatment of TB patients worldwide. Clinical trials on the combined effects of vitamin C and anti-TB medications are, limited, particularly in Bangladesh. Therefore, the goal of this study was to investigate effects of vitamin C in combination with anti-TB medicines on M. tuberculosis, how rapidly patients recovered and early healing in TB patients.

Materials and Methods

Study design and participants

Information were collected from TB patients, who were diagnosed with TB recently. An experimental comparative study was carried out on TB patients to investigate effects of vitamin C with anti-TB drugs in TB patients. In general, Personal information, disease occurrences and, socio-demographic information were collected. This clinical trial was carried out in TB patients from Santosh Bokkhobyadhi Clinic, Tangail-1902, Bangladesh. Patients with TB from various regions of Tangail have been enrolled in this trial study. In this study, *M. tuberculosis* strains were isolated from routine TB patients. Permissions were received by the physicians and patients were classified into the following categories as inclusion criteria: new suspected pulmonary TB, suspected treatment failure, suspected recurrence and treatment default. Individuals, who did not have TB, were excluded from the study. Patients' clinical data were routinely collected and documented in research forms. The present randomized control trial (RCT) study was approved by the Board of Ethical Review Committee with the ethical approval number of MBSTU/FTNS/42/842/2019. Consort flowchart of the study is as follows.



Flowchart 1. Patient selection and follow-up chart (consort flowchart of the study)

Sample size calculation

In the current study, sample size was calculated using Raosoft sample size calculator (11) with a recommended sample size of 46 individuals based on 5% margin of error, 95% confidence level and 3.6% response distribution or TB prevalence rate in Bangladesh (12). Despite the fact that 46 participants were selected for the trial, 14 patients were disqualified after screening based on the exclusion criteria. Hence, 32 patients were assigned to the treatment group and completed study follow-up and analysis. Overall, TB patients, who attended Santosh Bokkhobyadhi Clinic in Tangail-1902, Bangladesh, within 3-m data collecting period were included in this study.

Data collection tools

A semi-structured questionnaire was developed to collect relevant information of the participants. After pre testing, a modified validated questionnaire was used to collect socio-demographic and anthropometric data through face-to-face interviews with the participants passively and cautiously, not affecting the participants. Collected questionnaires were checked every day after interviewing and rechecked carefully after completion of data collection. Data were edited if discrepancies occurred. Furthermore, microbial-sputum test results were collected for analysis.

Assessment of anthropometric measurement

Participants with minimal cloths and bare feet were weighed three times using bathroom weighing scale and the average weight was calculated and recorded to the nearest 0.5 kg. Standing height was measured using stature meter for height measurement scales with no shoes and the average height was calculated and recorded to the nearest 0.5 cm. Body mass index (BMI) was calculated by dividing the weight in kilograms by the square of height in meters (13).

Experimental design

In the first stage, information were collected from the TB patients and 32 participants were subdivided into intervention group [vitamin C and anti-tuberculosis drugs (ATD)] and control group (ATD), where the intervention group was treated orally with 1000 mg of vitamin C capsules daily on a specific time in addition to ATD and the control group was treated with ATD alone. In the second stage, the microbial count of TB patients was carried out using sputum smear test (14, 15) in control and intervention groups after a 7-d time interval.

Sputum smear test

The microbial count of TB patients was carried out using sputum smear test (14, 15). In general, characteristics of cid-fastness is based on the presence of mycolic acids in the mycobacterial cell wall. Primary stain (auramine) binds cell-wall mycolic acids. Intense decolourization (strong acids and alcohol) does not release primary stain from the cell wall and the mycobacteria retain the fluorescent bright-yellow color of auramine. Potassium permanganate is used to quench fluorescence in the background; however, it

provides little contrast for focusing and stains are therefore sometimes preferred; of which, blue ink may be the best.

Materials and equipment

In this study, alcohol sand jars, Bunsen burner/spirit lamps, diamond/lead pencils, filter papers, forceps, lens paper/soft tissue paper, plastic bags of waste disposal, bamboo/wooden sticks or wire loops, fluorescence microscope with objectives of 20/25 and 40× (ideally specific for fluorescence microscopy) and eyepieces of 10×, slide staining racks, slide boxes, timers, staining reagents, staining bottles of, 250 ml with spouts, beakers for rinsing water, sinks and water supplies and disinfectant solutions were used.

Reagents and solutions

Auramine staining (0.1%), acid-alcohol decolorizing (0.5%), counterstaining and potassium permanganate (0.5%) solutions, as well as blue ink (10%) were used.

Preparation of smears

First, the working area was disinfected and slides were labelled appropriately using laboratory register serial numbers marked on the sputum containers and slides. Second, slides were proceeded close to the flame of a Bunsen burner or spirit lamp. For direct sputum smears, a small portion of purulent or mucopurulent materials was selected using stick/loop and transferred onto the slide. Third, material was carefully spread over an area of nearly 2–3 × 1–2 cm using repeated circular movements without touching the edge of the slide. This technique continued until no thick parts remained. Then, smear was air-dried at room temperature with no heat use. When humidity was high, gentle warming was used. After drying, slide was held using forceps and fixed by passing slowly through the flame of a spirit lamp or quickly through a flame of a Bunsen burner for three times. In general, smears were not overheated.

Staining method

Slides were set upwards on a staining rack over a sink, nearly 1 cm apart. A fresh filter paper was placed into a small funnel over the slides and the funnel was filled with auramine staining solution. Thus, solution was filtered through the paper covering slides completely for 20 min. Slides were tilted to drain off extra stain solution. Then, slides were rinsed well with indirect distilled water or clean tap water. Acid solution was poured over the smears, covering them completely for 3 min. Slides were tilted to drain off extra acid-alcohol solution. Slides were gently rinsed with distilled water or clean tap water. Then, smears were flooded with potassium permanganate or blue ink solution for 1 min. Slides were tilted to drain off extra counterstain solution and gently rinsed with distilled water or clean tap water. Then, slides were removed from the rack and dried.

Statistical analysis

Descriptive statistics were calculated for the variables and statistical analysis was carried out using SPSS Software v.25.0 (IBM, Armonk, New York, USA). Results were expressed as mean ±SD (standard deviation). Moreover, ANOVA and T-test were used for statistical analysis. In general, $p<0.05$ was considered statistically significant.

Results

Socio-demographic characteristics and BMI statuses of the participants

A majority of the participants were men (56.3%) rather than women (43.8%). More than 6% of TB patients were under the age of 20 years old with 21.9% aged 20–40 years old, 40.6% aged 40–60 years old and 31.3% aged 60–80 years old as the most common age groups. Statistics revealed that 43.80% of the patients were smokers, whereas more than 56% were non-smokers. Nearly 59.38% of TB patients were underweight, 31.25% had a normal BMI and 9.37% were overweight.

Table 1. Frequency distribution of the age categories and smoking statuses of the participants

	Frequency	Percentage (%)
Gender Category		
Female	14	43.8
Male	18	56.3
Total	32	100.0
Age category (year)		
<20	2	6.30
20-40	7	21.90
40-60	13	40.60
60-80	10	31.30
Total	32	100
Smoking status		
Smoker	14	43.80
Non-smoker	18	56.30
Total	32	100
BMI category		
<18.50	19	59.38
18.50-24.99	10	31.25
>25.00	3	9.37
Total	32	100

Effects of vitamin C in intervention (vitamin C and anti-tuberculosis drugs) and control (anti-tuberculosis drugs) groups of TB patients

Participants were detected with more TB bacteria in ATD-only treatment group than vitamin C and ATD group (Table 2). Week 2 of drug therapy included significant associations with the dependent variable (p = 0.018), whereas Weeks 3 and 4 included no significant associations (p = 0.341 and p = 0.226). Patients with TB, who received a combination of vitamin C and ATD medication showed decreases in TB germs from Week 1 to Week 4. The dependent variable included significant correlations with Weeks 2 and 3 treatments (p = 0.009 and p = 0.034, respectively), whereas Week 4 treatment was not significant (p = 0.533).

Table 2. Microbial count for the intervention (vitamin C and anti-tuberculosis drugs) and control (anti-tuberculosis drugs only) groups

Statement (Respondent's sputum test results)	Weeks	N	Minimum	Maximum	Mean ± SD
Control groups	1st	16	20	350	147.31±115.2
Intervention groups			6	310	155.31±110.9
Control groups	2nd		14	310	126.5±110.2
Intervention groups			0	250	116.12±99.2
Control groups	3rd		7	290	111.5±106.9
Intervention groups			0	180	69.94±70.8
Control groups	4th		1	240	94.38±88.6
Intervention groups			0	110	26.06±39.1

Table 3. Regression coefficients for the intervention (vitamin C and anti-tuberculosis drugs) and control (anti-tuberculosis drugs only) groups

Model (respondent's Sputum test results)	Weeks	Unstandardized coefficients		Standardized coefficients	t	P value
		Beta	Std. Error	Beta		
Constant	Constant	12.688	4.652		2.727	0.018
Constant		17.127	5.499		3.114	0.009
Control groups	2nd weeks	1.052	0.200	1.007	5.273	0.000
Intervention groups		1.768	0.231	1.579	7.657	0.000
Control groups	3rd weeks	0.289	0.291	0.268	0.991	0.341
Intervention groups		-1.023	0.427	-0.653	-2.398	0.034
Control groups	4th weeks	-.325	0.255	-0.278	-1.276	0.226
Intervention groups		0.172	0.268	0.061	0.642	0.533

Discussion

The present study was carried out on TB patients (n = 32) in a selected hospital to investigate effects of vitamin C and anti-TB drugs (vitamin C and ATD) in TB patients. Vitamin C has been treated as a functional food, which have clinically verified and documented with health benefits for the prevention, management and treatment of chronic diseases (16). Studies have shown that supplementation with vitamin C can prevent and treat respiratory infections (17) and increase immune responses (18). Vitamin C possibly prevents neutrophil oxidation that plays critical roles against a variety of microbial pathogens and needs dietary vitamin C intakes, optimizing cell and tissue levels (17, 19). Vitamin C and N-acetylcysteine (NAC) include significant effects on the antibacterial activity of ATD against *M. tuberculosis* (20). The current study investigated that when vitamin C was used in combination with anti-TB medications, microbiological counts of the patients decreased more quickly. However, microbial counts of the patients, who used ATD only decreased more slowly. Various studies have been shown various results for TB patients. These studies used vitamin C with drugs, medicinal plants or other combinations to treat TB patients. Based on the certain studies, ethanolic extracts of *P. granatum* and *P. harmala* demonstrated anti-TB effects equivalent to that isoniazid and rifampin did as promising candidates for innovative and safe natural TB treatments (21). Data showed that the minimum inhibitory concentration (MIC) of vitamin C for the prevention of *M. tuberculosis* development was 1 mM (22), while other studies declared that high vitamin C concentrations were needed to show their positive effects (23). Another study demonstrated that when ascorbic acid-containing media were used at greater doses (10 and 100 mM), no microorganism growths were reported in TB patients (24), supporting the present study. The present study revealed that vitamin C with ATD significantly ($p < 0.05$) decreased microbial counts of *M. tuberculosis* up to Week 3 of treatment. Whereas, patients treated with drugs only demonstrated non-significant results, indicating positive effects of this combined treatment. However, other studies verified that good antioxidant therapies might be beneficial and nutritional antioxidant supplements might be unique strategies to assist TB patients recuperate faster (25). However, induction of Fenton reaction has been suggested by Vilcheze and his colleagues as an explanation for vitamin C favorable supplementary effects against *M.*

tuberculosis (22). Such prooxidant activity has been seen in mycobacteria under various circumstances (26, 27). The present study revealed that vitamin C with ATD significantly ($p < 0.05$) decreased microbial counts of *M. tuberculosis* up to Week 3 of treatment. Whereas, treatment of patients with drugs only showed non-significant results, indicating positive effects of this combined treatment. These finding were partially supported by findings from a previous study; where, vitamin C supplementation improved TB patient healing processes as evidenced by a greater sputum conversion rate after eight weeks ($p = 0.02$) (28). Comparing between the intervention and control groups, it can clearly be identified that the microbial counts in TB gradually decreased with the intake of vitamin C and anti-TB drugs. However, further studies are necessary to describe functions of vitamin C in clinical care of TB, particularly in ideal dosages for therapeutic benefits with no toxicities.

Conclusions

The study model was significant for the intervention group, who were treated with vitamin C and ATD up to Week 3 of the treatment, compared to the control group. It is concluded that the combined therapy of drugs and vitamin C included dramatic effects on TB, playing important roles in early healing of TB.

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References

1. World Health Organization. Global tuberculosis report 2016. Geneva: World Health Organization; 2016. WHO/HTM/TB/2016.08.
2. Stewart GR, Robertson BD, Young DB. Tuberculosis: A problem with persistence. Nature Reviews Microbiology. 2003:1(2):97–105.
3. Ahmad S. New approaches in the diagnosis and treatment of latent tuberculosis infection. Respiratory Research. 2010:11(1):1–17.
4. Bhatt CP, Timalsina B, Kutu B, Pradhan R, Maharjan B, Shrestha B. A Comparison of Laboratory Diagnostic Methods of Tuberculosis and A etiology of Suspected Interventions of Pulmonary Tuberculosis. SAARC Journal of Tuberculosis, Lung Diseases and HIV/AIDS. 2014:11(2):1–6.
5. Sarker M, Homayra F, Latif AHM, Barua M, Saha A, Paul S, et al. A matched intervention-control study to identify risk determinants of tuberculosis in Bangladesh. GSTF Journal of Nursing and Health Care. 2017:5(1):1-12.
6. Leibert E, Rom WN. New drugs and regimens for treatment of TB. Expert Review of Anti-Infective Therapy. 2010:8(7):801–813.
7. Guy ES, Mallampalli A. Managing TB in the 21st century: Existing and novel drug therapies. Therapeutic Advances in Respiratory Disease. 2008:2(6):401–408.
8. Taneja NK, Dhingra S, Mittal A, Naresh M, Tyagi JS. Mycobacterium tuberculosis transcriptional adaptation, growth arrest and dormancy phenotype development are triggered by vitamin C. PloS One. 2010:5(5): e10860.
9. Vilchèze C, Kim J, Jacobs Jr WR. Vitamin C potentiates the killing of Mycobacterium tuberculosis by the first-line tuberculosis drugs isoniazid and rifampin in mice. Antimicrobial Agents and Chemotherapy. 2018:62(3): e02165-17.
10. Narwadiya SC, Sahare KN, Tumane PM, Dhumne UL, Meshram VG. In vitro anti-tuberculosis effect of vitamin C contents of medicinal plants. Asian Journal of Experimental Biological Sciences. 2011:2(151154):22.
11. Raosoft Sample Size Calculator. Available online: http://www.raosoft.com/samplesize.html (accessed on 22 October 2016).
12. TB statistics (2020). TB Incidence in high Burden Countries. TBFacts.org. retrieved from https://tbfacts.org/tb-statistics/.
13. Islam MF, Linkon KMM, Shyfullah M, Hassan M, Aoual ME, Alam MJ. Association of Dietary Patterns with Glycated Hemoglobin (HbA1c) among Type 2 Diabetes Patients: A Cross-sectional Study. Current Nutrition & Food Science. 2021:17(7):716–726.
14. Kanaya AM, Glidden DV, Chambers HF. Identifying pulmonary tuberculosis in patients with negative sputum smear results. Chest. 2001:120(2):349–355.
15. Shen F, Sergi C. Sputum Analysis. [Updated 2020 Oct 18]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2021 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK563195.
16. Martirosyan DM. Introduction to Functional Food Science: Textbook. Functional Food Center. 2014. CreateSpace Independent Publishing Platform; 3rd edition (2015).
17. Carr AC, Maggini S. Vitamin C and immune function. Nutrients. 2017:9(11):1211.
18. Asli MM, Hosseini SA, Lotfollahian H, Shariatmadari F. Effect of probiotics, yeast, vitamin E and vitamin C supplements on performance and immune response of laying hen during high environmental temperature. International Journal of Poultry Science. 2007:6(12):895–900.
19. Levy R, Schlaeffer F. Successful treatment of a patient with recurrent furunculosis by vitamin C: Improvement of clinical course and of impaired neutrophil functions. International Journal of Dermatology. 1993: 32(11):832–834.
20. Khameneh B, Bazzaz BSF, Amani A, Rostami J, Vahdati-Mashhadian N. Combination of anti-tuberculosis drugs with vitamin C or NAC against different Staphylococcus aureus and Mycobacterium tuberculosis strains. Microbial Pathogenesis. 2016: 93: 83–87.

21. Jahanpour S, Ghazisaidi K, Davoodi H, Mazandarani M, Samet M, Jahanpour N, Ghaemi EA. Antimicrobial effects of folk medicinal plants from the North of Iran against Mycobacterium tuberculosis. Archives Of Pediatric Infectious Disease. 2015:28(3) (3 TB).

22. Vilchèze C, Hartman T, Weinrick B, Jacobs WR. Mycobacterium tuberculosis is extraordinarily sensitive to killing by a vitamin C-induced Fenton reaction. Nature communications. 2013:4(1):1-10.

23. Pichat P, Reveilleau A. Comparison between the in vivo and in vitro bactericidal action of vitamin C and its metabolite and ascorbic acid level. InAnnales de l'Institut Pasteur 1951: 80(2):212-213.

24. Nikita ST, Delia BD, Savio R, Kulkarni MS. In vitro effect of vitamin C on the laboratory isolates of mycobacterium tuberculosis with known sensitivity and resistance to the first line anti tubercular drugs: an experimental pilot study. International Journal of Medical Research & Health Sciences. 2015:4(2):396-400.

25. Reddy YN, Murthy SV, Krishna DR, Prabhakar MC. Role of free radicals and antioxidants in tuberculosis patients. Index Medicus for South-East Asia Region (IMSEAR). 2004:51:213-218.

26. Mishra A, Sarkar D. Qualitative and quantitative proteomic analysis of Vitamin C induced changes in Mycobacterium smegmatis. Frontiers in microbiology. 2015:6:451.

27. Sikri K, Duggal P, Kumar C, Batra SD, Vashist A, Bhaskar A, Tripathi K, Sethi T, Singh A, Tyagi JS. Multifaceted remodeling by vitamin C boosts sensitivity of Mycobacterium tuberculosis subpopulations to combination treatment by anti-tubercular drugs. Redox biology. 2018:15:452-66.

28. Susanto L, Siregar Y, Kusumawati L. Vitamin C supplementation improve the sputum conversion culture rate in pulmonary tuberculosis treatment while rifampicin susceptible. InIOP Conference Series: Earth and Environmental Science. 2018:125(01):121-40.