

Effect of adding inhalation of sodium bicarbonate 8.4% to the usual treatment on smear-positive pulmonary tuberculosis: a prospective controlled study

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Background Pulmonary tuberculosis (TB) lesion is acidic, and changing this acidic pH may affect growth of TB bacilli and response to therapy. We aimed to assess the effect of adjuvant inhalation of sodium bicarbonate (SB) 8.4% on clinical, radiological, and microbiological responses in patients with sputum-positive drug-sensitive pulmonary TB.

Patients and methods One hundred and three patients with pulmonary TB completed the study, and they were classified into two groups: group I included 55 patients who received standard anti-TB regimen plus SB inhalation, and group II included 48 patients who received anti-TB regimen only. The responses in both groups were evaluated clinically, microbiologically, and radiologically.

Results There was no statistically significant difference between both groups in baseline bacillary load, clinical picture, and radiology. Both groups improved clinically 1 month after start of therapy. In group I only, there was a statistically significant improvement in chest radiograph after 1 month ($P < 0.001$). The median duration of smear conversion for group I was 3 weeks (1–8) compared with 9.5 (2–17) in group II, with a statistically significant difference ($P < 0.001$). Moreover, the median duration of culture conversion for group I was 1 month (1–3) compared with 3

months (1–4) in group II, with a statistically significant difference ($P < 0.001$).

Conclusion Adjuvant inhalation of SB in smear-positive pulmonary TB to standard anti-TB drugs accelerates smear conversion, culture conversion, and clinical and radiological improvement.

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Introduction

In 2014, tuberculosis (TB) infected nine millions people and killed 1.5 million [1]. Pulmonary TB infection occurs via inhalation of droplet nuclei from open pulmonary TB cases [2].

Drug-sensitive pulmonary TB is treated with the standard treatment regimen. Infectious patients become less infectious within 10–14 days from the start of treatment, and most patients with sputum smear-positive TB will become smear negative within 2 months [3]. Poor compliance to anti-TB treatment regimens is a major barrier to effective management of TB, increasing multidrug resistance and treatment failure [4].

Mycobacterium tuberculosis (MTB) bacilli are protected by the acidic media within human body as its classical location inside phagosomes of alveolar macrophages and the centers of caseating granulomas found in rabbit models of TB [5,6].

Sodium bicarbonate (SB) is commonly used as a pH buffering agent. It is used in patients with renal tubular

acidosis syndromes, diarrhea, acute lactic acidosis, and ketoacidosis [7]. Bronchoalveolar lavage (BAL) with SB 8.4% affects staining of TB bacilli with Ziehl–Neelsen stain and is inhibitory for TB bacilli in culture [8].

Rationale and aim

Application of SB to the infected lobe or lung with TB will temporally change the acidic medium within the TB lesion into neutral or alkaline. This may be inhibitory to MTB bacilli and may affect the structure of its cell wall as well as its response to anti-TB drugs. So, the aim of this study was to assess the effect of adjuvant inhalation of SB 8.4% to the standard anti-TB drugs on clinical, radiological, and microbiological responses in patients with sputum-positive drug-sensitive pulmonary TB.

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Patients and methods

This study included 111 patients with smear-positive drug-sensitive pulmonary TB who were admitted to/or treated as outpatients during the period from June 2014 to June 2016. The study was carried out chronologically in two successive periods (Fig. 1):

- (1) Control group: between June 2014 and June 2015, 52 consecutive patients with drug-sensitive smear-positive pulmonary TB were treated with the standard anti-TB regimen according to WHO, alone [9]. However, four patients were excluded as they died owing to pulmonary TB or associated chronic obstructive pulmonary disease at the initial phase of treatment. So, the data of 48 patients only completed the study.
- (2) Study group: between June 2015 to June 2016, 59 consecutive patients with drug-sensitive smear-positive pulmonary TB received the standard anti-TB regimen according to WHO [9] in addition to SB 8.4% inhalation in a dose of 5 ml/6 h using electric nebulizer for 1 month after sputum smear conversion into negative with continuation of the anti-TB drugs till the end of its course. However, two patients were lost to follow-up and two patients died owing to associated lymphoma, so 55 patients completed the study.

Inclusion criteria

Patients with smear-positive drug-sensitive pulmonary TB with or without extrapulmonary TB were included.

Exclusion criteria

The following were the exclusion criteria:

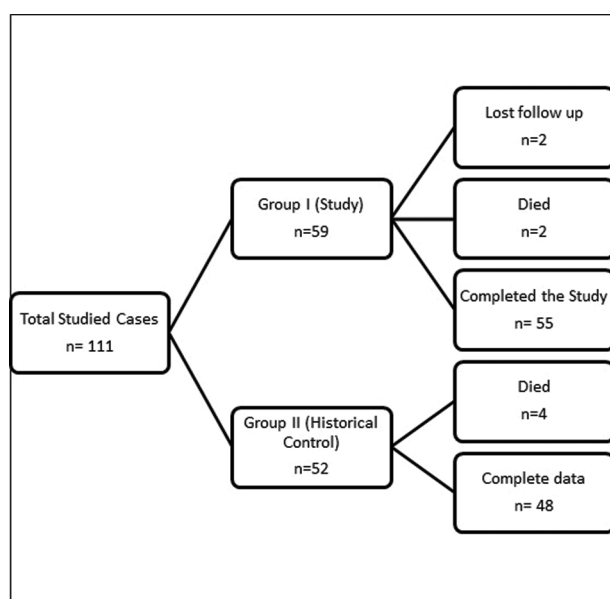
- (1) Patients with isolated extrapulmonary TB or drug-resistant pulmonary TB.
- (2) Patients refused to be included in the study.
- (3) Patients with known contraindications of SB as chronic heart failure, severe renal impairment, visible water retention, kidney problems causing a decreased amount of urine to be passed, or known allergy to the compound.

Ethics approval had been obtained from Institutional Research Board, Mansoura University, code number MS/15.06.32, and the research unit in Ministry of Health, Egypt, code number 19-2015/8. It was also registered on PACTR with unique identification number for the registry PACTR201508001234317. All patients signed their written informed consent forms to be included in the study.

Both groups were subjected to the following:

- (1) Clinical evaluation with stress on smoking, drug abuse, respiratory symptoms, fever, and clinical examination, which were evaluated monthly till the end of treatment.
- (2) Laboratory investigations such as complete blood count, blood glucose, liver function tests, and serum creatinine, which were also evaluated monthly.
- (3) Microbiological examination:
 - (a) For both groups, three morning sputum samples on 3 successive days for Ziehl–Neelsen staining that was repeated weekly after the start of anti-TB treatment till smear conversion then monthly to the end of the recommended anti-TB regimen. Bacillary load was graded according to Lohmann *et al.* [10].
 - (b) TB culture on Lowenstein–Jensen medium at the start of the study and then monthly to the end of treatment.
 - (c) GeneXpert was done before the start of treatment, and the patients with rifampicin resistance were excluded.
- (4) Radiological investigations:
 - (a) Chest radiograph: posteroanterior view was repeated monthly. The disease extent was classified radiologically into either minimal, moderately advanced, or far advanced according to the National Tuberculosis Association of the USA [11].
 - (b) CT chest was done at the start of treatment and repeated if there was no response or reported complications.

Figure 1



Schematic representation for the studied cases.

- (5) Treatment regimens for TB: For patients in both groups, they received standard first-line anti-TB regimen for 6 months according to WHO [9].
- (6) SB 8.4%: Patients in study group only (group I) received SB 8.4% inhalation using electric nebulizer in a dose of 5 ml/6 h until sputum smear conversion into negative.

Monitoring of the patients was done for the adverse effects of the prescribed anti-TB drugs or the inhaled SB. Both groups were followed up for 1 year for the TB relapse.

Outcomes of the treatment were cured, treatment completed, treatment failure, or default according to WHO definitions [9].

Statistical analysis

Statistical analysis of data was done using Excel and SPSS program version 16.0. The normality of data was first tested with one-sample Kolmogorov–Smirnov test. Categorical data were presented as numbers (percentage). For data with normal distribution, data were presented as mean \pm SD; independent-samples *t*-test was used to compare the results between two groups. For data without normal distribution, data were presented as median (minimum–maximum); nonparametric two-related-samples test (Wilcoxon type) was used to compare the results in the same group, and

Mann–Whitney *U*-test was used to compare the results between two groups. χ^2 -Test was used to compare paired proportions (or Fisher exact test when needed). Statistical significance was defined as *P* value less than 0.05.

Results

Demographic data and comorbidities of both groups are illustrated in Table 1. With the exception of sex, there was no statistically significant difference between both groups.

Table 1 shows also the baseline bacillary load. The median baseline bacillary load was higher in study group [2 (1–5)] compared with the control group [1 (1–3)], but this difference was statistically insignificant (*P*=0.282).

Before the start of treatment, there was no statistically significant difference in clinical symptoms between both groups. Both groups showed significant improvement in symptoms without statistically significant difference between them (Table 1).

Regarding CXR, there was no statistically significant difference between both groups at the start of therapy or after 1 month (*P*=0.437 and 0.888, respectively). However, within the group, only group I showed

Table 1 Baseline characteristics of both groups

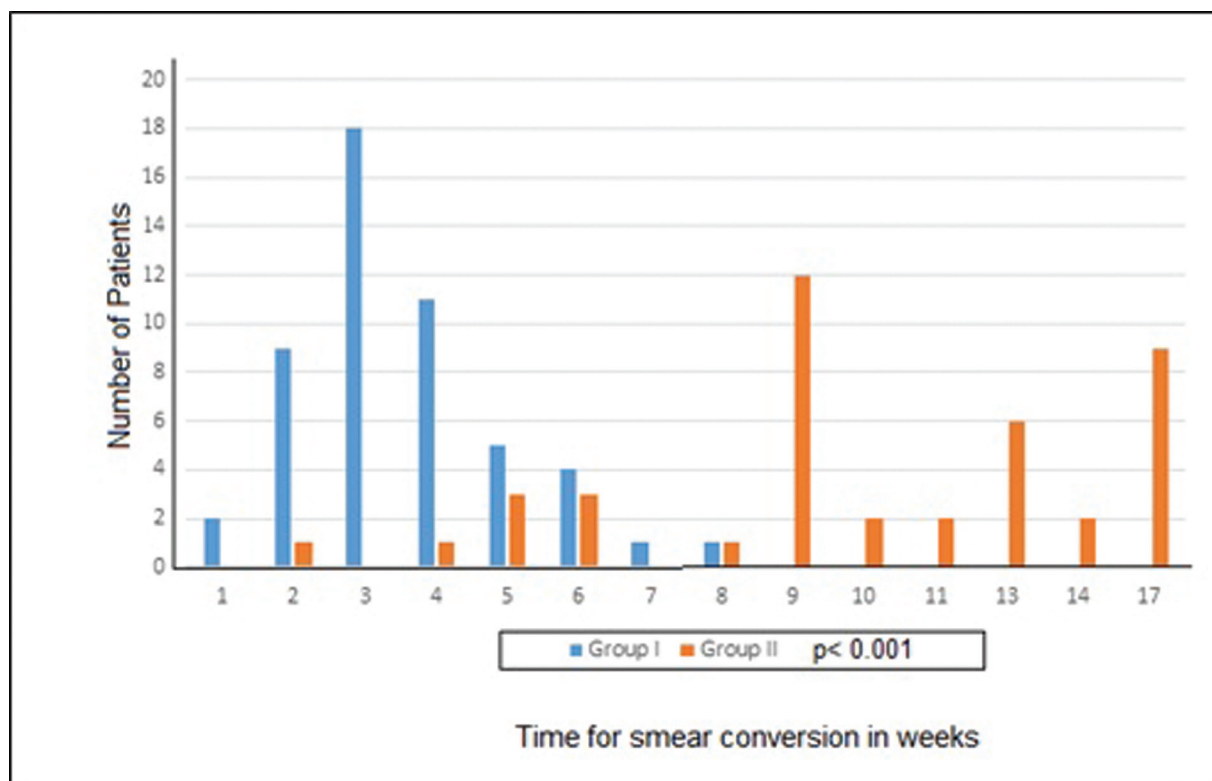
Variables	Study group (<i>n</i> =55)	Control group (<i>n</i> =48)	<i>P</i> value
Demographics			
Age	40.72 \pm 15.06	43.81 \pm 13.85	0.285
Sex			
Male	47 (85.5)	48 (100)	0.007 ^a
Female	8 (14.5)		
Weight (kg) (mean \pm SD)	63.77 \pm 12.44	63.53 \pm 11.31	0.924
Diabetes mellitus	12 (21.8)	9 (18.8)	0.665
Hypertension	2 (3.6)	3 (6.2)	0.664 ^a
Smoking	23 (41.8)	30 (62.5)	0.055
Clinical presentation and MMRC			
Cough	55 (100)	48 (100)	–
Expectoration	55 (100)	48 (100)	–
Hemoptysis			
Blood tinged	9 (16.4)	4 (8.3)	0.394
Frank	10 (18.2)	12 (25)	
Wheezes	5 (9.1)	6 (12.5)	0.576
Night fever	55 (100)	48 (100)	–
Night sweating	55 (100)	48 (100)	–
Weight loss	55 (100)	46 (95.8)	0.215 ^a
Anorexia	55 (100)	46 (95.8)	0.078 ^a
MMRC [median (minimum–maximum)]	3 (1–4)	3 (2–4)	0.983
Bacillary load [median (minimum–maximum)]	2 (1–5)	1 (1–3)	0.282
CXR score [median (minimum–maximum)]	3 (1–3)	3 (1–3)	0.437

MMRC, modified medical research council. ^aFisher exact test.

Table 2 Follow-up characteristics of both groups' clinical presentation and radiology

Variables	Study group (n=55)	Control group (n=48)	P value
Clinical presentation and MMRC			
Cough	1 (1.8)	0	—
Expectoration	0	0	—
Hemoptysis			
Blood tinged	0	3 (6.2)	0.092*
Frank	—	—	
Wheezes	2 (3.6)	3 (6.2)	0.660
Night fever	0	0	—
Night sweating	0	0	—
Weight loss	0	1 (2.1)	0.463
Anorexia	0	2 (4.2)	0.077*
MMRC [median (minimum–maximum)]	2 (2–2)	2 (0–3)	0.097
CXR score [median (minimum–maximum)]	1 (0–3)	1 (1–2)	0.888

MMRC, modified medical research council. *Means Fisher exact test.

Figure 2

Time for smear conversion in weeks [the median duration of smear conversion for group I was 3 weeks (1–8) compared with 9.5 weeks (2–17) in group II ($P<0.001$)].

statistically significant improvement in CXR after 1 month of treatment ($P<0.001$; Tables 1 and 2).

The median duration for smear conversion in weeks in study group was 3 (1–8) and 9.5 (2–17) in control group, with a statistically significant difference between both groups ($P<0.001$; Fig. 2).

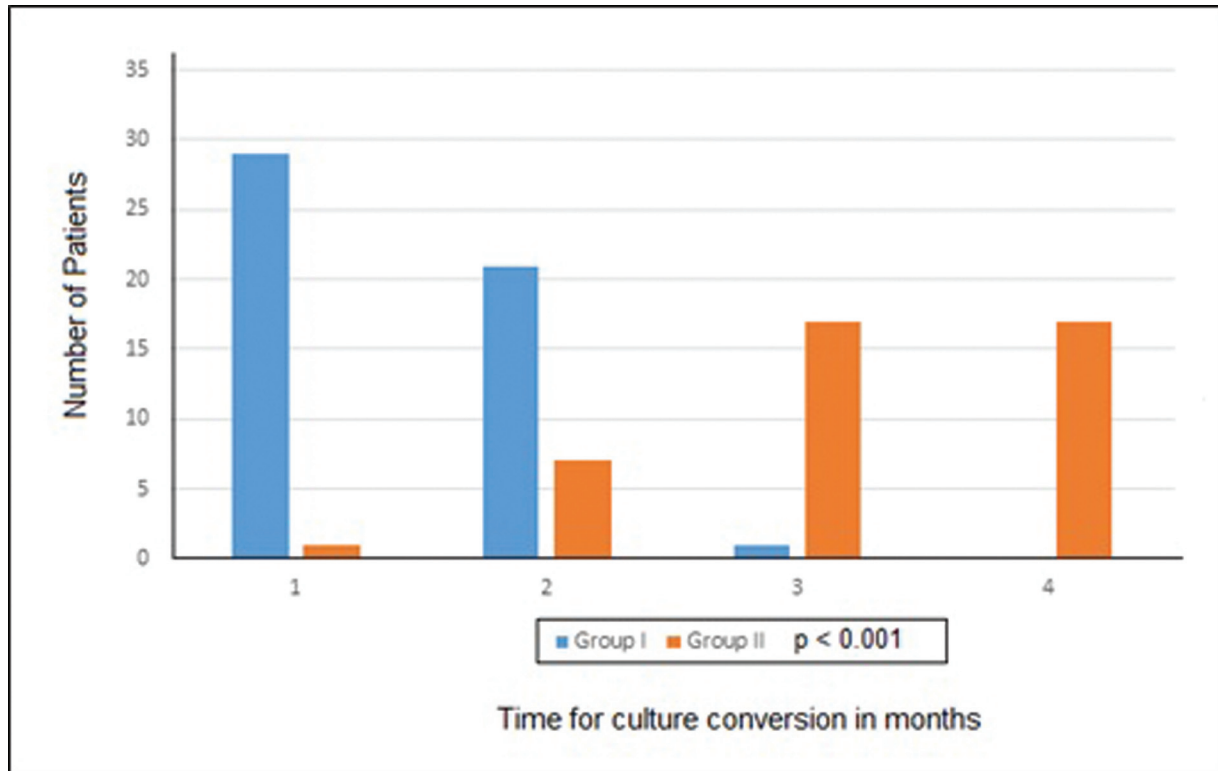
Moreover, the median duration of culture conversion for group I was 1 month (1–3) compared with 3 months (1–4) in group II, with statistically

significant difference between both groups ($P<0.001$; Fig. 3).

Regarding the adverse effects of SB inhalation, all patients after SB inhalation developed mild cough and salty taste for 5–10 min after SB inhalation session. No serious adverse effects were reported in all patients.

On follow-up of all patients for one year after the end of the study, no relapse was reported in all cases.

Figure 3



Time for culture conversion in months [the median duration of culture conversion for group I was 1 month (1–3) compared with 3 months (1–4) in group II ($P < 0.001$)].

Discussion

In healthy individuals, airway surface liquid lines the conducting airways of the lungs, and it has an acidic pH (about 6.6) [12,13]. Local pH is one of the effective host innate immunity lines against microorganisms. Pulmonary infections lead to local acidic pH, which promotes growth of bacteria and increases bacterial resistance [14]. It also causes inactivation of antibiotics and impairs function of alveolar macrophages [15,16].

Pulmonary TB is transmitted through inhalation of droplet nuclei of infected patients and spread through droplet nuclei which carry infectious bacilli. So, they are considered to be the most significant source of infection for TB [17]. Agarwal and Chauhan [18] reported that one untreated patient with infectious TB is likely to infect 10–15 persons annually.

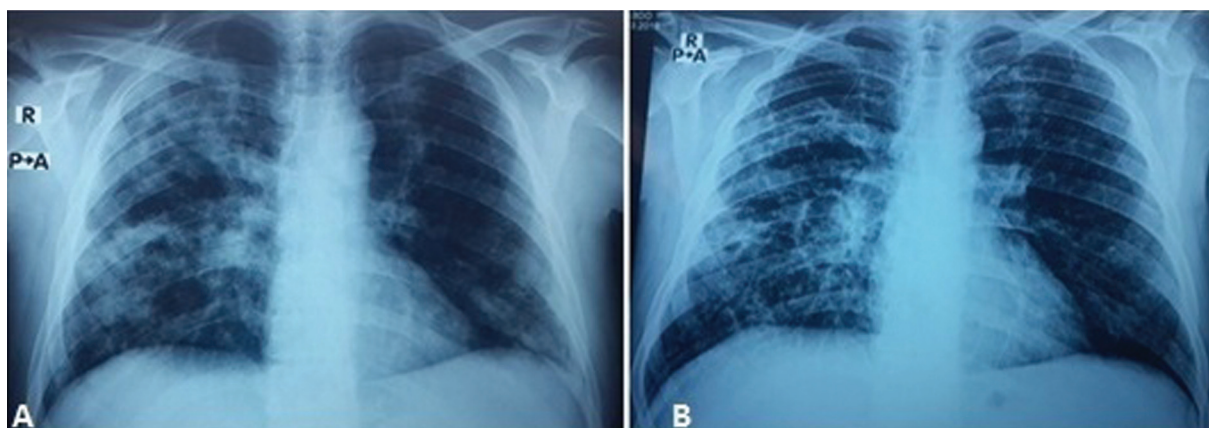
Sputum conversion usually occurs in ~80–90% of patients within 2–3 months of treatment [19]. Factors that lead to delay in smear and culture conversion include high initial bacillary load, TB cavities, diabetes mellitus, old age, multidrug resistance-TB, initial treatment with less than four anti-TB drugs, and non-rifampicin-based treatment regimens [15,20].

Exhaled breath condensate pH in patients with active pulmonary TB was significantly lower than control. The authors speculated that airway epithelium acidifies in response to various insults [21]. Therefore, they theorized that changing the acidic pH of airway fluid secretions into alkaline or neutral pH may change the acidic medium in the TB lesions, which may disturb the structure and/or multiplication of MTB and enhance its response to anti-TB treatment. The target was to augment the effect of anti-TB drugs to convert the sputum-positive patients into smear negative in shorter periods and decrease the inflammatory response in the lungs that may be useful in prevention of drug resistance and abortion of TB transmission to other susceptible persons.

We selected for the study patients with sputum-positive drug-sensitive pulmonary TB because they represent a worldwide health problem as the chance of transmission of TB to the contacts is high, and till now, there are no new effective drugs that can shorten the duration of TB treatment, and the incidence of TB resistance to any newly introduced drug is high.

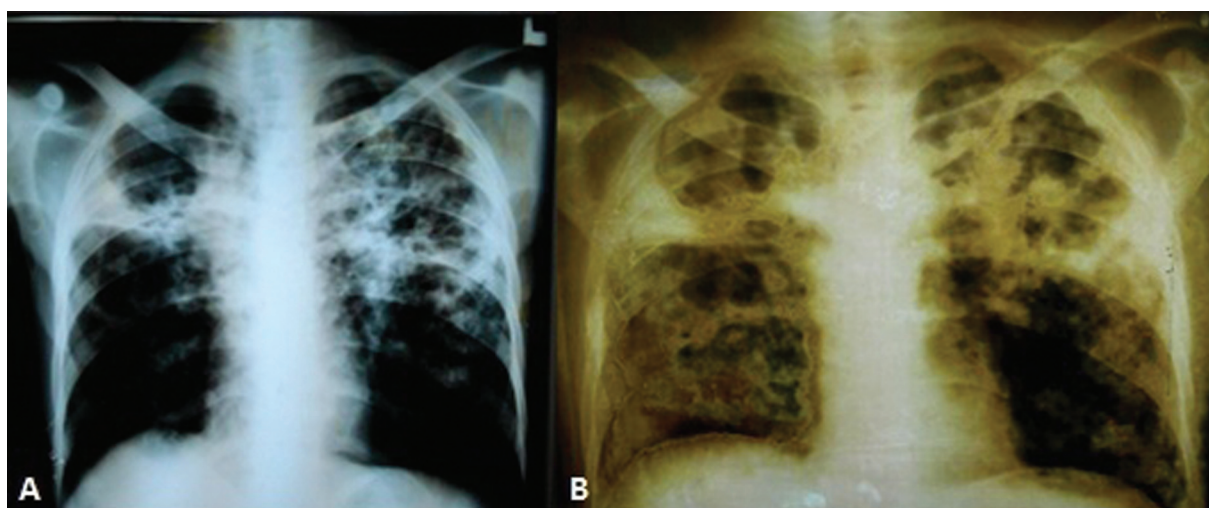
Most MTB bacilli are rapidly metabolizing and killed within the first 8 weeks of treatment; however, there are semidormant bacilli that require longer durations of

Figure 4



A 45-years-old male patient with pulmonary tuberculosis from group I. (a) CXR before starting treatment showing alveolar infiltrates in all right lung zones (far advanced). (b) CXR after one month of treatment showing marked improvement.

Figure 5



A 34-year-old male patient with pulmonary tuberculosis from group II. (a) CXR before starting treatment showing bilateral alveolar infiltrates with cavitation (far advanced). (b) CXR after one month of treatment showing no improvement.

treatment and the intracellular bacilli that require drugs that act in the acidic medium [3]. If treatment is not continued for a long enough duration, the surviving bacteria may cause the patient to become ill and infectious again, potentially with drug-resistant disease [22].

We selected SB 8.4% in this study owing to its safety to human and its high alkaline pH. It is commonly used as a pH buffering agent. Chronic bicarbonate replacement is used safely for patients with renal tubular acidosis syndromes or diarrhea. In patients with acute lactic acidosis and ketoacidosis, however, bicarbonate therapy must be individualized [7]. It has also an inhibitory effect to the respiratory pathogens including TB in-vitro as reported in a study conducted by Abdalla *et al.* [8], as they found a statistically significant inhibitory effect of BAL with SB on TB

cultures when compared with BAL with saline ($P=0.031$).

We used nebulizer for delivery of SB as it is noninvasive, can be repeated many times per day at home or hospital, and can transform the fluid SB into inhalable particles. The given dose was 5 ml, because with the use of nebulizer for delivery of medication, part of drug remains inside, called dead volume and ranges from 1 to 3 ml. So, a nebulizer fill volume of 4–6 ml is recommended according to Hess *et al.* [23]. All patients in group I received SB inhalation for 1 month after sputum conversion.

Regarding symptoms, there was no significant difference between groups in respiratory symptoms or toxemic manifestations (Table 1).

Only patients in group I showed a significant improvement in follow-up CXR ($P < 0.001$) 1 month after start of treatment (Table 2 and Figs 1 and 2).

The median duration of smear conversion for group I was 3 weeks (1–8) compared with 9.5 weeks (2–17) in group II ($P < 0.001$; Fig. 2), and the median duration of culture conversion for group I was 1 month (1–3) compared with 3 months (1–4) in group II, with statistically significant difference between both groups ($P < 0.001$; Fig. 3).

Iivanainen *et al.* [24] studied the occurrence of MTB in aerobic brook sediment. They found that the culturable counts of MTB correlated negatively with water and sediment pH and with alkalinity of water and that acidity increases the count of MTB. Moreover, Parashar *et al.* [25] in their research about neutralization of the gastric aspirate with SB divided the gastric aspirate in two containers, and one of them was neutralized with SB with a concentration of 1%. After doing smear and culture examinations for the aspirate, there were no differences in smear positivity. However, the culture result was significantly lower in the neutralized samples [16.3% (38/232)] than in the non-neutralized samples [21.5% (50/232); $P = 0.023$].

MTB bacilli can live in acidic media inside macrophages. The estimated pH of the macrophage compartment, in which MTB resides, ranges from pH 6.2 to 4.5, depending on the activation state of the macrophage [26–28]. Optimal growth of MTB in enriched liquid medium was observed at a slightly acidic pH, between 5.8 and 6.7 [29].

Jackett *et al.* [30] reported that a variety of strains of MTB are resistant to killing at a pH of 4.5 in phosphate-citrate buffer. The bacilli are also able to maintain a near-neutral intrabacterial pH when placed in phosphate-citrate buffer at pH 4.5, indicating that they are able to counter the entry of protons [31]. Moreover, other different studies had addressed survival of MTB in acidic media [32,33]. In the early 1900s, Metchnikoff [34] speculated that the waxy MTB cell wall serves as an important guard against acid stress present in phagocytes. MTB has a lipid-rich cell wall that consists of a typical bilayered plasma membrane followed by a layer of peptidoglycan-arabinogalactan covalently linked to mycolic acids. In 2008, researchers demonstrated the existence of an additional outer lipid bilayer surrounding MTB [35,36]. This complex cell envelope acts as a major permeability barrier for antibacterial effectors, including protons. Indeed,

studies examining the physiology of mycobacteria at low pH reported that the cell wall plays a critical role in resistance to acid [29].

From the aforementioned data, we speculated that changing this acidic media in which MTB live may lead to changes in the wall of TB bacilli leading to its destruction, which may explain the shorter time of smear conversion in study group compared with control group.

Regarding radiological changes 1 month after starting anti-TB drugs in this study, in group I, there was a significant improvement in disease extent in chest radiograph regarding parenchymal infiltration and cavitation. This may be explained by rapid smear conversion and regression of the inflammatory response to TB bacilli. Mesquita *et al.* [37] studied the relation between bacillary load, inflammatory markers, and radiological findings before treatment and after 2 months. They found that individuals presenting with pretreatment AFB smear less than 2+ and CRP levels less than 4.7 mg/l were 10 times more likely to exhibit radiographic improvement of lung disease compared with those with higher values of those parameters ($P = 0.002$). In group II, there were no significant radiological changes in the disease extent. By increasing the local bronchial pH, SB weakens the bonds between the side chains of the mucus molecule, which decreases mucus viscosity and elasticity [38].

Finally, this study has some limitations. First, we did not address changes in airway fluid pH after inhalation of SB. Second, we did not examine structural changes of the MTB bacilli that may explain the results. Moreover, titration of the most suitable dose of SB, frequency of its use, and the most suitable mode of administration were not evaluated. Application of SB inhalation all over the course of anti-TB drugs may be more effective.

Conclusion

Inhalation of SB 8.4% in drug-sensitive pulmonary TB as adjunctive therapy to standard anti-TB drugs shortens the duration required for smear and TB culture conversion and achieves rapid clinical and radiologic improvements.

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Conflicts of interest

There are no conflicts of interest.

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