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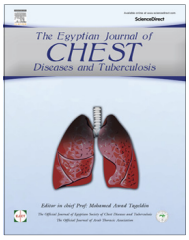

ORIGINAL ARTICLE

The role of supplementary vitamin D in treatment course of pulmonary tuberculosis

Essam Gouda Hassanein [a], **Enas El Said Mohammad** [a], **Ayman Ibrahim Baess** [a,*], **Eman Tayae EL-Sayed** [b], **Ahmad Madi Yossef** [c]

[a] *Department of Chest Diseases, Alexandria Faculty of Medicine, Egypt*
[b] *Department of Clinical and Chemical Pathology, Alexandria Faculty of Medicine, Egypt*
[c] *EL-Maamoura Chest Hospital, Alexandria Governorate, Egypt*

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KEYWORDS

Vitamin D;
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Abstract *Background:* Vitamin D insufficiency/deficiency is associated with impaired immune function and increased risk of active pulmonary tuberculosis (TB).

Objectives: To evaluate the role of vitamin D as supplementary treatment with the first line anti-tuberculous drugs (rifampicin, izoniazide, ethambutol and pyrazinamide) in treatment course of patients with active pulmonary tuberculosis.

Methods: We conducted a case-control study in El Maamora chest hospital, Alexandria governorate, Egypt, including 60 adult patients with active pulmonary TB of 30 patients each. Patients in group I (cases) received vitamin D (200,000 IU) intramuscular injection once besides anti-tuberculous drugs, while patients in group II (controls) were randomly selected from the hospital registry who received the first line anti-tuberculous treatment only. The primary outcome was evaluation of conversion time of sputum smear. The secondary outcome was clinical improvement as assessed by TB score.

Measurements and main results: Mean ± SD age of all patients was 41.55 ± 14.91 years. The study included 44 (73.3%) males and 16 (26.7%) females. Vitamin D deficiency/insufficiency was detected in 54 (90%) patients. Comparing the two groups, there was a rapid decline in sputum conversion time and severity classes of TB score in group I compared to group II ($p < 0.001$ and $p = 0.02$, respectively). No complications secondary to supplementary vitamin D were met all through the study.

Conclusion: Vitamin D accelerates the improvement observed in vitamin D supplemented TB therapy. Vitamin D is safe when added to anti-tuberculous drugs. Vitamin D deficiency/insufficiency is common among TB patients. Further studies are required to validate this observation and define a cut off of vitamin D level to prevent immunological alterations.

* Corresponding author at: Chest Department, Secretary Office, Alexandria Faculty of Medicine, Champillion St, Alexandria, ElAzareita 21131, Egypt. Tel.: +20 1006822068.
E-mail address: ayman.baeis@yahoo.com (A.I. Baess).

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Introduction

Tuberculosis (TB) is a chronic specific bacterial infection caused by bacteria of the *Mycobacterium tuberculosis* [1]. Human monocytes have receptors for 1,25 dihydroxyvitamin D that activates anti mycobacterial responses in human monocytes and macrophages by enhancing phagocytosis and granuloma formation [2].

Vitamin D is synthesized in the skin during exposure to ultra violet B (UV-B) radiation and is also present in foods. Vitamin D is readily metabolized in the liver, to form 25 hydroxy-vitamin D [25(OH)D], the accepted measure of vitamin D status [3,4]. Calcitriol, the active metabolite of vitamin D, induces anti mycobacterial activity in vitro [2]. This metabolite modulates the host response to Mycobacterial infection by induction of reactive nitrogen and oxygen intermediate, [5,6] suppression of matrix metalloproteinase enzymes implicated in the pathogenesis of pulmonary cavitation [7] and induction of anti microbial peptide catheliciden [8,9] which induces autophagy [10].

The human vitamin D receptors (VDRs) are polymorphic. Carriage of the t allele of taq I VDR polymorphism is associated with an increase in calcitriol-induced phagocytosis of *M. tuberculosis* in vitro [11] and more rapid sputum conversion in patients with pulmonary tuberculosis [12]. While, carriage of f allele of the fok I VDR polymorphism is associated with a reduction in transcriptional activity, [13] reduction of calcitriol-induced phagocytosis, and slower sputum smear or culture conversion in pulmonary tuberculosis [11].

Lower vitamin D level was reported to be associated with a higher risk of developing active pulmonary tuberculosis [14]. The role of vitamin D in modifying the treatment course of pulmonary tuberculosis is still a matter of debate [15,16]. We aimed this work to evaluate the role of vitamin D as supplementary treatment when added to the first line anti-tuberculous drugs, (rifampicin, izoniazide, ethambutol, and pyrazinamide) in the treatment course of patients with active pulmonary tuberculosis.

Methods

Study site and time

El-Maamoura chest hospital in Alexandria governorate, Egypt, from January to August 2015.

Study design

We conducted a randomized, case control clinical trial, including adult ($\geqslant$18 years old) patients who had active pulmonary TB diagnosed by sputum examination (smear microscopy or mycobacterial culture) or by World Health Organization clinical criteria [17]. Exclusion criteria were corticosteroids or immunosuppressive treatment, human immunodeficiency virus (HIV), multi-drug resistant TB (MDR-TB), Liver cirrhosis, renal failure, vitamin D supplementation, malignancies, hypercalcemia.

Patients

This study enrolled sixty patients with active pulmonary tuberculosis divided into two groups of thirty patients each. Group I (cases) included patients who received both the first line anti tuberculous treatment besides supplementary vitamin D [200,000 IU of vitamin D (25(OH)vitamin D) intramuscular injection once, (Devarol-S-ampoule, Memphis for pharmaceuticals & chemicals industries, El-Amirya, Cairo, Egypt). Group II (controls) included patients who were randomly selected from the hospital registry who received the first line anti tuberculous treatment only.

Follow-up

Patients were followed up weekly by microscopic examination of sputum for acid fast bacilli of pulmonary tuberculosis, [18,19] and calculation of TB score [20].

Adverse effects

We questioned the patients for the following adverse effects related to hypercalcemia e.g. nausea, vomiting, excessive thirst, anorexia, symptoms of kidney stones, and confusion.

Measurements

Body mass index (BMI) was assessed using the following formula: BMI = weight/(height)^2. Height was measured by a meter scale; weight was measured in kilograms, using the same weight scale at each patient visit. Mid–upper arm circumference was measured at the midpoint between the acromion and olecranon over the biceps of the non dominant arm, using a non stretchable measuring tape. Severity of TB disease was assessed by the TB score [20]. TB score has been validated in another cohort and has been grouped in severity classes as follows: I (0–5 points), II (6 or 7 points), or III (8 points or more).

Serum level of 25-hydroxyvitamin D [21] was measured at time of diagnosis and after 2 months of anti-tuberculous therapy with chemiluminescence immunoassay technology using ADVIA Centaur immunoassay system (Siemens Healthcare Diagnostics, 1717 Deerfield Road, Deerfield, IL 60015-0778, USA). Serum vitamin D was defined as deficient ($<$20 ng/ml), insufficient (20–30 ng/ml), sufficient (30–100 ng/ml), or toxic ($>$100 ng/ml).

Serum levels of Calcium as well as albumin, both were measured at time of inclusion and at the end of 2nd month. Serum albumin was measured in order to correct serum calcium using the following equation: Corrected calcium (mg/dL) = serum calcium + 0.8 (4 − serum albumin (g/dL)) [22] Hypocalcemia, normo-calcemia or hypercalcemia were defined when corrected serum calcium levels were below 8.5 mg/dl, 8.5–10.10 mg/dl or exceeding 10.10 mg/dl, respectively [22].

Outcome

The primary outcome was evaluation of conversion time of sputum smear. The secondary outcome was clinical improvement as assessed by TB score [20]. The TB score is a newly developed tool aimed at assessment of change in the clinical state in patients with TB. It is based on points assigned to signs and symptoms, including cough, hemoptysis, dyspnea, chest pain, night sweating, anemia, tachycardia, lung auscultation finding, fever, low body mass index, and low mid–upper arm

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circumference, giving patients a TB score from 0 to 13. Change in TB score has been shown to detect clinical change as well; a high TB score correlates well with mortality and low TB scores correlate with favorable outcomes, cure, and completed treatment [20].

Statistical analysis

Data were fed to the computer and analyzed using IBM SPSS software package (IBM SPSS Statistics for Windows, Version 20.0., IBM Corp., Armonk, NY) Qualitative data were described using number and percent. Quantitative data were described using range (minimum and maximum) mean, standard deviation and median. Comparison between different groups regarding categorical variables was tested using Chi-square test. When more than 20% of the cells have expected count less than 5, correction for chi-square was conducted using Fisher's Exact test or Monte Carlo correction. McNemar–Bowker and Marginal homogeneity test was applied for ordinal data .The distributions of quantitative variables were tested for normality using Kolmogorov–Smirnov test, Shapiro–Wilk test and D'Agstino test, If it reveals normal data distribution, parametric tests was applied. If the data were abnormally distributed, non-parametric tests were used for normally distributed data, comparison between the two studied groups were done using independent *t*-test, also paired *t*-test is used to analyze two paired data. For abnormally distributed data, comparison was done using Mann Whitney test. To compare between the different periods, Wilcoxon signed rank test was applied. Correlations between two quantitative variables were assessed using spearman coefficients regarding normality of the data. Significance of the obtained results was judged at the 5% level.

Results

Group I included 22 (73.3%) males and 8 (26.7) females. Sex-matched controls were selected from the hospital registry where 22 males and 8 females were included in group II. In group I, mean ± SD age was 41.70 ± 14.87 years. Age-matched controls were selected as group II from the registry with a mean ± SD age of 41.40 ± 14.94 years. There was no statistically significant difference between the two studied groups regarding age ($p = 0.938$). Regarding smoking status, Group I included 11 (36.7%) non-smokers and 19 (63.35%) smokers. Group II included 8 (26.7%) non-smokers and 22 (73.3%) smokers. There was no statistically significant difference between the two groups regarding smoking status ($p = 0.405$).

In group I, on commencing the anti-TB treatment, Serum vitamin D deficiency was defined in 29(96.6%) patients, serum vitamin D insufficiency was detected in a single (3.4%) patient while there was no cases where vitamin D sufficiency or serum vitamin D toxicity was found. Mean serum vitamin D level at the start of treatment was 9.83 ± 5.88 ng/dl. At the end of 2nd month, serum vitamin D deficiency was detected in 7 (23.3%) patients, serum vitamin D insufficiency was traced in 17 (56.7%) patients, serum vitamin D sufficiency was found in 6 (20%) patients, while there were no detected patients with serum vitamin D toxicity. Mean serum vitamin D level at the end of second month of treatment was 23.76 ± 7.09 ng/dl. Notably, there was a statistically significant difference between the cases at the start of treatment and the end of 2nd month of anti-TB treatment regarding levels of serum vitamin D ($p < 0.001$) Fig. 1.

At the start of anti-TB treatment, group I included 22 patients (about 73.3%) who were hypocalcemic, 8 patients (26.7%) who were normo-calcemic, and there were no patients with hypercalcemia. The mean corrected serum calcium level at the start of treatment was 8.19 ± 0.71 mg/dl. At the end of 2nd month of anti-TB treatment, detected 4 patients (about 13.3%) who were hypocalcemic, 15 patients (50%) who were normo-calcemic, 11 patients (36.7%)who were hypercalcemic. The mean corrected serum calcium level at the 2nd month of anti-TB treatment was 3.15 ± 0.55 mg/dl. There was a statistical significant difference in serum calcium level at the start and after 2-months of initiating anti-TB treatment ($p < 0.001$) Fig. 2.

Group I included 17 patients (56.7%) who were sputum smear negative for acid fast bacilli at 3rd week, 9 patients (30%) who were sputum smear negative at 4th week and 4 patients (13.3 %) who were sputum smear negative at 5th week. Mean conversion time ± SD = 3.57 ± 0.73 weeks. While group II included 3 patients (10%) who were sputum smear negative for acid fast bacilli at 5th week, 8 patients (26.7 %)who were sputum smear negative at 6th week, 5 patients (16.7%) who were sputum smear negative at 7th week and 14 patients (46.7 %) who were sputum smear negative at 8th week. Mean conversion time ± SD = 7.0 ± 1.08 weeks. There was a statistical significant difference between the two groups regarding the time of sputum conversion ($p < 0.001$) Figs. 3 and 4.

At the start of anti-TB treatment for patients in group I, 14 patients (46.7%) who were severity class (SC II) and 16 patients (53.3%) who were (SC III), Mean TB score was 2.53 ± 0.51. Group II included 13 patients (about 43.3 %) who were (SC II) and 17 patients (about 56.7 %) who were (SCIII), Mean TB score was 2.57 ± 0.50. There was no statistical significant difference between the two groups regarding TB score at the start of treatment with anti tuberculous drugs ($p = 0.795$) Fig. 5.

At the time of conversion of sputum smear or end of 2nd month, group I included 26 (86.7%) patients who were severity class (SC I) and 4 (13.3%) patients who were (SC II). Mean TB

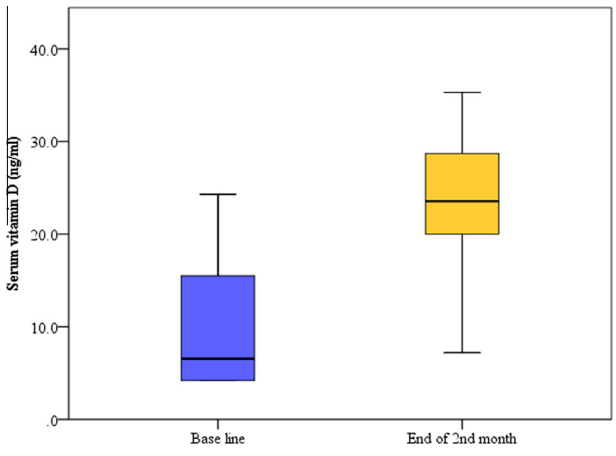

Figure 1 Serum vitamin D measurement in patients in group I at base line and 2 months after initiation of supplemented treatment.

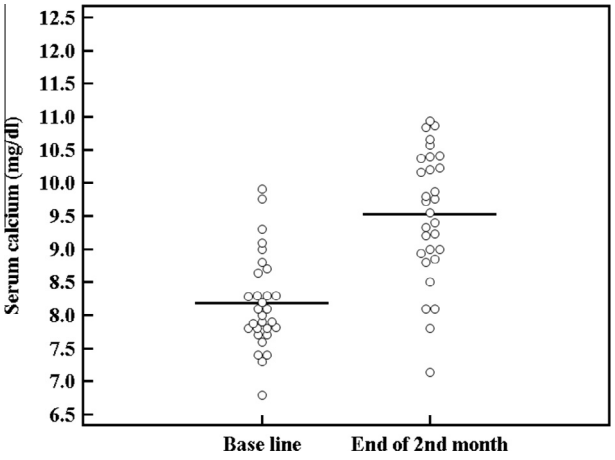

Figure 2 Corrected serum calcium level in patients in group I at baseline and 2 months after initiation of supplemented therapy.

score was 1.13 ± 0.35. Group II included 18 (60%) patients who were severity class (SC I) and 12 (40%) patients who were (SC II), Mean TB score was1.40 ± 0.50. There was a statistical significant difference between the two groups regarding TB score at the time of conversion of sputum smear or end of 2nd month (p 0.020) Fig. 6.

Discussion

Vitamin D insufficiency/deficiency is associated with impaired immune function and increased risk of active TB. It has recently been proposed that vitamin D accelerates resolution of host inflammatory responses and this may contribute to the improvement observed in vitamin D supplemented TB therapy [23].

In the present study, the mean age was 41.70 ± 14.87 years in patients in group I while it was 41.40 ± 14.94 years in the control group (II).Similarly, in El Maamora chest hospital, Mohamed et al. [24] studied the clinical presentation, complication and outcome of patients suffering from multi-drug resistant tuberculosis (MDR-TB) and extensively drug resistant (XDR-TB) and found that the mean age was 40.36 ± 14.74 years. Similarly, Sayed et al. [25] studied the role of interferon gamma release assays (IGRAs) in the diagnosis of pulmonary TB and found the mean of age was 38.75 ± 10.45 years.

This may be explained by fact that TB is a disease of young and middle-aged adults with most cases occurring between the ages of 20 to 40 years due to increase of exposure to infection in this active age group and the effect of physical and mental stress [26].

The present study revealed that the majority of patients were males (73.3%) in both groups. Similarly, Mohammed et al. [24] reported males were 70% of patients, Elrefaey et al. [27] reported that males were 79.3% of cases.

This coincides with the epidemiological picture of tuberculosis where males are more exposed to infection in the community than females. Male and female rates of infection are nearly the same from childhood through young adult life. Thereafter, the rate of infection in males becomes increasingly greater than in females in the absence of widespread HIV infection. Also in some instances, women may have poorer access to the diagnostic facilities [26,28].

Of note, the same or near-same age and gender in both groups is due to the fact that after enrollment of all patients in group I, Age and sex-matched controls were chosen from the hospital registry.

In the current study, the percentage of smokers was (63.35%) in group (I) and (73.3%) in group (II). The role of active smoking in development of TB is well known as described by WHO reports from different countries [29].

Similarly, Oztürk et al. [30] who studied the effect of smoking and indoor air pollution on the risk of tuberculosis found that patients who smoke had a fivefold ($p < 0.0001$) higher odds of having active tuberculosis compared with patients who do not smoke.

Also Mohammed et al. [24] found that 50% of MDR patients were tobacco smokers and 46.8% of tuberculous non-MDR patients were smokers.

Tobacco smoking increases risk of *M. tuberculosis* infection by several means namely; alteration of muco-ciliary clearance,

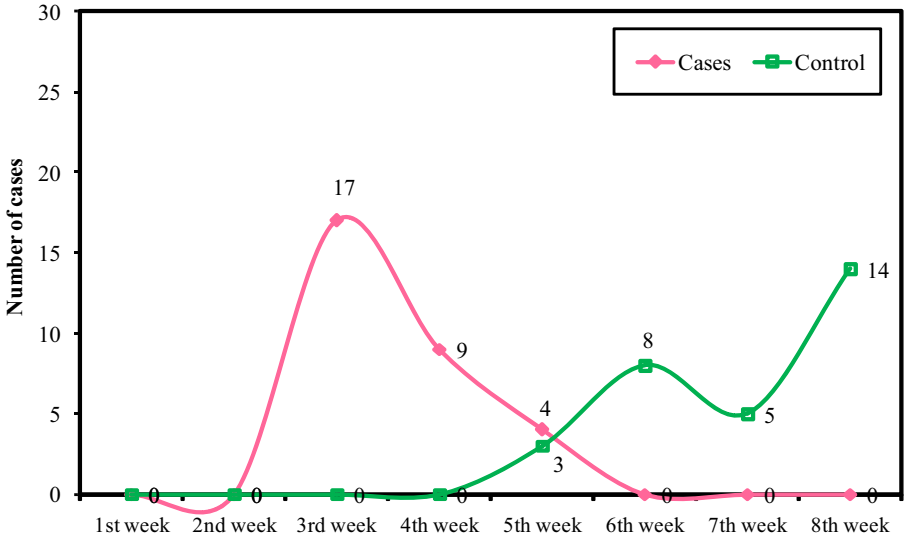

Figure 3 Comparison between the two studied groups according to sputum conversion time.

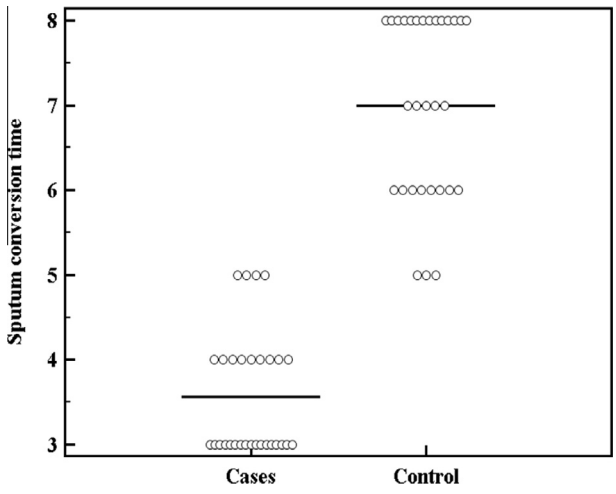

Figure 4 Mean sputum conversion time in both studied groups.

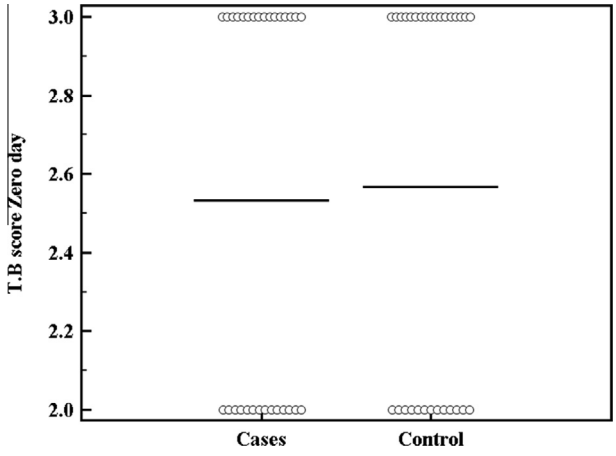

Figure 5 Comparison between the two studied groups according to TB score at day zero.

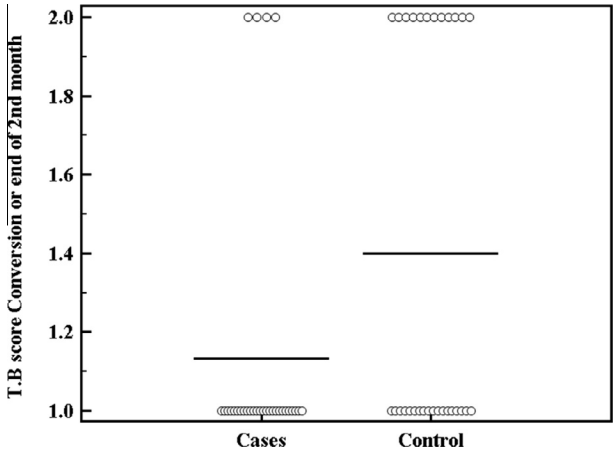

Figure 6 Comparison between the two studied groups according to TB score at sputum conversion or at the end of 2nd month of initiating supplemented treatment.

reduced alveolar macrophage activity; immune-depression of pulmonary lymphocytes, reduction of cytotoxic activity of natural killer cells and alteration of the activity of the pulmonary dendritic cells [31].

In the current study, there was a statistical significant difference between serum vitamin D at the start of anti-TB treatment and that at the end of 2nd month after initiation of anti-Tb treatment ($p < 0.001$) .This signifies the good response of patients with active pulmonary TB to vitamin D supplementation regardless of the vitamin D state in serum before initiation of vitamin D therapy. Of note, no single case experienced manifestations of hypercalcemia, 2 months after initiation of vitamin D therapy, although the presence of elevated serum calcium level was seen in 11 (36.7 %) patients.

In accordance with our finding, Sita-Lumsden et al. [32] had reported that patients with active TB had lower serum vitamin D levels than controls from similar ethnic and social backgrounds; moreover, even with comparable dietary intake and sun exposure, they did not show the expected seasonal variation .Also Nnoaham et al. [14] reported earlier that 25-hydroxyl cholecalciferol levels were low in patients with TB compared with that of the healthy controls. Also Arnedo-Pena et al. [33] reported that vitamin D deficiency is associated with TB incidence.

Compared to our results, Wejse et al. [34] reported that vitamin D insufficiency was common among patients with TB in the study area, but severe vitamin D deficiency was rare.

Vitamin D deficiency or insufficiency in our patients is not fully explained. This may be attributed to low vitamin D intake and lack of sun exposure as most of our patients are of low socioeconomic class.

In the present study, we observed that, there was a significant decrease in sputum conversion time in group I (cases) compared to group II (control) ($p < 0.001$). Sputa of most patients (56.7%) in group I were converted at the 3rd week of initiation of anti-TB drugs while in group II, sputa of most patients (46.7%) were converted at the 8th week after the start of anti-TB therapy.

Our later finding is supported by many studies in the literature. Sato et al. [35] reported that in cured patients, serum 25 hydroxy-vitamin D levels showed a significantly negative correlation with duration until sputum conversion. This relationship suggests that a low serum vitamin D level may not only be a risk factor for the development of active TB but it may also be related to poor treatment outcomes in active TB patients. Furthermore, Kota et al. [36] who studied the effect of vitamin D supplementation in type 2 diabetes patients with pulmonary tuberculosis, reported that in subjects receiving 60,000 IU cholecalciferol per week along with anti-TB treatment, duration of sputum conversion to 100% negative for AFB was 6 weeks compared to 8 weeks in subjects not receiving cholecalciferol. Also, Nursyam et al. [37] reported that pulmonary TB patients given 420,000 IU of vitamin D over 6 weeks had significantly higher sputum conversion rates as compared to placebo (p 0.002).

Compared to our finding, Martineau et al. [38] demonstrated that administration of four doses of 2.5 mg vitamin D (100,000 IU, 1 mg = 40,000 IU) did not affect time to sputum culture conversion in the whole study group. However, improvement of the serum vitamin D level was experienced both safely and quickly and a faster conversion to negative sputum for AFB was encountered in patients with *tt* genotype

of the *Taq* I vitamin D receptor polymorphism suggesting that this subgroup of patients with TB might derive clinical benefit from vitamin D supplementation.

Unfortunately and to the best of the authors' knowledge, no available data exist regarding the genotypes of vitamin D receptor polymorphism in Egyptian tuberculous patients.

In the current study, there was a statistical significant difference between serum calcium at the start of anti-TB treatment and that measured at the end of 2nd month after initiation of anti-TB treatment ($p < 0.001$). Narang et al. [39] reported similar results. Hypercalcemia was detected in 19 of 30 patients with smear positive pulmonary tuberculosis taking a daily dose of 10–95 mg vitamin D.

Compared to our results, Martineau et al. [38] showed that serum corrected calcium concentration declined in both intervention and control arms after initiation of antimicrobial treatment. Such a decline might have resulted from a reduction in granulomatous burden in patients responding to treatment, leading to a decrease in extra-renal 1-alpha hydroxylation of 25-hydroxy-vitamin D and a fall in serum 1, 25-dihydroxyvitamin D concentrations.

TB score assessed at the end of treatment also strongly predicted subsequent mortality. The TB score is a simple and low-cost tool for clinical monitoring of tuberculosis patients in low-resource settings and may be used to predict mortality risk. Low TB score or fall in TB score at treatment completion may be used as a measure of improvement [40].

In the current study, there was a statistical significant difference between the two groups regarding TB score at the time of sputum conversion or at the end of the 2nd month after the start of anti-tuberculous treatment (p 0.020).

Wejse et al. [34] found that TB score declined for 96% of the surviving patients from initiation to end of treatment, and declined with a similar pattern in HIV-infected and HIV-uninfected patients, as well as in smear negative and smear positive patients. The risk of dying during treatment increased with higher TB score at inclusion. For patients with a TB score of >8 at inclusion, mortality during the 8 months treatment was 21% (45/218) versus 11% (55/480) for TB score <8 ($p < 0.001$).

On contrary, Janols et al. [41] reported that in TB patients (53.6% of whom were HIV co-infected), the median TB score declined from week 0 to week 2 (8 (IQR 6–9) vs 4 (IQR 2–6)) and dropped to a low level at week 8, which was still significantly higher than that found in blood donors (2 (IQR 1–4) vs 0 (IQR 0–1), $p < 0.0001$). Patients who died had a significantly higher TB score at week 0, week 2, and week 8 than survivors. Mortality was associated with a failure to achieve a decrease greater than 25% in the TB score at 2 weeks. Baseline CD4+ cell counts (< 200 cells/mm^3) were associated with mortality but not with initial TB score results. They concluded that the TB score was increased during the first 2 months of treatment among patients who died. Failure to achieve a greater than 25% decrease in TB score after 2 weeks of treatment was associated with increased mortality. Repeated clinical scoring during the intensive phase of TB treatment could be useful to identify high-risk patients.

In conclusion, supplementary vitamin D when added to first line anti-tuberculous drugs was of benefit in treatment of active pulmonary TB. This results in shorter conversion time and less severe TB clinical manifestation without any notable manifestations of hypercalcemia. Therefore, it is recommended to add vitamin D as supplementary treatment to first-line anti-tuberculous drugs in the treatment course of active pulmonary TB.

Conflict of interest

We have no conflict of interest to declare.

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