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Original article

Effect and possible mechanisms of metformin as adjuvant therapy in the management of tuberculosis: A prospective study

Ritu Mishra [a], Shri Krishan [b], Pradeep Kumar Rai [c], Prem Kapur [d], Khalid Umer Khayyam [e], Md Azharuddin [f], Kanika Sharma [g], Manju Sharma [h,*]

[a] Department of Pharmacy Practice, SGT College of Pharmacy, Shree Guru Gobind Singh Tricentenary University, Gurgaon, 122505, India
[b] Department of Drug Safety and Pharmacovigilance, Syneos Health Gurgaon, 122002, India
[c] Department of Life Sciences, BD Biosciences, Gurgaon, 122002, India
[d] Department of Medicine, Hamdard Institute of Medical Sciences and Research & Hakeem Abdul Hameed Centenary Hospital, Jamia Hamdard, New Delhi, 110062, India
[e] Department of Epidemiology & Public Health, National Institute of Tuberculosis & Respiratory Diseases, New Delhi, 110030, India
[f] Department of Pharmaceutical Medicine, Division of Pharmacology, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, 110062, India
[g] Department of Pharmacy, Ram-Eesh Institute of Vocational and Technical Education, Greater Noida, Uttar Pradesh, 201310, India
[h] Department of Pharmacology, School of Pharmaceutical Education and Research, Jamia Hamdard, New Delhi, 110062, India

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ABSTRACT

Objective: Tuberculosis (TB) is a deadly infectious ailment causing mortality and morbidity globally. The study objectives were to investigate the effect and possible mechanisms of metformin as adjuvant therapy in the management of tuberculosis.

Methods: TB patients attending HAHC hospital, New Delhi (India) and had T2DM (Type-2 diabetes mellitus) comorbidity were enrolled. Study participants were categorized into metformin non-users and metformin users depending on the presence of metformin in their prescription. Whole blood was used for immunophenotyping of total T-Cells ($CD45^+CD3^+$), Helper T-Cells (Th) ($CD45^+CD3^+$ $CD4^+$) and Cytotoxic T Cells (Tc) ($CD45^+CD3^+CD8^+$) through flow cytometry. BD FACS Verse and BD LSR II were used for sample acquisition.

Results: Estimation of T-Cells ($CD45^+CD3^+$), Helper T-Cells (Th) ($CD45^+CD3^+$ $CD4^+$) and Cytotoxic T Cells (Tc) ($CD45^+CD3^+CD8^+$) levels at 2nd visit showed a larger T-Cells ($CD45^+CD3^+$) ($p < 0.001$), Helper T-Cells (Th) ($CD45^+CD3^+$ $CD4^+$) ($p = 0.004$) and Cytotoxic T Cells (Tc) ($CD45^+CD3^+CD8^+$) ($p = 0.001$) in metformin users in comparison with metformin non users. The use of metformin had significant effect on the conversion of sputum smear ($p = 0.0318$, unpaired *t*-test) in comparison with metformin non-users.

Conclusion: Metformin has potential to develop long-term immunity against TB infection and responsible for its protective impact in TB + T2DM comorbid patients. Metformin therapy improved T cells, Helper T cells and cytotoxic T cells in TB + T2DM comorbid patients and it can be prescribed as an adjuvant antitubercular medication unless contraindicated.

1. Introduction

An approximate 10 million new TB cases have been reported globally in 2019.[1] TB is the most widespread communicable and infectious ailment which leads to morbidity and mortality worldwide. Individuals with compromised immune function like diabetes mellitus (DM) are expected to progress from latent TB to active tubercular infection.[2] The relationship between TB and DM is well recognized. DM is related with adaptive immune and decreased innate responses which is crucial to prevent *Mycobacterium tuberculosis* (*Mtb*) proliferation.[3–5] Host cell identification in diabetics is diminished resulting in reduced immune response, making diabetics more vulnerable to bacterial infections. There are evidences which show that DM is a crucial risk factor for the development of TB and may influence presentation of the ailment, clinical outcomes and reduces TB control.[6–8] Furthermore, TB causes hyperglycemia, glucose resistance as well as exacerbates glycaemic control in diabetic patients.[9–12] Although there are safe and efficient treatment regimens available for TB management with over 95%

* Corresponding author.
E-mail address: msharma@jamiahamdard.ac.in (M. Sharma).

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treatment success but the lengthy period of such treatment duration has created a problem for effective TB management.[13] Thus, there is also a need to ponder alternative adjuvant drugs which can activate host immune system and can eliminate TB bacilli.[14] The greater cost and tedious character of anti-TB medication screening coupled with low profit leads to repurposing and revival of drugs such as metformin which can be used effectively in the management of TB and can contribute as a new intervention and pharmaceutical approach.[15] An effective and functional immune system is vital for restraining and curbing TB bacilli in the host. Host-targeted adjunctive therapies not only improves defensive host immune responses but additionally minimize possibility of microbial resistance. Currently, among the drug which regulates DM, metformin has grossed considerable recognition as a host-directed adjunctive therapy (HDT). The effects of metformin comprise an increase in macrophage effectors, decrease in inflammation, and prevent lung damage. The antidiabetic drug metformin is an AMPK modulator which hampers *Mtb* intracellular development, increases traditional anti-TB drug potency as well as confines ailment immunopathology.[16] Proposed mechanisms of metformin favorable treatment outcomes in TB involves escalating mROS (mitochondrial reactive oxygen species) as well as *Mtb* elimination. Metformin treatment augments Cytotoxic T Cells (Tc) ($CD45^+CD3^+CD8^+$), Helper T Cells (Th) ($CD45^+CD3^+$ $CD4^+$) and T Cells ($CD45^+CD3^+$) pool which combats against *Mtb* infection and hence control Mtb infection in patients.[2,16] In TB patients, the protective role of metformin could be due to its ability to produce immunity against TB infection.[17] Promising results and findings of metformin as antitubercular drug inspires us to evaluate whether the current antidiabetic drug metformin could be repurposed as effective ATT drug.

2. Methods

2.1. Study design and setting

This non-interventional, prospective study was performed at Hakeem Abdul Hameed Centenary Hospital (HAHC), Jamia Hamdard, New Delhi, India at respiratory medicine out-patient department from April 2018 to July 2019. Patient enrollment is done and followed up until completion of their continuation phase of their TB treatment.

2.2. Ethical issues

The research protocol had been approved by the "Jamia Hamdard Institutional Ethics Committee (JHIEC-2018/05-01)" prior to the study. The study participants voluntarily gave written informed consent. Patients were ensured that their identity is confidential and ambiguous.

2.3. Study population and sample size

A total of 120 patients have been included in the study according to the inclusion and exclusion criteria of the study protocol. Patients with age 30 years or greater than 30 of both the sex groups who had confirmed T2DM as per their medical reports and microbiologically confirmed pulmonary TB by sputum smear microscopically or/and CBNAAT were included. The sputum smear examinations reports were collected from the DOT center at HAHC hospital. It was done at baseline (1st visit) and every week till the termination of intensive phase (2nd visit). For acid fast bacilli (AFB) sputum was evaluated for pulmonary tuberculosis (PTB) patients in DOT cum microscopy laboratory through Ziehl-Neelsen staining technique. Patients were categorized into 2 groups: metformin user and metformin non-user depending on their anti-diabetic drugs in their recent prescription. Metformin users were taking ATT+500 mg metformin BD and metformin non users were taking ATT + antidiabetic drugs other than metformin. Patients who have been on metformin for more than 6 months are classified as metformin users. Because of the clinical difficulties to rule out Type-1 diabetes, patient aging ≤30years were not included. Additionally, patients diagnosed with sputum negative, multi Drug Resistant (MDR) TB, extra pulmonary tuberculosis and category II patients were excluded. Patients who were diagnosed with disease other than T2DM and TB were also excluded.

2.4. Diagnosis of TB and blood glucose measurements

For acid fast bacilli (AFB) sputum was evaluated in pulmonary tuberculosis (PTB) patients through Ziehl-Neelsen staining technique. The sputum positive cases of PTB were recorded. The cases of PTB and extrapulmonary tuberculosis (EPTB) were differentiated by the physician. The sputum positive confirmed cases of PTB were screened for blood glucose level at the initiation of treatment, completion of intensive phase and at end of treatment (EOT). HBA1c (Blood glucose control) was evaluated. According to American Diabetes Association (ADA), individuals having HbA1c $\geq$ 7% were considered as uncontrolled diabetics whereas individuals having HbA1c <7% were considered as controlled diabetics.[18]

2.5. Blood collection

Blood sample was collected in plastic whole blood tube with spray coated K2EDTA for immunophenotyping. Blood was also collected in anti-coagulant free plastic tubes for serum separation. Serum was used for cytokine analysis through ELISA.

2.6. Serum isolation

Blood samples were drawn into anti-coagulant free plastic tubes. Samples was incubated in an upstanding position at room temperature (RT) for 30–45 min to facilitate clotting followed by centrifugation at 2000 RCF at RT for 15 min. Supernatant was cautiously aspirated (serum) and transferred into a centrifuge tube. Serum was aliquoted into cryovials and was stored at −80 °C.

2.7. Flow cytometry staining by lyse-wash method

100 μl of blood sample (about 1 million cells) was taken in a 12 × 75 mm FACS tube. Recommended volume of antibodies were added and vortexed. Samples and antibody cocktail were incubated in dark at RT for 5 min 10X BD FACS Lysing solution (Cat no. 349202) were diluted to 1X with DDW and 2 ml of working 1X solution was poured into each tube and vortexed. At RT the tubes were incubated for 10 min in the dark and cells were centrifuged at 300g for 5 min. Supernatant was aspirated. Pellets were broken by gentle vortex. Cells were washed twice with 2 ml of stain buffer by centrifugation for 5 min at 300g as well as supernatant was separated. Cells were again resuspended in 500 μl of stain buffer and immediately obtained on a flow cytometer. BD FACS Suite v 1.3 was used for data analysis.

2.8. ELISA

Blood samples were drawn into anti-coagulant free plastic tubes and serum was collected from patients so as to evaluate the levels of IL-17A before and after the treatment. IL-17 A was obtained from Bioassay Technology Laboratories (Cat. No E0142Hu). All chemical reagents, standard working solutions and patient's samples were composed according to standard operating procedure. 50 μl of standard working solution was poured in standard wells. 40 μl of sample was poured into sample wells. 10 μl anti-IL-17 antibody was mixed into sample wells followed by addition of 50 μl of streptavidin-HRP into standard well and sample wells. Plate was enclosed and incubated for an hour at 37 °C. Plate was given a deep wash with wash buffer for 5 times. Soake wells in 350 μl wash buffer for 1 min after each wash. Plate was blotted over the top of paper towels. 50 μl of solution A substrate was poured and afterwards 50 μl of solution B substrate was poured to all wells. Plate was

again incubated at 37 °C in the dark for 10 min 50 μl of stop solution was poured into all well, the blue color turns to yellow instantly. Optical density was assessed for all wells instantly with microplate reader at 450 nm after mixing the stop solution.

2.9. Statistical analysis

Demographic profiles were described by descriptive statistics. Continuous variable measured by standard mean and deviation. Categorical variable summarized as percentage and frequencies as well as interpreted with chi square tests. Also, Chi-square test are utilized for quantifying association and differences as well as comparison is performed between metformin non users and metformin users. Statistical analysis for immunological parameters was carried out employing an unpaired student *t*-test as well as a comparison is performed between metformin non users and metformin users. A p value is considered statistically significant when it is smaller than 0.05. Graph Pad Prism version 5.01and Statistical Package for the Social Sciences version 21.0 (SPSS, Inc., Chicago, IL, USA) was utilized for executing statistical analyses.

3. Results

3.1. Patient baseline characteristics

A total of 120 patients contributed in this research study, among them 16 patients were excluded due to unavailability of their sputum examination reports and blood samples either at first visit or at the time of follow up. In the metformin non users (n = 52) the patient's mean ± SD age were 47.60 ± 6.46 years as well as 55% were males. In the metformin users (n = 52) the patients mean ± SD age was 48.35 ± 6.58 years and 65% were males. In both the groups, baseline characteristics were same except for the addiction history i.e., consumption of alcohol, cigarette smoking and tobacco. (p = 0.002) and Literacy Level (p = 0.007) (Table 1).

Table 1
Baseline characteristics of the patients.

Characteristics	Metformin users (N = 52)	Metformin non users (N = 52)	P value
Age (mean ± SD)	48.35 ± 6.58	47.60 ± 6.46	0.35
≥40	37 (71.15%)	42 (80.76%)	
<40	15 (28.84%)	10 (19.23%)	
Gender			0.31
Male	35 (67.30%)	29 (55.76%)	
Female	17 (32.69%)	22 (42.30%)	
Family history of TB			0.21
Yes	14 (26.92%)	21 (40.38%)	
No	38 (73.07%)	31 (59.61%)	
Sputum Status			0.30
3+	11 (21.15%)	16 (30.76%)	
2+	19 (36.53%)	21 (40.38%)	
1+	22 (42.30%)	15 (28.84%)	
Chest X ray grades			0.35
Mild	11 (21.15%)	7 (13.46%)	
Moderate	24 (46.15%)	31 (59.61%)	
Advanced	17 (32.69%)	14 (26.92%)	
Glycaemic control			1.0
HbA1c % (mean ± SD)	7.38 ± 1.68	7.92 ± 1.79	
Poor (HbA1c > 7)	21 (40.38%)	21 (40.38%)	
Good (HbA1c < 7)	31 (59.61%)	31 (59.61%)	
Family history of DM			0.55
Yes	24 (46.15%)	21 (40.38%)	
No	28 (53.84%)	31 (59.61%)	
Substance used history			0.002**
Yes	11 (21.15%)	26 (50%)	
No	41 (78.84%)	26 (50%)	
Literacy Level			0.007**
Illiterate	7 (13.46%)	16 (30.76%)	
Primary School	10 (19.23%)	21 (40.38%)	
Middle School	12 (23.07%)	5 (9.61%)	
High School	9 (17.30%)	5 (9.61%)	
Intermediate	9 (17.30%)	3 (5.76%)	
Graduate and professional degree	5 (9.61%)	2 (3.84%)	
Socioeconomic status			0.28
Lower	16 (30.76%)	21 (40.38%)	
Upper lower	14 (26.92%)	19 (36.53%)	
Lower middle	11 (21.15%)	5 (9.61%)	
Upper middle	9 (17.30%)	5 (9.61%)	
Upper	3 (5.76%)	2 (3.84%)	
BMI			0.16
<15	16 (30.76%)	19 (36.53%)	
15–18	17 (32.69%)	24 (46.15%)	
18–23	12 (23.07%)	5 (9.61%)	
>23	7 (13.46%)	4 (7.69%)	
Duration of DM			0.21
>5 years	37 (71.15%)	31 (59.61%)	
<5 years	15 (28.84%)	21 (40.38%)	

All data was presented as percentages and statistically analyzed using Chi square test and compared between metformin users and metformin non users in TB + T2DM patients. *P < 0.05 was considered as significant.

3.2. Metformin therapy increases proportions of total T cells ($CD45^+CD3^+$), helper T cells (Th) ($CD45^+CD3^+CD4^+$) and cytotoxic T cells (Tc) ($CD45^+CD3^+CD8^+$) and IL-17 levels in TB + T2DM patients

Th cells has a significant part in controlling *Mtb* infection by releasing cytokines IFNγ, TNFα, IL-17A and IL-2.[19,20] It has been observed that metformin users expressed a trend of larger total T Cells $CD45^+CD3^+$ (p < 0.001), Helper T Cells (Th) $CD45^+CD3^+CD4^+$ (p = 0.004) and Cytotoxic T Cells (Tc) $CD45^+CD3^+CD8^+$ (p = 0.001), cell percentages at 2nd visits when compared with metformin non users group. (Fig. 1). Furthermore, metformin users showed significant decrease in IL-17 level at the end of intensive phase in comparison to metformin non users (p=<0.001 and r2 = 0.94). (Fig. 2).

3.3. Effect of metformin therapy on sputum conversion in TB + T2DM patients

Sputum examination was done at baseline (1st visit) and every week till the termination of intensive phase (2nd visit). Sputum assessment at every week showed that remarkable number of study participants were sputum negative in metformin user in comparison with metformin non user. The mean time needed for sputum conversion in metformin users was 3.7 ± 2.23 week whereas 5.3 ± 2.47 week in metformin non users. (Table 2).

3.4. Change in T cell proportions in controlled and uncontrolled diabetics

Total T Cells $CD45^+CD3^+$, Helper T Cells (Th) $CD45^+CD3^+CD4^+$ and Cytotoxic T Cells (Tc) $CD45^+CD3^+CD8^+$ proportions were estimated at baseline and at the end of intensive phase in controlled and uncontrolled diabetic patients in metformin non-users and metformin users. Controlled diabetics in metformin users indicated a substantial enhancement in Total T Cells $CD45^+CD3^+$ CD3 (p = 0.0005), and Cytotoxic T Cells (Tc) $CD45^+CD3^+CD8^+$ (p = 0.0001) levels at the end of intensive phase than patients with uncontrolled diabetics in metformin non users. (Fig. 3).

3.5. Adverse events

Adverse drug reaction (ADR) was collected and a comparison is performed between metformin non-user and metformin user and there was no statistically significant difference in both the groups. Every reported adverse event was basically mild. ADR incidence between metformin non-users as well as metformin users were shown in Table 3.

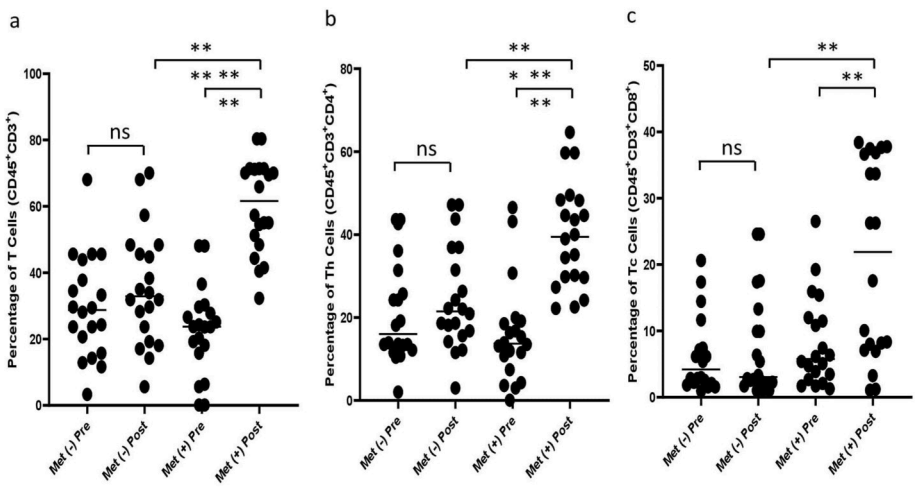

Fig. 1. T cells, Th and Tc cells level increases in TB patients on Metformin therapy. Whole blood of TB patients of Metformin users and non users were stained for CD45, CD3 and CD4 by stained lyse wash method at pre and post treatment stages. The blood samples were analyzed for the (**a**) total T cells ($CD45^+CD3^+$), (**b**) total Th cells ($CD45^+CD3^+CD4^+$) and (**c**) total Tc cells ($CD45^+CD3^+CD84^+$).The data depicted as median. $^{**}P \leq 0.001$, $^{***}P \leq 0.0004$, $^{****}P \leq 0.0001$, ns = not significant. Th: Helper T Cells, Tc: Cytotoxic T Cells. Met (-) Pre: Metformin non user group Pre-treatment, Met (-) Post: Metformin non user group Post-treatment, Met (+) Pre: Metformin user Pre-treatment, Met (+) Post: Metformin user Post-treatment.

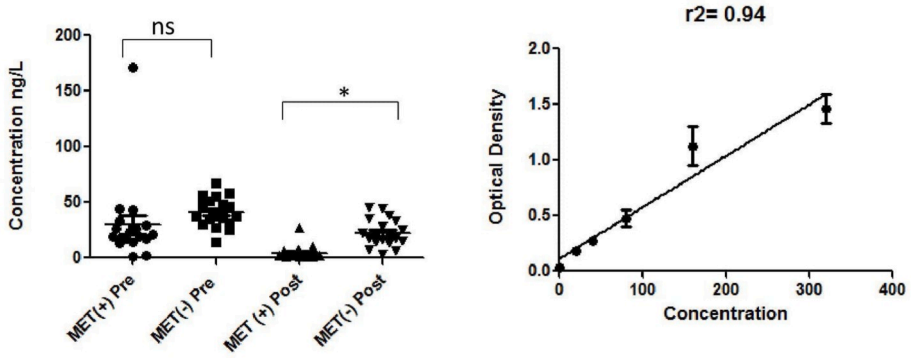

Fig. 2. IL-17 level decreases during metformin therapy: Serum obtained from blood of TB patients of Metformin users and non users were analyzed for IL-17 levels. The data depicted as median. $^{***}P \leq 0.0001$, ns = not significant. MET (+) Pre:Metformin users group Pre-treatment, MET (-) Pre: Metformin non user group Pre-treatment. MET(+) Post:Metformin user Post-treatment, MET(-) Post. Metformin non user group Post-treatment.

Table 2
Sputum smear conversion in Metformin users and Metformin non users.

No of weeks	Metformin users (n = 52)	Metformin non users (n = 52)	P-value
1	7 (13.46%)	3 (5.76%)	0.31
2	14 (26.92%)	6 (11.53%)	0.07
3	21 (40.38%)	7 (13.46%)	0.003**
4	28 (53.84%)	12 (23.07%)	0.002**
5	35 (67.30%)	18 (34.61%)	0.001**
6	38 (73.07%)	22 (42.53%)	0.002**
7	42 (80.76%)	28 (53.84%)	0.006**
8	45 (86.53%)	31 (59.61%)	0.003**

All data was presented as percentages and statistically analyzed using unpaired *t*-test and compared between metformin users and metformin non users in TB + T2DM patients. *P < 0.05 was considered as significant.

4. Discussion

Despite the presence of effective antitubercular medications, TB is still among foremost causes of morbidity and mortality all over the world. The existing ATT has a problem of lengthy period and adverse effects that creates a problem of drug resistance and patient non-compliance.[20–23] Recognition of new adjunctive therapies that can ameliorate the clinical results of TB should be pondered as a priority and repurposing of the adjuvant drugs with antitubercular properties should be carried out. Such therapeutic strategies and pharmaceutical interventions might (i) improve the potency of antibiotic-based treatment (ii) reduces TB management duration (iii) decreases the immunopathology related with TB (IV) increase the immune responses of the host thus augmenting bacterial elimination from the system.

In recent years, there has been growing attention in the metformin use to improve results of TB treatment, as it is widely used anti-diabetic drug. Despite metformin's remarkable potency in TB treatment, it remains to be investigated how the defensive immunity and immune responses plays an important function in managing *Mtb* infection. We therefore sought to assess the effect of metformin therapy on TB patients with T2DM as a co-morbidity. Our data clearly suggests that maintaining immunity and enhancing immune responses had beneficial effects on *Mtb* infection because it increases host defensive immunity, facilitates disease resolution and improves TB treatment outcomes.

In our study, we found that there is a substantial increase in T Cells ($CD45^+CD3^+$), Helper T Cells (Th) ($CD45^+CD3^+CD4^+$) and Cytotoxic T Cells (Tc) $CD45^+CD3^+CD8^+$ cell count in metformin users than those of metformin non users and these results are in accordance with the study conducted by Singhal et al., 2014.[16] Research study carried out by Singhal et al., 2014[16] also showed a rise in *Mtb*-specific T cells i.e., Cytotoxic T Cells (Tc) $CD45^+CD3^+CD8^+$ and $^+$), Helper T Cells (Th) ($CD45^+CD3^+CD4^+$) in metformin treated diabetic patients with tubercular infection.

At the end of intensive phase patients with controlled diabetics in metformin users reported a substantial increase in T Cells $CD45^+CD3^+$ and Cytotoxic T Cells (Tc) $CD45^+CD3^+CD8^+$ levels than those of patients with uncontrolled diabetics. Impaired cell-mediated immunity may have induced poor clinical outcomes in metformin non users. It has

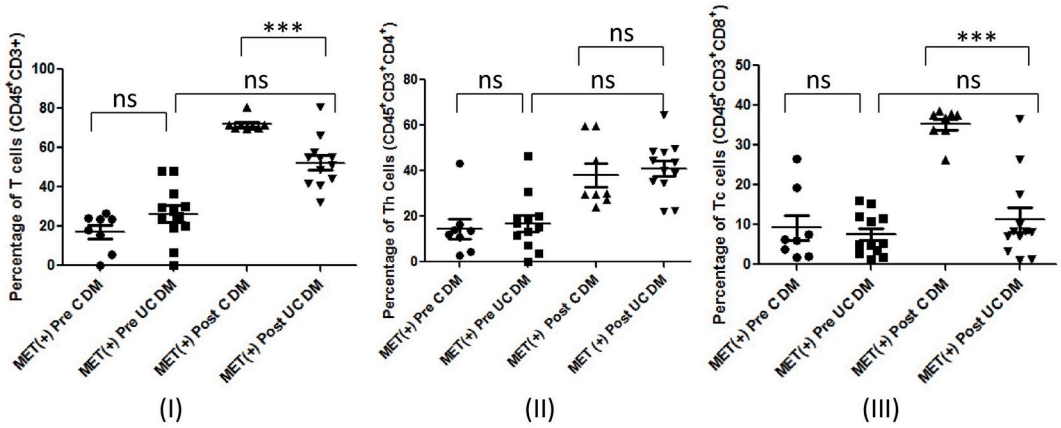

Fig. 3(a). T cells, Th and Tc cells level increases in TB patients on Metformin therapy with controlled DM. Whole blood of TB patients of Metformin receiver with controlled DM were stained for CD45, CD3 and CD4 by stained lyse wash method at pre and post treatment stages. The blood samples were analyzed for the (**I**) total T cells ($CD45^+CD3^+$), (**II**) total Th cells ($CD45^+CD3^+CD4^+$) and (**III**) total Tc cells ($CD45^+CD3^+CD8^+$).The data depicted as median. $^{***}P \leq 0.0005$, $^{***}P \leq 0.0001$, ns = not significant. Th: Helper T Cells, Tc: Cytotoxic T Cells. MET (+) Pre C DM: Metformin users group Pre-treatment, with controlled DM MET (+) Pre UC DM: Metformin user group Pre-treatment, with uncontrolled DM. Met (+) Post C DM: Metformin user Post-treatment, with controlled DM.MET(+) Post UC DM. Metformin user Post-treatment group with uncontrolled DM.

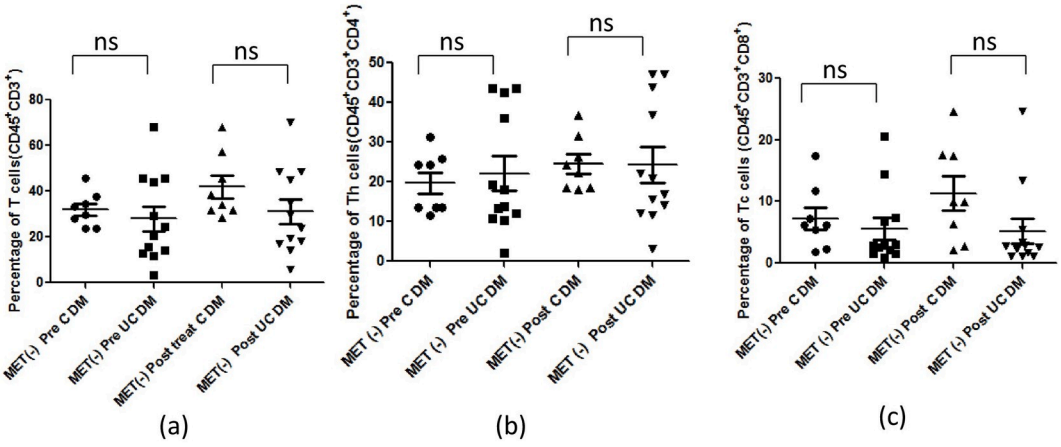

Fig. 3 (b). Estimation of T cells, Th and Tc cells levels in Metformin non users with controlled and uncontrolled DM. Whole blood of TB patients of Metformin users with controlled DM and uncontrolled DM were stained for CD45, CD3 and CD4 by stained lyse wash method at pre and post treatment stages. The blood samples were analyzed for the (**I**) total T cells ($CD45^+CD3^+$), (**II**) total Th cells ($CD45+CD3^+CD4^+$) and (**III**) total Tc cells ($CD45^+CD3^+CD8^+$). The data depicted as median. ns = not significant. Th: Helper T Cells, Tc: Cytotoxic T Cells. MET (-) Pre C DM: Metformin non user group Pre-treatment, with controlled DM MET (-) Pre UC DM: Metformin non user group Pre-treatment, with uncontrolled DM. Met (-) Post C DM: Metformin non user Post-treatment with controlled DM.MET(-) Post UC DM. Metformin non user Post-treatment group with uncontrolled DM.

Table 3
ADR incidence in metformin users and metformin non users in TB + T2DM patients.

ADR	Metformin users (n = 52)	Metformin non users (n = 52)	P-value
Gastrointestinal	9 (17.30%)	12 (23.07%)	0.62
Liver and Biliary	7 (13.46%)	9 (17.30%)	0.78
Musculoskeletal	7 (13.46%)	12 (23.07%)	0.31
Cutaneous	9 (17.30%)	11 (21.15%)	0.80
Hearing and Vestibular	2 (3.84%)	4 (7.69%)	0.67
Central and Peripheral Nervous system	3 (5.76%)	6 (11.53%)	0.48
Peripheral Neuropathy	11 (21.15%)	13 (25%)	0.81
Cardiorespiratory	3 (5.76%)	5 (9.61%)	0.71
Occular	2 (3.84%)	4 (7.69%)	0.67
Hypoglycaemia	3 (5.76%)	6 (11.53%)	0.48

All data was presented as percentages and statistically analyzed using Chi square test and compared between Metformin users and Metformin non users in TB + T2DM patients. *P < 0.05 was considered as significant.

been already reported that sustaining HbA1c within a reasonable range could lead to T Cells ($CD45^+CD3^+$), Helper T Cells (Th) ($CD45^+CD3^+CD4^+$) and Cytotoxic T Cells (Tc) $CD45^+CD3^+CD8^+$ improvements in TB+T2DM patients.[23] Particularly, multiple studies have documented the effect of metformin therapy on glycaemic control.[24,25] When compared to placebo it was discovered to decrease levels of mean HbA1c through 1.1%.

The combination of metformin adjunctive therapy with standard ATT has shown improved clinical results in TB. We further examined the impact of metformin on cytokine responses. IL-17 (Interleukin 17) is a significant pro-inflammatory cytokine released by Th17 lymphocytes and plays an important immunoregulatory role through the development of wide spectrum of pro-inflammatory cytokines.[26] These cytokines can provoke the stimulation and recruitment of neutrophils, macrophages, and Th1 lymphocytes at the location of infection, resulting in the demarcation of the injured area in lung tissue. In our study we concluded that use of metformin contributes to significant decrease in IL-17 release when compared to metformin non-user that was almost similar with the study conducted by Lachmandas et al., 2019.[26] In that

study it was investigated that metformin intake contributes to substantial decrease in IL-17, IFN-γ, IL-6, IL-1β, TNF-α, release.[27]

Metformin has recently attracted considerable attention as a HDT because it has an inhibitory impact on mitochondrial complex I and has been established to amplify the AMP/ATP ratio.[28,29] The protective and beneficial outcomes of metformin are due to mROS'samplified host cells generation as well as mycobacterial phagosome's augmented acidification.

The research has a few shortcomings. First, as a patient-reported prospective outcome analysis, we relied solely on information given by patients about duration of metformin therapy, which we believe is a significant information that may influence the interpretations of study outcomes. Second, because we limited our investigation to one site and used a smaller sample size due to patients falling in exclusion criteria, these findings may not be applicable. Thirdly the precise dose of metformin remains to be determined in the management of TB. The current study was performed with 52 patients in both the group. Although early conversion of sputum smear and increase in T helper cells are in support of metformin therapy, a bigger sample size and multicenter patient enrollment would have provided much better clinical and statistical support. Moreover, these findings must be confirmed in well-designed research study with bigger sample size from different sites.

5. Conclusion

Metformin has favorable effect in enhancing clinical outcomes of TB patients as shown by early sputum conversion and increased T helper cells in TB and T2DM comorbid patients. The metformin's favorable and protective properties in patients of TB may be due to its capacity to maintain extended immunity. Metformin which can concurrently act as an anti-diabetic and antitubercular drugs can improve the clinical outcomes of TB + T2DM patients. Unless contraindicated, doctors must prescribe metformin in all TB and T2DM patients.

Declaration of competing interest

The authors declare no potential conflict of interest.

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