

A Comparative Study in Patients of Sputum Positive Pulmonary Koch's on ATT With and without Metformine- Randomized Control Trial

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Abstract

Aim: To compare the adjuvant effect of Metformin with ATT to that of ATT alone

Methods: A comparative study was conducted in the Department of Medicine, Darbhanga Medical College and Hospital, India. 200 patients were included in this study. Patients with new smear positive pulmonary tuberculosis, Aged above 13 years, patients who never received treatment with multidrug anti-Tb therapy for more than a weekend Is willing to attend a treatment centre for supervised treatment and remain within in the study.

Results: The mean age of the patients in control group was 42 (± 11.8) years and in Metformin group, it was 40.3 (± 10.1) years. In control group, there were 70 males and 30 females and in Metformin group, 75 males and 25 females. The average time taken for sputum smear conversion was significantly lower in the Metformin group in comparison with the control group ($p = 0.015$, unpaired t-test). It was about 3.6 (± 1.84) weeks in Metformin group while it was 4.8 (± 2.41) weeks in the control group. All the subjects enrolled in the study were non-diabetics. At the time of enrollment, their fasting and post prandial blood sugar and HbA1c values were measured and only those who were having normal values were selected for the study. The mean fasting blood sugar was 97.5 ± 8.8 mg/dl and 93.2 ± 11.4 mg/dl and the mean sugar values at post prandial state was 128.22 ± 24.15 mg/dl and 126.98 ± 30.11 mg/dl in control and Metformin groups respectively at the time of enrollment. In control group, the baseline HbA1c was 4.82 ± 0.61 % and it was 4.95 ± 0.83 % in Metformin group. Adverse events were seen in 10 patients (10%) in control group and 13 patients (13%) in Metformin group. The difference was not statistically significant (p value = 0.744, chi square test). All of the adverse events were only minor in nature and gastrointestinal related problems like nausea, vomiting and gastritis.

Conclusion: The supports Metformin added to standard ATT potentially benefiting TB patients as evidenced by significant reduction in the time needed for sputum smear conversion and reduction in the occurrence of drug resistance.

Keywords: Metformin, ATT, drug resistance

Introduction

Globally in 2016, there were an estimated 10.4 million new cases of tuberculosis (TB) with five countries, India, Indonesia, China, Philippines and Pakistan, accounting for 56% of the total cases.¹ There were an estimated 1.3 million TB deaths in 2016 among HIV-negative people and an additional 374 000 deaths among people living with HIV.¹ Though effective regimens are available for the treatment of drug-sensitive TB with more than 95% cure, the long duration of such regimens has posed problems for TB treatment and control. This, along with drug toxicity, results in poor adherence to treatment resulting in the emergence of drug resistance.

All these have led to an urgent need for more efficient anti-TB drugs, regimens as well as for newer modalities of treating TB. Drugs targeting the TB bacilli can result in the emergence of drug tolerance and resistance, thereby worsening the overall treatment outcomes. Thus, there exists a need to consider alternate modalities such as enhancing the host immune system for a faster and complete elimination of the TB bacilli.^{2,3} An efficient and functional immune system is essential to restrain and curb the growth of TB bacilli in the host. Yet, the TB bacilli can still elude the host immune responses, infect the host cells and either multiply or maintain long-term latency in those cells.^{4,5} 'Host-targeted' adjunct therapeutic strategies not only augment protective host immune responses but also reduce the chance of development of microbial resistance.

One of the host cell innate antimicrobial arsenals includes the capacity to destroy intracellular pathogens using the phagosomes machinery or autophagy pathway. Intracellular pathogens are effectively controlled by autophagy that is regulated by adenosine monophosphate-activated protein kinase (AMPK).^{6,7} The *Mycobacterium tuberculosis* virulence results from perturbations in the autophagy network and AMPK signalling.⁸ The antidiabetic drug metformin (MET; 1, 1-dimethyl biguanide) is an AMPK modulator that inhibits the intracellular growth of *M. tuberculosis*, restricts disease immunopathology and enhances the efficacy of conventional anti-TB drug.⁹ Given these promising findings, we plan to test whether the existing approved antidiabetic drug, metformin added to the existing anti-TB regimen, with its defined effects on host cell functions could be repurposed for effective and faster treatment of TB as compared with the current standard of care anti-TB regimens.

Materials and Methods

A comparative observational study was conducted in the Department of Medicine, Darbhanga Medical College and Hospital, India, after taking the approval of the protocol review committee and institutional ethics committee. Total 200 patients were included in this study.

Inclusion Criteria:

- Patients with new smear positive pulmonary tuberculosis.
- Aged above 13 years.
- patients who never received treatment with multidrug anti Tb therapy for more than a week
- Is willing to attend a treatment centre for supervised treatment and remain within in the study.

Exclusion Criteria:

- Patients having extra pulmonary TB or Patients drug-resistant TB.
- Patients having a poor history of exposure to anti-TB treatment for more than a week. Patients with concomitant diabetes mellitus or random blood sugar level >200 mg/dl. or fasting blood sugar level >140 mg/dl.
- Patients with serum creatinine level >1.2 mg/dL or blood urea level >43 mg/dL EIA HIV positive patients.
- Patients with acidosis

Methodology

Written informed consent will be taken from the patient or relatives, Study participation will last for 6 months: during the first 2 months, participants will receive the randomly assigned regimen of either daily anti TB treatment with metformin or only anti TB Regimen.

Patients will be randomized into two groups; Group A and Group B

Group A : control group

Group B: study group

Demographic Data

The mean age of the patients in control group was 42 (± 11.8) years and in Metformin group, it was 40.3 (± 10.1) years. In control group, there were 70 males and 30 females and in Metformin group, 75 males and 25 females. There was no significant difference seen in age and gender distribution of the patients between two groups, as evidenced by the p value more than in unpaired t test for age and chi square test for gender. Hence, both the groups were comparable in terms of age and gender.

Sputum Smear Conversion

Sputum smear examination was done at baseline and once a week till it became negative. Weekly sputum smear assessment showed that significant number of patients attained smear negativity in the Metformin group compared to the control group. The number of patients who attained sputum smear conversion in both the groups is shown in table 1. In metformin group, one patient remained sputum positive after completion of intensive phase and in control group 9 patients remained sputum positive.

The average time taken for sputum smear conversion was significantly lower in the Metformin group in comparison with the control group ($p = 0.015$, unpaired t-test). It was about 3.6 (± 1.84) weeks in Metformin group while it was 4.8 (± 2.41) weeks in the control group.

Table 1: Sputum smear conversion (positive to negative)

| Week | Control=100 | Metformin=100 | p-value |
|------|-------------|---------------|---------|
| 1 | 7 (7%) | 17 (17%) | 0.12 |
| 2 | 15 (15%) | 33 (33%) | 0.035* |
| 3 | 27 (27%) | 48 (47%) | 0.039* |
| 4 | 53 (53%) | 77(77%) | 0.015* |
| 5 | 67 (67%) | 85 (85%) | 0.066 |
| 6 | 77 (77%) | 95 (95%) | 0.023* |
| 7 | 81 (81%) | 98 (98%) | 0.01* |
| 8 | 83 (83%) | 98 (98%) | 0.018* |

Drug Resistance Pattern

Drug susceptibility testing was performed at the end of intensive phase for patients who remained sputum positive, in both the groups using GeneXpert. In Metformin group, one patient who remained sputum positive had resistance for Rifampicin. In control group, out of 20 patients who remained sputum positive, 7 patients had resistance for Rifampicin and 2 patient had indeterminate result in GeneXpert. The sputum of the patient who had indeterminate result in GeneXpert was analysed in LPA and found to have INH resistance. The other 11 patients in control group who were sputum positive showed sensitivity to the standard ATT and hence they were continued on the same medications and eventually they became sputum negative. The difference in the development of drug resistance between the two groups was not statistically significant (p value=0.361, chi square test). The drug resistant patients were removed from the study and appropriate alternate drug regimens were provided to them.

Table 2: Complete Blood Count and Biochemical parameters

| | Baseline (mean±SD) | Control End (mean±SD) | p-value # | Baseline (mean±SD) | Metformin End (mean±SD) | p-value # | p value \$ (intergroup) |
|----------------------|-----------------------|--------------------------|--------------|-----------------------|-------------------------------|--------------|----------------------------|
| Hb | 12.16±1.08 | 12.08±0.86 | 0.52 | 12.27±1.97 | 12.45±1.73 | 0.11 | 0.08 |
| T.RBC | 4.32±0.53 | 4.35±0.48 | 0.77 | 4.30±0.69 | 4.33±0.60 | 0.41 | 0.45 |
| T.WBC | 8636.96±1239.24 | 7506.52±847.32 | <0.0001* | 9216.33±1431.11 | 7767.35±1090.95 | <0.0001* | 0.09 |
| N | 52.19±5.76 | 51.71±6.5 | 0.613 | 54.88±7.38 | 54.57±7.17 | 0.61 | 0.02* |
| E | 2.43±1.08 | 2.41±0.98 | 0.309 | 2.42±1.55 | 2.38±1.43 | 0.16 | 0.47 |
| L | 39.78±5.79 | 40.43±6.55 | 0.48 | 39.89±10.31 | 40.20±7.19 | 0.28 | 0.44 |
| M | 3.10±1.12 | 3.15±1.19 | 0.96 | 3.78±2.18 | 3.53±1.90 | 0.15 | 0.12 |
| Platelets | 2.52±0.78 | 2.57±0.77 | 0.21 | 2.77±0.59 | 2.80±0.57 | 0.18 | 0.05 |
| ESR | 57.47±16.39 | 24.69±7.82 | <0.0001* | 68.02±16.33 | 28.28±6.06 | <0.0001* | 0.47 |
| Renal function tests | | | | | | | |
| BUN | 9.97±3.46 | 10.06±2.5 | 0.79 | 10.02±3.9 | 10.34±4.19 | 0.07 | 0.33 |
| Creatinine | 0.79±0.23 | 0.80±0.22 | 0.61 | 0.77±0.24 | 0.78±0.23 | 0.14 | 0.36 |
| Liver function tests | | | | | | | |
| AST | 36.19±5.65 | 50.30±17.52 | <0.0001* | 38.08±6.08 | 51.98±13.84 | <0.0001* | 0.31 |
| ALT | 37.43±6.11 | 55.69±21.27 | <0.0001* | 35.76±5.76 | 57.47±21.23 | <0.0001* | 0.35 |
| ALP | 88.71±15.99 | 108.26±19.03 | <0.0001* | 87.94±16.46 | 110.16±20.52 | <0.0001* | 0.33 |
| T.Bil | 0.72±0.20 | 0.74±0.22 | 0.07 | 0.80±0.29 | 0.83±0.32 | 0.42 | 0.44 |
| D.Bil | 0.24±0.10 | 0.26±0.11 | 0.05 | 0.26±0.15 | 0.28±0.12 | 0.58 | 0.28 |

N-Neutrophils, E-Eosinophils, L-Lymphocytes, M-Monocytes, BUN-Blood Urea Nitrogen, AST- Aspartate transaminase, ALT- Alanine transaminase, ALP- Alkaline phosphatase, T.Bil- Total bilirubin, D.Bil- Direct bilirubin. Statistics:# Control group (Baseline vs End) and Metformin group (Baseline vs End)- paired t test \$ Control vs Metformin- unpaired t test * p-value <0.05 was considered statistically significant.

Complete Blood Count (CBC)

The blood parameters such as Haemoglobin, total RBC count, total WBC count, Differential count and platelet count were measured at the baseline and at the end of the study. The difference noted between the values observed before and after treatment was not statistically significant between Metformin and control groups. The analysis was done by using unpaired t test (between group analysis) and the p value was more than 0.05. Within group analysis was done by using paired t test which showed that there was a reduction in total WBC count and ESR within control and Metformin groups and the reduction was statistically significant (p-value less than 0.05). The other parameters did not show significant changes in the within group analysis.

Renal Function Tests (RFT)

Renal function tests which include Blood urea nitrogen (BUN) and serum creatinine did not show any significant differences within the groups and between the groups.

Liver Function Tests (LFT)

Liver function tests showed significant increase in the liver enzymes- AST, ALT and ALP, at the end of the study when compared with baseline values. The increase was seen in both control and Metformin groups but inter group comparison did not show any statistically significant difference in the enzyme levels. There was no significant difference in the total and direct bilirubin values both within the groups and between the groups.

Random Blood Sugar

All the subjects enrolled in the study were non-diabetics. At the time of enrollment, their fasting and post prandial blood sugar and HbA1c values were measured and only those who were having normal values were selected for the study. The mean fasting blood sugar was 97.5 ± 8.8 mg/dl and 93.2 ± 11.4 mg/dl and the mean sugar values at post prandial state was 128.22 ± 24.15 mg/dl and 126.98 ± 30.11 mg/dl in control and Metformin groups respectively at the time of enrollment. In control group, the baseline HbA1c was 4.82 ± 0.61 % and it was 4.95 ± 0.83 % in Metformin group.

After the initiation of treatment, random blood sugar was measured once in 7 days for first two months and once in a month thereafter. Within group analysis was done using repeated measures ANOVA and between group analysis was done by using one-way ANOVA to detect the differences in random blood sugar values. There was no statistically significant difference noted in the RBS values within the groups in both control and Metformin groups. When RBS values of control and Metformin groups were compared, it showed significant difference between the groups ($p < 0.001$). Though statistically significant, there was no clinical significance as the mean values were within the normal range.

Adverse Events

Adverse events were seen in 10 patients (10%) in control group and 13 patients (13%) in Metformin group. The difference was not statistically significant (p value = 0.744, chi square test). All of the adverse events were only minor in nature and gastrointestinal related problems like nausea, vomiting and gastritis.

Discussion

Sputum smear examination is the test which is usually done to assess the treatment outcome in pulmonary tuberculosis patients. It is an inexpensive and easy method when compared to sputum culture. Sputum smear examination is usually done at the end of intensive phase and if

it becomes negative, it indicates good prognosis. If the sputum smear remains positive despite treatment, it might result in treatment failure, relapse and increase the chance of drug resistance.^{10,11} Sputum smear positive patients are highly infectious and one of the important goals of anti-tubercular therapy is to render the patients non-infectious as a smear positive patient can infect more than 10 persons annually.²²

In our study, the average time taken for sputum smear conversion was significantly lower in the Metformin group in comparison with the control group ($p = 0.015$, unpaired t-test). It was about $3.6 (\pm 1.84)$ weeks in Metformin group while it was $4.8 (\pm 2.41)$ weeks in the control group, which was almost similar to the results obtained from a prospective study done by Parikh *et al.*, in 2012.¹²

In this study, Metformin added to standard therapy was found to have significant effect on sputum smear conversion. The number of patients who had become smear negative was significantly high in the Metformin group when compared to control. This difference was observed every week and at the end of 8 weeks, 98 patients (98%) in Metformin group attained smear negativity as against 83 patients (83%) in the control group.

The role of Metformin in tuberculosis has been studied only in diabetic patients so far. Singhal *et al.*, in their study found that tuberculous patients, who were taking Metformin for Diabetes showed reduced number of pulmonary cavities when compared to the patients who were on other anti-diabetic medications.¹³ Ye-Jin Lee *et al.*, in their retrospective study found that pulmonary tuberculosis patients with cavitary TB taking Metformin for Diabetes showed significantly higher sputum culture conversion rates at the end of two months.¹⁴ .Y. Ma *et al.* (2018), in their retrospective cohort study involving TB patients with Diabetes, found out that Metformin treatment had a favourable effect on treatment success rate, sputum culture conversion at the end of two months and also the relapse rates when compared to the diabetic patients who were not on Metformin.¹⁵

In the present study, drug resistance pattern also showed changes between the control and Metformin group. Drug sensitivity testing was done using the molecular methods, GeneXpert and/ or LPA at the end of 2 months. Drug susceptibility testing was performed at the end of intensive phase for patients who remained sputum positive, in both the groups using GeneXpert. In Metformin group, one patient who remained sputum positive had resistance for Rifampicin. In control group, out of 20 patients who remained sputum positive, 7 patients had resistance for Rifampicin and 2 patient had indeterminate result in GeneXpert. The sputum of the patient who had indeterminate result in GeneXpert was analysed in LPA and found to have INH resistance. The other 11 patients in control group who were sputum positive showed sensitivity to the standard ATT and hence they were continued on the same medications and eventually they became sputum negative. The difference in the development of drug resistance between the two groups was not statistically significant (p value=0.361, chi square test).

One of the reasons for antibiotic resistance in tuberculosis is the formation of persister phenotypes of Mycobacteria which can survive even in the presence of antibiotics. These are slow growing and genetically similar to susceptible bacteria.¹⁶ The main mechanism of persister formation is utilisation of the NAD (Nicotinamide adenine dinucleotide) pathway and NDH-I (NADH dehydrogenase-I) for ATP synthesis. NDH-I is similar to human mitochondrial complex-I. Metformin is an inhibitor of mitochondrial complex-I and hence it could also inhibit NDH-I of Mycobacteria and prevent the formation of persister phenotypes, thereby preventing resistance.¹⁷

Along with antibiotics, host immune mechanisms are very important in destroying the TB bacilli. In animal models of TB, Metformin treatment increased the production of CD4⁺ and CD8⁺ T-lymphocytes and there are also an increased percentage of Interferon- γ secreting CD8⁺ cells. By inhibiting mitochondrial complex-I, Metformin increases the production of mitochondrial ROS and damages the bacterial cell.¹³ Mycobacteria, on entering the host cells by phagocytosis, prevents the maturation of phagosome and starts replicating within the cell. Phagosome maturation is essential for eliminating the pathogen. Autophagy is a defense mechanism which involves the formation of autophagosome, a double membrane vesicle engulfing the cellular components along with the microbes and this autophagosome then fuses with the lysosome, leading to degradation of the cellular components.¹⁸ Metformin was found to induce autophagy and phagolysosome fusion in the host cells.¹³ In the present study, adverse drug reactions were seen in 10 patients (10%) in the control group and 13 patients (13%) in the Metformin group and the difference noted between the groups was not statistically significant. The adverse reactions seen in both the groups were only mild and most of them were gastrointestinal related symptoms like nausea, vomiting and gastritis. These adverse events are not specific to Metformin and could occur with anti TB drugs also. Hypoglycaemia was not reported in any of the patients in the Metformin group.

Conclusion

The supports Metformin added to standard ATT potentially benefiting TB patients as evidenced by (i) significant reduction in the time needed for sputum smear conversion and (ii) reduction in the occurrence of drug resistance. However, further studies with large sample size and with varied outcome measures are needed to confirm the observations noted in this study.

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