Journal of Advanced Medical and Dental Sciences Research

@Society of Scientific Research and Studies

Journal home page: www.jamdsr.com doi: 10.21276/jamdsr ICV 2018= 82.06

(e) ISSN Online: 2321-9599; (p) ISSN Print: 2348-6805

Original Research

To examine the adjuvant impact of Metformin with ATT and ATT alone

Balaji Tuppekar

Assistant Professor, Department of Pulmonary Medicine, D.Y. Patil Deemed To Be University-School of medicine, Nerul, Navi Mumbai, India

ABSTRACT:

Aim: To examine the adjuvant impact of Metformin with ATT and ATT alone. **Methods:** A comparative observational study was conducted in the Department of Respiratory Medicine. 120 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 week and Is willing to attend a treatment centre for supervised treatment and remain within in the study. **Results:** 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 3.5 (±1.82) weeks in Metformin group while it was 4.6 (±2.42) 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 97.7±8.5mg/dl and 93.4±11.2mg/dl and the mean sugar values at post prandial state was 128.31±24.21mg/dl and 126.87±30.41 mg/dl in control and Metformin groups respectively at the time of enrollment. In control group, the baseline HbA1c was 4.67±0.63 % and it was 4.83±0.81% in Metformin group. **Conclusion:** We concluded that 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: Adjuvant, Metformin, ATT

Received: 16-06-2019 Accepted: 19-07-2019

Corresponding author: Balaji Tuppekar, Assistant Professor, Department of Pulmonary Medicine, D.Y. Patil Deemed To Be University-School of medicine, Nerul, Navi Mumbai, India

This article may be cited as: Tuppekar B. To examine the adjuvant impact of Metformin with ATT and ATT alone. J Adv Med Dent Scie Res 2019;7(8):340-344.

INTRODUCTION

An estimated 10.4 million new cases of tuberculosis (TB) were reported globally in 2014, with India, Indonesia, China, the Philippines, and Pakistan accounting for 56% of all cases. [1] Despite the fact that there are successful regimens for the treatment of drug-sensitive TB that cure more than 95% of cases, the lengthy nature of these regimens has complicated TB therapy and management. Along with medication toxicity, this leads to poor treatment compliance and the development of drug resistance.

All of these have created an urgent need for innovative, more effective TB treatment techniques, anti-TB medicines, and regimens. Drugs that target the TB bacilli may cause the development of drug tolerance and resistance, worsening the course of therapy as a whole. Therefore, it is essential to investigate alternative treatment methods, such as boosting the immune system of the host, in order to achieve a more rapid and comprehensive eradication of the tuberculosis bacilli. [2,3] It is very necessary to have an immune system that is effective and operational in order to control and curb the expansion of TB bacilli in the host. However, the tuberculosis bacilli are still able to avoid the host's immune reactions, infect the host cells, and either grow or remain dormant in those cells for an extended period of time.[4,5] Host-targeted' adjunct therapy strategies not only boost the protective immune responses of the host but also lessen the likelihood that the microbe will become resistant to the treatment.

It is possible for a host cell to eliminate intracellular infections by using either the phagosomal machinery or the autophagy pathway, which is one of the host cell's innate antibacterial arsenals. The process of autophagy, which is controlled by an enzyme called adenosine monophosphate-activated protein kinase, is an efficient means of combating intracellular infections (AMPK).[6,7] The virulence of Mycobacterium TB is caused by disturbances in the

autophagy network and AMPK signalling.[8] Metformin (MET; 1, 1-dimethyl biguanide), a medication used to treat diabetes, is a kind of AMPK modulator. As such, it suppresses the intracellular development of Mycobacterium tuberculosis, controls disease immunopathology, and increases the effectiveness of traditional anti-TB drugs. [9] In light of these encouraging findings, we intend to investigate whether the diabetes medication metformin, which is already on the market and approved for use, could be repurposed for the treatment of tuberculosis in a manner that is more effective and takes less time than the anti-TB regimens that are currently considered to be the gold standard.

MATERIALS AND METHODS

A comparative observational study was conducted in the Department of Respiratory Medicine, after taking the approval of the protocol review committee and institutional ethics committee.
120 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 40 (±10.2) years and in Metformin group, it was 38 (±10.4) years. In control group, there were 40 males and 20 females and in Metformin group, 35 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 8 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 3.5 (±1.82) weeks in Metformin group while it was 4.6 (±2.42) weeks in the control group.

Table 1: Sputum smear conversion (positive to negative)

Week	Control=60	Metformin=60	p-value
1	5 (8.3%)	11(18.3%)	0.12
2	13 (21.6%)	24 (40%)	0.035*
3	25 (41.6%)	32 (53.3%)	0.039*
4	31(51.6%)	41(68.3%)	0.015*
5	38(63.3%)	48 (80%)	0.066
6	41 (68.3%)	51(85%)	0.023*
7	44 (73.3%)	56 (93.3%)	0.01*
8	50(83%)	59(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 15 patients who remained sputum positive, 4 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 9 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	12.32±1.06	12.06±0.95	0.52	12.25±1.76	12.31±1.71	0.11	0.08
T.RBC	4.44±0.64	4.41±0.41	0.77	4.31±0.71	4.31±0.60	0.41	0.45
T.WBC	8636.89±1239.31	7506.59±847.41	<0.00001*	9216.21±1431.23	7767.39±1090.86	<0.00001*	0.09
N	52.16±5.68	51.83±6.7	0.613	54.67±7.35	54.62±7.15	0.61	0.02*
E	2.31±1.06	2.32±0.89	0.309	2.31±1.72	2.35±1.41	0.16	0.47
L	39.78±5.79	40.21±6.67	0.48	39.71±10.23	40.21±7.15	0.28	0.44
M	3.12±1.31	3.15±1.19	0.96	3.67±2.13	3.51±1.91	0.15	0.12
Platelets	2.51±0.76	2.61±0.71	0.21	2.65±0.53	2.81±0.55	0.18	0.05
ESR	57.45±16.21	24.72±7.71	<0.00001*	68.04±16.11	28.22±6.09	<0.00001*	0.47
Renal function tests							
BUN	9.86±3.31	10.09±2.8	0.79	10.04±3.7	10.34±4.19	0.07	0.33
Creatinine	0.83±0.41	0.81±0.41	0.61	0.79±0.21	0.78±0.23	0.14	0.36
Liver function tests							
AST	36.16±5.71	50.31±17.61	<0.00001*	38.06±6.05	51.98±13.84	<0.00001*	0.31
ALT	37.44±6.12	55.65±21.29	<0.00001*	35.82±5.57	57.47±21.23	<0.00001*	0.35
ALP	88.67±15.86	108.29±19.01	<0.00001*	87.67±16.48	110.16±20.52	<0.00001*	0.33
T.Bil	0.74±0.21	0.72±0.21	0.07	0.83±0.26	0.83±0.32	0.42	0.44
D.Bil	0.21±0.11	0.29±0.14	0.05	0.27±0.17	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 97.7±8.5mg/dl and 93.4±11.2mg/dl and the mean sugar values at post prandial state was 128.01±24.21 mg/dl and 126.87±30.41 mg/dl in control and Metformin groups respectively at the time of enrollment. In control group, the baseline HbA1c was 4.67±0.63 % and it was 4.83±0.81 % in Metformin group.

After the initiation of treatment, random blood sugar was measured once in 15 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 5 patients (8.3%) in control group and 8 patients (13.3%) 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 3.5 (±1.82) weeks in Metformin group while it was 4.6(±2.42) 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, 59 patients (98%) in Metformin group attained smear negativity as against 50 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 cavitatory TB taking Metformin for Diabetes showed significantly higher sputum culture conversion rates at the end of two months.[14] Motta AB et al[15] 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 15 patients who remained sputum positive, 4 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 9 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 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.[17] Metformin was found to induce autophagy and phagolysosome fusion in the host cells.[13] In the present study, adverse drug reactions were seen in 5 patients (8.3%) in the control group and 8 patients (13.3%) 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

We concluded that 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.

REFERENCE

1. Van Rie A, Warren R, Richardson M, Gie RP, Enarson DA, Beyers N, Van Helden PD. Classification of drug-resistant tuberculosis in an epidemic area. The Lancet. 2000 Jul 1;356(9223):22-5.
2. Kuijl C, Savage ND, Marsman M, et al. Intracellular bacterial growth is controlled by a kinase network around PKB/AKT1. Nature 2007;450:725–30.
3. Ejim L, Farha MA, Falconer SB, et al. Combinations of antibiotics and nonantibiotic drugs enhance antimicrobial efficacy. Nat Chem Biol 2011;7:348–50.
4. Bhatt K, Salgame P. Host innate immune response to Mycobacterium tuberculosis. J Clin Immunol 2007;27:347–62.
5. Behar SM, Divangahi M, Remold HG. Evasion of innate immunity by Mycobacterium tuberculosis: is death an exit strategy? Nat Rev Microbiol 2010;8:668–74.
6. Gutierrez MG, Master SS, Singh SB, et al. Autophagy is a defense mechanism inhibiting BCG and Mycobacterium tuberculosis survival in infected macrophages. Cell 2004;119:753–66.
7. Kim J, Kundu M, Viollet B, et al. AMPK and mTOR regulate autophagy through direct phosphorylation of Ulk1. Nat Cell Biol 2011;13:132–41.
8. Kumar D, Nath L, Kamal MA, et al. Genome-wide analysis of the host intracellular network that regulates survival of Mycobacterium tuberculosis. Cell 2010;140:731–43.
9. Singhal A, Jie L, Kumar P, et al. Metformin as adjunct antituberculosis therapy. Sci Transl Med 2014;6:263ra159.
10. Singla R, Bharty SK, Gupta UA, Khayyam KU, Vohra V, Singla N, Myneedu VP, Behera D. Sputum smear positivity at two months in previously untreated pulmonary tuberculosis patients. International journal of mycobacteriology.; **2**(4):199-205 (2013).
11. Liang L, Wu Q, Gao L, Hao Y, Liu C, Xie Y, Sun H, Yan X, Li F, Li H, Fang H. Factors contributing to the high prevalence of multidrug-resistant tuberculosis: a study from China. Thorax. 2012 Jul 1;67(7):632-8
12. Parikh R, Nataraj G, Kanade S, Khatri V, Mehta P. Time to sputum conversion in smear positive pulmonary TB patients on category I DOTS and factors delaying it. J Assoc Physicians India. **60**(22):6 (2012).
13. Singhal A, Jie L, Kumar P, Hong GS, Leow MK, Paleja B, Tsenova L, Kurepina N, Chen J, Zolezzi F, Kreiswirth B. Metformin as adjunct antituberculosis therapy. Science translational medicine; **6**(263):263ra159- (2014).
14. Lee YJ, Han SK, Park JH, Lee JK, Kim DK, Chung HS, Heo EY. The effect of metformin on culture conversion in tuberculosis patients with diabetes mellitus. The Korean journal of internal medicine.; **33**(5):933 (2014).
15. Motta AB. Mechanisms involved in metformin action in the treatment of polycystic ovary syndrome. Current pharmaceutical design. 2009 Sep 1;15(26):3074-7
16. Zhang Y, Yew WW, Barer MR. Targeting persisters for tuberculosis control. Antimicrobial agents and chemotherapy.; **56**(5):2223-30 (2012).
17. Singla R, Bharty SK, Gupta UA, Khayyam KU, Vohra V, Singla N, Myneedu VP, Behera D. Sputum smear positivity at two months in previously untreated pulmonary tuberculosis patients. International journal of mycobacteriology. 2013 Dec 1;2(4):199-205