Original Research Article

Efficacy of metronidazole to prevent active pulmonary tuberculosis in people living with HIV/AIDS on highly active anti-retroviral therapy: a prospective cohort study

Ketut Suryana*

Department of Internal Medicine, Merpati Clinic, Wangaya HIV Study Group (WHSG), Allergy and Clinical Immunology Services Unit at Wangaya Hospital in Denpasar, Bali, Indonesia

Received: 03 September 2020
Accepted: 08 October 2020

*Correspondence:
Dr. Ketut Suryana,
E-mail: ketutsuryana@gmail.com

Copyright: © the author(s), publisher and licensee Medip Academy. This is an open-access article distributed under the terms of the Creative Commons Attribution Non-Commercial License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

ABSTRACT

Background: People living with HIV/AIDS (PLWHA) were more susceptible of Active Pulmonary Tuberculosis (APT) than non-PLWHA. Whether Metronidazole Preventive Therapy (MPT) may prevent APT, remain unclear. The objective of the study was to investigate efficacy of MPT and other associated risk factors of APT among PLWHA on Highly Active Anti Retroviral Therapy (HAART).

Methods: A prospective cohort study included 182 PLWHA on HAART and asymptomatic tuberculosis (TB), 62 received MPT (first group) and 120 PLWHA did not receive MPT (second group). APT were diagnosed among the first group (4 participants) and the second group (26 participants). Monthly visit to replenish pills and to confirm APT. Efficacy of MPT to prevent APT, socio-demography and laboratory, were analyzed using Chi-square with significance p<0.05.

Results: Of 112 participants (62.20%) were males, 70 (37.80%) females, mean age (year) 37.31±9.83. Four (2.20%) of participants (first group) and 26 (14.47%) (second group) were confirmed APT (p=0.003). In bivariate analysis, sex (p=0.020), alcohol consumption (p=0.000); smoking (p=0.000), CD4 cell counts (<70 cell/µl) (p=0.001), previous history of TB (p=0.000) were the significant factors associated with APT. Participants who received MPT had a significantly lower risk of APT than participants who did not receive MPT (p=0.003). Other factors; weight, Hb, WBC; neutrophil, lymphocyte, Neutrophil Lymphocyte Ratio (NLR) were not significantly associated with APT.

Conclusions: We found, a significant protective effect of MPT, prevent APT. Other significant associated risk factors of APT were sex (male), smoking, alcohol consumption, previous TB history, lower CD4 counts.

Keywords: Active pulmonary tuberculosis, Human immunodeficiency virus, Metronidazole preventive therapy

INTRODUCTION

The world’s population are estimated almost a quarter infected with Mycobacterium tuberculosis (MTB). About 2 million people die each year and 2 billion people are asymptomatic or have latent MTB infection with 10% of them potentially reactivating /APT.1,2

There are physiologic stages of MTB in granulomatous lesions varies from actively replicating (AR) bacilli to dormant, non replicating (NR) bacilli coexist in the lungs of TB patients. Low oxygen pressure limits the growth of MTB to dormant state. Dormant is a non-replicating state, especially in the caseous nodules of the lungs, hypoxic / anaerobic conditions.2

The strongest risk factor for progression to active disease is co-infection TB and human immunodeficiency virus (HIV).3 PLWHA had higher risk (15-22 times) to develop
active disease than non-PLWHA and TB is the main cause of death among PLWHA.\textsuperscript{3,4}

The recent study reported that MTZ avoids reactivation of dormant MTB infection.\textsuperscript{2} Anti-mycobacterial agents are very effective against the growing bacilli.\textsuperscript{3} Chemoprophylaxis can reduce the risk of active disease by as much as 90\%, thus give the drugs that kill dormant MTB is an urgent. Bactericidal activity of MTZ on hypoxic / anaerobic conditions, can prevent active disease / TB reactivation.\textsuperscript{2,6-10}

MTZ, is an affordable broad spectrum antimicrobial agent used to treat opportunistic infections in PLWHA, Wayne and colleagues (1998) in vitro demonstrated the presence of MTB in the oxygen-free layer. They also demonstrated the bacilli not only rejected the bactericidal effect of anaerobiosis but also represented partial or complete resistance to the bactericidal effect of isoniazid or rifampicin and it also indicated that MTZ could act on this bacilli.\textsuperscript{5,11}

The aim of this study is to assess the efficacy of MPT to prevent APT in PLWHA on HAART.

METHODS

Study population and design

A prospective cohort study was conducted from March 2018 to April 2020. Total participants in this study were 182 PLWHA on HAART that consisted of 62 participants who received MPT and 120 participants did not receive MPT. Participants routinely visits every month but also visits for acute illnesses occasionally. We evaluated the efficacy of daily MPT prospectively for 12 weeks which reduced the risk of APT in PLWHA on HAART, socio-demography, laboratory (haemoglobin, CD4, WBC, neutrophil, lymphocyte and neutrophil/lymphocyte ratio). Structured questionnaire was used to collect the data, after completed by physicians. We assessed the APT during 2 months taking Metronidazole 500 mg twice daily and 3 months after. APT was confirmed by TB screening, Chest X ray and laboratory findings (Acid Fast Bacilli / AFB).

Interview, TB screening (current cough, fever, night sweat, and weight loss) and giving information about MPT, Cell Blood Count (CBC), the obedience to the appointed schedule were performed to all of eligible participants (PLWHA aged \( \geq 18 \) years).\textsuperscript{12-14} If there was a suspicion for TB (at least one of the positive symptom screening components), then a radiological and bacteriologic examination (AFB) was done to identify the MTB. If it was confirmed, they were given standard Anti Tuberculosis Drugs (ATD). Participants who were unconfirmed TB continued to the clinical condition examinations such as nausea/vomiting. Metronidazole hypersensitivity. Participants with abnormalities were excluded. Participants who were unconfirmed TB or without any clinical abnormality were divided into 2 groups. The first group: participants who were unconfirmed APT and accepted MPT. The participants who were unconfirmed APT but refused MPT as a second group. During 2 months MPT administration and 3 months after, a TB screening was done for every participants, also for the participants who refused MPT as a control. The flowchart of participants in this study is described in Figure 1.

The independent variables were age, sex, body weight, laboratory result (CBC: haemoglobin, White Blood Cells, Neutrophil, Lymphocyte, CD4+).

Eligible participants received a package containing 60 MTZ tablets 500 mg. Participants were educated to take twice daily of MTZ tablets for 30 days and were given monthly refill appointments. The dependent variables were APT, confirmed by presumptive diagnostic (routine diagnostic procedure). APT were compared between participants received MPT and participants did not receive MPT.

Statistical analysis

The effectiveness of MPT on APT in PLWHA on HAART was analyzed using Chi-square. A p-value of < 0.05 was considered statistically significant. Statistical software package SPSS 20.0 was used for statistical analysis.
RESULTS

A total of 182 participants (PLWHA) were included in this study. The mean of age was 37.29-9.81 years old, 112 (62.20%) of the participants were males and 70 (37.80%) females, majority of participants 140 (77.30%) on secondary education, 58 (32.70%) smokers, 67 (36.80%) alcohol consumption, 75 (41.00%) previous history of TB or presence of a TB patient in the family, the mean of weight 52.25-9.02 kg, and baseline CD4 cell counts: 73.54±52.52 (Table 1).

To investigate the effect of MPT to prevent APT among PLWHA on HAART, the participants were divided into 2 groups, the first group 62 participants accepted MPT, 120 participants refused MPT (second group).

In this study we confirmed APT 4 (2.20%) among the first group during the 3 months follow up, but 26 (14.47%) among the second group were confirmed APT (p=0.003; OR=0.251; 95% CI=0.092-0.673) (Table 2). In the bivariate analysis (Chi-square) we found the statistically significant association between the other factors with APT, male was more likely than female (p=0.020; OR=2.749; 95% CI=1.146-6.578). A higher proportion of APT was in participants who were smoking (p=0.000) and had alcohol consumption (p=0.000) compared with the controls. A previous history of TB or presence of a TB patient in the family was one of the most important risk factors of APT (p=0.000). Lower CD4 cell counts was risk factors of APT (p=0.001) (Table 3).

Table 1: The characteristics data of the participant (n=182).

| Variable                                      | N (%) / mean±SD                                      |
|-----------------------------------------------|-----------------------------------------------------|
| Age (year)                                    | 37.29±9.81                                          |
| Sex                                           |                                                     |
| Male                                          | 112 (62.20%)                                        |
| Female                                        | 70 (37.80%)                                         |
| Education level                               |                                                     |
| No Formal education                           | 4 (2.00%)                                           |
| Primary education                             | 21 (11.60%)                                         |
| Secondary education                           | 140 (77.30%)                                        |
| Tertiary education                            | 17 (9.20%)                                          |
| Smoking                                       |                                                     |
| Yes                                           | 58 (32.70%)                                         |
| No                                            | 124 (67.30%)                                        |
| Alcohol consumption                           |                                                     |
| Yes                                           | 67 (36.80%)                                         |
| No                                            | 115 (63.20%)                                        |
| Previous history of TB or presence of a TB patient in the family |             |
| Yes                                           | 75 (41.00%)                                         |
| No                                            | 107 (59.00%)                                        |
| Weight (kg)                                   | 52.25±9.02                                          |
| Cell blood count                              |                                                     |
| Haemoglobin (g/dl)                            | 11.76±2.50                                          |
| WBC (10⁹/µl)                                  | 5.32±2.39                                           |
| Neutrophil (10⁶ cells/µl)                     | 3.33±1.97                                           |
| Lymphocyte (cells/mm³)                        | 1.02±0.50                                           |
| Neutrophil lymphocyte ratio (NLR)             | 4.48±5.18                                           |
| CD4cell counts (cells/µl)                     | 73.54±52.52                                         |

WBC=White Blood Cells; NLR=Neutrophil Lymphocyte Ratio; CD4=Cluster Differentiation-4

Table 2: The effect of MPT to prevent APT among PLWHA on HAART (N=182).

| Variables | Accepted MPT (n=62) | Refused MPT (n=120) | P value | Odd Ratio | CI 95%  |
|-----------|---------------------|---------------------|---------|-----------|---------|
| APT       |                      |                     |         |           |         |
| Yes       | 4 (2.20%)           | 26 (14.47%)         | 0.003*  | 0.251     | 0.092-0.673 |
| No        | 58 (31.87%)         | 94 (51.53%)         |         |           |         |

Bivariate analysis (Chi-square) with Significant p<0.05* MPT= Metronidazole Preventive Therapy APT=Active Pulmonary Tuberculosis
Table 3: The Association between other factors with active pulmonary tuberculosis (n=182).

| Variables                      | APT (n=30) | No APT (n=152) | P value | Odds Ratio | CI 95% |
|--------------------------------|------------|----------------|---------|------------|--------|
| Sex                            |            |                |         |            |        |
| Male                           | 20 (10.98) | 93 (51.09)     | 0.020*  | 2.049      | 1.146-6.578 |
| Female                         | 10 (5.49)  | 59 (32.52)     |         |            |        |
| Age (year)                     |            |                |         |            |        |
| <35 years                      | 9 (4.94)   | 51 (28.02)     | 0.436   | 1.327      | 0.646-2.720 |
| ≥35 years                      | 21 (11.54) | 101 (55.50)    |         |            |        |
| Smoking                        |            |                |         |            |        |
| Yes                            | 24 (13.20) | 34 (18.68)     | 0.000*  | 4.944      | 2.736-12.898 |
| No                             | 6 (3.29)   | 118 (64.83)    |         |            |        |
| Alcohol consumption            |            |                |         |            |        |
| Yes                            | 25 (13.74) | 42 (23.07)     | 0.000*  | 5.214      | 2.371-11.455 |
| No                             | 5 (2.75)   | 110 (60.44)    |         |            |        |
| Previous history of TB or presence of a TB patient in the family | | | | | |
| Yes                            | 22 (12.09) | 55 (30.22)     | 0.000*  | 2.751      | 1.135-4.026 |
| No                             | 8 (4.40)   | 97 (53.29)     |         |            |        |
| Weight (kg)                    |            |                |         |            |        |
| < 51 kg                        | 13 (7.14)  | 66 (36.02)     | 0.329   | 1.427      | 0.697-2.920 |
| ≥51 kg                         | 17 (9.34)  | 86 (47.50)     |         |            |        |
| Haemoglobin (gr%)              |            |                |         |            |        |
| < 11.4 gr%                     | 16 (8.79)  | 69 (37.91)     | 0.145   | 1.698      | 0.822-3.498 |
| ≥11.4 gr%                      | 14 (7.69)  | 83 (45.61)     |         |            |        |
| White blood cell (10³/μl)      |            |                |         |            |        |
| < 4.80                         | 11 (6.04)  | 79 (43.40)     | 0.402   | 0.736      | 0.356-1.516 |
| ≥4.80                          | 19 (10.44) | 73 (40.12)     |         |            |        |
| Neutrophil (10³/μl)            |            |                |         |            |        |
| < 3.17                         | 17 (9.34)  | 80 (43.95)     | 0.210   | 0.631      | 0.303-1.307 |
| ≥3.17                          | 13 (7.14)  | 72 (39.57)     |         |            |        |
| Lymphocyte (10³/μl)            |            |                |         |            |        |
| < 0.95                         | 18 (9.89)  | 78 (42.86)     | 0.834   | 1.079      | 0.526-2.206 |
| ≥0.95                          | 12 (6.59)  | 74 (40.66)     |         |            |        |
| NLR                            |            |                |         |            |        |
| < 3.39                         | 14 (7.69)  | 81 (44.50)     | 0.104   | 0.549      | 0.261-1.147 |
| ≥3.39                          | 16 (8.79)  | 71 (39.02)     |         |            |        |
| CD4 cell counts (cell/μl)      |            |                |         |            |        |
| <70                            | 23 (12.64) | 72 (39.56)     | 0.001*  | 3.414      | 1.526-7.630 |
| ≥70                            | 7 (3.84)   | 80 (43.96)     |         |            |        |

Bivariate analysis (Chi-square) with Significant p<0.05* NLR=Neutrophil Lymphocyte Ratio CD4=Cluster Differentiation 4 TB=Tuberculosis

DISCUSSION

MTZ shows considerable bactericidal activity for MTB under hypoxic conditions in non-replicating persistence model. Bactericidal activity of MTZ depends on the formation of a redox intermediate metabolite from the reduction of the nitro group in MTZ under the oxygen-free conditions. This metabolite oxidizes DNA and causes extensive breakage of DNA strands and subsequent cell death, and also inhibits DNase I, which has a function as a repair endonuclease in bacteria. Reduced MTZ exerts a dual action by destroying DNA strands and inhibiting the enzyme responsible for repairing strand breaks in DNA. The risk of developing active tuberculosis and mortality are higher in PLWHA (5-15%) compared with immunocompetent 5-10%. MTZ alone given for 2 months is almost as effective as INH/RIF for 2 months in preventing from reactivation.

Therefore MPT has been recommended as part of the essential treatment and support package for PLWHA at Wangaya Hospital in Denpasar, Bali, Indonesia.
In this study MPT showed the significant protective effect for the first group (accepted MPT) to prevent APT (p=0.003; OR=0.251; 95% CI=0.092-0.673). Other associated factors of APT; sex (male) were significantly more likely associated with APT than female (p=0.020; OR=2.049; 95% CI=1.146-6.578).

Smoking might cause immune disruption, ciliary clearance break down, and it might increase the risk of APT. This study also revealed that smoking was significantly associated with APT (p=0.000; OR=4.944; 95% CI=2.736-12.898).

Long-term alcohol consumption can disrupt immunity, modulate the immune response, and have a higher risk of developing APT. This study reported that alcohol consumption was significant associated with APT (p=0.000; OR=5.214; 95% CI=2.371-11.455).

This study found that previous history of TB or TB patient in the family was significantly associated with APT (p=0.000; OR=2.751; 95% CI: 1.135-4.026).

MPT has beneficial effects in improving immunity (increasing CD4 cells count and reducing viral load). MPT reduces mortality in PLWHA with pulmonary tuberculosis and CD4 counts less than 200 cells/μl were independent factor for increased APT. PLWHA with low CD4 counts were more likely to develop APT or other opportunistic infection. In this study the CD4 level <70 (Cell/μl) reported has significant association with APT (p=0.001; OR=3.414; 95% CI=1.526-7.630).

Limitations of study: The limitations of this study, the diagnostic of APT cases were confirmed with TB screening, a radiological and bacteriologic examination (AFB). Without confirmation the Mycobacterium Tuberculosis (MTB) positive culture results.

Recall bias might have influence the exactness of data related to the substance use in cigarette smoking and alcohol consumption.

CONCLUSION

Our study found that MPT has a significant protective effect prevented APT. Another risk factors that significantly associated with APT were sex (male), alcohol consumption, smoking, lower CD4 cell count, and previous history of TB or presence of a TB patient in the family.

ACKNOWLEDGEMENTS

Authors would like to thank the volunteer doctors: Dwijo Anargha Sindhughosa, William Ray Cassidy and all of team work of Wangaya HIV Study Group (WHSG) staff for their cooperation during the data collection. We are grateful to Wangaya Hospital Director for the support.

Funding: No funding sources

Conflict of interest: None declared

Ethical approval: The study was approved by the Institutional Ethics Committee No: 07/RSUDW/Litbang/2018 from the local ethical committees. We collected data from PLWHA who visited Wangaya Hospital, Merpati Clinic of Denpasar, Bali, Indonesia and completed at least 3 months (this period is considered the time period in which MPT is effective) follow-up from the ethical clearance granted date

REFERENCES

1. Bares SH, Swindell S. Latent tuberculosis and HIV infection. Curr Infect Dis Rep. 2020;22:17.
2. Piccaro G, Giannoni F, Fillippini P, Mustazzolu A, Fattorini L. Activities of drug combinations against Mycobacterium tuberculosis grown in aerobic and hypoxic acidic conditions. Antimicrobial agents and chemotherapy. 2013;57:1428-33.
3. Pawloski A, Jansson M, Skold M, Rottenberg ME, Kallius G. Tuberculosis and HIV Co-Infection. PlosPathog. 2012;8:e1002464.
4. Getahun H, Matteelli A, Chaisson RE, Raviglione M. Latent Mycobacterium tuberculosis infection. N Engl J Med. 2015;372:2127-35.
5. Wayne LG, Sramek HA. Metronidazole is bactericidal to dormant cells of Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 1994;38:2054-8.
6. Lacobino A, Piccaro G, Giannoni F, Mustazzolu A, Fattorini L. Fighting tuberculosis by drugs targeting nonreplicating Mycobacterium tuberculosis Bacilli. Int J Mycobacteriol. 2017;6:213-21.
7. Peddireddy V, Doddam SN, Ahmed N. Mycobacterial Dormansy systems and host responses in tuberculosis. Front Immunol. 2017;8:1-19.
8. Zhang Y. Metronidazole validates drugs targeting hypoxic bacteria for improved treatment of tuberculosis. PNAS. 2012;109:13890-91.
9. Filippini P, Iona E, Piccaro G, Peyron P, Neyroles O, Fattorini L. Activity of drug combinations against dormant Mycobacterium tuberculosis. Antimicrobial agents and chemotherapy. 2010;54:2712-5.
10. Iona E, Giannoni F, Pardini M, Brunori L, Orefici G, Fattorini L. Metronidazole plus Rifampin sterilizes long term dormant Mycobacterium tuberculosis. Antimicrobial Agents and Chemotherapy. 2007;51:1537-40.
11. Paramasivan CN, Kubendiran G, Herbert D. Action of metronidazole in combination with isoniazid & rifampicin on persisting organisms in experimental murine tuberculosis. Indian J Med Res. 1998;108:115-9.
12. Solomon S, Asmare Y, Taddesse B, Negah S, Mamuye Y, Yitayew B, et al. Prevalence of tuberculosis among HIV positive individuals with asymptomatic disease states at St. Paul’s Hospital.
Millennium Medical College, Addis Ababa, Ethiopia. J Med Microb Diagn. 2015;4:1-6.
13. Swaminathan S, Padmapriyadarsini C, Narendran G. HIV-associated tuberculosis: clinical update. Clinical Infectious Disease. 2010;50:1377-86.
14. Sterling TR, Pham PA, Chaisson RE. HIV infection-related tuberculosis clinical manifestations and treatment. Clinical infectious disease. 2010;50(3):S223-30.
15. Carroll MW, Jeon D, Mountz JM, Lee JD, Jeong YJ, Zia N, et al. Efficacy and safety of Metrodinazole for pulmonary multidrug resistant tuberculosis. Antimicrobial agents and chemotherapy 2013;57:3903-9.
16. Klinkenber LG, Sutherland LA, Bishai WR, Karakouisis PC. Metronidazole lacks activity against Mycobacterium tuberculosis in an in vivo Hypoxic Granuloma model of latency. J Infectious Dis. 2008;198:275-83.
17. Padgilvar SS, Manmode S, Sahare AY, Kadam M, Manwar JV, Warade PP, et al. Recent advances in treatment for tuberculosis: A review. Int J Pharm Sci Res. 2016;33:162-72.
18. Alsaaad N, Williert B, Altena RV, Lange WCM, Werf TSVD, Kosterink JGW, et al. Potential antimicrobial agents for the treatment of multidrug resistant tuberculosis. Eur Respir J. 2014;43:884-97.
19. Lin PL, Dartois V, Johnston PJ, Janssen C, Via L, Goodwin MB. Metronidazole prevents reaction of latent Mycobacterium tuberculosis infection in macaques. PNAS. 2012;109:14188-93.
20. Bruchfeld J, Neves MC, Kallenius G. Tuberculosis and HIV Coinfection. Cold Spring HarbPerspect Med. 2015;5:a017871.
21. Ai JW, Ruan QL, Liu QH, Xhang WH. Updates on the risk factors for latent tuberculosis reactivation and their management. Emerging Microbes and Infections. 2016;5:e10.
22. Sullivan ZA. Latent and active tuberculosis infection increase immune activation in individuals co-infected with HIV. Ebiomedicine. 2015;334-40.
23. Person AK. Treatment of latent tuberculosis infection in HIV: shorter or longer?Curr HIV/AIDS Rep. 2012;9:259-66.
24. Granich R, Akolo C, Gunneberg C, Getahun H, Williams P, Williams B. Prevention of tuberculosis in people living with HIV. Clinical Infectious Disease. 2010;50:S215-22.
25. Djomo PN, Rodrigues LC, Smith PG, Abubakar I, Mangtani P. Drug misuse, tobacco smoking, alcohol and other social determinants of tuberculosis in UK-born adults in England: a community-based case-control study. Scientific Reports Nature Research. 2020;10:5639.
26. Horne DJ, Campo M, Ortiz JR, Oren E, Arentz M, Crothers K, et al. Association between smoking and latent tuberculosis in the US population: an analysis of the national health and nutrition examination survey. Plos One. 2012;7:e49050.
27. Fiao WH, Campagnolo D, Dayao C, Lukas RJ, Wu J, Shi FD. Nicotine and inflammatory neurological disorders. Acta Pharmacol Sin. 2009;30(6):715-22.
28. Szabo G, Soho B. Alcohol’s effect on host defense. Alcohol Res. 2015;37(2):159-70.
29. Lomnroth K, Williams BG, Stadlin S, Jaramillo E, Dye C. Alcohol use a risk factors for tuberculosis – a systematic review. BMC Public Health. 2008;8:289.
30. Skogmar S, Schon T, Balcha TT, Jemal ZH, Tibbeso G, Bjork J, et al. CD4 cell levels during treatment for tuberculosis (TB) in Ethiopian adults and clinical markers associated with CD4 lymphocytopenia. Plos One. 2013;8:e83270.

Cite this article as: Suryana K. Efficacy of metronidazole to prevent active pulmonary tuberculosis in people living with HIV/AIDS on highly active anti retroviral therapy: a prospective cohort study. Int J Res Med Sci 2020;8:3826-31.