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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

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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 significancy $p<0.05$.
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.[3,4]

The recent study reported that MTZ avoids reactivation of dormant MTB infection.[2] Anti-mycobacterial agents are very effective against the growing bacilli.[5] 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.[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.[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 paticipants did not receive MPT. Participants routinely visits every month but also visites for acute ilnesses 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 ≥ 18 years).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 hypersensitifity. 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.

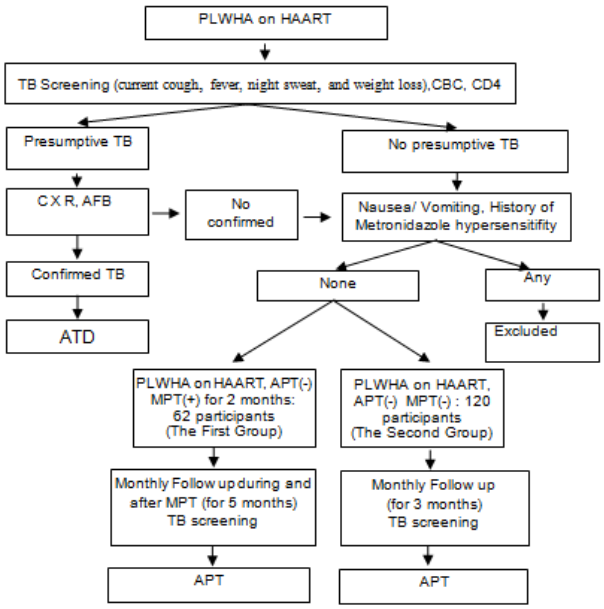

Figure 1: Participants flowchart.

PLWHA=People living with HIV/AID; HAART=Highly Active Anti Retroviral Therapy;TB=tuberculosis; CBC= Cell Blood Counts; CD4= Cluster Differentiation;CXR=Chest X Ray; AFB=Acid Fast Bacilli; ATD=Anti Tuberculosis Drugs; APT=Active Pulmonary Tuberculosis; MPT= Metronidazole Preventive Therapy.

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 37.29-9.81years 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 (10^3/µl)	5.32±2.39
Neutrophil (10^9cells/l)	3.33±1.97
Lymphocyte (cells/mm^3)	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%
	N (%)	N (%)			
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) N (%)	No APT (n=152) N (%)	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 (10^3/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 (10^3/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 (10^3/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.[8,15,16] 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 1, 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.[17-19]

The risk of developing active tuberculosis and mortality are higher in PLWHA (5-15%) compared with in immunocompetent 5-10%.[3,20-24]

MTZ alone given for 2 months is almost as effective as INH/RIF for 2 months in preventing from reactivation.[8]

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 significant 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.[25-27] 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.[25,28,29] 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.[30] 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 historyof TB or presence of a TB patient in the family.

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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

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