International Journal of Sciences: Basic and Applied Research (IJSBAR)

ISSN 2307-4531
(Print & Online)

http://gssrr.org/index.php?journal=JournalOfBasicAndApplied

First Line Anti-Tuberculosis Drug Resistance Among Human Immunodeficiency Virus Infected Patients Attending Maryland Comprehensive Care Centre Mathare 4a Nairobi Kenya

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Abstract

TB is a major cause of death among people living with human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS). Multi drug resistant tuberculosis (MDR-TB) accounts for up to 14 % of all these T.B cases. İn this study; Sputa from patients with bacteriologically confirmed pulmonary tuberculosis (PTB) were cultured on Mycobacterium Growth Indicator Tube (MGIT) media. Strains of MTB complex from MGIT were subjected to drug susceptibility testing for isoniazid, Rifampicin, Streptomycin, and Ethambutol using the proportional method on (MGIT). The CD4 cell counts were obtained from the Maryland laboratory registers. The results show that the Median CD4 count was 286 . A total of 51 (37.0%) patients had CD4 count (<200) while 87 (63.0%) had CD4 count ≥200.

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Patientswith CD4 count <200 were 42 (82.4%) and 70 (80.5%) with CD4 count ≥200 were fully sensitive to all anti-tuberculosis drugs tested. Resistance patterns among patients with CD4 count of<200 was as follows; isoniazid 6 (11.8%), rifampicin 5 (9.8%), ethambutol 4 (7.8%), streptomycin 3 (5.9%). Among patients with CD4 count ≥200 the resistance pattern was isoniazid 10 (11.5%), ethambutol 7 (8.0%), rifampicin 4 (4.6%), and streptomycin 4 (4.6%) (Table 1). Three (5.9%), and 3 (3.4%) isolates from patients with CD4 count <200, and those with CD4 count ≥200 respectively, had multidrug resistant TB (MDR TB) defined as resistant to both isoniazid and rifampicin. Our study concluded that there were no significant associations between the various resistant patterns and levels of CD4.

Keywords: Tuberculosis; First Line Drug Resistance; HIV; Kenya; Multi drug resistant tuberculosis

1. Introduction

TB is a major cause of death among people living with the human immunodeficiency virus/acquired immune deficiency syndrome (HIV/AIDS) [13]. Multi drug resistant tuberculosis (MDR-TB) accounts for up to 14 % of all T.B cases, with TB being the leading cause of death among people living with H.I.V/A.I.D.S [7]. Although there have been several well documented outbreaks of MDR-TB in institutional settings, little evidence indicates that H.I.V is associated with MDR-TB among the general population [10-17]. Most studies conducted in the general population have very little power, are not methodologically rigorous, and have many potential confounders [2]. The treatment of tuberculosis is becoming increasingly more complex and difficult to treat in H.I.V infected patients due to the rising incidence of MDR-TB [15-19]. As immune suppression progresses, disseminated and extra-pulmonary forms of T.B become more frequent [4]. Occurrence of drug resistant T.B does not correlate with the cluster of differentiation (CD4) counts, although TB is more commonly seen in severely immune-compromised patients [5].

Several recent studies showed that resistance to additional first-line drugs other than isoniazid and rifampicin, were independently associated with unfavorable treatment outcomes [7]. The risk of developing tuberculosis after an infectious contact has been estimated to be 5-15% / year in H.I.V infected patients [4]. H.I.V induced immune-suppression modifies the clinical presentation of T.B. In the early stages of immune- suppression, most tuberculosis patients with infection present in the same fashion as others with tuberculosis not infected with H.I.V [1]. As immune suppression progresses, disseminated and extra-pulmonary forms of tuberculosis become more frequent [1]. The treatment of tuberculosis is also becoming increasingly more complex and difficult in H.I.V infected patients due to the rising incidence of MDR-TB. MDR-TB and extremely drug resistant tuberculosis (XDR-TB) are associated with very high mortality rates and their transmission both in community and health care settings remains an ongoing challenge in resource limited settings and in countries with high rates of HIV co-infection [18].

The true magnitude of drug resistance is not well described [8]. There are several limitations to adequate assessment of this problem, especially in developing countries. In many areas there are few facilities for culture of *mycobacterium tuberculosis* and where they are antimicrobial susceptibility testing is not performed [13].

Standardized laboratory methodologies have not been followed uniformly and in some surveys small or unrepresentative populations have been sampled, thus difficult to accurately monitor trends [13].

This study was undertaken to determine *M. tuberculosis* resistance patterns against first-line drugs with respect to CD4 counts among patients attending Maryland comprehensive care centre.

2. Materials and Methods

2.1 Setting

The study was conducted in Mathare 4A, Nairobi the capital city of Kenya. The population of Mathare is nearly 180,000 and is steadily growing due to rural /urban migration. This poses a lot of problems socially and economically. A significant proportion of the residents of Mathare live below the poverty line, with high population densities. Mathare valley is approximately 6km to the north east of Nairobi's central business district. It is bordered by Thika road to the north and Juja road to the south. The study was cross sectional, eligible patients (new and retreatment) randomly sampled during the intake period, who gave consent were enrolled for the study. The intake period was between April and November 2013.

2.2 Specimen Collection and Transport

A spot sample and one early morning sputum were collected in sterile 50 milliliters falcon tubes. Genexpert was done on the spot sample to confirm T.B diagnosis of suspected patients. Second samples from the *MTB* positive patients were then transported weekly by smith-line courier service, to central reference laboratory (CRL) for culture and drug susceptibility testing (DST). The CRL is located within the centre for respiratory diseases research, Kenya Medical Research Institute (CRDR-KEMRI) at Kenyatta National Hospital.

2.3 Culture of M. Tuberculosis and Drug Susceptibility Testing

Sputum culture and drug susceptibility testing (DST) for *M. tuberculosis* was conducted in the central reference laboratory. Primary culture of *M. tuberculosis* was performed using non radiometric method Mycobacterium growth indicator tube (MGIT) 960. The sputa were decontaminated with NAOH solution (40%w/v) combined with 2.9% sodium citrate solution and N-acetyl -L-cystein (NALC) powder. Sterile phosphate buffer was added and the organisms concentrated by centrifugation at 3,000rpm for 15 minutes. The supernatant was decanted and the sediment suspended with phosphate buffer and inoculated in liquid MGIT media and incubated along with a growth control and an external control H37Rv at 37 degrees centigrade in BACTEC 960 systems (BD Diagnostic Systems, Sparks, MD, USA). The MGIT tubes were incubated until the instrument flagged them positive. After a maximum of six weeks, the instrument flagged the tubes negative if there was no growth at 37 degrees centigrade. A positive culture of *M. tuberculosis* confirmed the diagnosis of active disease.

2.4 Sensitivity Testing of M. tuberculosis

All culture positive tubes were tested for contamination before sensitivity tests using the standard method used in Kenya for drug susceptibility BACTEC MGIT 960 liquid culture system (Becton Dickson Company Sparks, MD, USA. A total of four first line drugs collectively referred to as SIRE Streptomycin (S)-1.00ug/ml; Isoniazid (H)-0.10ug/ml; Rifampicin (R)-1.00ug/ml and Ethambutol (E)-5.00ug/ml were tested for sensitivity. A control tube was matched with all the isolates tested. An external control of H37Rv was also set in all culturing and sensitivity testing processes. All readings were performed inside the machine and results were printed as susceptible, resistant or indeterminate.

3. Ethical Approval

The research proposal was approved and ethically cleared by the national ethical review committee (ERC) and Scientific Steering Committee (SSC) at the Kenya Medical Research Institute (KEMRI). Each patient who consented to enroll was required to complete an informed consent form.

4. Results

Of the 138 patients who had valid results for analysis, 79(57.2%) were male and 59(42.8%) were female. Analysis of CD4 count among the patients revealed that median CD4 count was 286 ranging between 2 and 859. A classification of CD4 was done using a cut-off of 200. A total of 51 (37.0%) patients had low CD4 count (<200) while 87 (63.0%) had CD4 count>200.

In this study, 42 (82.4%) of patients with CD4 count <200 and 70 (80.5%) of patients with CD4 count $\geq$200 were fully sensitive to all anti-tuberculosis drugs tested. Resistance patterns among patients with CD4 count of<200 was as follows; isoniazid 6 (11.8%), rifampicin 5 (9.8%), ethambutol 4 (7.8%), streptomycin 3 (5.9%). Among patients with CD4 count $\geq$200 the resistance pattern was isoniazid 10 (11.5%), ethambutol 7 (8.0%), rifampicin 4 (4.6%), and streptomycin 4 (4.6%) (Table 1; table 1 is at the end of the paper). Three (5.9%), and 3 (3.4%) isolates from patients with CD4 count <200, and those with CD4 count $\geq$200 respectively, had multidrug resistant TB (MDR TB) defined as resistant to at least both isoniazid and rifampicin. There were no significant associations between the various resistant patterns and levels of CD4

5. Discussion

HIV pandemic has changed tuberculosis from an endemic disease to a worldwide epidemic (WHO 2011). The risk of developing TB after an infectious contact has been estimated to be 5-15% /year in HIV infected patients compared to 5-10% during the lifetime of non HIV infected patients [3]. The risk of drug resistant TB is higher among those infected with H.I.V this is because of decreased immunity. T.B drug resistance is usually related to non adherence to therapy, severe immunodeficiency, diarrhea, and concurrent antifungal therapy [6]. Worldwide incidence of T.B is increasing, particularly in areas where H.I.V is prevalent [14].

The effect of CD4 count on T.B drug resistance is varied in various studies and it is often difficult to compare data because of relatively small patient numbers in previous studies and few documented data [9]. In the present study, Three (5.9%), and 3 (3.4%) isolates from patients with CD4 count <200, and those with CD4

count ≥200 respectively, had multidrug resistant TB (MDR TB) defined as resistant to at least both isoniazid and rifampicin. The median CD4 count was 286 ranging between 2 and 859. This contrasts with a study carried out in South Africa's Tugela Ferry from 2005 to 2007 and found that of the 272 MDR-TB and 382 XDR-TB cases, 90% and 98% were co-infected with HIV with median CD4 counts of 41 cells/µl and 36 cells/µl.

In another study carried out by Gandhi et al. in Kwazulu Natal South Africa, of the 1,539 patients tested, 542 (35%) had culture-positive TB, with MDR-TB in 221 (41%) of those with culture-positive TB. Of the MDR-TB cases, 53 (24%) had XDR-TB, of which all of the 44 patients who were tested for HIV were infected with HIV, with a median CD4 count of 63 cells/µl. The CD4 count does not predict the occurrence of drug resistant TB, because there were no significant associations between the various resistant patterns and levels of CD4.

Some studies show that CD4 count does not have significant effects on MDR TB development based on there being no difference found in sputum cultures of H.I.V positive and negative individuals with MDR TB [16-12]. Instead, these studies propose that MDR TB is greatly impacted by previous antibiotic treatment, with

individuals who have had previous treatment being five times more likely to develop MDR TB [11].

6. Study limitations

This study was conducted over a limited period of seven months and survey was conducted only in Mathare. Similar studies should be undertaken in other regions.

7. Conclusion

The effects of CD4 count on T.B drug resistance are varied in various studies and it is often difficult to compare data because of relatively small patient numbers in previous studies. CD4 count does not have a direct effect in development of T.B drug resistance among the immune-compromised patients. Immediate detection of drug resistance cases through rapid identification and DST is a key element and this benefits interruption of disease transmission. Rapid diagnosis of drug resistant T.B will have several benefits: earlier treatment of patients which will save lives and reducing the time spent on ineffective patient treatment. Diagnosis of MDR-TB and XDR-T.B now requires the scaling up of culture and drug susceptibility testing capacity, which is limited in disease endemic countries where H.I.V rates are high, and the expanded use of technology assays for rapid determination of resistance.

Acknowledgement

We thank the Maryland comprehensive care center, particularly the hospital laboratory staff for their support throughout the study period. We also in a special way thank the patients for accepting to participate in the study and also for their patience throughout the study.

Table 1: Presents Patterns of resistance to first line anti-tuberculosis drugs in relation to CD4 levels

	Total (n=138)		CD4<200 (n=51)		CD4>=200 (n=87)		95% CI			p
Antibiotic	**N**	**%**	**N**	**%**	**N**	**%**	**OR**	**Lower**	**Upper**	**value**
Sensitivity to all	112	81.2%	42	82.4%	70	80.5%	1.13	0.46	2.77	0.784
Any resistance										
Isoniazid (H)	16	11.6%	6	11.8%	10	11.5%	1.03	0.35	3.01	0.962
Rifampicin (R)	9	6.5%	5	9.8%	4	4.6%	2.26	0.58	8.82	0.290
Ethambutol (E)	11	8.0%	4	7.8%	7	8.0%	0.97	0.27	3.50	1.000
Streptomycin (S)	7	5.1%	3	5.9%	4	4.6%	1.30	0.28	6.04	0.709
Monoresistance TB										
Isoniazid (H)	6	4.3%	1	2.0%	5	5.7%	0.33	0.04	2.89	0.413
Rifampicin (R)	2	1.4%	2	3.9%	0	0.0%	UD	UD	UD	0.135
Ethambutol (E)	4	2.9%	0	0.0%	4	4.6%	UD	UD	UD	0.296
Streptomycin (S)	3	2.2%	1	2.0%	2	2.3%	0.85	0.08	9.61	1.000
Multi drug resistance TB (MDR TB)										
H+R	2	1.4%	1	2.0%	1	1.1%	1.72	0.11	28.11	1.000
H+R+E	2	1.4%	1	2.0%	1	1.1%	1.72	0.11	28.11	1.000
H+R+S	1	0.7%	0	0.0%	1	1.1%	UD	UD	UD	1.000
H+R+E+S	1	0.7%	1	2.0%	0	0.0%	UD	UD	UD	0.370
Total MDR TB	6	4.3%	3	5.9%	3	3.4%	1.75	0.34	9.01	0.670
Other resistant Patterns										
H+E	2	1.4%	1	2.0%	1	1.1%	1.72	0.11	28.11	1.000
H+S	1	0.7%	0	0.0%	1	1.1%	UD	UD	UD	1.000
H+E+S	1	0.7%	1	2.0%	0	0.0%	UD	UD	UD	0.370
R+E	1	0.7%	0	0.0%	1	1.1%	UD	UD	UD	1.000
E+S	0	0.0%	0	0.0%	0	0.0%				
R+S	0	0.0%	0	0.0%	0	0.0%				
R+E+S	0	0.0%	0	0.0%	0	0.0%				

Table 1: Patterns of resistance to first line anti-tuberculosis drugs in relation to CD4 levels

UD-Undefined

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