IDENTIFICATION OF MULTI DRUG RESISTANT TUBERCULOSIS AMONG HIV/AIDS PATIENTS BY BACTEC CULTURE METHOD.

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Summary

The worldwide epidemics of tuberculosis (TB) and HIV/AIDS have been joined by an insidiously developing third epidemic of TB drug resistance, the presence of TB drug resistance, particularly Multiple Drug Resistance, is a growing concern worldwide. The work is aimed at investigating prevalence of multi drug resistant tuberculosis in HIV individuals with an objective to prevent the risk of subsequent development of disease by early diagnosis using rapid sensitivity tests. Between November 2009-february 2010 sputum specimens (n=87) from HIV Seropositive individuals attending ART centre, Salem were examined for mycobacterium tuberculi and resistance strains to first line drugs using BACTEC 460 method . Bacterial etiology was observed in 15(17.24%) cases. Among them 7 (8.046%) were both Smear and Culture positive (s+ c+), 3 (3.448%) were Smear negative and culture positive (s-c+)**.** Mycobacterium Other Than Tuberculosis grown in 3(3.448%) of Smear positive specimens and 2(2.298%) of Smear negative specimens. Multi Drug Resistant Tuberculosis Strains were observed in 2(2.30%) cases during the study period. Study results reveals that effective treatment and prevention of MDR TB relay upon the prompt availability of drug susceptibility testing results and anti-TB drug susceptibility testing should be performed for all patients who are culture positive for M. tuberculosis.

Key words: Multi Drug Resistant Tuberculosis, Bactec 460 method, HIV-individuals.

Introduction

Tuberculosis (TB) is a pervasive and deadly disease of the respiratory system discovered by Robert Koch in 1882 is one of the main challenges in public health. [1, 2] After HIV/AIDS, TB is the second most deadly infectious disease. A high prevalence of HIV / TB co-infected patients has spurred the WHO to declare a global sanitary emergency since 1993. [3] Infection with HIV leads to both greatly increased rate of reactivation of latent tuberculosis infection and enhanced susceptibility to progression to active tuberculosis following new infection.[4, 5] The stigma of HIV has increased the existing stigma surrounding tuberculosis[11]. The interaction between TB and HIV infection is bidirectional. HIV increases the chances of TB reactivation by 20-fold and increases the risk of re-infection with potentially resistant *M. tuberculosis* strains.[6] On the other hand, TB causes cellular activation and excessive cytokine and chemokine production that stimulates replication of HIV and contributes to an accelerated course of acquired immunodeficiency syndrome and a shorter overall survival. HIV and TB represent a fatal combination, since both are more destructive together than either is alone.

The immune suppression caused by HIV is the major risk factor for the transition of latent TB into actively transmissible TB and accelerating the spread of the disease. Multi-Drug Resistance Tuberculosis
(MDR-TB) has been a particular concern among HIV-infected persons as this account for considerably rapid disease progression, which may result in rapid transmission. Diagnosis of TB depends on the isolation and identification of Mycobacterium and the control of MDR tuberculosis depend on rapid sensitivity results. Culture of *Mycobacterium tuberculosis* from clinical material is the "GOLD STANDARD" for diagnosis of tuberculosis (TB). Considering the emerging problem of drug resistance in TB, there is need to shorten the duration of time required for culture of *Mycobacterium tuberculosis* from sputum samples, so that it can be used for rapid drug susceptibility testing aimed at early diagnosis of Treatment Failure and MDR TB. Therefore use of sensitive and faster culture method like Bactec 460 system is necessary.
The work is aimed at investigating prevalence of multi drug resistant tuberculosis in HIV individuals with an objective to prevent the risk of subsequent development of disease by early diagnosis using rapid sensitivity tests.

Materials and Methods

Isoniazid and Rifampicin were obtained from Becton Dickinson, MD, USA. Middle brook 7H12 broth with carbon14 {14c} labelled palmitic acid, PANTA- antibiotic mixture solution were obtained from Sigma, St. Louis, Mo, USA. Para nitro –α-acetyl amino-β-hydroxy propiophenone (NAP) was a gift sample from Qualigens fine Chemicals, Mumbai. All other chemicals and reagents used in the study were of analytical grade.

Determination of Body mass index (BMI):

Body weight was determined to the nearest 0.1 kilogram using adult balance and standing weight was determined to the nearest one centimeter (cm) using Standiometer provided by National AIDS control Organization (NACO). Body mass index results were generated using BMI software What Health BMI calc, version 1.1.

Staging of HIV disease according to WHO:

The revised WHO clinical classification of HIV-associated disease was designed to use in patients with confirmed HIV infection. Along with measurement of the CD4 T lymphocytes count, BMI was used to stage the HIV disease based on severity of weight loss. [7]

Table1. Clinical staging of HIV disease based on severity of weight loss.

Clinical stages	Symptoms
Asymptomatic stage	Asymptomatic PGL (persistent generalized lymphadenopathy)
Mild stage	Unexplained moderate weight loss (<10% of presumed or measured body weight)
Moderate stage	Unexplained severe weight loss (>10% of presumed or measured body Weight), Pulmonary TB
Severe stage	HIV wasting syndrome, Extra Pulmonary TB (EPTB).

Specimens Collection and Processing:
Three sputum samples were taken for testing using a specific sampling technique called "spot early morning spot" according to RNTCP (Revised National TB Control Programme} guidelines, i.e. First sputum sample collected when the patient comes to the doctor initially .On instructions the patient collects the second sputum sample early in the morning and brings the same on the next day. The third sputum sample collected on the same day from him as per the procedure. Early morning spot sputum specimens were collected in sterile disposable 50 ml polypropylene container and were refrigerated at 2-8^{0}C. Quantity of the sample collection was <3-5 ml. Specimens collected for 1 week were transferred to department of microbiology, YRG Centre for AIDS Research and Education, VHS Campus, Chennai (YRG CARE) for Drug Susceptibility Testing (DST).
This study included 110 clinical specimens (collected from 110 patients), samples consecutively sent for mycobacterial culture in TB laboratory. Investigated specimens included 110 respiratory specimens (almost entirely represented by sputa). Blood specimens were not included in the study. [8]

Culture using Radiometric BACTEC 460 TB system:
Sputum specimens were collected and processed following the standard procedure recommended by the BACTEC 460 TB (BECTON DICKINSON) operations manual. Specimens were decontaminated using a final concentration of 1% NaOH and concentrated at 3000×g for 15 minutes. The sediment was reconstituted to 2.5ml with phosphate buffer p^H 6.8 and used to prepare smears and cultures on middle brook 7H 10 agar and inoculated in BACTEC 12B vials (BECTON DICKINSON). An initial smear was prepared and stained by Zheil- Neilson method and observed under oil-immersion for Acid fast bacilli (AFB). The medium used in BACTEC 460 TB system was 4ml of middle brook 7H12 broth with carbon14 {14c} labelled palmitic acid. The clinical specimen was inoculated along with an antibiotic mixture containing solution- PANTA (polymyxin B, amphotericin B, nalidixic acid, trimethoprim and azlocillin). All the inoculated bottles were incubated at 37^{0}C and observed for growth. BACTEC 12B bottles were read alternate days for the first week, the ninth and twelfth day and there after weekly once up to six weeks using the BACTEC 460 instrument. The time taken for detection and total number of positive cultures recorded. All the mycobacterial isolates were differentiated by NAP (Para nitro – α-acetyl amino-β- hydroxy propiophenone) test. The average time for identification / differentiation between mycobacterium and Non- Tubercular mycobacterium (NTM) was four days. Vials were pre-incubated before testing on the BACTEC 460 instrument, depending on the degree of smear positivity. 1+ and 2+ smears were held 2 days 3+ and 4+ smears were held 4 days before testing commenced. [9]

BACTEC 460 TB DST (Drug Susceptibility Testing):
Isolated M. tuberculosis colonies from BACTEC 12B media were subjected to DST using BACTEC 460 TB system. The modified critical concentrations of provided drugs (BECTON DICKINSON, MD, USA) were adopted: 0.1µg/ml for isoniazid (INH) ; 2.0 µg/ml for RIF. In short, actively growing BACTEC 12B cultures vials were incubated until the growth index > 500 was reached and was used to inoculate 12B vials containing single drug concentrations, while antibiotics free vials were seeded with a 1/100 dilution of the same culture. Vials were read daily until a control growth index (GI) of at least 20 was reached. Susceptibility to Rifampicin was determined by comparing the change in GI values between the Rifampicin containing vial and the control vial and Susceptibility to isoniazid was determined by comparing the change in GI values between the containing vial and the control vial when the GI in the drug vial decreased in relation to the GI of the control vial the organism was considered "susceptible". Conversely when the GI of the drug vial increased in relation to the GI of the control vial the organism was considered

"resistant" This method was used as a gold standard for evaluation of *mycobacterium Tuberculosis.* [9]

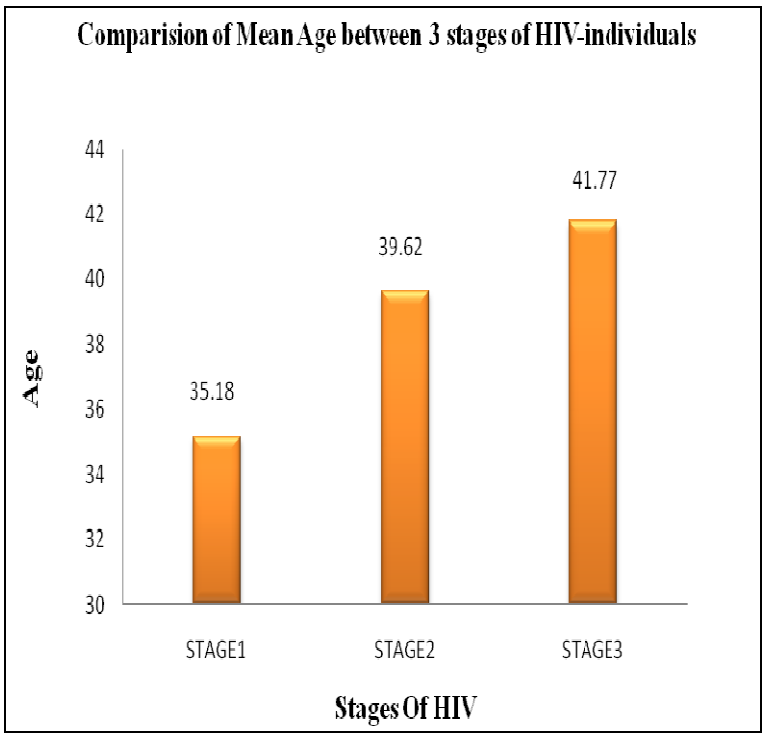
Statistical Analysis:
Data was analyzed using SPSS software version 17.0. Normal distribution of data were assessed using kolmogorov-Smirnov test and independent sample t-test was used to compare numeric variables such as age, weight, height, CD4 count between different stages of HIV infected patients.

Results

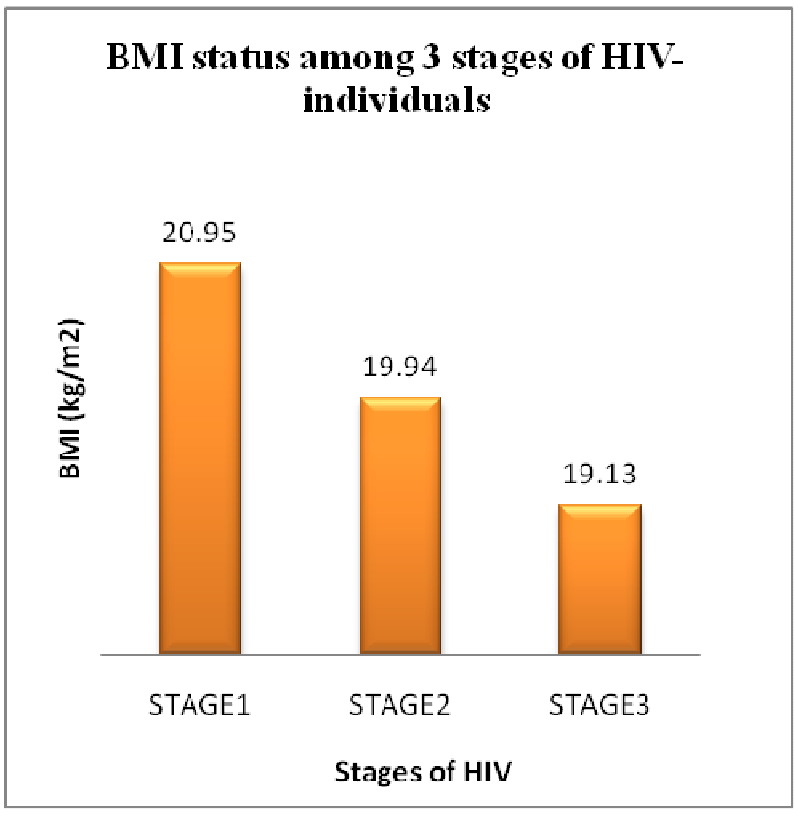
Results revealed that, 110 HIV-infected adult subjects of which 11 patients were asymptomatic with the mean age of (35.18 ± 12.6 years) and 72 mild HIV diseased subjects with mean age of (39.62 ± 9.97) and 27 severe HIV disease patients with the mean age of (41.77 ± 13.74) years were included in the study. TB Symptoms and signs of patients were presented as follows: fever (102°C) (54%), cough >3 weeks (93%), night sweats (61%), dyspnea (68%), chest pain (63%) and loss of appetite (76%). Of the cases, a total of 110 specimens collected from 110 patients were evaluated. The radiographic signs of patients were as follows: 5 patients had bilateral infiltration, 3 had pulmonary cavities.

Table 2. The Study Groups Base line Characteristics Age, BMI, CD4 count among different stages of HIV infection.
SD- Standard deviation, p< 0.99 compare to control

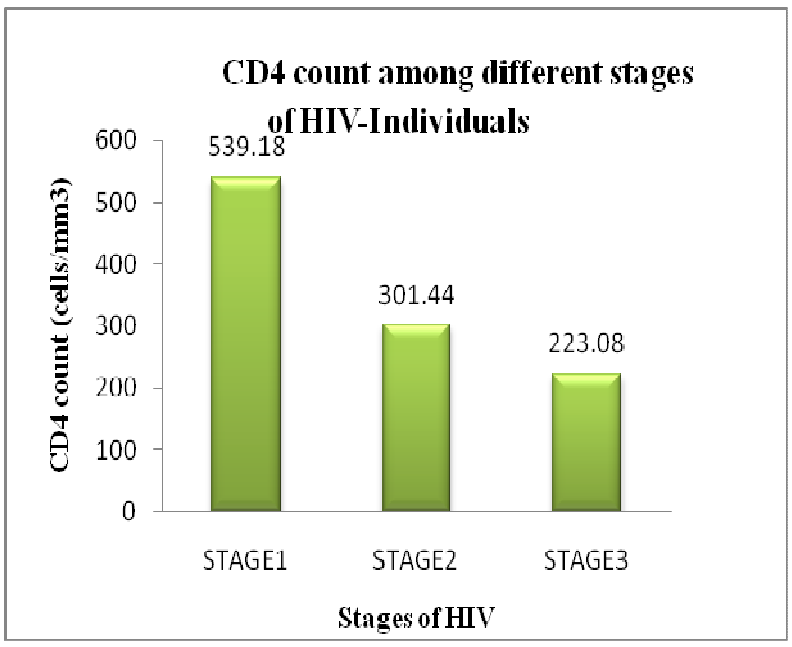
Parameters	**HIV Infected Patients**			**p- value**
	Stage 1	**Stage 2**	**Stage 3**	
Number of subjects	11	72	27	< 0.99
Age (year) (mean ± SD)	35.18 ± 12.60	39.62 ± 9.97	41.77 ± 13.74	
BMI (kg/m^2) (mean ± SD)	20.95 ± 2.602	19.94± 2.11	19.13 ± 2.35	
CD4(Cell/mm3) (mean ± SD)	539.18 ± 213.82	301.44 ± 128.8	223.08 ± 216.25	



Graph 1. Comparison of mean age between three stages of HIV-individuals



Graph 2. BMI status among 3 stages of HIV-individuals

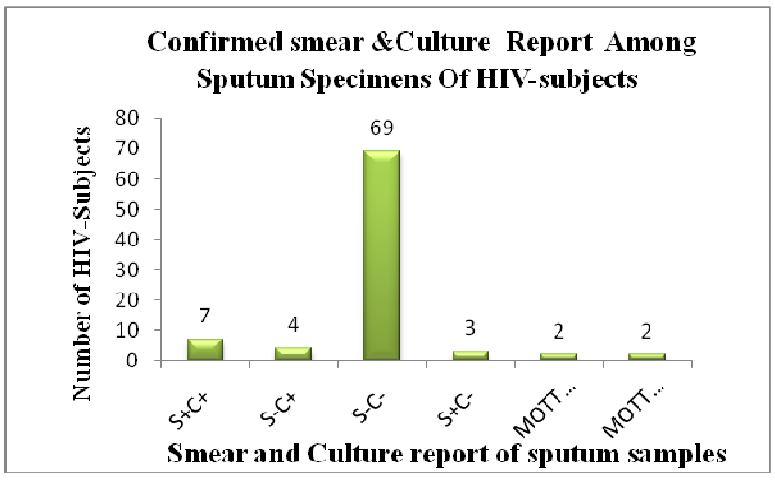


Graph 3. CD4 count among different stages of HIV-Individuals

Table 3. Particulars of smear and BACTEC 460 culture reports of samples

	Total no-of Samples sent	Samples received at testing centre		Results received from testing centre as					
		As Contam-inated	As Intact	S+C+	S-C+	S-C-	S+C-	MOTT	
								S+	S-
N	110	23	87	7	3	69	3	3	2
%	100	20.9	79	8.046	3.448	79.31	3.448	3.448	2.298

% - Percentage, N-Number of cases, MOTT- Mycobacterium Other Than Tuberculosis
S - Smear, C - Culture, + Positive, **-** Negative.

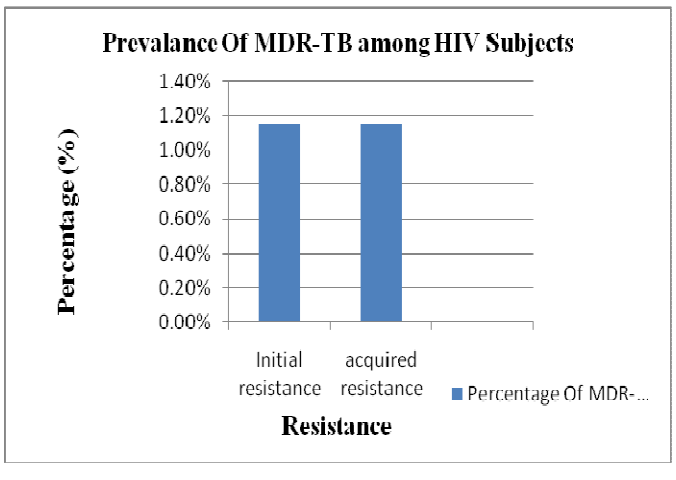


MOTT- Mycobacterium Other Than Tuberculosis, S -Smear, C -Culture, + Positive, - Negative.

Graph 4. Confirmed smear & culture report among sputum specimens of HIV subjects

Table 5. Drug resistance profile of M. tuberculosis isolates with initial and acquired resistance to primary anti-tuberculosis drugs.

Resistance profile	Number of isolates	
	With initial resistance (n=1)	With acquired resistance (n= 1)
Resistance to Isoniazid and Rifampcin (MDR-TB)	1	1
Percentage of MDR-TB	1.15%	1.15%



Graph 5. Prevalence of MDR-TB among HIV subjects

Discussion

In the present study DST by BACTEC 460 TB used as a rapid screen for antimicrobial sensitivity. In this study there was 2.3% resistant to anti- mycobacterial drugs and 9.19% were INH and RIF sensitive as evident by BACTEC 460 TB system. The resistance to INH and RIF was found to be (1.15%) and the prevalence of Multi Drug Resistant Tuberculosis (MDR-TB) among HIV subjects for two drugs was found to be (2.3%). HIV and TB represent a fatal combination, since both are more destructive together than either is alone. The immune suppression caused by HIV is the major risk factor for the transition of latent TB into actively transmissible TB, accelerating the spread of the disease. [10] The present study reveals the prevalence of TB in HIV-infected patients in the lower side, there by highlighting the importance of effective guidelines developed by WHO to control the emergence of TB co-infection in HIV/AIDS. MDR-TB has been a particular concern among HIV-infected persons as this account for considerably rapid disease progression, which may result in rapid transmission. [11] Based on the results it was observed that the rates of acquired resistance were equal to those of initial resistance. The rate of acquired resistance may be due to easy accessibility to the anti-TB drugs to the patients, indiscriminate use of Rifampcin, improper and inadequate treatment regimens and poor adherence of regimen by the patients. [12, 13]

Conclusion

Study results reveals that effective treatment and prevention of MDR TB relay upon the prompt availability of drug susceptibility testing results and anti-TB drug susceptibility testing should be performed for all patients who are culture positive for M. tuberculosis. The Present study
emphasizes the need for strengthening collaboration between TB and AIDS control programmes to counteract the impact of HIV on TB.

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