e-ISSN: 0975-5160, p-ISSN: 2820-2651

Available online on www.ijtpr.com

International Journal of Toxicological and Pharmacological Research 2023; 13(2); 124-130
Original Research Article

The Incidence of Initial INH and Rifampicin Resistance in Untreated Cases of Tuberculosis at a Teaching Hospital Providing Tertiary Care

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Received: 05-11-2022 / Revised: 16-11-2022 / Accepted: 29-12-2022
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Conflict of interest: Nil

Abstract

Aim: With the underlying cases of tuberculosis, it is imperative to detect issue of drug resistance that is prevailing amongst the newly diagnosed cases of tuberculosis. The existing cross-sectional study was structured to find the occurrence of primary INH and Rifampicin resistance among treatment Naïve tuberculosis cases in tertiary care teaching hospitals.

Material and Methods: This research was carried out at the Department of Tb and Chest, Lord Buddha Koshi Medical College, Saharsa, Bihar, India for one year and 50 participants were there. Prospective and cross-sectional design method. Patients diagnosed newly with pulmonary tuberculosis cases. The specimen samples of the patients (with informed consent) were subjected to MGIT 960 and further the positive cultures were subjected to the INH (0.1 µg/ml) and Rifampicin (1 µg/ml) testing. At the same time, for control sampling, a non-drug MGIT was used.

Result: Isoniazid (INH) resistance was found in 8 out of 50 samples, none had resistance to rifampicin.

Conclusion: All of the samples were showing mycobacterium tuberculosis growth and out of the fifty samples, only 8 showed resistance to the INH. None of the sample showed resistance towards rifampicin.

Keywords: Isoniazid, Tuberculosis, Drug Resistance, and Rifampicin.

Introduction

Tuberculosis is an infectious ailment which is majorly triggered by the bacteria Mycobacterium Tuberculosis. Aerosols are the primary source of contamination which when enters inside the human respiratory tract can lead to infection. As per the international tuberculosis report of 2017, issued by the World Health Organization, it is assessed that contribution of India in the global burden and multi-drug resistant TB is 27% and 25% respectively [1,2]. Even before the appearance of the COVID-19 pandemic, tuberculosis was the leading infectious disease which accounts for approximately 1.6million death per annum.

Primarily, an intrinsic resistance is observed mainly due to the presence of a thick hydrophobic cell envelope, which contains drug degrading and modifying enzymes. Whenever poor adherence is observed by the patient, in the absence of the drug, chromosomal mutation takes place, as a result of which overexpression

can be observed. Currently, a number of promising methods are evaluated including Fluorimetry-based liquid culture detection system and MGIT 960 (also termed as Mycobacterial Growth Indicator Tube MGIT-960). MGIT-960 is referred to as the gold standards which was majorly introduced for the diagnosis of the Mycobacterium Tuberculosis. Over the time, one issue that is faced by the doctors and the patients world-wide is the poor adherence towards the medicine, which is mainly because of the long duration or treatment time [3,4]. Mycobacterium Tuberculosis is resistant to numerous antibiotics which makes the treatment strategies very limited. In the light of the major problematic issue of drug resistance amongst the naïve diagnosed cases of tuberculosis patients, the current study was structured and planned in manner to find the prevalence or persuasiveness of INH and Rifampicin resistance in the treatment of the newly diagnosed cases. In addition to this, we tried finding out a relation between the sputum grading, resistance patterns, and sociodemographic factors [4,5].

Isoniazid is an antibiotic medication used in treating tuberculosis and other mycobacterial infections. By disrupting this process, isoniazid prevents the growth and replication of the bacteria. Isoniazid resistance can develop in a number of ways. One of the utmost mutual mechanisms is through the acquisition of mutations in the bacterial genes that are accountable for the synthesis of mycolic acids. These mutations can alter the structure of the mycolic acids, making them less susceptible to the inhibitory effects of isoniazid. Resistance can also occur through the upregulation of efflux pumps in the bacterial cell membrane, which can pump out isoniazid and other drugs before they have a chance to exert their therapeutic effects. Additionally, bacteria can produce enzymes such as catalase-peroxidase, which can break down isoniazid and render it ineffective. Isoniazid resistance can be detected through laboratory testing, and treatment options for resistant strains of tuberculosis typically involve the use of alternative drugs or combination therapies [6].

Rifampicin is a broad-spectrum antibiotic that is commonly used in the treatment of tuberculosis and other bacterial infections. It works by inhibiting the bacterial enzyme RNA polymerase, which is responsible for transcribing genetic information from DNA into RNA [7].

Material and Methods

This research was carried out at the Department of Tb and Chest, Lord Buddha Koshi medical College , Saharsa, Bihar, India for one year and 50 participants were there., wherein initially the ethical clearance was obtained from the ethical committees i.e., Institute Ethics Committee (Human Studies).

Criteria for inclusion: While setting the inclusion criteria, various factors were taken into consideration. All the patients who attended the Out Patient Department for their pulmonary disease and were detected majorly with the sputum smear-positive tuberculosis. Also, it was considered that all those patients aren't diagnosed with the Anti-tuberculosis drugs for more than 2 weeks.

Inclusion Criteria: Patients who all were suffering from the smear positive tuberculosis were included in the criteria. In addition to this, the patients who have administered the Anti-tuberculosis drug for a time span of less than two weeks.

Exclusion criteria: All the patients who were previously subjected to the anti-tuberculosis drug for more than two weeks. Also, the patients who were not willing to participate in the study weren't taken into consideration.

Research Question: What is the Prevalence of primary INH and Rifampicin resistance

among treatment Naïve tuberculosis cases in tertiary care teaching hospital

Populance Size: A populace size of 50 was taken into consideration, which was calculated using the openepi.com, wherein a hypothesized frequency of the outcome factor in the population (naïve smear patients) was considered to be 3+/1 3. Also, the confidence limits were considered 3% as mentioned in the below figure.

Sample Size for Frequency in a Population				
Populace size (N):				50
Hypothesized % frequency of outcome factor in the population (p):				3%+/-3
Confidence limits as % of 100(absolute +/- %) (d):				3%
Sample Size(n) for Various Confidence Levels				
	Confidence	Level (%)	Sample Size	
	95%		36	
	80%		27	
	90%		33	
	97%		38	
	99%		41	
	99.9%		44	
	99.99%		46	

Collection of the Specimen: The suspects who matched the inclusion criteria were processed on daily basis. A wide-mouth bottle was used for collecting the sputum.

Specimens were collected two times (spotted and early morning), which were further subjected to fluorescent stains. These stains were selected as per NTEP guidelines where the collected sputum was studied under the light as well the fluorescent microscopy and further the results was documented as per the above-mentioned guidelines.

After getting the consent from the willing patients, the procedure was started, wherein the sputum was collected in sterile Falcon bottle tubes for checking the presence of MGIT and DST.

The samples were then transferred to the laboratories wherein they were processed using the instructions and procedure laid down by the FIND diagnostics.

Mycobacterial Growth Indicator culture tubes were prepared and the samples were subjected to the drug sensitivity testing, where INH (0.1 µg/ml) and Rifampicin (1 µg/ml) was taken as the testing samples which were parallelly tested with a nondrug MGIT which served as a control sample.

The results were analysed and recorded [8-10].

Processing of the samples**:** The samples were then subjected to the test as per the guidelines prepared by the FIND diagnostics, manufacturer of Mycobacterial Growth Indicator Tube.

NaOH-NALC testing procedure: Equivalent amounts of sputum samples and sodium citrate solution was poured and stirred casually for a shorter time span of 15-30seconds. Further NaOH-NALC solution was poured to this mix and allowed to rest for 25 minutes, wherein after every 5minutes, stirring must be done lightly. After 25minutes, phosphate buffer (Ph 6.8.) was added slowly. This mixture should be centrifuged at 3000 rotation per minutes for 20minutes and then the tube was allowed to settle down for 5minutes.

The supernatant liquid containing the mycobacterial disinfectant was discarded carefully. Further phosphate buffer was added and the leftover suspension was resuspended using the help of pipette. Further PANTA mixture (comprising of Polymyxin B, Amphotericin B, Nalidixic acid, Trimethoprim, and Azlocillin) was reconstituted as per the manufacture explanation and then incorporated into the MGIT tubes [11,12].

Inoculation: For inoculation, 0.8ml of MGIT growth supplement and PANTA was added with 7ml MGIT. Further 0.5ml of the well-prepared mixture was mixed and the tube was processed to inversion several times. The tube and caps were disinfected using the disinfectant and inoculation tubes were placed in the normal surrounding for a timespan of 30minutes.

The scanning was done using the BACTEC MGIT 960 instruments, where in the tubes were kept inside and were incubated at further 37 degrees Celsius. They were incubated till one of the tubes flagged positive. After that the tubes were manually observed.

Using Auramine Rhodamine, the smear was made and stained and left for a timespan of six weeks. After six weeks, if no growth is observed in the tube, the instrument flag turns negative. In a similar manner, the latter sample was also exposed for line probe assay.

Statistical Method: Outcome variable expected: Resistance of the Mycobacterium Tuberculosis to INH and Rifampicin.

The data was staggered and prepared by considering numerous variables including sputum spear results, smoke history, age, gender, weight, medical conditions like presence and absence of HIV seropositivity, anorexia, fever, cough, weight loss, past of anti-tubercular drugs, were articulated in terms of frequency and percentage. The dispersal table was prepared using the Kolmogorov-Smirnov Test. Further one-way anova testing with Bonferronic correction was implemented in order to find the correlation of resistance with numerous factors like socioeconomic status, lifestyle habits, age, sex, diabetes, and previous medical history. The test analysis was supported at 5% p-value and significance [9, 13,14].

Result

50 naïve cases detected with pulmonary tuberculosis were considered for this particular study. Majority of infected patients were men (n = 35). Total of 28 cases (56%) were lying between the age group of 20 to 50 years with their mean of 44.46 (SD±14.14) years. The average weight was 45.06 kg (SD±8) and most patients (n = 38, 76%) were in the range of 40-60kg.

Table 1: Further tabulates the over-all features of the cases.

Characteristic	N (%age)
Men	35 (70%)
Women	15 (30%)
Non-Smoker	40 (80%)
Smoker	10 (20%)
HIV Seropositive	0(0%)
Diabetic	8 (16%)
Asthmatic	2 (4%)
Former history of ATT	0 (0%)
Hypertension	4 (8%)

Socioeconomic Category	N (%)
Lower	27(54)
Upper Lower	14 (28%)
Lower Middle	5 (10%)
Upper Middle	4 (8%)
Upper	0

As per the Modified Kuppuswamy socioeconomic scale, 27 cases belonged to the lower socioeconomic group which were the part of our studies. 14 patients were belonging to the upper lower group, followed by 5 cases of lower middle, and 5 cases of upper middle.

Out of the fifty patients, forty-five (90%) showed the symptoms of cough, followed by forty-two (84%) who suffered from fever, twenty-four (48%), and twenty (40%) showed loss of weight as the major symptom. Out of 50 cases, minor proportion (n = 8, 16%) were suffering from diabetes, which can be observed in the table 1. None of the cases were HIV-seropositive and no cases had a previous history of anti-tubercular drug intake [15-17].

Sputum Smear grade results: 18 patients provided samples belonging to grade 2 whereas other 22 patients provided the samples relating to grade 3. 9 samples belonged to the grade 1 category and one was smear negative.

Results and resistance pattern: All of the samples were showing mycobacterium tuberculosis growth and out of the fifty samples, only 8 showed resistance to the INH. None of the sample showed resistance towards rifampicin. These results were accurately as when compared with the line probe essay. All of the females were tested insensitive towards the INH whereas 8 males were resistant towards the INH.

Discussion:

In conclusion, a subtotal of 50 patients were included in these studies, where mail predominant cases were there. The male to female ratio turned out to be 7:3, which reflected the study distribution as a whole. While considering other review articles, a similar kind of study was observed. The major reason for higher cases of tuberculosis cases observed in male gender was mainly due to the external environment and the related work. The mean age of the patients suffering from tuberculosis turned out to be 44.46 years (SD±14.14). Taking into consideration the age factor, majority of the infected patients were male (n = 35). A total of 28 cases (56%) were lying between the age of 20 to 50 years with their mean of 44.46 (SD±14.14) years. The mean weight was 45.06 kg (SD±8) and most patients (n = 38, 76%) were in the range of 40-60kg. The major reason is that the age group between 20-50 years is the most productive time period wherein due to socio-economic implications, the disease can be majorly observed. Underlying factors such as poor health and compliance towards medication are the other significant factors that enriches the threat of the drug resistance. While comparing the same with other studies, it can be observed that co-morbidities are also found in patients. Out of the fifty samples, only 8 (16%) showed resistance to the INH. None of the sample showed resistance towards rifampicin. These results were accurately as when compared with the line probe essay. All of the females were tested insensitive towards the INH whereas 7 males were resistant towards the INH [18-23].

The major limitation observed was the lesser consent. The majority of the patients from the other states didn't provide the consent due to their logistics and personal

reasons. The next major issue was that none of the patient suffered from HIV seropositivity which restrained us from further studying the effect of HIV on primary drug resistance. Mono-resistance to rifampicin isn't observed but with isoniazid (16%) n= 8 cases were observed.

Conclusion

It is crucial to obtain a detailed record of a patient's prior use of anti-tuberculosis treatment (ATT) to rule out the possibility of primary drug resistance in cases of newly diagnosed patients with tuberculosis. To ensure proper treatment, it is recommended to provide newer diagnostic techniques such as CB-NAAT at the outset. Conducting routine assessments of drug resistance is also essential to understand the issue and preparing accordingly.

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