ORIGINAL RESEARCH PAPER
VOLUME-6 | ISSUE-5 | MAY - 2017 | ISSN No 2277 - 8179 | IF : 4.176 | IC Value : 78.46
INTERNATIONAL JOURNAL OF SCIENTIFIC RESEARCH

Prevalence of Drug Resistance in Mycobacterium tuberculosis from Isolates of younger patients in a TB Hospital, Nashik, India.

Microbiology

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ABSTRACT

Background: Prevention and treatment of drug resistance TB are major concerns before the Government and Health workers all together. To evaluate the various measures and TB control programs undertaken by the government agencies, regular surveillance of prevalence and incidence of drug-resistant TB is carried out.

Objectives: The objectives of this research works was to find out if any difference between prevalence of drug susceptible and drug resistant TB in different age groups of male and female patients exists.

Materials and Methods: 750 Smear positive sputum samples in 2008 and 427 smear positive sputum samples in 2015 were processed for drug susceptibility testings using proportion method and data was tabulated age-wise and gender-wise.

Results: During 2008, the proportions of drug resistance to any single anti-tuberculosis drug in male and female patients of all age-groups were 24.18% and 35.0% respectively. The proportion of MDR-TB in male and female patients of all age-groups were recorded as 22.00% and 30.50% respectively during 2008.

During 2015, the proportions of drug resistance to any single anti-tuberculosis drug in male and female patients were 20.20% and 20.74% respectively. The proportion of MDR-TB in male patients during 2015 was recorded as 13.01% whereas, in female patients of all age-groups, it was recorded as 19.25%

Conclusions: Prevalence of drug-resistance to anti-tuberculosis drug is more in female patients than in male patients, in 2008 and 2015.

KEYWORDS:

Drug resistance; Prevalence; Incidence; Annual Risk of Infections(ARI); MDR-TB.

Introduction:

In India prevalence of smear and culture positive pulmonary tuberculosis(PTB) varies between 2.1 and 12.7/1000 in time period of 1958 to 2001 in different study regions[1].

During 2000 to 2003 study, the prevalence of MDR-TB in new cases is reported to be 0.5 to 5.3% which is 0.4 to 28% in HIV seropositive TB cases and is 8% to 67% in previously treated TB patients[1].

According to the estimation by WHO in 2015, in India the prevalence of TB is 2.5 million and incidence of the disease is 2.2 million cases[2].

As per the RNTCP (Revised National Tuberculosis Control Program) data for 2015, the prevalence of TB is 1.11/1000 cases in India[3]. Therefore one can conclude that the prevalence of TB is remarkable reduced during 2003 and 2014.

The reduction in prevalence of TB is due to efforts of Government of India to eradicate TB by implementing different TB control programs. RNTCP is such a major TB control program.

There are number of epidemiological indices to measure the success rate of these TB control programs in terms of prevention and treatment success of the disease[3].

Prevalence of Tuberculosis in BCG-vaccinated as well as non-vaccinated patients aged between 0 to 14 yr. can be used to obtain incidence and annual risk of infections[4]. Prevalence of TB in this age group can also indicate the situation of the infection in a community. It can also be used to monitor the success rate of TB control program in term of incidence of the infection in a community.

Therefore the present study was undertaken to evaluate the prevalence of all forms of tuberculosis in community from semi-urban region, in younger aged patients to assess the success of TB control program.

In the present study, prevalence over two time span of an interval of 7 yrs among younger patients were evaluated. The prevalence of Tuberculosis during 2008 was compared with the prevalence during 2015 in younger patients visited a TB hospital in Nashik and the results were evaluated statistically.

Materials and Methods:

Sampling:

The present research work was undertaken to do the cross sectional survey for prevalence of drug resistance among male, female patients of different age-groups visiting Dr. Gangurde's TB Hospital, Nashik, from semi-urban population. Semi-urban community is population of those people who live in slum and low-income resistances of the city.

Majority of the patients under study, especially female patients, have availed anti-tuberculosis treatment previously in Government or other hospitals and are not recovered completely.

The two consecutive samples were collected from each patient over a period of one week before starting anti-tuberculosis treatment. Sample size for this cross sectional survey was estimated using "Epicalc" R statistic analysis software[5, 6]. Since Samples size was

less than actual available samples for drug susceptibility test, data of all the samples were recorded.

For the year 2008, 750 samples' drug susceptibility data produced by proportional method was available. This data is compared with the drug susceptibility data for male, female patients of different age-groups with the data of year 2015.

Since the research project has not used any personal information except age and gender of the patients, no approval from ethical committee was necessary.

Bacteriological Investigation:

Microscopy, strain identification and drug susceptibility testing were carried out as per the accepted standard protocols.[7] Smears were made from all the collected samples and stained using acid fast staining method and observed under microscope. The positive smears were graded as 1+, 2+, 3+.[8]

Culture Preparation. Sputum samples were decontaminated by sodium hydroxide-N-acetyl-L-cysteine method.[9] 250µL aliquots were inoculated onto each of the two slopes of L-J medium. The inoculated L-J media were incubated at 36^0C and weekly examined for visible growth till 8^{th} weeks. Culture identification were carried out using isolated colonies from each slope on the basis of cord formation, niacin test, catalase test at 68^0C and pH 7.0 and growth on L-J medium with p-nitrobenzoic acid.[10, 11]

Drug Susceptibility Testing.

Results of previously processed sputum samples during 2008, in the TB hospital, Nashik were obtained and the results were tabulated, grouping the patients age-wise and gender wise with corresponding prevalence data of drug resistance and MDR for each group as shown in table No.1. Similarly the sputum samples, obtained during the year 2015, were processed for drug susceptibility testing using SL023 Tuberculosis First line Kits manufactured by HiMedia Laboratories Pvt. Limited, Mumbai-400 086, India, as per the manufacturers protocol and as described by More AP et.al.[5]. 1% or more than 1% ratio of colonies on drug containing tube to the colonies on control tube was taken as resistance and less than 1% ratio was taken as sensitivity to that drug.

The drug susceptibility testings were done using following anti tuberculosis drugs: streptomycin(STR)(4.0 µg /mL), rifampicin(RIF) (40.0 µg /mL), isoniazid(INH)(0.2 µg /mL), pyrazinamide(PZA) (200.0 µg /mL), ethionamide(ETHD) (40.0 µg /mL), P-aminosalicylic acid(PAS) (1.0 µg /mL), roxythromycin(ROX) (1.0 µg /mL)[12].

All the statistical analysis were carried out using the R package[13] and "Epicalc"[6] package in the R software on Linux operating system using LaTeX packages.

Results:

Data of prevalence of single drug resistance and multi-drug resistance found in male and female patients of different age groups during the year 2008 and 2015 are recorded as shown in Table No.1 and Table No.2. respectively.

In 2008, total 750 cases of TB were evaluated; out of 750 cases, 550 were male patients and 200 were female patients.

In 2015, total 427 cases of TB patients were evaluated; out of these total 427 cases 292 were male patients whereas 135 were female patients.

During 2008, the proportion of drug resistance to any single anti-tuberculosis drug in male patients of all age-groups was 24.18% whereas in female patients of all age-groups, it was recorded as 35.0%. The proportion of MDR-TB in male and female patients of all age-groups were recorded as 22.00% and 30.50% respectively during 2008. Total male to female TB patients ratio during this period recorded as 2.75.

During 2015, the proportion of drug resistance to any single anti-tuberculosis drug in male patients was 20.20% whereas in female patients it was recorded as 20.74%. The proportion of MDR-TB in male patients during 2015 was recorded as 13.01% whereas, in female patients of all age-groups, it was recorded as 19.25%. The ratio of male to female TB patients of all age-groups was recorded as 2.16 in 2015.

Table No.1 Age group wise prevalence of drug resistant TB cases in 2008

Variables	No. of TB Cases(N)(%)	Any Drug Resistant Cases(%)	P-value	MDR Cases(%)	P-Values
Total Cases	750	203(27.068)	2.2e-16	182(24.26)	2.2e-16
Sex:					
Male	550	133(24.18)	2.106e-10	121(22.00)	2.2e-16
Female	200	70(35.0)	3.02e-05	61(30.50)	5.189e-08
Age Groups:					
Male:					
0-24	90	19(21.11)	7.621e-08	16(17.77)	1.874e-09
25-44	230	53(23.04)	5.046e-16	64(27.82)	2.743e-11
45-54	135	28(20.74)	1.904e-11	26(19.26)	1.696e-12
55-64	45	14(31.11)	0.01707	13(28.88)	0.00729
>65	50	19(38.0)	0.1198	12(24.0)	0.000407
Female:					
0-24	125	42(33.6)	0.0003466	36(28.8)	3.303e-06
25-44	75	28(37.33)	0.03767	25(33.33)	0.005584
45-54	*	*			

* Data for this age-group was not available.

Table No.2 Age group wise prevalence of drug resistant TB cases in 2015

Variables	No. of TB cases(%)	Any Drug Resistant cases(%)	P-Values	MDR Cases	P-Values
Total Cases	427	87(20.37)	2.2e-16	64(14.99)	2.2e-16
Sex:					
Male	292	59(20.20)	1.904e-11	38(13.01)	2.2e-16
Female	135	28(20.74)	6.109e-11	26(19.25)	1.696e-12

Age Groups:					
Male:					
0-24	29	6(20.69)	0.002967	4(13.79)	0.0002041
25-44	102	20(19.60)	1.542e-09	13(12.74)	1.118e-13
45-54	122	21(17.21)	8.532e-13	15(12.29)	2.2e-16
55-64	23	7(30.43)	0.09529	4(17.39)	0.003509
>65	16	5(31.25)	0.2113	2(12.5)	0.00596
Female:					
0-24	47	9(19.14)	4.423e-05	8(17.02)	1.209e-05
25-44	88	19(21.59)	1.757e-07	18(20.45)	5.43e-08
45-54	*	*			

* Data for these age-groups was not available.

Discussion:

The prevalence of any single drug-resistance is increasing with the age of male and female patients in both the years 2008 and 2015, the findings which is in agreement with one such study done by R. Wood et.al.[14] and Chakraborty AK[4].

The prevalence of drug resistance to any drug tested has reduced by 3.98% in male TB patients and it has reduced by 14.26% in female patients in time period from 2008 to 2015 during 7 years' duration. Whereas prevalence of MDR-TB has reduced by 8.99% in male TB patients and by 11.25% in female TB patients of all age-groups in during the same period.

Age-wise there is remarkable decrease in drug resistance during 0 to 54 age groups patients especially during 0 to 24 age group patients. The drug resistance in male TB patients of 0 to 24 years age group has decreased from 21.11% in 2008 to 20.69% in 2015. The drug resistance in male TB patients of 25 to 44 years age group has decreased from 23.04% in 2008 to 19.60% in 2015. Thus there is 3.44% decrease in drug-resistance prevalence in this age-group.

In the present study the male to female TB patients ratios is almost same during the years 2008 and 2015(ratio in 2008 was 2.75, in 2015 it was 2.16), suggesting that the male patients are almost two times more infected with TB infections in 2008 as well as in 2015.

In our study, researchers found that the female patients were visiting in number which is in proportional to their population in the community(unpublished data) and therefore, the researchers found it appropriate to considered data of number of female patients to prove that there are some factors other than the poor accessibility of female patients to health care sectors responsible for the gender inequality to prevalence of TB in the community, in disagreement with some previous studies[15,16].

In most of the world regions, men are 70% more infected with TB and die from it than women; the reason of this gender inequality toward TB infection is unknown[15]. In Pimpri region of Pune city of India, the gender ratio for male to female, infected with TB, was reported as 2:1 in 2008(Shilpa Lagali, 2009, unpublished data).

Unlike in other poor countries where the low prevalence of TB in female patients is due to poor access of these patients to health care settings[16, 17], in India female patients between 15–24 age groups use private medical sector more than the public sector health care sector[18].

In the present study, it has been observed that though the both male and female patients unequally infected in their adulthood, the male patients are two times more infected than female patients; however the prevalence of drug resistance to *M. tuberculosis* is observed many folds in female patients than in male patients and its fall also is more in these patients i.e. approximately 15% for any single drug resistance and 10% for MDR. Whereas in male patients, any single drug-resistance has decreased by 4% and MDR has reduced by 9% from 2008 to 2015 years.

In the present study, the researchers also tried to find out the inferential reasons for gender inequality towards TB and found that the unequal gender ratio of the infection is not because of the poor access of women to health care settings(unpublished data) but may be due to some epidemiological factors such as risk of infection, rate of transmission, progression from infection to disease, unbalanced nutrition and biological difference in immunological responses between male and female patients.

In the present study, the prevalence of MDR-TB is higher than the 4% to 10% proportion of MDR-TB in the region. The one reason for this can be the higher number of TB patients, especially female patients, who had received treatment for TB and not recovered completely.

Since the TB infection has slow dynamics, the present type of study of assessing prevalence of drug resistance should be taken over a long window of time regularly such as the present study.

The present type study of prevalence of TB among different age group and genders help to monitor any deviations in the usual trends in epidemiology of drug resistance in *M. tuberculosis*.

Limitations of the Research:

One limitation of this research works is that the data for higher age-group of female patients was not available. The factors contributing to the higher prevalence of drug-resistance in female patients could not be evaluated due to scanty data in this context.

The exact number of patients who received precious treatment for TB and not responded to it was not available.

Authors Contributions:

Dr. Arun Punaji More has conceived, designed the experiments, carried out isolation, identification, drug susceptibility testing for number of sputum samples and statistical analysis using free software, R-package and "Epicalc" to draw significant conclusions.

Dr. Aniket Gangurde has contributed extensively to this research project by diagnosing patients clinically, aseptically collecting consecutive sputum samples from suspected TB patients and collecting medical and treatment history of the patients and classifying these data for statistical analysis. He has also edited this paper.

Mr. Jagganath D. Andhale helped in carrying out isolation, identification and drug susceptibility testing for number of samples by proportion method. He has also tabulated the data obtained for statistical analysis.

Dr. R.P. Nagdawane has immensely guided throughout this research project helping designing the experiments and writing this research paper.

Dr. Vijay N. Patole has recorded the data for prognosis of TB patients and help Aniket Gangurde in clinical diagnosis of TB patients.

The authors have no competing interest in the research project.

Acknowledgments

The researcher, Arun Punaji More, gratefully acknowledge the inspiration and precious guidance of Dr. R. P. Nagdawane. The researcher also gratefully acknowledge the immense help and contribution of Dr. Aniket Gangurde in collecting sputum samples and treatment and medical history of suspected TB patients. The researcher also acknowledge the active participation by Mr. Jagganath D. Andhale for carrying out experimental works. The researcher is also thankful to Dr. Vijay N. Patole, for his help in recording the follow-up prognosis data during treatment of TB patients.

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