CHARACTERIZATION OF MULTIDRUG RESISTANT MTB IN HYBRIDIZATION AND MUTATION

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ABSTRACT

Tuberculosis is one of the major public health problems, particularly in developing countries (TB). India accounts for one-fifth of the global TB burden in terms of the total number of incident cases that occur each year. Multidrug-resistant tuberculosis (MDR-TB), which is characterized by resistance to the two most potent antituberculosis drugs, isoniazid and rifampicin, has made TB control more challenging (RMPR). India is the country with the second-highest population in the world. The country with the highest new TB cases each year is India (TB India, 2016). India contributed for 2.0–2.5 million cases, or 26% of all cases worldwide, of the anticipated 8.8 million cases in 2010. It is estimated that the TB bacillus is present in around 40% of Indians. Measures of the global TB burden according to the 2019 WHO report (rates per million persons). TB has existed for a very long period. Surprisingly, poor tuberculosis control management and a lack of fundamental knowledge might result in the development of MDR-TB and XDR-TB. Regular updates on the prevalence of drug-resistant TB and tuberculosis in general in the nation are required to guarantee that the national treatment plan for TB is successful. Given the vastness of the nation, it is conceptually challenging to survey the various geographic and environmental conditions at the national level. Resistance to different medications is the biggest threat posed by M. tuberculosis. This study is conducted to determine the prevalence of TB and multidrug-resistant tuberculosis. This study aims to analyze the genes associated with MDR-TB and to pinpoint the mutations associated with pathogenic MDR-TB strains.

***Keywords**: MDR-TB, XDR-TB, Drugs*

Introduction

In 1993, the WHO declared TB a global emergency in recognition of its growing importance as a public health problem in many parts of the world. Governments in many high-burden countries have neglected TB control in the past. TB programmes have failed to achieve high detection and cure rates for infectious patients. In 2019, there were an estimated 10.4 million new cases of TB worldwide (including 1.2 million HIV-positive people), of which 5.9 million (56%) were among men, 3.5 million (34%) among women and 1.0 million (10%) among children. There were an estimated 4,80,000 new cases of multidrug-resistant TB (MDR-TB) and additional 100,000 people with Rifampicin-resistant TB(RR-TB) also newly eligible for MDR-TB treatment. There were an estimated 1.4 million TB deaths, most occurring in developing countries and affecting mostly young adults in their more productive years (age group 15-50 years). In 2000, Sub-Saharan Africa had the highest TB incidence rate (290/100000) and the highest annual rate of increase of cases (6%) (WHO, report 2005).

Salient features of WHO report

Global incidence estimated at 10.4 million cases. Most of them occurred in Asia (59%) and Africa (26%). The 22 high tuberculosis burden countries that have

been given highest priority at the global level since 2000 accounted for 81% of all estimated cases worldwide. The Six countries with the largest number of incident cases in 2019 were India (2.0-2.5 million), China (0.9-1.2 million), Nigeria, South Africa, Indonesia and Pakistan.

India and China combined accounted for 38% of all cases. Estimated 12.0 million prevalent cases of TB of the 8.8 million incident cases, 1.0-1.2 million (12-14%) were among people living with HIV. The proportion of TB cases co-infected with HIV is highest in countries in the African Region (accounted for 82%). Approximately 1.4 million deaths (1.1 million cases in HIV negative cases of TB and an additional 0.4 million in HIV positive cases of TB).

Tuberculosis problem in India

One of the biggest issues with public health, especially in underdeveloped nations, is tuberculosis (TB). In terms of the absolute number of incident cases that occur each year, India represents one-fifth of the worldwide TB burden. TB control has been made more difficult by the introduction of multidrug-resistant TB (MDR-TB), which is characterized by resistance to the two most effective anti tuberculosis medications, isoniazid and rifampicin (RMPR). The nation with the second-highest population in the world is India. India has the most new TB cases per year of any other nation (TB India, 2016). The projected 8.8 million cases in 2010 India accounted for 2.0–2.5 million cases, or 26%, of all cases globally (WHO report, 2019). It is believed that roughly 40 percent of Indian people is infected with TB bacillus. Measures of the burden of TB WHO worldwide report from 2019 (rates/one million people).

Global Epidemiology of TB drug resistance

Almost all of the nations studied have multi-drug resistant tuberculosis (MDR-TB) (WHO report, 2016). Up until recently, it was difficult to pinpoint where drug-resistant TB was present throughout the world. The Global Project on anti-tuberculosis drug resistance monitoring was started in 1994 by the WHO and the International Union against Tuberculosis and Lung Disease.

The Global Project has assisted national TB control programs in launching medication resistance monitoring initiatives around the globe since 1994. In 2019, there were about 6,50,000 cases of MDR-TB worldwide. Nine percent or so of these patients are thought to have XDR-TB. Each year, 1,50,000 people die from MDR-TB, which affects about 4,40,000 people. Eighty countries and eight territories reported surveillance data, according to data from the World Health Organization's (WHO) Global Project on Anti-Tuberculosis Drug Resistance Surveillance from 2007 to 2010 (Zignol, 2016).

The percentage of new TB cases with multidrug resistance ranged from 0% to 28.9%. The Russian Federation has the most new cases of MDR-TB (Murmansk oblast, 28.9 percent) More than 10% of MDR-TB infections in three former Soviet Union nations and South Africa were very drug-resistant.

Tuberculosis in the human history

More people have been murdered by tuberculosis than by any other infectious illness in the history of the world (more than one billion in the last two hundred years). TB is one of the oldest human diseases (1). Knowing the history of tuberculosis means learning the history of humanity. There was evidence of Mtb, the bacterium agent that causes the sickness, in the spines of Egyptian mummies that dated back more than 5,000 years. These findings were discovered in Neolithic bones (2). There was a specific kind of the illness that affects the vertebrae that was seen in Egyptian mummies.

This condition, which was named Pott's disease and is tuberculous spondylitis, was observed in Egyptian mummies. In spite of these discoveries, allusions to tuberculosis have not been identified in any Egyptian papyri. The 'white plague,' as it was often referred as during the epidemic period in Europe that lasted over two centuries, is a disease that is extraordinarily resilient, and once it enters a society, it remains there. This was the case for over two centuries.

Study Area

In the present study 1,07,722 numbers of patients were enrolled in various DMC centers of Bidar, Raichur, Gulbarga,Belgaum, Yadgir and Bijapur districts of Karnataka based on symptoms and radiographic evidence.

I. Collection of Sputum specimens, Transportation and storage

From each patient three Sputum specimens preferably one in early morning and one spot specimen were collected in sterile, wide mouth leak–proof containers (50 ml Falcon tubes). Thus collected specimens from six districts, DMC (Designated Microscopy Centers)were refrigerated by proper packing with cool gel packs in the thermacol boxes and transported to laboratory as soon as possible and processed within 48 hours. The samples were clearly labelled with patient name, age, sex, area of district and history of patient.

a. Staining and Microscopy

The thick, muco purulent part of the sputum was used for the preparation of smear. Microscopy is the easiest and most rapid procedure to detect the presence of Acid-fast bacilli. Usually, the acid fastness could be determined by Ziehl-Neelson (Z-N) staining (or) Fluorescence staining procedure (Auramine- Phenol method). In our study we used Fluorescence staining method.

b. Preparation of Auramine-Phenol stain:

- 0.3% Auramine phenol solution: Auramine-O (Sigma) = 3g, Ethanol= 350ml, Phenol (crystals) AR=30 ml, Distilled water =620 ml)
- 1% Acid Alcohol: (NaCl AR 10g, HCl -10ml, Ethanol – 750ml, Distilledwater-250ml)
- 0.1% Potassium Permanganate solution:($KMnO_4$ AR -1g , Distilled water-1000ml)

Procedure

All the slides were labelled with Lab Number / Unique ID Number. A very small amount of sputum sample was removed using a loop and gently rubbed into one drop of sterile saline on a slide. The smear was allowed to dry by placing the slide on a electric slide warmer at 80°C for 20 min and cooled to room temperature.

Then the slides were placed on the

staining rack and flooded with freshly prepared Auramine-phenol stain and left for 10-15 min and again washed under running tap water. Finally, the slides were counter stained with 0.1% $KMnO_4$ for 30sec, then washed under tap water and air dried. Thus, prepared smears were observed under Fluorescent microscope (Carl Zeiss, Primostar iLED, Germany).

A minimum of 50 fields were examined systematically and the slides were categorized as Negative, Scanty or Positive. Under Fluorescent microscope bacilli will appear as slender bright yellow fluorescent rods standing against a dark background. Smears found positive graded as per RNTCP guidelines.

PROCEDURE

A clean sterile screw capped centrifuge tube was used for decontamination procedure. A 4.0 ml of sputum sample was transferred into centrifuge tube then an equal volume of 4% NaOH was added and vortexed for 15 to 30 sec. This liquefied mixture was incubated at room temperature for 15 minutes and then equal volume of sterile distilled water was added to stop further action of alkali and centrifuged for 15 min at 3600 rpm in a refrigerated centrifuge. The supernatant was discarded carefully. Then collected sediment was washed twice by adding 8.0 ml distilled water. Thus concentrated sputum sample was further used for isolation of Mycobacterium.

Result & Conclusion

- According to a comprehensive study that was carried in the entire Karnataka region, the number of cat-I cases recorded was three times higher than the number of cat-II cases. New sputum cases account for 44.6 percent of RNTCP category I cases, whereas relapse and TAD patients made up a greater percentage of category II cases. Only 2.09 percent of instances are considered failures; however, 7.82 percent are classified as other.
- The age group between 45 and 54 years old was the one that was impacted the most (22.3 percent), followed by the age group between 35 and 44 years old (19.9 percent). Children were less affected (0.8%).
- The age group 45-64 years old was the most afflicted age group in the Gulbarga district, while the age group 35-54 years old was the most impacted age group in the other districts.
- The incidence of tuberculosis was three times more common in males than in women.
- Co-infections with HIV and tuberculosis are also significant in cat-II patients.
- In 2016, there were a total of 25,320 patients who were registered at various DTC centers. There was a possible MDR strain present in 970 samples, or 3.8 percent of the total.
- A smear and culture test confirmed each and every one of the 970 cases, and 86.8 percent of the cultures proved to be positive (n = 842)
- Out of these 842 M.tb isolates, 51.1% (n = 430) were drug sensitive, 14.1% (n = 119) were INH mono resistant, 6.8% (n = 57) were Rifampicin Mon resistant, and 20.9% (176) were multi-drug resistant isolates.
- The Belgaum district had the highest

reported rate of INH mono resistance (21.3 percent), the Gulbarga district had the highest documented rate of rifampicin mono resistance (19.4 percent), and the Belgaum district had the highest recorded rate of multidrug resistance (42.6 percent).

• Among the three forms of drug resistance, MDR is the most prevalent, and the male population of all three types of drug resistance was high.

• The people aged 21 to 40 years old had the highest number of cases documented for all categories of medication resistance, followed by the age group aged 41 to 60 years old. In the course of our research, we came across no instances of medication resistance in children of any age (0-12 years).

• Out of 842 MDR suspects that were culture positive, multiplex PCR was used to identify 176 multidrug resistant isolates that were resistant to both rifampicin and isoniazid. These isolates were shown to be resistant to both drugs.

• It was discovered that 78.9 percent of drug resistance is related with genes called rpoB+katG, 18.2 percent of drug resistance is associated with genes called rpoB+inhA, and 2.8 percent of drug resistance is associated with all three genes at the same time.

• The drug resistance associated with rpoB+katG+inhA was found to be present in the districts of Yadgir, Raichur, and Bijapur, but was entirely nonexistent in the districts of Bidar, Gulbarga, and Belgaum.

• According to the findings of our research, the rpoB gene, which is connected with rifampicin resistance, has the most prevalent mutation at site 531 (52.8%), while site 516 had the rarest mutation.

• In cases of isoniazid resistance, the number of mutations linked with the katG gene was high, whereas the number of mutations in the inhA gene was low. The point mutation that occurred most often in katG was at the 315 nucleotide location, and it changed the amino acid Serine to Threonine. In the case of the inhA gene, the frequency of the C-15T mutation was the greatest.

• Our research demonstrates that throughout all of Karnataka's districts, the MDR genotype with the point mutation at the 531 site of the rpoB gene and the 315 site of the katG gene is the most prevalent.

• Another finding that emerges from this research is the confirmation that there are no substantial regional disparities in the distribution of resistance-related mutations.

• It was shown that of the three patterns of drug resistance, MDRs accounted for the largest percentage of patients in Bidar, followed by INH mono resistance. This was the case regardless of gender.

• The Gulbarga region had the greatest percentage of MDR cases overall, including those involving both men and girls. Resistance to Rif-mono was much higher in men than resistance to INH-mono.

• A significant percentage of drug resistance to several drugs was found in both men and females in the Belgaum area. INH mono resistance was shown to be significantly higher in men compared to Rif- mono resistance.

• The Yadgir area showed the greatest percentage of multidrug resistance among M.tb isolates from both male and female patients. This was higher than the percentage of monodrug resistance. INH mono resistance was found to be rather

high in men, followed by Rif-mono resistance.

• M.tb isolates from men in the Bijapur area exhibited the greatest INH mono resistance, followed by multidrug resistance, and then Rif-mono resistance. In the same way, M.tb strains isolated from females exhibited the greatest percentage of resistance to several drugs, followed by Rifampicin resistance and INH resistance.

• As a result, our research found a significant incidence of tuberculosis and multidrug-resistant tuberculosis, which highlights the need for more efforts and research to expand treatment expertise and eradicate poverty in order to avoid MDR-TB.

• There is an immediate want for more expedient diagnosis. In our research, we used the Genotype MTBDR plus kit, which assists in early diagnostics and the initiation of appropriate therapy, hence lowering the risk of MDR tuberculosis transmission and progression.

References

1. Agarwal, A. K., Chugh, I. M., & Shah, A. (1998). Multi-drug resistant tuberculosis: successful treatment with an unconventional regimen. INDIAN JOURNAL OF TUBERCULOSIS, 45, 227-230.

2. Albert, H., Bwanga, F., Mukkada, S., Nyesiga, B., Ademun, J. P., Lukyamuzi, G., & O'Brien, R. (2010). Rapid screening of MDR-TB using molecular Line Probe Assay is feasible in Uganda. BMC infectious diseases, 10(1), 41.

3. Almeida, D., Rodrigues, C., Udwadia, Z. F., Lalvani, A., Gothi, G. D., Mehta, P., & Mehta, A. (2003). Incidence of multidrug-resistant tuberculosis in urban and rural India and implications for prevention. Clinical infectious diseases, 36(12), e152-e154.

4. Dorman, S. E., Chihota, V. N., Lewis, J. J., van der Meulen, M., Mathema, B., Beylis, N., & Churchyard, G. J. (2016). Genotype MTBDRplus for direct detection of Mycobacterium tuberculosis and drug resistance in strains from gold miners in South Africa. Journal of clinical microbiology, 50(4), 1189-1194.

5. Dye, C., Garnett, G. P., Sleeman, K., & Williams, B. G. (1998). Prospects for worldwide tuberculosis control under the WHO DOTS strategy. The Lancet, 352(9144), 1886-1891.

6. Dye, C., Watt, C. J., Bleed, D. M., Hosseini, S. M., & Raviglione, M. C. (2005). Evolution of tuberculosis control and prospects for reducing tuberculosis incidence, prevalence, and deaths globally. Jama, 293(22), 2767-2775.

7. Singh M, Jadaun GP, Ramdas, Srivastava K, Chauhan V, Mishra R, Gupta K, Nair S, Chauhan DS, Sharma VD, Venkatesan K, Katoch VM. Effect of efflux pump inhibitors on drug susceptibility of ofloxacin resistant Mycobacterium tuberculosis isolates. Indian J Med Res 2019 May;133:535e40.

8. Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, Cole ST, Jacobs Jr WR, Telenti A. Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB genes and detection of quinolone resistance mutations. Antimicrob Agents Chemother 1994 Apr;38(4):773e80.

9. Tondani A. Mboneni, Owen O. Eales, Ntsoaki L. Mosina and P. Bernard Fourie,2020 "Molecular detection of Mycobacterium tuberculosis in poor-quality cough specimens" Journal of Medical Microbiology 2020;69:1179–1182. tuberculosis genotyping" Rev. salud pública. 12 (3): 510-521, 2010.

10. Jassal M, Bishai WR. Extensively drug-resistant tuberculosis. Lancet Infect Dis 2009;9: 19–30.

11. Jiao WW, Mokrousov I, Sun GZ, Li M, Liu JW, Narvskaya O, Shen, AD 2007. Molecular characteristics of rifampin and isoniazid resistant Mycobacterium tuberculosis strains from Beijing, China.

12. Juan C. Rozo-Anaya and Wellman Ribón," Molecular tools for Mycobacterium