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MONITORING OF RIFAMPICIN RESISTANT TUBERCULOSIS IN NEW AND TREATED PATIENTS IN HODEIDAH, YEMEN BY USING RT – PCR

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ABSTRACT

Background: Rifampicin Resistant Tuberculosis (RR - TB) is detected by using phenotypic or genotypic methods, with or without resistance to other anti-TB drugs. RR is a risk factor poor outcome in tuberculosis. **Objectives**: Therefore, the study aims to analyze the RR – TB in patients of Hodeidah, Yemen based on real time - polymerase chain reaction (RT - PCR) in Hodeidah, Yemen. **Methods**: Based on a cross sectional study, 201 subjects were collected from adults aged from 18 – 80 years of age, classified into two groups, the first group were new cases that are suffering from cough, fever, chest pain and the second group that were treated with first line of anti-TB. The RR – TB of both groups were detected by RT- PCR. **Results**: The results showed that 10 cases (4.97%) were RR. 9 cases were under treatment group and 1 case (0.97%) was new. **Conclusion:** The finding data showed that analysis of RR is very important for the determination the guideline in treatment of Tb.

KEYWORDS: Tuberculosis, Rifampicin, Resistance, Yemen.

1. INTRODUCTION

Rifampicin (RIF) is one of the most potent and broad spectrum antibiotics against bacterial pathogens and is a key component of anti-tuberculosis therapy. RIF is bactericidal, by inhibition of the beta – subunit of RNA polymerase of *Mycobacterium tuberculosis* (*M. tuberculosis).*[1,2] It is highly effective and extremely valuable in tuberculosis (TB) treatment, as RIF is capable of killing both actively dividing and dormant bacilli. Rifampicin - resistant tuberculosis (RR- TB) is caused by *M. tuberculosis* bacteria that do not respond to RIF, one of the most effective anti- TB medicines, requiring longer treatment and more medication than patients with RIF susceptible disease.[3]

In Hodeidah, Yemen, RR – TB is diagnosed using sputum culture in center of National TB Control Program limits access and results in delayed treatment. In 2015, the center started using the Xpert MTB/RIF test that has the potential on detection of RR- TB cases and reduction the time in treatment initiation, through diagnosis of RR simultaneously with Tb diagnosis. Therefore, we conducted this study to detect the RR- TB in patients of Hodediah, Yemen, using real time - polymerase chain reaction (RT–PCR).

2. MATERIALS AND METHODS

2.1. Study design

A cross sectional study conducted by recruiting adults aged from 18 – 80 years of age and diagnosed in TB center in Hodeidah, Yemen. The study was conducted from September 2015 to March 2016. 201 samples of patients were collected, diagnosed and treated. RR- TB was detected in new cases (non – treated) and previously cases (under - treated). Patients received a simple explanation of the aim of the study and asked to participate. If they agreed, the sample was collected and an interview was conducted. Confidentiality of the collected data was achieved by keeping data record in a locked room with limited access to the research team only. Clinical information was obtained from the patients. Information included the sex, age, symptoms, accommodation.

2.2. Treatment of Tb

Patients were classified into two groups, the first was new cases (non – treated) and the second was previously cases (under - treated). The second group was treated by directly observed treatment, short-course (DOTS Program) taken in TB Center namely Isoniazid 75mg, RIF 150 mg, pyrazinamide 400 mg and ethambutol 275 mg in combination tablet form for two months. You must monitor on efficacy of therapeutic. Then treatment

course was completed for four months with Isoniazid 75 and RIF 150 mg.

2.3. Samples collection

1 ml sputum per specimen was collected. The patient have inhale deeply, cough vigorously, and expectorate the material into sterile screwed –capped specimen collection. The specimens directly were diagnosed by microscopic and transferred to the department of molecular biology for analysis of RR – TB using RT–PCR method.

2.3. Tuberculosis detection based on microscopic method

Tb infection status was ascertained by microscopic method (Campus, Germany). 201 sputum specimens were obtained from the medical laboratory department for which smear results for acid-fast bacilli (AFB).[4]

2.4. Analysis of Rifampicin - Resistant Tuberculosis (RR – Tb) based on PCR

On the other hand, the samples were analyzed by RT - PCR of Xpert MTB/ RIF to simultaneously detect TB and RR- TB in a single-use-cartridge hands-free step. The Xpert MTB/ RIF assay consists of two main components, namely, a Xpert MTB/ RIF plastic cartridge (containing the liquid sample processing , PCR buffers, and lyophilized RR-PCR reagents with internal sample processing and PCR probe quality controls) and the automated Xpert MTB/ RIF machine (which controls the advanced automated portion of the procedure involving the engagement of the fluidics system within the cartridge, automated ultrasound lysis, and the performance of the real-time PCR analysis).[5,6]

2.5. Statistical methods

Demographic and laboratory results data were entered and analyzed using Excel Software 2010. For all statistical analyses, a p -value of less than 0.05 was considered statistically significant.

3. RESULTS

Samples were collected from 201 adults from 18 to 40 years old diagnosed with TB by two techniques namely microscopic and RT – PCR (Table 1) during the period from September 2015 to March 2016. RR-Tb was detected in 10 cases using RT –PCR. The age range of patients with RR- TB was from 28 to 40 years old. RR-TB was represented in the males as 60% while in the females it was represented as 40% (Table 2). However, this difference was not statistically significant (p > 0.05%). On the other hand, the results showed that 7/42 cases (16.66%) were under treatment group and 3/52 cases (5.76%) were new TB cases.

Table 1: Diagnosis of TB by microscopic and RT- PCR methods.

	Microscopic method		RT - PCR		
Cases	**Positive**	**Negative**	**Positive**	**Negative**	**RR**
New cases	23	124	52	95	3
Previously cases	35	19	42	11	7
Total	58	143	94	106	10

New cases : The patient did not use any treatment
Previously cases: The patient used the treatment.

Table 2: RR- TB in males and females based on PCR method.

	PCR		
Cases	**Male**	**Female**	**RR**
New cases	2	1	3
Previously cases	4	3	7
Total	6	4	10

New cases : The patient did not use any treatment
Previously cases: The patient used the treatment.

4. DISCUSSION

Drug-resistant TB can occur when the drugs used to treat TB are misused or mismanaged that include people do not complete a full course of TB treatment, health care providers prescribe the wrong treatment, drugs for proper treatment are not available, drugs are of poor quality.[7] On the other mean, in TB, there are two ways that people get drug - resistant TB. Firstly, people get acquired drug resistant TB when their TB treatment is inadequate. This can be for a number of reasons, including the fact that patients fail to keep to proper TB treatment regimes, the wrong TB drugs are prescribed, or sub-standard TB drugs are used for treatment. Secondly, transmitted or primary drug - resistant TB, results from the direct transmission of drug - resistant TB from one person to another.[8]

On the other hand, the mechanism of RR– TB was reported in several previous studies. Study in India reported the mutation in rpoB, the beta subunit of DNA-directed RNA polymerase of MTB, was reported to be a major cause of RR. Amongst mutations in the well-defined 81-base-pair central region of the rpoB gene, mutation at codon 450 (S450L) and 445 (H445Y) is mainly associated with RR. The rpoB mutants interacted with RIF with positive binding energy, revealing the incapableness of RIF inhibition and thus showing resistance.[9] In Australia, on a molecular level, analysis

of the rpoB gene revealed that 97% of the RR isolates had missense mutations within a conserved region of the gene and eight types of missense mutations were detected. Of the 31 RR isolates that were typed by restriction fragment length polymorphism (RFLP) analysis, 28 distinct patterns were obtained by RFLP analysis with IS6110 and three clusters of genetically related isolates were identified.[10]

In Kuwait and Dubai, mutations conferring RR in clinical TB isolates occur mostly in the 81 bp RR-determining region (RRDR) of the rpoB gene. These analyses identified 8 different mutations within RRDR of the rpoB gene including one novel mutation (S522W) that has not been reported so far. The genotyping performed on the isolates carrying similar mutations showed that majority of these isolates were unique as they exhibited varying DNA banding patterns. Correlating the ethnic origin of the infected TB patients with the occurrence of specific mutations at three main codon positions (516, 526 and 531) in the rpoB gene showed that most patients (11 of 15) from South Asian region contained mutations at codon 526 while majority of isolates from patients (6 of 11) of Middle Eastern origin contained mutations at codon 531.[11]

Also in previous study recorded the majority of resistant isolates involved base changes at codon 531 of the rpoB gene.[12] Other previous study in New York by using commercial line probe assay kit (Inno-LiPA Rif.TB) for rapid identification of mutations in the rpoB gene associated with RR in TB was evaluated with a collection of 51 RR- strains. Nine distinct rpoB mutations were identified. Concordances with automated sequence results for five wild-type kit probes and four probes for specific mutations were 94.1 and 100%, respectively. Overall concordance of the line probe assay kit with phenotypic RIF susceptibility testing results was 90.2%.[13]

In addition, several risk factors for RR – TB have been reported in literature scientific, that found that male gender; history of TB treatment; and adult age compared with either children or the elderly were risk factors associated with high TB detection amongst symptomatic, across the TB program unit. While treatment history was found be a significant risk factor for RR - TB, elderly people have significantly lower risk than other age.[14]

Finally, the recommendations for the dosages, duration, and combinations of drugs for treatment of drug-susceptible TB are based on sound evidence-based principles derived from multiple randomized trials. Adherence to authoritative guidelines for treatment and ensuring that all doses are taken correctly is unarguably the most effective means of preventing drug resistance.[15]

5. CONCLUSION

In conclusion, these finding demonstrate the feasibility of using PCR directly to detect the presence of *M. tuberculosis* and to determine RR – TB. Also the finding data showed that analysis of RR is very important for the determination the guideline of TB treatment.

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