

Gene Xpert MTB/RIF-----A Novel Diagnostic Tool for Tuberculosis in Pulmonary Samples.

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Abstract: Objective: To access diagnostic usefulness of Gene Xpert MTB/RIF assay technique in management of tuberculosis. **Study Design:** Observational Study.

Setting: Pathology (Microbiology) Department Sheikh Zayed Medical College/Hospital Rahim Yar Khan

Period: November 2012 to February 2013. **Materials and Methods:** 224 Sputum samples from pulmonology department. All samples were tested on Gene Xpert for MTB/RIF detection after AFB microscopy. **Results:** Chi-Square test applied; P value is <0.001. all results are highly significant. **Conclusion:** (I) Gene Xpert is more specific and sensitive technique. (II) It helps to avoid injudicious use of anti tuberculosis drug.

Key words; AFB, Gene Xpert, MTB/RIF, TB and ZN staining .

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I. Introduction

Tuberculosis is an infectious and highly transmissible disease.¹ Around nine million people worldwide are infected with Mycobacterium Tuberculosis and develop active disease. Out of these, two million people lose their lives. There is a troublesome drug resistance issue found in anti tuberculosis drugs. Though rarely encountered in Rifampicin (RIF) but usually indicate resistance in other 1st line drugs Isoniazid (INH), Ethambutol (EMB), and Pyrazinimide (PZA).²

Multidrug resistance is a reflection of either mismanagement of tuberculous patients' wrong diagnosis, delay in diagnosis, wrong or interrupted treatment and mistreatment of both first and second line drugs. Pakistan ranks 6th among the countries with a highest burden of tuberculosis in the world and contributes about 44% of tuberculosis weigh down in the Eastern Mediterranean Region, where resistance to anti tuberculosis drugs is a common problem. Injudicious use of drugs is to be avoided in better interest of patients.³

Molecular detection of mutated gene is a very important diagnostic area in metabolic, congenital or neoplastic diseases. For this purpose, a novel tool namely Gene Xpert MTB/RIF is available. Gene Xpert test is a semi-quantitative nested real-time PCR *in-vitro* diagnostic test for: (1)The detection of Mycobacterium tuberculosis complex DNA in sputum samples or concentrated sediments prepared from induced or expectorated sputa that are either acid-fast bacilli (AFB) smear positive or negative; and (2) The detection of rifampin resistance associated mutations of the *rpoB* gene in samples from patients of rifampin resistance.^{4,5} Among the most important diagnostic techniques one is the Gene Xpert which detects gene mutation (*rpoB*) associated with RIF resistance. Hence it is of enormous importance in the diagnosis of both drug susceptible and drug resistance cases.

Gene Xpert test can be performed on sputum or bronchial washing samples. Results becomes available in less than 2.5 hours. The rapid detection of Mycobacterium tuberculosis and its resistance to Rifampicin (RIF's) allows the physician to make critical patient management decisions regarding therapy during the same medical encounter.

II. Materials and Methods

2.1 Study Type Observational Study

2.2 Study Setting Study was conducted in the department of Pathology (Microbiology) Sheikh Zayed Medical College /Hospital Rahim Yar Khan.

2.3 Duration November 2012-February 2013

2.4 Materials

GeneXpert Dx system, an automated instrument which works on the principle i.e, sample processing, nucleic acid amplification, and detection of the target sequences in simple or complex samples using real-time PCR and reverse transcriptase PCR.

2.5 Methods

2.5.1 Sample Collection

We collected 224 sputum Samples from Pulmonology department. Early morning, deep coughed sputum specimens in sterile containers were included in the study. Specimen were stored at 2-8⁰C in freezer till further processing. However, the specimen can be safely stored at 35⁰C for three days. After collection Hot-Ziehal-Neelsen (ZN) staining on all samples was done then each samples was run on Gene Xpert.

2.5.2 Standard Assay Procedure of Gene Xpert

Each Xpert MTB/RIF cartridge was labeled with the sample identity (case number). Transferred at least 0.5 mL or 1ml sample of the total resuspended pellet to a conical, screw-capped tube for the Xpert MTB/RIF using a transfer pipette. Incubated for 10 minutes at room temperature, and then shaken the specimen vigorously 10 to 20 times or vortex for at least 10 seconds. Incubated the sample at room temperature for an additional 5 minutes. Started the test within 4 hours of adding the sample to the cartridge. Opened the cartridge lid, and then opened the sample container. Using the provided transfer pipette, aspirated the liquefied sample to the line on the pipette. Transferred the sample into the sample chamber of the Xpert MTB/RIF cartridge. Dispensed the sample slowly to minimize the risk of aerosol formation. Closed the cartridge lid firmly. Loaded the cartridge into the Gene Xpert Dx instrument and started the test within 5 hours of preparing the cartridge.

Turned on the GeneXpert instrument. GeneXpert Dx instrument, first turn on the GX Dx instrument, and then turned on the computer. The GeneXpert software was launched automatically. The Scanned Sample ID dialog box appeared. In the Sample ID box, typed the sample ID. The Scanned Cartridge Barcode dialog box appeared. Scanned the barcode on the Xpert MTB/RIF cartridge. The Create Test window appeared. Using the barcode information, the software automatically filled the boxes for the following fields: Selected Assay, Reagent Lot ID, Cartridge SN, and Expiration Date. Opened the instrument module door with the blinking green light and loaded the cartridge. Closed the door. The test started and the green light stops blinking. When the test is finished, the light turns off. Waited until the system releases the door lock at the end of the run, then open the module door and remove the cartridge.

II. Results & Discussion

We divided the samples in to two categories. In 1st category 188 samples of the patients having history of ATT, In 2nd category 36 samples of the patients having no history of ATT. We did ZN staining of both categories.

In 1st category samples 135(72%) were AFB smear positive and 53(28%) were negative. In 2nd category samples 21(71%) were AFB smear positive and 15(29%) were negative. Then all samples were performed on GeneXpert and their results were mentioned in tables I, II, III, IV.

Gene Xpert	ZN Staining on Smear		Total samples
	AFB+Ve	AFB-Ve	
MTB +ve	135	5	140
MTB -ve	0	48	48
Total samples	135	53	188

Table, I. Samples of the patient having history of ATT. (P<0.001)

Gene Xpert	ZN Staining on Smear		Total samples
	AFB+Ve	AFB-Ve	
MTB +ve	21	2	23
MTB -ve	0	13	13
Total samples	21	15	36

Table, II. Samples of the patient having no history of ATT. (P<0.001)

RIF Resistance	MTB RESULTS		Total samples
	MTB+Ve	MTB-Ve	
RIF NOT DETECTED	130	48	178
RIF DETECTED	10	0	10
Total samples	140	48	188

Table, III. Results of ‘Gene Xpert MTB/RIF, samples of the patient having history of ATT.

RIF Resistance	MTB RESULTS		Total samples
	MTB+Ve	MTB-Ve	
RIF NOT DETECTED	23	13	36
RIF DETECTED	0	0	0
Total samples	23	13	36

Table, IV. Results of ‘Gene Xpert MTB/RIF, samples of the patient having no history of ATT.

The results of Gene Xpert and ZN staining are compared in our study. it is evident from table I that 140 out of 188 are MTB positive on Gene Xpert while 130 out of 188 are AFB positive on ZN staining. In table II 23 out of 36 are MTB positive on Gene Xpert while 21 out of 36 are positive on ZN staining. It is cleared that Gene Xpert MTB/RIF is more sensitive and specific technique as compare to ZN staining it can detect MTB even in 1ml of sputum.

The second important advantage of Gene Xpert is that it also detects (RIF) rifampin resistance and we labeled the patients as MDR. In table III 140 positive MTB samples RIF resistance is 14%.In table IV Rif resistance is zero because the samples of the patients having no history of ATT.

III. Statistical analysis

All results were analyzed statistically by applying chi-square test.

$$\chi^2 = \sum \frac{(O_i - E_i)^2}{E_i}$$

P value was <0.001 all results are highly significant.

IV. Conclusion

We concluded that as compared to AFB microscopy, Gene Xpert is more sensitive and specific not only for acid fast bacilli (AFB) detection but also for rifampicine (RIF) resistance. It also helps to avoid injudicious use of anti tuberculosis drugs.

V. Suggestion

We recommend health departments to supply this instrument in every tertiary care hospital and if possible at tehsil level.

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