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GENEXPERT AND MYCOBACTERIUM TUBERCULOSIS

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Abstract: Mycobacterium tuberculosis is the causative agent of tuberculosis (TB), an infectious disease that affects the skin, abdomen, and lungs. Numerous methods can be used to diagnose tuberculosis, including direct sputum smears for microscopy, culture-based testing, serology, and nucleic acid amplification techniques (NAATs). Chest radiography is also crucial for the diagnosis, disease prognosis, and treatment monitoring . Recently, new diagnostic techniques have emerged, including the geneXpert assay and interferon-gamma release assays [3]. In order to successfully eradicate tuberculosis, the right technique must be chosen for precise and rabid detection, which lowers death and transmission rates [4].

Key words: Tuberculosis, transmission rates, tests, GeneXpert, treatment monitoring.

In the majority of developing nations, ZN stain is utilized for tuberculosis diagnosis due to its affordability and convenience of use, while culturing once thought to be the gold standard method is not frequently employed. False negativity and, to a lesser extent, false positivity are major mistakes from the ZN approach [2]. The use of tests that improve identification sensitivity and aid in therapy selection is crucial for disease control [3].

A modified nucleic acid amplification method called GeneXpert has been developed to distinguish between the rpob resistance gene producers and to specifically identify the species MTB [4] [10] [11]. Although the WHO has approved the test as the primary method for identifying drug-resistant TB worldwide, rpob resistance does not apply to all forms of resistance [4]. As TB is seen as a public health priority in Sudan, the government has been working to identify cases, treat patients, and include other sectors in control efforts through the national TB program.

GeneXpert assay has become an important part of tuberculosis diagnostic algorithms in many low- and middle-income countries [5] [6]. However, despite this effectiveness, smear for microscopy remains the primary diagnostic method for tuberculosis. In the context of negative tuberculosis detection, sensitivity and specificity of smear microscopy have been recorded to be 30% - 89% and 93% - 100% , noted that, lower specificity has been observed in tuberculosis prevalence surveys. Cross positivity may be due presence of other acid fast bacilli like actinomycetes and nocardia. In addition, laboratory errors such as analytic errors or sample mix-ups may lead to false positive smear microscopy results. Knowing that, smear microscopy enables to differentiate between Mycobacterium tuberculosis complex (MTBC) and non-tuberculous mycobacteria (NTM). This study was conducted to assess ZN stain, ZN stain with concentrated sputum and geneXpert assay for detection of MBT.

Methods

A cross-sectional laboratory based was followed in the period from March 2022 to March 2024 in Andijan Tuberculosis dispancer. This study included all patients attending Tuberculosis Center during study period; suffer from chronic pulmonary symptoms and had tuberculosis suspected chest X-ray report. Patients under anti-tuberculous treatment, extra-pulmonary tuberculosis and known multi-drug

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resistance cases were excluded. To achieve liquefaction and deactivation of specimens; using screw-capped tube two ml of sample reagent were added to one ml of sputum. After shaking for 20 times tubes were incubated at room temperature for 15 min as the follows; 10 minutes firstly then additional 5 minutes. Two ml of liquefied sputum samples were loaded into MTB\RIF cartridges and inserted into the geneXpert chamber. Programmed machine was adjusted to finish after 1 hour and 52 minutes. Lastly the results were read and interpreted according the load of bacilli and rifampicin resistance gene detection [2].

Results

A total of 200 sputum specimens from pulmonary tuberculosis suspected subjects were included, most of participants came from Fergana valley. Socio-demographic and clinical characteristics of enrolled patients. Positive ZN staining without concentration for acid fast bacilli obtained from 18% (51/300) while centrifugation technique revealed positivity of 30% (59/300). Degree of ZN stain positivity for staining without concentration and with concentration. Sensitivity of 47.28% and 49.51% recorded for ZN stains with and without centrifugation respectively. Mycobacterium tuberculosis was detected in 34% (103/300) by geneXpert assay. The relationship between geneXpert result levels and ZN stain with and without centrifugation expressed a significant association (Chi square 0.000) at most levels while the very low level result recorded in 9 by geneXpert and at the same time showed negative by ZN stains. The rpob gene was documented with frequency of 9.7% (10/103) (data not shown).

Discussion

Low level of income in Andijan, increase in poverty and insecurity especially in the places of boarder disputes led to an increase in the spread of diseases such as tuberculosis, in addition to the poor quality and continuation of health care services provided to citizens. Despite the endemicity of tuberculosis in Sudan, information like the actual prevalence, diagnostic data and monitoring of drug-resistance situations are limited. Early diagnosis of tuberculosis cases achieves cure and reduces the areas of spread of drug resistant cases and thus, enables control [3]. In view of results of participants whose were positive in the tests used in the present study, the small proportions (17%, 20% and 34%) that showed positive results necessitates a re-evaluation of the criteria for expectation of pulmonary tuberculosis. From the results obtained ZN stain without concentration revealed lowest sensitivity for detecting MTB from sputum; false negative results may occur due to sputum contamination with saliva diluents, personnel competence and or poor quality of equipment and reagents used. Thus, in the current study 49.5% (51/103) of geneXpert positive cases were negatively detected by ZN stain without concentration. The findings revealed sensitivity and specificity of ZN stain without centrifugation of 49% and 100% for detecting MBT, near results were concluded in France [4] and Pakistan [5]. ZN stain with centrifugation gave better sensitivity when compared to that without, thus false negative results were 42.7% (44/103). By looking to the given time required to complete centrifugation method, which is approximately 15 minutes, and simplicity of this technique, negative results of ZN satin without concentration should be repeated using a concentration method especially in case of no geneXpert availability. In line, other study recommended concentration approaches for examine pre-bronchoscope sputum in order to enhance sensitivity [6]. Since ZN stain without concentration can detect MTB amount of 5000 - 10,000 CFU/ sputum ml [7], centrifugation method actually detect less than 5000 CFU/ml. So far, the geneXpert assay has the best results for rabid pulmonary tuberculosis diagnosis but, beside possibility of contamination during sample collection and processing the high infrastructures requirements remain major factors that affect diagnosis by this method.

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