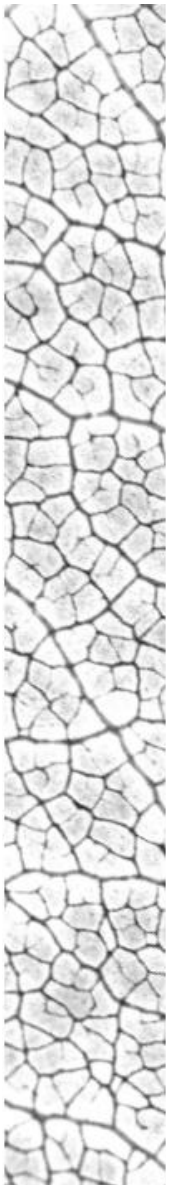

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Efficiency of molecular GeneXpert MTB/RIF system and conventional test as rapid detection of TB associated lung cancer

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Abstract
Lung cancer is main cause of morbidity and mortality; it includes 12.4% of all new cancer cases, and about 29% of all cancer deaths. There is evidence about persons infected with tuberculosis have increased lung cancer risk. GeneXpert System new diagnostic technique firstly, for TB infection secondly, detect RIF *–based rpoB* gene mutations related resistance tuberculosis. The rapid detection allows the doctors take critical decisions about therapy. This study aimed to find relationship between lung cancer and TB using GeneXpert MTB/RIF assay compared to conventional Z-N stain. 50 cases suspected cancer was enrolled to procedure of Cephid GeneXpert system manufacture also conventional stain was done for all samples. Out of 33 positive lung cancer, 7 (21.22%) MTB/Rif sensitive was positive detected. Significant difference (p-value 0.0001**) was found between detected MTB/Rif (sensitive and resist) and not detected MTB/Rif by Molecular gen expert method. In addition, Z-N stain was positive in 6 (18.18%) BAL cancer cases. In lung cancer cases, there is no significant difference (p-value 0.828) between two rapid methods. Present study data showed the high percentage of M. tuberculosis/RIF sensitive in cancer bronchoalveolar lavage. Gene Xpert had a higher diagnostic yield in patients beside the routine work to diagnosis and detection of TB/ RIF related *rpoB* gene mutation as a cancer risk factor.

Keywords: molecular GeneXpert MTB/Rif, smear test, lung cancer, Mycobacterium tuberculosis

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INTRODUCTION

Tuberculosis (TB), an infectious disease caused by *Mycobacterium tuberculosis*, was confirmed as global problem in 1993 and is still because the increasing number of immunedeficient patients and the development of multi-drug resistant TB (WHO 2007, Rieder 2002).

Pulmonary *tuberculosis* found in 85% of clinical TB cases, it is a chronic inflammation that may induce carcinogenesis effect for lung tissue (Dheda *et al*. 2005). Lung cancer represent important cause of morbidity and mortality; it about 12.4% of all new cancer cases, and 29% of all cancer deaths (Parkin 2005). In contrast coexistence of TB and lung cancer in thoracic surgery is rare. Coexistence was diagnosed in 2.1% from total 2218 patient's lung cancer (Saulius and Vladislavas V 2007). Pulmonary *tuberculosis* significantly had high risk of lung cancer related with diabetes mellitus and chronic obstructive pulmonary disease (Yang-Hao *et al*. 2011).

A recent systematic review of many studies was performed to determine whether preexisting tuberculosis increased the risk of developing lung cancers. Association of tuberculosis with lung adenocarcinoma group was noted particularly in non-westernized countries (Liang *et al*. 2009). Study provides a compelling evidence of increased lung cancer risk among individuals with tuberculosis. The risk may increase further with coexisting COPD or other smoking related cancers (Yang-Hao *et al*. 2011).

The TB rate for patients with lung cancer varied widely (52 – 320) cases per 100,000 persons). Wu and colleagues (Wu *et al*. 2011). Chen *et al.* (2011) cited that TB is a important risk factor for lung cancer, and the association be strong by smoking. While Carry and Greer studied 140 cases of bronchogenic carcinoma complicated by pulmonary TB and felt that there was no relationship between TB and bronchogenic carcinoma (Carey and Greer 1958, Goli *et al*. 2019).

MDR-TB is known as a *tuberculous* infection caused by a certain bacteria which is resistant to first-line drugs

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Table 1. Distribution of MTB/Rif sensitive, resistant and not detected in cancer BAL

Method	MTB/Rif sensitive detected NO. (%)	MTB/Rif resistant detected NO. (%)	MTB not detected NO. (%)	total
Molecular gen expert assay	7 (21.22%)	0 (0.00%)	26 (78.78%)	33

P-value (χ^2:) = 0.0001 (12.66 **).
** (P≤0.01)

include Isoniazid (INH), Rifampicin (also known as Rifampin, RIF), Ethambutol (EMB), and Pyrazinimide (PZA) making cure much more difficult to achieve. These data usually indicate the need for full susceptibility testing, also against second-line agents (WHO 2008, Morris *et al*. 1995, Ashok *et al*. 1998, Francis 2008, Josiane *et al*. 2018).

Molecular technique GeneXpert R System is a semi-quantitative nested real-time PCR *in-vitro* diagnostic test which detects DNA of TB different samples. At the same time recognize abnormalities in the *rpoB* gene correlated RIF resistance (Ethiopian Public Health Institute 2014). The GeneXpert method consists a computer, a barcode scanner and disposable cartridges that have test reagents. By this system, TB and *rpoB* gene mutations accelerate rapidly the diagnosis of MDR & drug-susceptible tuberculosis, this make the physician take decisions regarding therapy (WHO 2014, p. 17).

Study aims to find the relationship between lung cancer and TB because it is not obvious till now at the same time determine the Rifampcin resistant -TB using update molecular Cephid GeneX pert System.

MATERIALS AND METHODS

Specimen Collection

5 ml of bronchoalveolar lavage (BAL) from 50 suspected lung cancer patients was transported to Microbiology lab. as soon as possible and Specimens should be without food or other solid particulates. The bronchoalveolar lavage were diagnosis of Mycobacterium tuberculosis by conventional Ziehl-Neelsen stain and by GeneXpert MTB/RIF in Pulmonary diseases center in Baghdad. Also, patient's information was recorded include sex, age as well as types of cancer. After BAL collection, most of sample centrifuged at1400rpm for 15 min. the supernatant was discarded. The pellet stored in Eppendorf tube with 50% ethanol at -20C till use for molecular GeneXpert MTB/RIF analysis. Thirty-three from total cases 50 were positive lung cancer confirmed by cytology and pathology lab. the other cases were acute infection considered as normal. All patients underwent chest computed tomography (CT) and X-ray.

A Bacteriology Confirmed TB Method

Concentrated the samples by centrifugation at 3000/g for 15 min. and supernatant was discarded. Sediments were mixed with 3–5 ml of sterile DW. The concentrate was decontaminated and digested using the NaOH (modified Petroff). smear on slides were made and examined according to the conventional Ziehl-Neelsen stain method under light Microscop (Youness *et al*. 2019).

Molecular GeneXpert MTB/RIF System Method

Protocol was done according to the manufacturer (Cepheid Inc., Sunnyvale, CA, USA). The stored samples were washed by 1ml Bps from ethanol and discarded. Bps again added to the pellet with mixing. 1 mL gen expert Reagent solution was added to the specimen and vortex carefully for 10s. Tube was standing for 15min at room temperature with vortex carefully then proceeded to inoculate cartridge. Specimen transferred into the chamber of the Xpert MTB/RIF cartridge which have a barcoded for each sample then entered into the GeneXpert instrument. This instrument automates for all next steps, including sample workup, DNA amplification, detection of the target sequence for drug resistant and data analysis. The run took about 2 hours.

The primers in the Xpert MTB/RIF assay were amplified a segment of the *rpoB* gene containing the core region "81 base pair". The probes are able to differentiate between the mutations and wild-type sequence in the core region that are related with RIF / resistance. Automatically, internal quality control found within the test cartridge for each sample. The GeneXpert give the results via measured fluorescent signals and calculation algorithms will detect the results on computer screen either Both the MTB target and the *rpoB* gene mutation have been **detected** or The MTB target has been detected but no abnormalities in the *rpoB* gene has been detected or The MTB target is **not detected** within the sample (Cepheid 2011, QA Committee 2018, WHO 2014).

RESULTS

Cancer BAL Cases Analysis by Molecular Gene Expert System

According to **Table 1**, out of a total of 33 positive cancer cases, only 7 (21.22%) cases was MTB/Rif sensitive detected while there are no cases with MTB/Rif resistant detected as well as all positive cancer were squamous cell carcinoma. There is significant difference (p-value 0.0001**) between detected and not detected MTB/Rif by Molecular gen expert assay.

Different Rapid Detection Methods Comparison

Two different methods used to rapid detect TB in BAL of lung cancer and normal cases, Z-N stain and molecular method gene expert MTB/Rif. 6 (18.18%)

Table 2. BAL positive and negative TB using rapid gene expert MTB/Rif and Z-N stain in cancer and normal cases

Rapid methods	BAL cancer cases NO. (%)	BAL normal cases NO. (%)
Gene expert assay		
MTB detected	7 (21.22)	1 (5.88)
MTB not detected	26 (78.78)	16 (94.12)
Z-N stain for TB		
BAL positive	6 (18.18)	1 (5.88)
BAL negative	27 (81.82)	16 (94.12)
Total	33	17

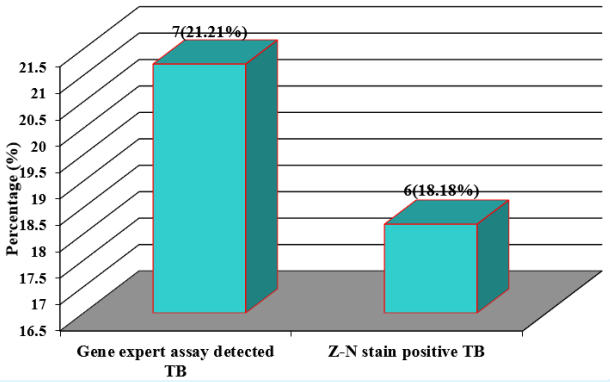

Fig. 1. Distribution of of positive MTB Gene expert assay and Z-N stain positive TB related lung cancer cases

cases was positive by Z-N compared to 7 (21.22) cancer in lung cancer. While one normal case 1 (5.88%) was positive by these methods. There is no significant difference (p-value 0.828) between two rapid methods in positive cancer cases (**Table 2** and **Fig. 1**).

DISCUSSION

Because geneXpert MTB/RIF does not require advanced or additional infrastructure within the laboratory like culture, smear-positive to be *M. tuberculosis*, in site of GeneXpert with smear-positive allow to rapid diagnosis. This will ensure that those with TB receive suitable medicine and prevent unnecessary treatment of individuals without TB (WHO 2011).

Table 1 illustrated that just 7 (21.22%) positive bronchial wash TB /Rif sensitive associated with lung cancer and TB /Rif resistant was 0% .This result close the study which cited that out of 714 samples tested by GeneXpert MTB/RIF, 20.59% cases were detected to be positive, while 79.41% cases were negative. Resistance rate to Rifampin was 6/ 0.84%, which is close to the WHO estimate (Youness *et al*. 2019).

Another study found 18.40% bronchial wash samples positive TB using Xpert MTB/ RIF while 22.38% cases was Rifampicin resistance from all 67 samples types including Sputum, Pleural, biopsies and BAL (Li *et al*. 2017).

Bronchial wash for MTB detected 62/250 (24.8%), in Gene Xpert MTB/RIF assay MTB genome was positive in 228/250 (91.2%) and Rifampicin resistance was positive in 12/250 (4.8%), It is alternative to conventional tests as it is reliable, cost effective and can be performed on all bronchoscopic samples (Opota *et al*. 2019).

Rufai et al study, who reported that Poor detection of GeneXpert MTB/RIF (Rufai *et al*. 2014). Notably, that Rifampin resistance about 5% caused by mutations not in *rpoB* gene as a result not detected by the GeneXpert MTB/RIF assay (Blakemore *et al*. 2010).

Many studies investigated the relationship between lung cancer and TB one of them, Inflammation and scarring due to chronic TB results in metaplasia, dysplasia, and cancer. On the other hand, reactivation of latent TB in patients with cancer can occur because of immunosuppression due to malnutrition, aggressive chemotherapy, and immunomodulatory therapy (Pesut and Marinkovic 2009).

Stimulate innate immunity and inflammation process lead to production of cytokines that can induce tumor progression (Lin *et al*. 2007). In addition**,** activated leukocytes that participate due to inflammatory steps produce reactive oxygen species (ROS), which can bind to DNA and cause genomic modifications (Weitzman *et al*. 1990). Whereas Carry and Greerstudied 140 cases of bronchogenic carcinoma complicated by pulmonary TB and felt that there was no relationship between TB and bronchogenic carcinoma (Carey and Greer 1958).

Table 2 appeared the differences between Z-N stain and gene Xpert MTB/RIF to detection TB methods in cancer and normal cases. There is no significant difference between two methods related lung cancer **Fig. 1**. Detection bronchoalveolar lavage *Mycobacterium tuberculosis* by Molecular GeneXpert MTB/RIF and convensional microscopic methods, showed parallel and a good agreement between these two techniques.

Lung cancer may cause TB infection, because it can reactivate of latent TB through reduce the immunity (Feld *et al*. 1976, Libshitz *et al*. 1997).

A WHO Global TB Programme commissioned review for Rif/resistance finding reported that Xpert MTB/RIF sensitivity and specificity were 95%, 98% respectively (Public Health Institute 2014). Whereas Opota et al. (2019) reported that sensitivity of positive smear 100% versus 66.67% of negative smear (Li *et al*. 2017, Opota *et al*. 2019). In Iranian study, based on microscopic methods and cultural, *M. tuberculosis* infection was high incidence (6.8%) within lung cancer (Amir *et al.* 2013). Generally. molecular Xpert MTB/RIF may be used as an alternate test for standard practice including conventional microscopy, culture, and histopathology. If results from this molecular method are not available at that time the conventional TB procedures will be used (WHO 2014).

CONCLUSION

Present study data showed the high percentage of *M. tuberculosis*/RIF sensitive in bronchial wash of lung

cancer patients using rapid methods , molecular and smear stain without significant difference, take in account that Gene Xpert MTB/RIF important assay to detect mutation *rpoB* gene with high sensitivity and specificity. Thus, Bronchoalveolar lavage gene Xpert had a higher diagnostic yield in patients beside the routine work to diagnosis and detection of TB/ RIF related *rpoB* gene mutation as a cancer risk factor.

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