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Prevalence of tuberculosis in Khyber Pakhtunkhwa Pakistan using GeneXpert assay and light microscopy

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Abstract

Tuberculosis (TB) is a common disease in developing countries including Pakistan which ranks sixth amongst highest tuberculosis reported countries. The present study aimed to determine the frequency of TB in Peshawar, Mardan and Swabi regions of Khyber Pakhtunkhwa province in Pakistan and also to evaluate the comparative performance of GeneXpert and microscopy analysis. In this cross-sectional study363 patients were included from Mardan (270), Nowshera (42) and Swabi (51). An early morning deep cough sputum of about 2ml was collected from each patient. Two aliquots of each sample were prepared one each for the analysis through GeneXpert and ZN staining based light microscopy. Among 363 cases 207 (57%) were males and 156 (43%) were females. The light microscopy method confirmed 198 (55%) while GeneXpert MTB/RIF assay confirmed 234 (64.4%) samples positive for Tuberculosis. The sensitivity of light microscopy was found 84.6% whereas for GeneXpert MTB/RIF it was 98%. Specificity of light microscopy and GeneXpert were found almost the same. Among the reported positive cases, Mardan constituted 74% of the cases while Nowshera and Swabi had 12% and 14% cases, respectively. Moreover, 83% of the cases had family history of tuberculosis. Maximum number of patients (117) was observed in the age group of 26-40 years. Frequency of TB was higher in Mardan than Nowshera and Swabi. GeneXpert method proved to be more efficient and sensitive.

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Introduction

Tuberculosis (TB) is a pulmonary disease which is caused by the accumulation of bacterium (*Mycobacterium tuberculosis*) in the lungs is among the top ten causes of death in the world (WHO, 2017). Recently WHO has reported 10 million infected people with *Mycobacterium tuberculosis* and 1.7 million deaths in 2016 (WHO, 2018). Pakistan ranks sixth amongst highest tuberculosis reported countries. In Pakistan per year reporting cases of tuberculosis are 420,000, while in every 100,000 of people 231 are infected individuals (Gilani and Khurram, 2012). In 2016 about 56% of TB cases have been reported from five countries including India, Indonesia, China, the Philippines and Pakistan (WHO, 2017).

The peculiar characteristics of *Mycobacterium tuberculosis* is their unique staining abilities. After staining with dyes i.e. Carbolfuchs in and fluorochrome, they resist decolourization of these dyes after washing with alcohols and acids and that is why they are known as "Acid-Fast Bacilli" (AFB). Mycobacteria retain pink colour with Ziehl-Nelson (Z-N) staining.

The worldwide occurrence of tuberculosis is 10 in 100,000 in northern sides of USA, in Asia, Africa and Russia it is 300 in 100,000 (Kochi, 2001). Pakistan ranks sixth amongst highest tuberculosis reported countries.

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Tuberculosis infected persons can exhale moisten droplets containing bacilli into air while coughing, sneezing etc. These droplets if inhaled by healthy personals can infect them. Larger droplets tend to settled down quickly on floor but smaller droplets remain suspended in air for long time period (Reichler *et al.,* 2002). When the moisture evaporates from the droplets it leaves residue (droplets nucleus) which is the basic infectious unit of tuberculosis and contain single or more bacterium. So it is understood that the factor of tuberculosis transmission is directly related to the concentration of droplets nuclei suspended in the air (Van Crevel *et al.,* 2002).

The commonly used detection techniques for this bacterium are collection of specimen, its digestion and liquefaction followed by the staining technique and light microscopy (Bassili *et al.,* 2008). The bacterium is also cultured specially for the purpose of its identification and isolation from other closely related bacterium like *Mycobacterium Bovis* and *Mycobacterium africanum*. Another technique known as GeneXpert MTB/RIF assay has been endorsed by WHO to be implemented for national tuberculosis programs in developing countries (WHO, 2011). The Xpert MTB/RIF (Cepheid Inc.) is an automated, user friendly and rapid test based on nested real-time PCR assay and molecular beacon technology for MTB detection and RIF resistance (Guenaoui *et al.,* 2016). It basically detects the DNA fragments of *Mycobacterium tuberculosis* in specimen and also identifies the mutations developed in DNA which leads to the drug resistance (Marlowe *et al.,* 2011). The results are obtained within a short period of time (2 h). Moreover this technique is not prone to cross-contamination, requires minimal Biosafety facilities and has a high sensitivity in smear-negative pulmonary TB (Alvarez-Uria *et al.,* 2012).

Though Pakistan has met 50% reduction in rates of mortality due to as per Stop TB target program of WHO till 2015, however Pakistan is still facing prevalence rate of 341 cases per 100,000 population and incidence rate of 270 cases per 100,000 (WHO, 2014; 2016). Numerous studies regarding prevalence and epidemiology of TB from different regions of Pakistan have been reported that are useful to inform about the TB reduction at different periods. Currently no such study has been reported to determine the prevalence of TB and routinely used techniques of diagnosis from Mardan, Swabi and Nowshera. Keeping in view this study aimed to determine the prevalence of TB and comparative performance of GeneXpert assay and light microscopy.

Materials and methods

Study design

This cross-sectional hospital based study was conducted at TB Laboratory of Mardan Medical Complex, Khyber Pakhtunkhwa during April 2017 to July 2017. Standard procedures were followed for analysis of the samples. Total 363 subjects were included in the study. Among 363 study subjects, 270, 42 and 51 were included from Mardan, Nowshera and Swabi, respectively.

Samples preparation

All the individuals having symptoms of tuberculosis were included in the study. Early morning deep cough Sputum samples of about 2ml were collected from both males and females of all ages. Only sputum having considerable mucoid content was processed for study. Specimens were stored at 4 °C until analysed. Two aliquots were prepared from each sample one for the analysis of GeneXpert and second for light microscopy using ZN staining. Slides were prepared for each sample and labelled.

Staining procedure

Each slide was processed for staining covered with Carbol fuchsin, heated and left for five minutes allowing the bacterium to absorb colour. Then decolourization was performed by applying 25% alcohol to each slide and rinsed with distilled water. The stained slides were then observed under light microscope. using 100 X magnification lens. Positive slides AFB were counted for quantification and recordings were categorized as follows. After Acid fast bacillus (AFB) staining, slides were subjected to light microscope using 100 X magnification, with emulsion oil. Pink rod shape AFB were observed and counted with blue background. The case was reported as positive where at least one red purple bacillus was seen with bluish background. The stained slides were observed under light microscope. Two drops of immersion oil were put on the left edge of the smear. Smears were observed under 100 X magnification lens. Positive slides AFB were counted for quantification and recordings were categorized as follows. Each sample was processed for GeneXpert after microscopy and then results were compared and efficiency of each technique was evaluated.

Results

A total 363 samples were collected, out of which, 207 (57%) patients were males and 156 (43%) were females. Out of all samples, 108 (30%) individuals were in the age range of 10-25 years, 117 (32%) aged 25-40 years, 75 (21%) aged 40-55 years, and 63 (17%) had age range from 55-70 years. The individuals under study from Mardan, Nowshera and Swabi were 74%, 12% and 14%, respectively (Table 1).

Table 1. Gender and Age wise distribution of Tuberculosis subjects in study area.

Gender-wise distribution	Frequency (%)
Males	207 (57%)
Females	156 (42.9%)
Age-wise distribution	
10-25 yrs	108 (30%)
26-40 yrs	117 (32%)
41-55 years	75(21%)
56-77 years	63 (17%)

AFB Staining/Microscopy

Pink rod shaped AFB were observed and counted with blue background. The case was reported as positive where at least one red purple bacillus was seen with bluish background. The AFB positive patients were 54.5% (198) while AFB negative cases were 45.4% (165) (Fig.1). Most of the AFB positive specimens were observed with medium concentration of the tubercle bacterium i.e. AFB++ (5-10 AFB counted under microscope), while the remaining sample had lower concentration i.e. AFB+ (less than 5 AFB counted under microscope) and very few samples

were noted to have high concentration of the bacterium i.e. AFB+++ (more than 10 AFB counted under microscope).Each sample was processed for GeneXpert after light microscopy and thus results were compared and efficiency of each technique was evaluated.

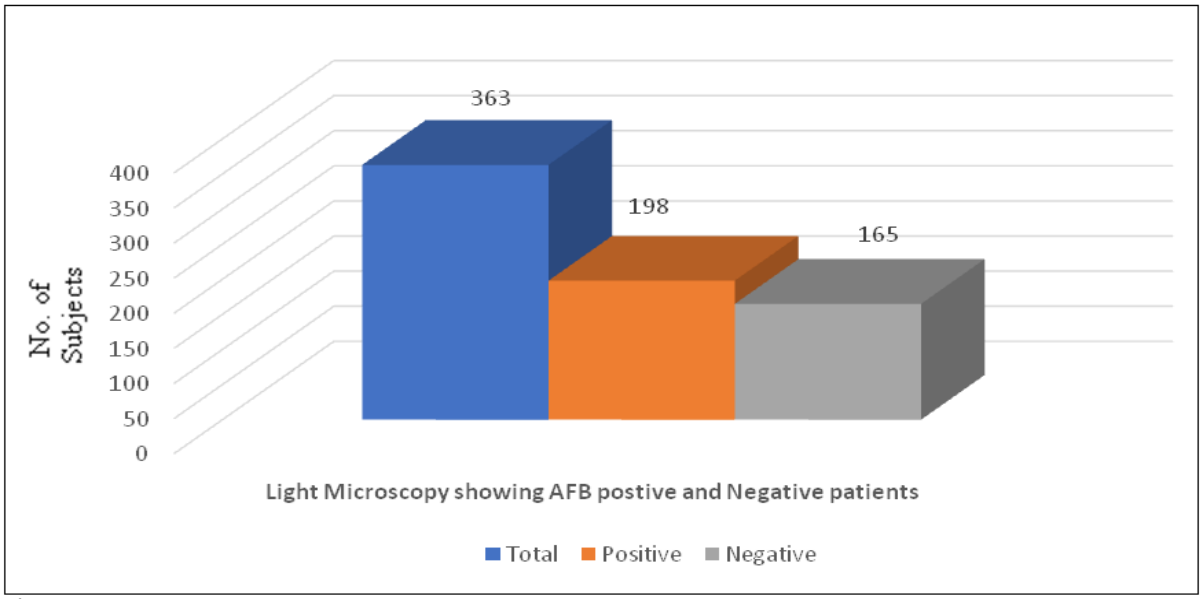

Fig. 1. Light microscopy showing AFB positive and negative patients.

GeneXpert MTB/RIF Assay

The GeneXpert MTB/RIF was performed for each specimen. Sample reagents were mixed with the clinical sample at ratio of 3:1. The closed container of specimen was vigorously agitated three times and was kept for 15-20 minutes at room temperature. About 2ml sample was transferred into test cartridge and were introduced into GeneXpert machine as advised by the manufacturer. For GeneXpert MTB/RIF assay buffer was added to each cartridge having sample. After ten minutes' cartridges were transferred to GeneXpert machine. In each cycle of PCR 4 cartridges were processed. GeneXpert MTB/RIF assay showed 234 (64%) of samples were positive for *Mycobacterium tuberculosis* while129 (35.5%) were negative (Fig. 2).

The sensitivity of light microscopy reported was 84.6% while its specificity was 100%. The low sensitivity of light microscopy in detection of tuberculosis is due to human errors in staining and smearing procedures. The probability of spotting the microbe under microscopic eye was not 100% which lowers the sensitivity of microscopic techniques for detection purposes. The specificity of light microscopy was reported as accurate as GeneXpert. The sensitivity of GeneXpert MTB/RIF assay for detection of *Mycobacterium tuberculosis* was found to be 16% higher than AFB light microscopy.

Discussion

TB is an increasing health burden in the developing countries with different rates in different regions worldwide. The present study reported the TB burden in Mardan Nowshera and Swabi along with the comparative performance of the two widely used techniques of diagnosis. Among the studied 363 TB samples in current study, 57% cases were male while 43% were female. Previously reported prevalence in males and females was 44.19% and 55.80%, respectively (Akhtar *et al.,* 2014). Gilani and Khurram (2012) reported 52% and 48% prevalence in male and female population, respectively while Ayaz *et al.* (2012) reported prevalence of 30% in male and 33% in female population.

These results indicate that there is no such remarkable difference in prevalence ratio between male and female population. In our study the most prevalent age group was 26-40 years reported 32%

prevalence. Previously reported prevalence in different age groups was 46% in age group of 31-45 years (Akhtar *et al.,* 2014), while another study has reported a high prevalence (68.96%) in age group 10-20 years and 35.29% prevalence in age group 21-40 years (Gilani and Khurram, 2012). Similarly a very high prevalence (82.72%) was reported in 15-64 years aged people (Ayaz *et al.,* 2012) which is in contradiction to our findings.

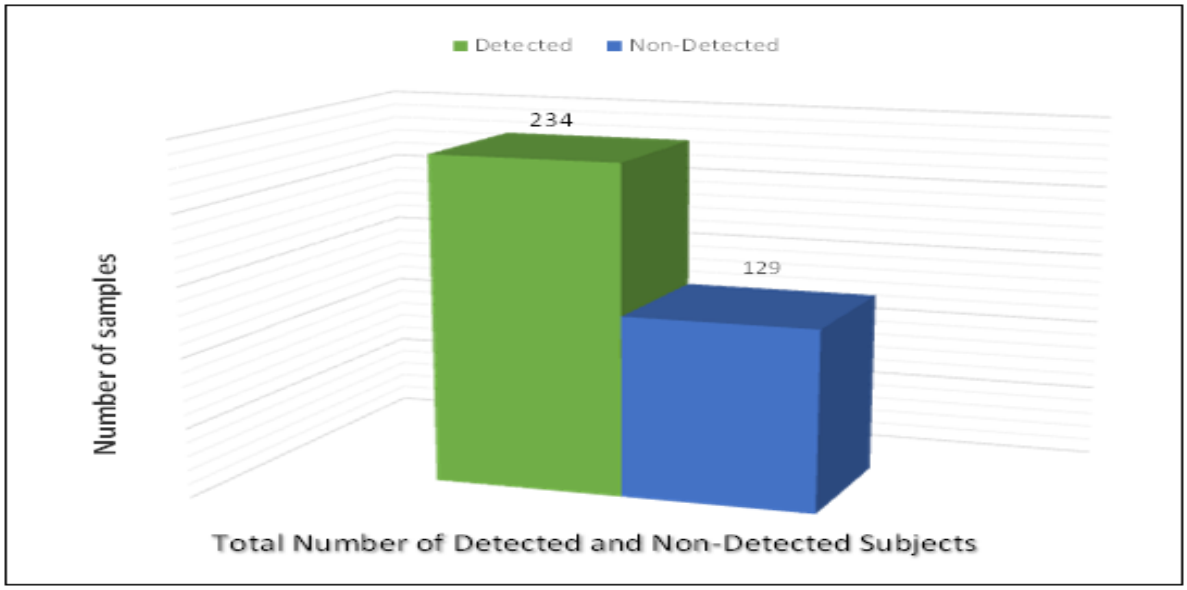

Fig. 2. GeneXpert results, showing number of samples for detected and non-detected M. *Tuberculosis* cases.

Our results showed that teenagers and youngsters most commonly suffer from tuberculosis which may be related to weak immunity and their unhygienic activities. Light microscopy is used thoroughly in clinical institutes of Khyber Pakhtunkhwa for detection of tuberculosis. Samples are stained with Z-N staining technique and then observed under light microscope. Though in local clinical institutes sample is collected only one time while according to World Health Organization (WHO) three samples should be collected from each patient for light microscopy.

Specificity of both the GeneXpert assay and light microscopy was reported the same (100%) while sensitivity of GeneXpert *MTB*/RIF assay for detection of *Mycobacterium tuberculosis* was found to be 16% higher than AFB light microscopy. Previous investigations showed that the specificity and sensitivity of GeneXpert MTB/RIF was 79.0% and 97.3% respectively (Tortoli *et al.,* 2012). Some other studies showed specificity and sensitivity of 95% and 100% respectively in 340 positive samples (Causse *et al.,* 2011). This higher ratio in term of sensitivity in comparison to our results may be due to sample size. The low sensitivity of light microscopy in detection of tuberculosis might be due to human errors in staining and smearing procedures. The probability of spotting the microbe under microscope was not 100% which lowers the sensitivity of microscopic techniques for detection purposes. GeneXpert test has been reported with highest sensitivity among all other techniques used for the detection of *Mycobacterium tuberculosis* (Zeka *et al.,* 2011).

In current study sensitivity of GeneXpert *MTB*/RIF assay for detection of *Mycobacterium tuberculosis* was found to be 16% higher than AFB light microscopy which makes the results of GeneXpert more accurate as compared to light microscopy.

Conclusion

Tuberculosis is one of the leading problems in most of the regions in Pakistan. GeneXpert appears to be valid and more accurate for the diagnosis of TB as compared to ZN staining followed by light microscopy.

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