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Prevalence of *Mycobacterium tuberculosis* in Suspected Tuberculosis Patients through ZN Staining and Gene Expert MTB/RIF: A Cross Sectional Study

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ABSTRACT: *A cross sectional study was conducted from January to May 2023 to determine the prevalence of Mycobacterium tuberculosis among tuberculosis suspected patients using ZiehlNeelsen staining and GeneXpert. Demographic information was also collected on excel sheet and statistical analysis was done on SPSS version 20.0 with confidence interval (IC) 95% and p value <0.05.A total of 200 patients were included in the study. A total of 38.1% (77/200) were females and 68.1% (123/200) were males. The mean age (±SD) was 44.3±20.3. The results indicate that prevalence of MTB was 52% (n=104/200) by Zn staining/smear microscopy and 58.5% (n=117/200) by GeneXpert. Zn staining showed 51.9 %(n=40/77) prevalence in females and 52 %(n=64/123) prevalence in males. GeneXpert results showed 58.4% (n=45/77) prevalence in females and 58.5% (n=72/123) in males. Age wise distribution showed that age group of 51 to 60 years had higher prevalence of MTB 66.7% (n=20/30) through Zn staining and 63.3% (n=19/30) by MTB/RIF GeneXpert. This study certifies the high prevalence of M. tuberculosis in District Okara. There is a dire need for extensive study to determine the burden of disease in the district along with effective preventive strategies to control the disease.*

Keyword: Mycobacterium tuberculosis, Prevalence, Gene xpert, ZN staining, Okara

INTRODUCTION

Tuberculosis, caused by the bacterium *Mycobacterium tuberculosis*, is a chronic disease predominately affecting the lungs (Hershkovitz et al., 2015). MTB infection affects approximately one third of the global population, with highest incidence observed in Eastern Europe, Southeast Asia and Sub-Saharan Africa (van Crevel and Alisjahbana, 2008). According to World Health Organization (WHO), Tuberculosis (TB) is ranking 13th among the death causing diseases. In 2021, 10.6 million people contracted with the disease, 1.6 million individuals lost their lives to TB and over 95% deaths occur in low to middle income countries (Organization 2022).The highest numbers of cases were reported in India, China, South Africa, Indonesia and Pakistan. India and China were accounted for 26% and 12% of all the TB cases respectively (Sharma and Sarkar 2018). Pakistan is ninth in the world and fifth among the top 22 high burden countries for tuberculosis (Organization 2017). Prevalence rate of 364 per 100,000 people with the infection of MTB and mortality rate of 34 per 100,000 people were observed in Pakistan (Malik et al., 2020).

Rifampicin is the most puissant anti-TB drugs used for the cure of TB. Antibiotic resistance in MTB is the major challenge in developing countries and it is due to frequent mutations in genetic loci, poor health infrastructure, and lack of awareness, poor reporting procedures and outdated data base system. The socio-economic burden of TB on developing countries is still increasing despite of WHO attempts to control the spread of disease (Steingart et al., 2006). The spread of tuberculosis is associated with many environmental and personal risk factors including smoking, poor sanitation, close contact with TB patients, intravenous drug abuse and co-morbidities (HIV, diabetes and cancer)

Diagnosis of MTB can be performed by different molecular assays and staining techniques like GeneXpert and ZiehlNeelsen staining respectively. Amplification of specific sequences leads to detection of MTB strains and rifampic in strains. Identification and treatment of sputum smear-positive cases can lead to control of drug resistant MTB. Additionally, prophylaxis and health education have a requisite role in controlling MDR-TB. In Pakistan, the implementation of Directly Observed Treatment Short-Course (DOTS) strategy for tuberculosis

(TB) began in 2001 and quickly expanded within five years to encompass a noteworthy portion of public health sector(Ajide, Igbabul and Kanyi 2019). Succeeding this success, National TB control program (NTP) shifted its focus to expanding DOTS coverage in private health sectors addressing childhood TB and implementing programmatic management of drug resistant TB. Although, National TB control program is running in the country, prevalence of MTB is still going at alarming level.

With this background we aim to determine the prevalence of MTB in district Okara in view of the fact that there was paucity of research conducted in this region and to best of our knowledge this is first reported study from the district.

METHODOLOGY

Study Area

This study was conducted to determine the prevalence of MTB in district Okara. It is the 23rd largest city in Pakistan in terms of population. Okara is located southwest of Lahore, with Faisalabad 100km away across River Ravi. Sahiwal, previously known as Montgomery, is the nearest major city of Okara, while Kasur is situated to the east. This city is renowned for its agriculture-based economy and cotton mills. Okara district is home to the Pakistan Military dairy farms, which are famous for their cheese production and annual temperature ranging from 20 to 40°C.

Sample collection

A total of 200 samples were collected from pulmonary tuberculosis suspected patients visiting DHQ Okara from January to May 2023. Sputum and blood samples were collected from the suspected patients aseptically in sterile containers and proceeded immediately for ZiehlNeelsen staining and Gene XpertMTB/RIF.

Sample processing

Briefly, sputum samples were applied on the clean slide, smear was fixed by passing through the flame, Carbolfuchsin stain was applied on the slide for 5 minutes, and flame was provided at the bottom of slide until fumes were produced. The slide was washed with distilled water. Acid alcohol was applied on the slide for 1 minute to decolorize. Slide was washed and methylene blue was applied on slide for 2 minutes. After that slide was washed and air dried (Steingart, Ng et

al. 2006). On microscopic examination, samples showed red/ pink rod-shaped bacteria were consider positive. ZN positive sample were subjected for GeneXpert and assay was performed as per manufacturer's recommendations. Briefly, 1 mL of the sputum sample was mixed with given buffered reagent in the kit and incubated at room temperature for 10 min. Then 2 mL was transferred into cartridge and loaded into GeneXpert analyser. The results were displayed on digital screen after 2hr and recorded (Ajide, Igbabul and Kanyi 2019).

Ethical Issue

Sample and data collection from suspected patients were obtained according to guidelines of University ethical review committee. Verbal consent was taken from patients to include their sample in the study.

Statistical Analysis:

The results obtained from ZN staining and GeneXpert were statistically analyzed by using statistical package for social science software version 20.0. Descriptive and bivariate levels of data analysis were used. Means and standard deviation were computed for quantifiable variables. Relationship between quantitative variable was determined by using Chi-square test with 95% of CI and $P < 0.05$ (Kirkpatrick and Feeney 2013).

RESULTS

This study analysed a total of 200 samples. The study population consisted of 38.1% (77/200) of females and 68.1% (123/200) of males. The data was divided into different age groups less than 20 years to above 70 years. The mean age of the study population was 44.3 with standard deviation (SD) of 20.4 (Table 1).

Table 1: Descriptive analysis of quantifiable variables

Gender	**Frequency**	**Percentage**	
Female	77	38.5	
Male	123	61.5	
Age			
Minimum	Maximum	Mean	S. Deviation

5	99	44.3	20.4

The results indicate that prevalence of MTB was 52% (n=104/200) by AFB staining and 58.5% (n = 117/200) by GeneXpert (Fig. 1).

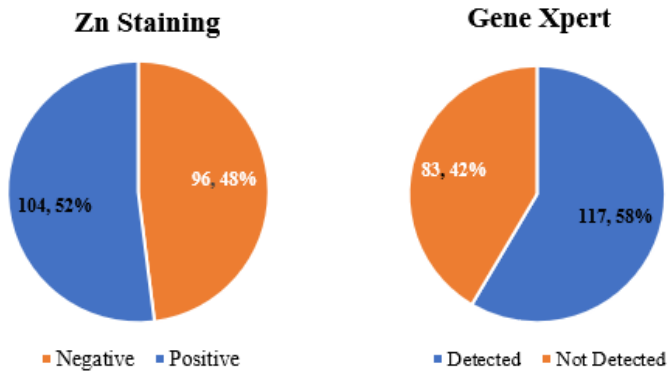

Fig. 1: Over all prevalence of rifampicin resistant *Mycobacterium Tuberculosis*

Comparison of GeneXpert and Zn staining showed that out of 117 detected samples by GeneXpert, 15 samples were those that was detected negative by Zn staining and out of 102 positive samples of Zn staining, 2 samples were not detected by GeneXpert as shown in Table 2.

Table 2: Bivariate analysis of quantifiable variables of RR-MTB

		ZN Staining		**Gene Xpert (MTB)**	
	Total Patients	**Negative**	**Positive N, (%)**	**Detected N, (%)**	**Not Detected**
	200	96	104 (52%)	117 (58.5)	83
Gender					
Female	77	37	40 (51.9)	45 (58.4)	32
Male	123	59	64 (52)	72 (58.5)	51
		P Value	0.991	**P Value**	0.989
		ZN Staining			
	MTB	Positive	Negative	Total	
	Detected	102	15	117	

	Not Detected	2	81	83	
	Total	104	96	200	P<0.001

Gender wise distribution showed that males and females were equally infected. Zn staining showed 51.9 %(n=40/77) prevalence in females and 52 %(n=64/123) in males. While the results of GeneXpert showed 58.4% (n=45/77) prevalence in females and 58.5% (n=72/123) in males.

In case of age wise distribution, the age group of 51 to 60 years was found highly susceptible for MTB as 66.7% (n=20/30) samples were positive through AFB smear staining and 63.3% (n=19/30) by GeneXpert as shown in Table 3.

Table 3: Prevalence of RR- MTB in pulmonary tuberculosis suspected patients based on Age

		Zn Staining		Gene Xpert (MTB)	
Age groups (years)	**Total Patients**	**Negative**	**Positive N, (%)**	**Detected N, (%)**	**Not Detected**
<20 Y	28	15	13 (46.4)	17 (60.7)	11
21-30 Y	36	22	14 (38.9)	20 (55.6)	16
31-40 Y	32	14	18 (56.3)	18 (56.3)	14
41-50 Y	34	17	17 (50)	20 (58.8)	14
51-60 Y	30	10	20 (66.7)	19 (63.3)	11
61-70 Y	20	10	10 (50)	11 (55)	9
Y 70>	20	8	12 (60)	12 (60)	8
		P Value	0.39	**P Value**	0.99

Then age group above 70 years had 60% (n = 12/20) positive results for both Zn staining and GeneXpert **(**Fig. 2).

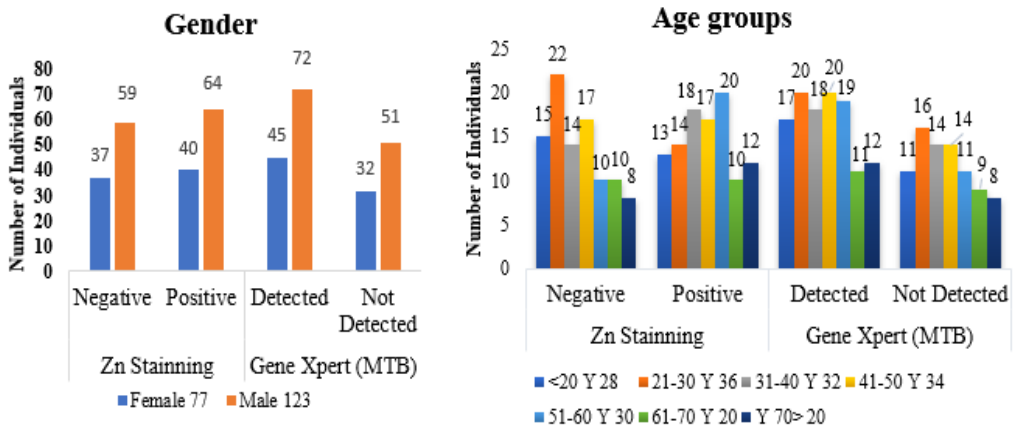

Fig. 2: Prevalence of *Mycobacterium tuberculosis* based on Gender and age

DISCUSSION

Tuberculosis is a chronic, inflammatory disease in humans caused by Mycobacterium tuberculosis. Tuberculosis can affect any part of the body, primarily it affects the lungs. Most of the tuberculosis (TB) cases was pulmonary and it is transmitted from one person to another by airborne bacterium droplets. A person becomes infected when they breathe in a few air drops containing *Mycobacterium tuberculosis* (Sharma and Sarkar, 2018). To the best of our knowledge, this was the first cross sectional study conducted to determine the prevalence of *Mycobacterium tuberculosis* in District Okara. Two diagnostic methods were used to find the presence of Mycobacterium tuberculosis, GeneXpert and Zn Staining. Our finding indicates 52% prevalence by Zn staining and 58.5% by GeneXpert. Approximately, 150,000 newly reported cases of TB in Pakistan are estimated to be linked to the malnutrition (WHO) (Organization and Staff 2013). The prevalence rate of MTB in Okara region is higher as compare to other regions of Pakistan either on provincial level or regional level (Khanzada et al., 2020). This finding of study was not aligned with other countries on higher prevalence basis (Organization and Staff 2013). In our finding, males and females were seemed to be equally infected with MTB (Table 2). The results of our findings correlate with the different gender base distribution studies in Pakistan as well as globally(Ullah et al., 2014; Tahseen et al. 2020). However, Chi square results showed no significant association between gender and MTB (P>0.05). The

higher prevalence of MTB was observed in this study among males and females may be attributed to their active lifestyles, which often hinders them from seeking health care particularly for condition like TB. TB symptoms such as persistent coughing may be mistaken for common respiratory issues. This delay in seeking medical attention can contribute to the progression from latent TB infection to active TB. Additionally, poor drug usage and lack of adherence to prescribed dosage further contribute to the development of drug resistance in TB cases.

Our results showed that age group of 51 to 60 years had higher prevalence of MTB followed by the age group above 70 years, While the age group less than 20 years also had higher prevalence. The results of the previous study also showed that age group of 40 to 60 years had higher prevalence of MTB as well as above 70 years also had higher prevalence (Ullah et al., 2020). A research study conducted in Mararaba also indicated that people with the age above 50 years are more likely to be infected with MTB (Ajide et al., 2019). The higher prevalence of MTB in elderly age groups may be due to lack of awareness about the spread and transmission of the disease. Moreover, Okara region lack sufficient health care resources to effectively diagnose and treat other communicable diseases such as HIV, HCV and HBV which can weaken the immune response and increases the susceptibility to MTB infection. Another contributing factor could be the practice of burning wood in rural areas, which can damage the lungs cell lining and impair the activity of macrophages, thereby increasing the risk of contracting the disease.

CONCLUSION

In this study a high prevalence of *Mycobacterium tuberculosis* in district Okara was reported. This highlights the need for the implication of effective control and preventative measurements such as early detection. Management along with awareness programs to reduce the disease burden in the district. There is also dire need of extensive study on factors responsible for such high burden of disease in the district.

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Conflict of interest

There is no conflict of interest among authors.

Authors' contribution

MH conceived and design the study, KG, SS, FM and SR collected the data, processed the sample, FM analysed the data, SR and SS drafted the manuscript, and MH edited the manuscript.

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