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DEMOGRAPHICS AND CRITICAL ANALYSIS OF SMEAR-POSITIVE TUBERCULOSIS IN-DISTRICT ABBOTTABAD, PAKISTAN: IMPLEMENTATIONS FOR FUTURE CHALLENGES

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Abstract

Tuberculosis is one of the prime reasons for disease and death worldwide. In terms of tuberculosis prevalence, Pakistan is ranked fifth globally. The World Health Organization estimates that 20,000 people develop tuberculosis every day, and 5,000 people die worldwide. Tuberculosis is caused by bacteria that spread through the air, just like a cold or flu. Common symptoms of active lung TB are cough with sputum and blood, chest pains, weakness, weight loss, fever, and night sweats. Many countries still rely on a long-used method called sputum smear microscopy to diagnose. Multidrug-resistant tuberculosis (MDR-TB) is a type of tuberculosis caused by bacteria that do not respond to isoniazid or rifampicin, the two most powerful first-line anti-TB medications available. It is treatable and curable by using second-line drugs. This cross-sectional study included sputum samples from 900 participants suspected of having tuberculosis. Out of 900 samples, 557 were males and 343 were females. The rate of occurrence of pulmonary tuberculosis was higher in males as compared to females, with 77 percent against 37 percent. It has been identified that the age group of 55 and above showed high smear-positive results while the 18-24 age group were more smear-negative results on testing. Therefore, the overall calculated TB prevalence was 13.7% in district Abbottabad. Serious measures should be taken by government to control TB in targeted areas.

Keywords: *Mycobacterium tuberculosis, Multi-drug resistance, Symptoms, Sputum smear positive, prevalence*

INTRODUCTION

There are 8 million new TB infections worldwide per year that bring about 2-3 million deaths, rendering TB the leading killer of all infectious diseases. Pakistan is among the top countries sharing the global burden of tuberculosis. The country stands in the fifth position amongst the highest incidence record countries for TB around the globe in Pakistan since 2014 by WHO. In the 17th and 18th centuries, *M. tuberculosis* was the cause of the "White Plague", which resulted in an almost 100% infection rate within the European population, and a 25% death rate. However, the bacillus causing tuberculosis, *M. tuberculosis*, wasn't identified and described until March 24, 1882, by Robert Koch. This pioneering tuberculosis research was facilitated by developing culture media to grow the organism and then demonstrate the mode of transmission of the disease (1). The bacteria that produce tuberculosis has affected approximately one-third of the worldwide population (TB). *Mycobacterium tuberculosis* is an aerobic infectious bacterium that causes respiratory infections. Two billion individuals are believed to be infected with non-eradicated intra-granulomatous tuberculosis (TB) bacilli as latent tuberculosis infection (LTBI), and one in ten is acquiring active TB over their life (2). Being unwell or lethargic, change in appetite and weight loss, shivering, fever, excessive sweating at night, persistent coughing takes about three weeks or more, and difficulty breathing are all possible symptoms. Through the bloodstream, tuberculosis can transmit to certain other regions of the body. This can cause spinal ache and joint problems; it could also cause meningitis in the brain; it can

affect waste filtering activities in the kidneys and liver, leading to blood in the urine; it also can decrease the heart's capacity to circulate blood, resulting in cardiac tamponade, a potentially deadly disease (3).

Symptoms may also include feeling sick or weak, loss of appetite and weight loss, chills, fever, and night sweats, a severe cough that lasts for three weeks or more, chest pain. TB can spread to other parts of the body through the bloodstream. There may be spinal pain and joint destruction; in the brain, it can lead to meningitis, in the liver and kidneys, it can impair the waste filtration functions and lead to blood in the urine; it can damage the heart's ability to pump blood, resulting in cardiac tamponade, a condition that can be fatal (4).

Droplet nuclei containing *Mycobacterium tuberculosis* must pass through structural obstacles, in a way to attain the alveolar spaces, ciliated respiratory epithelial cells and mucins must travel via the upper airways (5). *M. tuberculosis* is phagocytized by the alveolar macrophages when it enters the alveoli. For access into macrophages to occur, interactions with complement receptors, mannose receptors, and Fc receptors are involved. Various pattern recognition receptors (PRRs) on the macrophage's cell surface, phagosome, and cytoplasm identify distinct *Mycobacterium* TB compounds. Other immune cells that contribute to the early host response, in addition to neutrophils and dendritic cells, include mucosal-associated invariant T (MAIT) cells, CD1-restricted T lymphocytes, and natural killer (NK) cells, all of which participate in the inflammatory response (6). Depending on the conditions, the alveolar macrophage may destroy Mycobacteria via phagosome maturation, fusion with the lysosome, or autophagy. However, Mycobacteria may produce virulence factors that lead phagosome maturation to be retarded or phagosome disintegration. To persist inside macrophage phagosomes as delayed or inhibiting significant immune responses, *Mycobacterium tuberculosis* employs various strategies. Mycobacterial urease avoids acidification in the phagosome, limiting the efficiency of bactericidal enzymes in the bacteria. Apart from that, because the bacteria stay inside that phagosome during infection, it's doesn't immediately activate T-cell responses through the proteasome's pathway of antigen presentation. Superoxide dismutase, catalase, thioredoxin, and other antioxidants are abundantly secreted by the organism. Microbial antioxidants defend against oxidants produced by the host and reduce early immunological responses triggered by oxidants, such as macrophage activation and apoptosis (7).

The World Health Organization (WHO) developed a common TB control approach called "directly observed therapy, short-course" (DOTS) in 1993, in part because of this complicated and time-consuming medical therapy (9). A five-component TB control plan includes political commitment, direct smear microscopic identification, a method to assure medication supply, and standardized short-course medication with direct medical monitoring (10). The recommendation to use drug susceptibility testing (DST) for monitoring and guiding NTPs were made many years ago (11). Tuberculosis caused by bacteria resistant to isoniazid and rifampin, the two most effective first-line anti-tuberculosis medications, is known as multidrug-resistant tuberculosis (MDRTB). MDRTB is a severe public health problem that requires long-term therapy with more expensive and unnecessary drugs (12, 13). MDR-TB with additional resistance to any fluoroquinolone and one of the second-line injectable drugs kanamycin, amikacin, or capreomycin is extensively drug-resistant (XDR) TB (14, 15).

The major aims of TB therapy are to rehabilitate the particular patient and reduce the spread of *Mycobacterium tuberculosis* to the general population. The age-dependent use of anti-tuberculosis treatment varies according to national recommendations around the world.

Some major risk factors of TB are age, gender, socio-economic status and environment. Tuberculosis has the greatest prevalence and fatality rates in the 15-49 age group worldwide. In contrast, tuberculosis is more common in older people in Europe. In 2013, 1⁄2 a million people under the age newborn to 14 were diagnosed with tuberculosis (TB), with 80,000 of them dying as a result of the disease (16). The majority of notified tuberculosis cases are reported by men rather than women, according to research findings (17, 18), varying from 0.5 in Afghanistan to 3.0 in Viet Namand 1.0 in Pakistan (19, 20). Countries may differ in their epidemiology and access to and utilization of healthcare services associated with the national tuberculosis control program(NTP) (16). Studies have shown that the rate of tuberculosis cases in urban regions is higher than in rural areas, most likely due to the higher population density in urban areas and the greater

likelihood of immigration (20). Environmental variables linked to TB risk include genetic susceptibility, ethnic group, malnutrition, HIV infection, migration, high population, climate, alcoholism, smoking, and urbanization (21, 22).

A history of asthma, a family medical history of tuberculosis, single marital status, a poor education level, indoor air pollution, and immunosuppressive medicines have all been identified as key variables in the development of tuberculosis in several research (23)(24).

SOCIAL AND ECONOMIC IMPACT OF TB

The stereotype associated with tuberculosis has a wide range of social consequences. Many people avoid intimate contact with sick persons to prevent the risk of airborne transmission. Thus, the quality of life of infected individuals is significantly lower than that of non-infected individuals (25).The majority of the population in urban areas is aware of the current health care system for tuberculosis treatment (26). Tuberculosis is most prevalent in the world's poorest countries. These countries will suffer a loss of between $1 and $3 trillion over the next decade due to tuberculosis (27). According to the World Bank, some countries' gross domestic product (GDP) decreased by between 4% and 7% due to tuberculosis *(TB)* (28).

MATERIALS AND METHODS

This present study was a laboratory-based descriptive cross-sectional study. It was performed to offer a snapshot (one-point measurements) of tuberculosis prevalence (TB). All measurements for this design were made at one point in time to describe variables and their distribution patterns. It is the only design that gives the prevalence of TB. The design was used as the first step in laboratory experiments involving sputum analysis. This study was conducted over eight months, from April 2019 to November 2019, in the microbiology section of Ayyub Teaching Hospital Abbottabad. The study population comprised adult TB suspects (n=900) eighteen years and above. The hospital physicians did different medical examinations, including observing complex symptoms suggestive of TB such as fever, a cough that has lasted more than two weeks and is not responding to antibiotic therapy, night sweats, and weight loss are among symptoms that might occur. Different methods are used to diagnose tuberculosis, but the two main tests are sputum smear microscopy and chest X-ray.

SPUTUM COLLECTION AND PROCESSING

The investigation was presented to every TB suspect who visited a hospital care facility. Those who agreed to provide specimens for smeared microscopy examination are requested to use it. When patients were asked to provide a specimen, they were instructed to rinse their mouths twice with water. This assisted in removing food particles and any contaminating germs that may have been present in the mouth before presenting the specimen. To collect the substance, they are advised to take two long breaths, cough vigorously, and expectorate the contents into a sterile 50ml blue cap screw-capped vial. Sputum may be produced from deep within the lungs as a result of the procedure. Following the technique, after coughing out a large amount of TB sputum, TB patients were directed to maintain the sputum cup near their lips and softly throw into it. A total of nine hundred tuberculosis (TB) suspects provided sputum samples, collected under the direction of trained and experienced medical personnel. This study collected and examined three sputum specimens (spot, early morning, and spot). To accurately diagnose of tuberculosis in people who have been suspected of having it, at the regional laboratory, conventional methods were used to perform acid-fast (AFB) direct smear microscopy with Ziehl-Neelsen (ZN) staining on the first sputum specimen.

MICROSCOPIC EXAMINATION OF SPUTUM SPECIMENS

A smear was created for analysis using a clean, unused glass slide tagged with a laboratory number. There was just one smear on each slide, and the sample was collected using either a swab or a loop to ensure that it was representative of the entire sample. The fixing process was carried out with mild heating just after drying. The staining procedure was subjected to smeared slides placed on staining rack. To minimize extra material, transfer from one smear to the next and solutions from flowing away from slide surface, a

finger-thick spacing was left between borders of a slide. A 1% carbon fuchsin solution from Ziehl-Neelsen (ZN) was used to cover the whole slide. Each slide was gradually heated until steam began to form. Intermittent heat was used to sustain steaming for 5 minutes. Each slide was meticulously washed in a gentle spray of water until all traces of free stain had been eliminated. Tipping the slide allowed any excess rinsing water to flow away from it. After three minutes, the slide was sprayed with the decolorizer solution to remove the color (2 per cent sulphuric acid). Gentle cleaning with water was performed on the slide, and any remaining water was carefully emptied from it. Cotton soaked in a decolorizer was wiped off the rear of the slide, followed by a rinse with water and the tilting of the slide to drain any surplus water. For 60 seconds, the slide was soaked with 0.1 per cent methylene blue and counterstained. Afterwards, the slide was thoroughly cleaned, and any surplus water was dried off by placing the slide under a mild stream of flowing tap water. The slide was then left to dry on a holding block for many hours (36). After staining using the ZN technique, acid-fast bacilli (AFB) were examined in sputum smears to see whether they were found. It was determined that the degree of ZN smear-positive was 1+ when there were 10-100 AFBs per 100 fields, 2+ when there were 1-10 AFBs per field (50 fields), and 3+ when there were >10 AFBs per field (20 fields). When there were fewer than ten AFBs per 100 fields, the actual number of AFBs was specified. If at least one sample tested positive for ZN, for the rest of the examination, the suspect was considered to be ZN smear-positive.

RESULTS

GENDER DISTRIBUTION AMONG TOTAL SPECIMENS

In this study we analyzed n=557/900 (62%) male and n=343/900 (38%) female samples. The overall prevalence of tuberculosis suspects observed in this study were 133/900 (14.70%), as shown in Fig. 1.

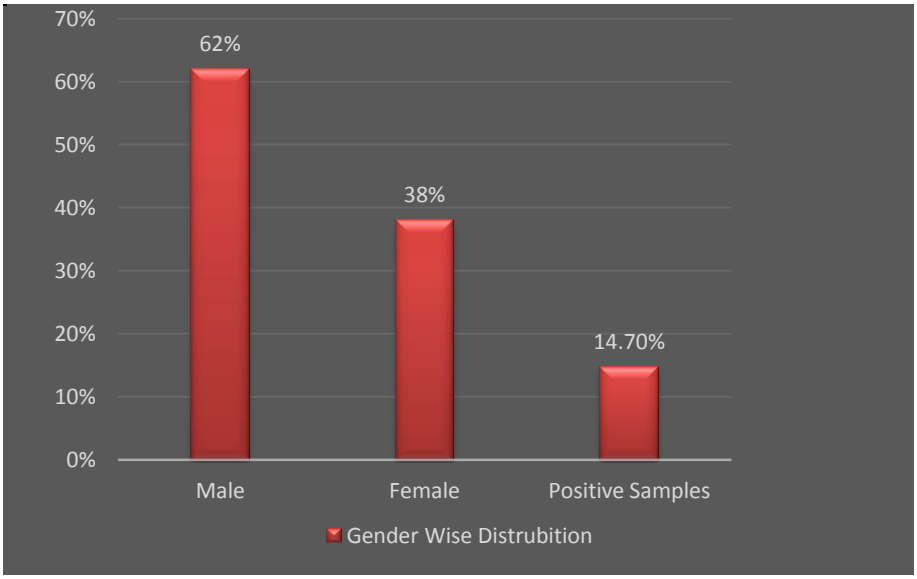

Fig. 1. Distribution of male and female participants included in the study; and prevalence of positive samples

ASSOCIATION OF GENDER WITH SITE OF TUBERCULOSIS

Tuberculosis is categorized into two groups on the basis of site of occurrence, i.e., pulmonary tuberculosis and extra pulmonary tuberculosis. From the entire statistics, it was found that the rate of occurrence of pulmonary tuberculosis was higher in males than females. While extra pulmonary tuberculosis was also prevalent in men at a significant rate.

According to the findings, 37 of the 343 females had pulmonary tuberculosis and rest of the subjects had extra pulmonary tuberculosis. While 13 of the 557 males tested positive for extra pulmonary tuberculosis, while the remaining subjects had pulmonary tuberculosis. According to the statistics, males had a greater overall ratio of extra pulmonary TB cases than females, and males appeared to have a higher incidence of pulmonary tuberculosis than females as shown in Table I.

Table I. Association of gender with disease site of tuberculosis

Gender	Total	Pulmonary TB	Extra pulmonary TB
Male	557	77 (13.82 %)	13 (2.33)
Female	343	37 (10.78%)	6 (1.74 %)

There are multiple reasons responsible for their prevalence, i.e., there are more chances of contracting extra pulmonary tuberculosis due to specific comorbidities in males. i.e., diabetes, cancer HIV, extreme age and due to the compromised immune system; whereas females are less co-infected with other diseases which are described earlier and the other reason is maybe the earlier diagnosis of tuberculosis.

ASSOCIATION OF GENDER WITH SMEAR RESULTS

A small amount of specimen (sputum) was mounted on the slide, either directly or processed for the microscopic examination. The presence of *Mycobacterium tuberculosis* in the sputum findings plays a significant role in the diagnosis of TB. The presence of *Mycobacterium tuberculosis* in specimen showed as smear-positive while the absence of *Mycobacterium tuberculosis* in the specimen results as smear-negative. Out of 900 cases, only 133 cases showed smear-positive outcomes while the remaining cases showed smear-negative outcomes. Only 81 males showed smear-positive outcomes while 476 males showed smear-negative outcomes. On the other hand, 43 females showed smear-positive results while 300 females showed smear-negative results.

As a result, the ratio of smear positive males to smear positive females was observed to be greater. (Fig. 1). There are two main causes for this: first, patients may have another co-infection, causing their immune system to become compromised, allowing *Mycobacterium tuberculosis* to escape the host defense system. The second thing that is responsible for it is environmental factors, i.e., smoking, air pollution, alcoholism, or the resources available for the diagnosis of tuberculosis etc.

ASSOCIATION OF AGE WITH SMEAR RESULTS

The clinical specimen (sputum) was studied on a glass slide for microscopic inspection, and it is the safest microscopic test for determining the presence of *Mycobacterium tuberculosis* in the specimen. To define those age groups that have smear-positive and smear-negative test results, the full data was split into distinct categories depending on age. As observed in the bar chart, the 55 and above age group had more smear-positive test findings, whereas the 18-24 age group had more smear-negative test results (Fig. 2).

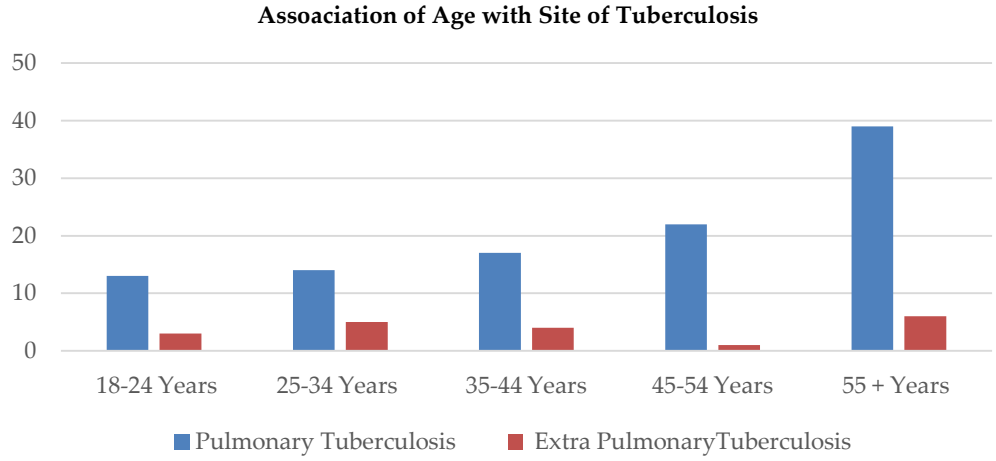

Fig. 2. An association between age and the type of tuberculosis

Fig. 2 depicts a strong link between age and the occurrence of tuberculosis; as shown in the bars, subjects in the age group of 55 and above were more susceptible to acquire TB, whereas those in the age groups of 45-54 years were at a higher risk of infection, because the subjects in these age groups were more vulnerable to disease due to environmental exposure (workplace, community, home, friends, etc.) or sometimes due to a suppressed immune system. While in age group 35–44 years, age group 25-34 years and above 18-24 years' cases were reported (Fig. 2)

DISCUSSION

The total number of estimated samples were 900, including 343 females and 557 males. Based on the total number of samples, it was determined that those aged 25-34 had a high chance of infection, while those aged 55 and higher have a low chances of risk of TB. because the subjects in these age groups were more vulnerable to disease due to environmental exposure (workplace, community, home, friends, etc.) While from the total calculated samples, males have a strong association with the smear-positive test while females have a weak association with the smear-positive test.

Tuberculosis is a social ailment that has medical consequences. Drug-resistant TB is frequent in Pakistan, although there is no complete data due to a shortage of diagnostic institutions. Most drug-resistant TB patients go undiagnosed because few laboratories perform AFB culture and drug sensitivity testing. Second, diagnostic laboratories are only available in major cities. Simultaneously, the bulk of these tuberculosis patients live in rural locations and so lack access to diagnostic facilities to conduct the necessary testing. As a result, efficient and accurate assays for detecting drug resistance are required.

People with a lower socioeconomic level (SES) are more likely to be exposed to congested, poorly ventilated spaces, as well as to have access to less healthful cooking and practice facilities (29, 30). Pakistan has one of the highest rates of tuberculosis in the world. Nearly half of all new TB infections occur in China, India, Bangladesh, Indonesia, and Pakistan (around 48 percent) (31, 32).

Males have a greater TB incidence rate than females over the world; males are 1.8 times more likely to have tuberculosis than females (33). Males had a greater incidence rate than females in our research. In Balochistan, Pakistan, males 57 percent and females 43 percent obtained comparable results (34) even detected by spoligotyping (35). Two-thirds of those diagnosed with tuberculosis do not finish their treatment cycle, according to study conducted in Lahore, Pakistan. Overcrowding in the home can expose people to infectious respiratory infections and raise the risk of transmission. People's misconceptions regarding the disease's impacts are the cause of this high frequency. Tobacco smoke, biomass fuel smoke, and coal fire are all linked to tuberculosis. According to an Indian research, biomass fuel exposure is an indigenous risk factor for pulmonary TB (36). Poor housing conditions, overcrowding, inadequate ventilation, and poor cleanliness are the primary reasons of tuberculosis spread, according to our research. Health-by-health department performance must be immediately addressed, and public-private partnership must be reinforced.

In our study out of 900 cases, only 133 cases showed smear-positive outcomes while the remaining cases showed smear-negative outcomes. Only 81 males showed smear-positive outcomes while 476 males showed negative outcomes. On the other hand, 43 females showed smear-positive results while 300 females showed smear-negative results.

CONCLUSION

It was observed that the rate of pulmonary tuberculosis was higher as in the case of extra pulmonary tuberculosis as well as pulmonary tuberculosis gave more smear-positive results on testing as compared to extra pulmonary tuberculosis. It has a high burden of disease nowadays, and it is necessary to take steps to eradicate the *Mycobacterium tuberculosis* from the environment. There is a need to focus on the risk factors responsible for recurrent tuberculosis to avoid mortality and morbidity rate. The development of drug-resistant tuberculosis is one of the major challenges faced by the tuberculosis Management Programs; knowing about the disease episode, treatment regimens, and the treatment duration will help us deal with the disease with proper regimens to cure as earlier as possible. Lifestyle medications, early diagnosis, well-equipped techniques, and proper treatment regimens are the best future perspective to monitor and reduce disease frequency. Prediction of the frequency and the treatment outcomes of *Mycobacterium tuberculosis* play an important role in the countries where the prevalence rate is very high. This research will be helpful in the future, especially for those working with the strategies to eradicate *Mycobacterium tuberculosis* from the

environment. It will help determine the occurrence rate of tuberculosis in patients who are more susceptible to *Mycobacterium tuberculosis.*

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Conflicts of interest:

The authors have no conflicts of interest.

References:

1. Cambau E, Drancourt M. Steps towards the discovery of *Mycobacterium tuberculosis* by Robert Koch, 1882. Clinical Microbiology and Infection. 2014;20(3):196-201.
2. Mancuso JD, Tobler SK, Keep LW. Pseudoepidemics of tuberculin skin test conversions in theUS Army after recent deployments. American journal of respiratory and critical care medicine. 2008;177(11):1285-9.
3. 3.Mangura BT, Reichman LB. Periodic chest radiography: unnecessary, expensive, but still pervasive. The Lancet. 1999;353(9149):319-20.
4. Cote A. Epidemiology of Tuberculosis. 2014.
5. Fitzgerald D, Sterling TR, Haas DW. Mycobacterium tuberculosis. Principles and practice of infectious diseases, 6th ed. Philadelphia. 2005;2852-2886.
6. Schlesinger LS, Azad AK, Torrelles JB, Roberts E, Vergne I, Deretic V. Determinants of phagocytosis, phagosome biogenesis and autophagy for Mycobacterium tuberculosis. Handbook of Tuberculosis: immunology and cell biology. 2008;1:22.
7. Stanley SA, Cox JS. Host–pathogen interactions during Mycobacterium tuberculosis infections. Pathogenesis of Mycobacterium tuberculosis and its interaction with the host organism. 2013:211-41.
8. Organization WH, Initiative ST. Treatment of Tuberculosis: guidelines: World Health Organization;2010.
9. Davies GR, Connolly C, Sturm AW, McAdam KP, Wilkinson D. Twice-weekly, directly observed treatment for HIV-infected and uninfected tuberculosis patients: cohort study in rural South Africa. Aids. 1999;13(7):811-7.
10. Kim DY, Ridzon R, Giles B, Mireles T, Garrity K, Hathcock AL, Crowder D, Jackson R, Taylor Z. A no-name tuberculosis tracking system. American journal of public health. 2003;93(10):1637-9.
11. Cohn DL, Bustreo F, Raviglione MC. Drug-resistant Tuberculosis: review of the worldwide situation and the WHO/IUATLD global surveillance project. Clinical infectious diseases. 1997;24(Supplement_1):S121-S30.
12. Organization WH. Anti-tuberculosis drug resistance in the world. World Health Organization; 2000.
13. Mukherjee JS, Rich ML, Socci AR, Joseph JK, Virú FA, Shin SS, Furin JJ, Becerra MC, Barry DJ, Kim JY, Bayona J. Programmes and principles in treatment of multidrug-resistant tuberculosis. The Lancet. 2004;363(9407):474-81.
14. Gandhi NR, Nunn P, Dheda K, Schaaf HS, Zignol M, Van Soolingen D, et al. Multidrug-resistant and extensively drug-resistant tuberculosis: a threat to global control of Tuberculosis. The Lancet. 2010;375(9728):1830-43.
15. Caminero JA, Sotgiu G, Zumla A, Migliori GB. Best drug treatment for multidrug-resistant and extensively drug-resistant Tuberculosis. The Lancet infectious diseases. 2010;10(9):621-9.
16. Organization WH. Digital health for the End TB Strategy: an agenda for action. World Health Organization; 2015.
17. Ottmani S, Uplekar M. Gender and TB: pointers from routine records and reports [Editorial][Special section on gender and TB]. The International Journal of Tuberculosis and Lung Disease. 2008;12(7):827-8.
18. Salim H, Declercq E, Van Deun A, Saki K. Gender differences in Tuberculosis: a prevalencesurvey done in Bangladesh. The International Journal of Tuberculosis and Lung Disease. 2004;8(8):952-7.
19. Sabawoon W, Sato H. Sex difference in Tuberculosis in Afghanistan: a national cohort study. Mycobac Dis. 2012;2(3):155-60.
20. De Vries G, Van Hest N, Baars H, Šebek M, Richardus JH. Factors associated with the high tuberculosis case rate in an urban area. The International journal of Tuberculosis and lung disease. 2010;14(7):859-65.

21. Lienhardt C. From exposure to disease: the role of environmental factors in susceptibility to anddevelopment of Tuberculosis. Epidemiologic reviews. 2001;23(2):288-301.
22. Sun W, Gong J, Zhou J, Zhao Y, Tan J, Ibrahim AN, Zhou Y. A spatial, social and environmental study of Tuberculosis in China using statistical and GIS technology. International journal of environmental research and public health. 2015;12(2):1425-48.
23. Shaviya N, Budambula V, Webale MK, Were T. Circulating interferon-gamma levels are associated with low body weight in newly diagnosed Kenyan non-substance using tuberculosis individuals. Interdisciplinary perspectives on infectious diseases. 2016;2016.
24. Shetty N, Shemko M, Vaz M, D'souza G. An epidemiological evaluation of risk factors for Tuberculosis in South India: a matched case control study. The International Journal of Tuberculosis and Lung Disease. 2006;10(1):80-6.
25. Dhuria M, Sharma N, Ingle G. Impact of Tuberculosis on the quality of life. Indian journal of community medicine: official publication of Indian Association of Preventive & Social Medicine. 2008;33(1):58.
26. Kilale A, Mushi A, Lema L, Kunda J, Mukasi C, Mwaseba D, Range NS, Mfinanga GS. Perceptions of Tuberculosis and treatment seeking behaviour in Ilala and Kinondoni Municipalities in Tanzania. Tanzania Journal of Health Research. 2008;10(2):89-94.
27. Knight GM, Griffiths UK, Sumner T, Laurence YV, Gheorghe A, Vassall A, Glaziou P, White RG. Impact and cost-effectiveness of new tuberculosis vaccines in low-and middle-income countries. Proceedings of the National Academy of Sciences. 2014;111(43):15520-5.
28. Raviglione MC. The TB epidemic from 1992 to 2002. Tuberculosis. 2003;83(1-3):4-14.
29. Srivastava K, Kant S, Verma A. Role of environmental factors in transmission of Tuberculosis. Dynamics of Human Health. 2015;2(4):12.
30. Hameed T, Yousaf M, Abbas F, Rafiq A, Khan A, Iqbal Q, Ali MM, Khan S, Rauf A. Hematological Study on Pulmonary Tuberculosis (PTB) Predominance in Quetta. Pak-Euro Journal of Medical and Life Sciences. 2019;2(2):37-40.
31. Zignol M, Gemert Wv, Falzon D, Sismanidis C, Glaziou P, Floyd K, Raviglione M. Surveillance of anti-tuberculosis drug resistance in the world: an updated analysis, 2007-2010. Bulletin of the world Health Organization. 2012;90:111-9.
32. Khurram M, Khaar HTB, Fahim M. Multidrug-resistant tuberculosis in Rawalpindi, Pakistan. The Journal of Infection in Developing Countries. 2012;6(01):29-32.
33. Kyu HH, Maddison ER, Henry NJ, Mumford JE, Barber R, Shields C, Brown JC, Nguyen G, Carter A, Wolock TM, Wang H. The global burden of Tuberculosis: results from the Global Burden of Disease Study 2015. The Lancet Infectious Diseases. 2018;18(3):261-84.
34. Ali F, Shafee M, Akbar A, Khan N, Khetran MA, Rahman F, Mengal MA. Frequency of Pulmonary Tuberculosis in Afghan assylum seekers dwelling in Balochistan, Pakistan. Rawal Medical Journal. 2018;43(4):577-80.
35. Shafee M, Abbas F, Tanveer Z, Whitelaw A, Tow LA, Ashraf M, Ahmad I, Patching SG, Jabbar A, Akbar A, Mustafa MZ. Predominance of Central Asian strain (ST 26) in Mycobacterium tuberculosis isolates from Balochistan by spoligotyping. Journal of Infection in Developing Countries. 2019; 13(7):619-625.
36. O'grady J, Maeurer M, Atun R, Abubakar I, Mwaba P, Bates M, Kapata N, Ferrara G, Hoelscher M, Zumla A. Tuberculosis in prisons: anatomy of global neglect. European Respiratory Journal. 2011;38(4):752-4.