Comparative Sero-Epidemiological Prevalence of TB with Acid Fast *Bacilli sputum* Positive Cases in TB Suspects of Lower Dir

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Abstract

Background: Tuberculosis is an infection of lungs accompanied by coughing more than two weeks, fever, and night sweat, loss of appetite, weight loss and anorexia. Approximately 33% of the entire world population has infected the incidence of TB is 9 lac new cases each year with mortality rate of 1.3 million. There are 22 high risk burden countries accounting for nearly 80% entire TB of the globe.

Materials and Methods: A cross sectional investigation was carried out to analyze patients by both immune chromatographic test and direct sputum smear microscopy. Patients were asked through a questionnaire mentioning the age, name, gender, location, and symptoms of TB. Thrusfield formula was used from veterinary microbiology to calculate the sample number. So, with 95% confidence interval, 5% absolute precision, I analyzed only 324 subjects of interest

Results: The entire positivity rate was 25% in which 79.5% was the rate of positivity by ICT and 20.5% by sputum smear microscopy. Higher positivity by Age range 20-40 showed 40.9% by ICT and by sputum it was found in age group 1-20 years 17.5%. Based on gender, higher positivity rate was found by both tests in females as compared to males. Females were 31.8% positive by ICT and 10% by sputum test. Males were 22.8% positive by ICT and 5% by sputum test. Marital status based higher positivity by ICT was found in married which is 29.11% while positivity in unmarried which is 12.5%.

Conclusion: Tuberculosis is still a prevalent disease in lower Dir Khyber Pukhtunkhwa province of Pakistan. The more positivity rate has found in females as compared to males. It requires further sophisticated investigation to acquire a more accurate result in case of tuberculosis prevalence in lower Dir region of KP province of Pakistan. As the DOTS strategy states, Directly observed treatment and short course is a better way to early case detection and treatment otherwise the disease can become more exaggerated in short period of time because the bacteria is an airborne pathogen so it can freely transmit to the lungs.

Introduction

TB pathogen is thought to be present from 10,000 to 25000 years ago and has transferred to humans. Some hard tissues like bone can preserve the specie up to 4000 years, Denmark, and countries in the Middle East also designates that TB was found throughout the world up to 4,000 years ago [1].The presence of mycobacteria is evident from 1.5 million years ago in Jurassic Era [2]. Egypt reports the presence of tuberculosis from 5000 years, 2300 years ago from china and 3300 years ago from India. The evidence of tuberculosis from spinal cord have been identified sense 3400 BC [3]. But now these bacteria are ubiquitous except the polar regions of the globe. Bacillus leprea is the first identified and known specie of mycobacteria in 1875 [4]. World health organization has called for an emergent inquiry of tuberculosis in all over the world sense 1992 [3]. There are total 22 countries in the world who are under the susceptible risk burden, among them Pakistan is on 6th position. 80 to 100/100,000 is the incident rate of infectious tuberculosis in Pakistan, this rate if multiplied with the total population so it gives of figure of 100,000 to 120,000 lakh of people infected with tuberculosis in Pakistan. This rate is even more high in northern area of Pakistan nearly 554/100,000 stated [5]. These twenty-two countries accounts for about 80% of entire world tuberculosis [6].Tuberculosis is

one of the major devastating pathogen which has infected 2 billion people with increase of 9 million cases each year and 1.5 million deaths every year. Poverty, malnutrition, displaced, are the main socio-epidemiological factors which account for wide spreads dispersal of TB particularly in Africa where the case is associated with HIV infection mostly [7]. TB is the second most causative agent of death, it is due to multiple cellular structure like glycogen, poly methylated polysaccharides and glucosylglycerate which resist nitrogen stress and osmotic stress. These glycoconjugates are present in their cell wall which serves as barrier against different biocides and render protection, rigidity, and unique staining characteristic to cell [Acid Fast Stain] [8]. The aerosols droplet nuclei contain 1 to 3 bacillus particles which provide sufficient invading capability to penetrate the alveoli with particle size less than 5 micrometer [9] and 1 or up to 10 micrometers in size according to [10].The tubercle bacilli first encountered by the alveolar macrophages which resist the macrophages action and acquires an access to the blood flow and lymphatic system of the human thus depicting a successful infection [11,12]. Pulmonary tuberculosis is accompanied by different signs and symptoms like fever, cough which exceed two weeks or more, hemoptysis, night sweat, anorexia, and weight loss [13]. A 2012 investigation in northern part of Pakistan showing mostly females were infected, while in eastern part of Pakistan there is a high prevalence found in males. 571958 were the entire study participants who were confirm positive cases for the presence of AFB in their sputum [14]. Investigation for Lower Dir location of Chakdara Pakistan which is under the susceptible threat of tuberculosis spread for several years. High prevalence found in females as 60% more than males. There were only 35 cases diagnosed in which 19 were positive for AFB and 16 were negative. The range of 41 up to 60 years old has the highest positivity rate approximately 75% [5]. A subsequent carried for three months study based on the prevalence of tuberculosis in lower Dir region in 2012. This study was based on primary data which is comprised of 340 suspected persons in 5 centers out to total eight centers in which there were 118 are sputum positive and 222 sputum negative. 38.80% were males and 62.20% are female. Laal Qilla, one of the 5 centers has shown the highest number of cases positive [15]. Another study has conducted from 2011 to 2014 based on the prevalence of tuberculosis in District Dargae and Malakand which is near to my study location. This study was a retrospective based on registered cases in the TB centers. Number of subjects was 736. There were 40% sputum positive and 15.63% sputum negative. As the previous study has the highest cases in females, in this study also the females were more positive, 57.63% females while 42.33% males [16]. The aim of this research investigation was to find an accurate and precise rate of tuberculosis infection in the area because this area lacking sufficient information regarding TB infection as I have mentioned only four relevant articles. Trustworthy and applicable research can help to advance tuberculosis control worldwide. In current years, a variety of organizations have assessed research requirements and planned priorities for the control of TB worldwide.

Materials and Methods

Study location

The entire research investigation has carried out in lower Dir Khyber Pukhtunkhwa of Pakistan. Lower Dir is the north-west of Khyber Pukhtunkhwa of Pakistan with an entire area of 1580 square kilometer. The climate is very temperate in summer and winter nearly -8°C in extreme winter like in December and January. The current population of lower Dir exceeds 12 lacks with an average increase of 3.37% in oneyear; it was 12 lacks in 2015. Health status of the region is concerned with the availability of resources and health practices but here is no proper organized article which could be helpful to render us a relevant data **(Table 1)**.

Table 1:. Combined prevalence of tuberculosis in lower Dir. through sputum microscopy and immune-chromatographic test.

Total samples No:	Positive cases	Negative cases
324	81	243
Percentage %	25%	75%

Suspects

The entire research is based on those people who are diagnosed as suspects of tuberculosis. All the patients were diagnosed in Ouch TB center and District head quarter hospital Timergarah [DHQ] through physical examination by OPD setting health worker Dr. Zabihullah Shinwari. According to WHO guidelines, suspect is a person who coughs at least two weeks or more than two weeks up to months with high fever **(Table 2)**.

Table 2: Individual AFB results.

Total number of samples	Positive cases	Negative cases
249	69	180
Percentage	27.71%	72.28%

Sample size

Sample size determination was done according to formula from veterinary microbiology which renders a possible sample size to be collected according to the previous data present. As the prevalence rate of tuberculosis was 34.71% according to (Ahmad and Ahmad 2013) in lower Dir, I will need approximately 348 samples according to the formula, Thrusfield, from veterinary microbiology

n=1.962 × Pexp (1-Pexp)

d2,(Thrusfield, 2007)

Where n=sample size

Pexp=Expected prevalence

d2=Desired precision

So, with 95% confidence interval, 5% absolute precision and 34.5% expected prevalence, there will be 348 samples required for this study but unfortunately due to shortage of time, I collected only 324 samples.

Sputum collection

Every patient referred by the Doctor was advised to collect the sputum in sputum collection box. A special plastic cup labeled with the name of patient was given to the suspect. Every patient individually informed about sputum collection procedure by the health worker. Spot sputum collection was preferred as it supervises the suspect or patient to expectorate the sputum in the presence of lab technician or health worker. Before the sputum collection each sputum box was named with marker. Children were unable to expectorate sputum so they were only diagnosed with ICT.

Centrifugation of the sputum

Once the sputum collected 2 ml of the sputum taken from the box and mixed with 4% NAOH in a 2 ml eppendorf tube. It was kept for 20 minutes of time and then added phosphate buffer saline. The tubes were then kept in centrifuge for 15 minutes at 3000 rpm in centrifuge machine. After centrifugation, the pellet mixed again with phosphate buffer saline to become ready for smearing.

Sputum examination

The sputum was stained with Ziehlneelsen and then made a 2 mm smear on the slide. The glass slide was then kept under the light microscope for the examination. The smear was observed with 100× oil immersion lens. Approximately 15 fields were observed in each examination either for the presence or absence of AFB **(Table 3)**.

Table 3: Total cases.

Total number of cases	Positive cases	Negative cases
268	20	248
Percentage	0.0746	0.9253

Blood collection and ICT

All the patients were informed about the study. Informed consent was taken from all the patients. The puncture sites were disinfected with the 70% and a tourniquet was built on the upper portion of the hand. The blood was sucked from all the patients including males and females but children as having small unobservable veins, so they were subjected to direct cut on the finger. The blood was then centrifuged at 3000 rpm for 5 to separate the serum. The serum obtained was then poured through the help micro pipette onto the rapid test cassette ICT strip. All procedures followed according to **(Table 4)**.

Table 4: Based on age groups.

Age group [years]	SampleSize ICT + Sputum	Positive cases ICT + sputum	%age of +ve ICT	%age of +ve Sputum	Chi-square	P-value
20-Jan	87+40	24+7	27.40%	17.50%	AFB	AFB
21 - 40	66+96	27+9	40.90%	9.37%	68.26	0.146
41 - 60	53+76	10+2	18.66%	2.63%		
61 - 80	39+51	7+2	17.94%	3.92%		
80 - 100	4+0	1+0	25%		ICT	ICT
Above 100	1+0	0+0			75.27	0.2

Results and Discussion

Combined prevalence of tuberculosis in lower Dir. through sputum microscopy and immune-chromatographic test **(Table 5)**.

Table 5: Prevalence based on gender.

Gender	Positive ICT + AFB	Negative	Positive by % ICT + AFB	Chi - square	P- value
Male	27 + 6	91+112	22.88% +5.04%	ICT 2.49	ICT 0.115
Female	42 + 14	90+126	31.81% + 10%	AFB 4.8	AFB 0.091

There were entirely 324 cases of suspected TB examined from different locations and sub-locations in Ouch TB center and District head quarter hospital Timergarah. All the patients were examined through sputum smear microscopy by acid fast staining and immune-chromatographic test cassette except the children under 15 who cannot expectorate the sputum. The combined result shows a higher rate of positive cases as compared separate diagnostic test either ICT or Sputum smear microscopy. There are 75% negativity rate while 25% positivity rate **(Table 6)**.

Table 6: Prevalence of positive cases based on marital status.

Marital status	No. of samples ICT + AFB	Positive cases ICT + AFB	% of positive cases ICT + AFB	Chi-square	P-value
Married	158 + 220	46 + 14	29.11% + 6.36%	ICT 323.55	ICT 0
Unmarried	91 + 48	23 + 6	25.27% + 12.5%	AFB 2.149	AFB 0.143

Combined prevalence of Mycobacterium tuberculosis by IgG and IgM rapid test cassette and sputum smear microscopy based on age.

Out of 324 patients, I tested 194 patients with both the diagnostics tests as the sputum smear microscopy and immune chromatographic test. Higher positivity rate was found in age group of 1 up to 20 which is 48.48% while age group of 61-80 there were 17.95% positivity rate. The p-value is non-significant because both tests have not depicted same result of positivity and negativity for everyone **(Table 7)**.

Table 7: Positivity rate based on area.

Main Location	Sub-Areas	Sample Size		+ve cases		%age + ve cases		Chi-square	P-value
		AFB		AFB		AFB			
		ICT		ICT		ICT			
Lower Dir	Area-01	63		4		6.34%			
	Chakdara	95		28		29.40%			
Lower Dir	Area-02	32		3		9.37%			
	Taalash	35		13		37.10%			
Lower Dir	Area-03	48		5		10.40%		ICT	ICT
	Timergara	34		8		23.50%		214.32	0
Lower Dir	Area-04	23		2		8.65%		AFB	AFB
	khal	17		6		35.20%		224.47	0
Lower Dir	Area-05	20		0		0%			
	Maidan	12		3		25%			
Lower Dir	Area-06	28		3		10.70%			
	Munda	12		2		16.60%			

Discussion

Tuberculosis is the word 2nd most cause of death. The incidence of TB is 9 million new cases a year with average death rate of 1.5 million people. The bacteria are so difficult to combat because it resides in air droplet nuclei. 22 countries of the world are high burden countries which accounts for about 80% of the world entire TB burden in which Pakistan is on 6th position. The annual incidence of TB in Pakistan is 100,000 to 120,000. The current study carried out in lower Dir KP Province of Pakistan to find out the rate of positive cases through immune chromatographic test and sputum smear microscopy in those people who are suspects of TB. There were totally 324 cases who were suspect to be TB positive were diagnosed by means of sputum smear microscopy and immune chromatographic tests. These 324 cases were from 3 different locations including Lower Dir, Upper Dir and Bajaur region. The cases from upper Dir were 43 and from Bajaur agency there were total 17 cases while the rest of cases were from lower Dir regions. Lower Dir subdivided in 5 main areas naming Chakdarra, Talash, Timergarah, Khal, Maidan, and Munda. As the previous study carried by, the prevalence of TB in lower Dir was 34.17% through case finding by questionnaire so this current study shows a tremendous difference with the previous investigation in case of smear microscopy, there are 27% positivity by ICT and 7.4% positivity by sputum smear microscopy in this study. Based on age groups this study has shown higher positivity in same range of age as shown by in

2012. The research investigation was composed of two tests, ICT, and sputum smear microscopy and four variables including age, gender, marital status, and area. All the result has scrutinized based on these four variables in one way or another. Out of 324 cases, only 194 cases were those who tested with both the tests ICT and sputum while the rest 130 cases were tested with only one test either ICT or sputum smear microscopy. The overall rate of positive cases in 324 cases is 25% while 75% is rate of negativity. Gender or sex based interpretation of results shows that there was high positivity rate in females 31.81% by ICT test while 10% by sputum smear microscopy as compared to the males in which the positivity rate by ICT was 22.8% while by sputum smear examination the positivity rate was 4.6%. Marital status-based interpretation of results revealed that the rate of positivity by immune chromatographic test or ICT was high in married nearly 29.11% and 25.27% in unmarried. Unlike the ICT, the positivity by sputum smear examination was higher in unmarried 12.5% while in married this rate was 6.36%. P-value in 10% of the result shows non-significant because there are different number of cases in married and unmarried categories. If we combine both the results based on marital status so there is 23.42% positivity in married and 28.43% positivity rate in unmarried cases. The overall result is non-significant in 10% of the result according to the P-value 0.334. The prevalence of TB was divided into 3 main locations, Lower Dir, Upper Dir and Bajaur region. The were 43 cases from upper Dir and 17 from Bajaur and the rest 264 from lower Dir region. In lower Dir all the cases subdivided by 5 areas in which the highest positivity was shown by sputum in Area 3 [10.41%] and Area 6 [10.71% while no positivity in Area 5. The result for ICT in these areas was high in Area 2 [37.29%] while low positivity in Area 6 [16.66%]. Both the results are highly significant in 1% of the result according to the P-value 0.000. In Area 1, age group 1-20 shows higher positivity rate which is 7.5% by sputum smear microscopy and 13.9% by immune chromatographic test. The lowest positivity rate in Area 1 has shown by age group 60-80 by immune chromatographic test it is 1.9% while on sputum smear examination it is 7.6%. In Area 2, the highest positivity rate by sputum smear examination has shown by age group 1-20 which is 2.5% while 4.6% positivity has shown by ICT in age group 20-40. The lower positivity in Area 2 by ICT has shown by age group 60-80 [2.5%] and based on sputum, the lower positivity depicted in age group 20-40 in Area 2. Area 3 has high positive rate on ICT in age group 20-40 which is 4.1% while based on sputum the higher positive rate found in same age group. Apart from age group 1-20, the rest of all the result are 0% in this Area. Area 4 has only 1.3% positivity by smear only in age group 40-60 while the rest of all groups depicts 0% positivity on sputum. Based on ICT in Area 4, age group 20-40 shows high positivity as 4.5% followed by 3.1% in age group 1-20 and the rest of all group are 0% positive for immune chromatographic test. Area 5, the highest positivity rate by sputum smear examination has found to be 5% in age group 1-20 followed by 1.3% in age group 40-60. The ICT positivity rate in Area 5 is higher in age group 40-60 [1.8%] followed by 1.1% in age group 1-20 and the rest of all the age groups shown no positivity or 0% positivity rate. Area 6, no positivity has found in all age groups in this area by sputum

smear examination while there is 2.5% positivity in age group 60-80 by immune chromatographic test followed by 1.8% in age group 40-60 and 1.5% in age group 20-40 years. No positive cases on sputum smear microscopy has been found in Bajaur region in all age groups but there is 33% positivity in age group 60-80 and 25% positivity in age group 20-40 based on immune chromatographic tests. In upper Dir location, only age group 20-40 shown the positivity rate of 15% by sputum smear examination while 30% positivity in age group 20-40 by immune chromatographic test followed by 25% in age group 40-60 and 25% in age group 60-80. No positivity rate has found by either sputum smear microscopy or immune chromatographic test in age above 80. Further the result has interpreted in different areas based on sex or gender. Females show the highest rate of positivity in Area 1 by immune chromatographic test 43% and 5.1% on sputum smear examination. The males have 3.5% positivity on sputum smear examination in this area as compared to females and 25% positivity of male by immune chromatographic test. In the second Area, again the females show more higher positive rate by sputum smear as 8.6% and 30% by immune chromatographic test, while the males 4.7% positivity by sputum smear examination while 28% positivity by immune chromatographic test. In Area 3, the females show 20% positivity by smear sputum examination and 25% by immune chromatographic test. The males in this area are 3.2% positive by sputum smear microscopy while 9.6% positive by immune chromatographic test. In Area 4, females show 12.5% positivity rate by sputum smear examination and 62% positivity rate shown by immune chromatographic test in this region in females while the males are equally positive by immune chromatographic test and sputum smear microscopy nearly 6.5% positivity rate for both the tests. In Area 5, females are equally positive for sputum smear examination and immune chromatographic test about 9.5% but no males are positive on immune chromatographic test while 12.5% positivity shown by males on sputum smear examination, this is the only area in lower Dir which shows higher positivity on sputum smear examination in males as compared to females. Area 6, no percentage of females are positive for either test, but males show only 20% by only immune chromatographic test. Same is the case with Bajaur region where no percentage of positivity has shown in females by either test but only male show 16% positivity by immune chromatographic test. In upper Dir region, the females show 13% positivity rate by sputum smear examination while males have no percentage of positive cases. Based on immune chromatographic test, 4% are males positive and 26% females. Sub-location based on marital status, the highest positivity by sputum smear examination found in Area 3 in married cases which are 4 cases out of total 40 cases becomes 10% positivity while lower positivity found in Area 1 where only 1 case is positive by sputum smear test out of total 53 cases so it becomes 1.8% positivity rate. In unmarried, even higher positivity found in Area 1 where 3 cases are positive out of 10 so it gives a figure of 30% positivity rate while the rest of all areas have 1 or zero positive cases. Based on immune chromatographic test, the Area 2 shows the higher positivity rate in married as well in unmarried, married are 8 cases out of 20 which is 40% positivity while unmarried are 5 out of 16

so it equals to 31%. In Bajaur region only 15.3% positive cases found in married by immune chromatographic test while there is no case in unmarried by either test. 10% are positive by smear in married in upper Dir while no positivity rate in unmarried. The immune chromatographic test in upper Dir shows 27% positivity in married while 22% positivity rate in unmarried cases.

Conclusion

Tuberculosis is still a prevalent disease in lower Dir Khyber Pukhtunkhwa province of Pakistan. The more positivity rate has found in females as compared to males. Age group 20-40 depicts the rate of positivity higher as compared to other groups in case of ICT and sputum smear microscopy both. Children are screened to be more positive for AFB as compared to old men and women. It requires further sophisticated investigation to acquire a more accurate result in case of tuberculosis prevalence in lower Dir region of KP province of Pakistan. A person who breathes is at risk of getting TB. As the DOTS strategy states, Directly observed treatment and short course is a better way to early case detection and treatment otherwise the disease can become more exaggerated in short period of time because the bacteria is an airborne pathogen so it can freely transmit to the lungs. Home to home visits are very crucial to aware the inhabitants of this devastating agent, and it is possible through community participation which will be fruitful if little bit incentives are bestowed to the active community members. Arrangement of seminars and conferences about tuberculosis infection can render better result in cases when the suspects are coughing more than 2 weeks and they do not make access to the TB diagnose and treatment centers in the area.

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