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Study on Accuracy and Efficiency of Molecular Diagnostic Techniques used for Tuberculosis and Analysis of Associated Risk Factors for Tuberculosis in Jail Inmates of Quetta, Pakistan

Niamatullah Kakar, Farhat Abbas, Muhammad Shafee and Tauseef Asmat*

Center of Excellence in Vaccinology and Biotechnology, University of Balochistan, Quetta

ABSTRACT

Tuberculosis (TB) is airborne infectious disease and poses a great threat to public health. Precisely and timely detection of *Mycobacterium tuberculosis* is prerequisite for the successful treatment of TB. Aim of this study was to evaluate the prevalence of tuberculosis along with associated risk factors in Quetta Jail, Pakistan. In this cross-sectional study, 186 sputum samples were collected randomly from jail inmates. Sputum was processed for smear microscopy and culture inoculation for the detection of *Mycobacterium tuberculosis* infection. Moreover, the suspected samples were further investigated by polymerase chain reaction (PCR). Three samples were found positive by PCR test while other detection methods were not that sensitive to detect the tuberculosis at that stage. Two patients were reported with history of tuberculosis but were effectively treated before imprisonment. On age basis, 128/186 (68.88%) patients were of younger age (18-30 years) and 10 (5.37 %) were above 50 year. Illiteracy, unemployment (daily wages) and tobacco smoking were some of the main risk factors common in inmates. The trend of sharing blankets, towels and utensils were 158/186 (84.49%), 152/186 (81.72%) and 165/186 (88.70%), respectively which likely increase the risk of disease spread. Smear microscopy revealed that 27.92% prisoners were involved in acute and chronic inflammatory disease. PCR proved to be more sensitive compared to the sputum smears or culture based tuberculosis diagnosis.

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Tuberculosis, Prisoners, AFB and Culture, PCR.

INTRODUCTION

Although developed countries have minimized if not eradicated Tuberculosis (TB) infection but it is still a one of the leading causes of death in underdeveloped countries (Raviglione and Sulis, 2016). The prevalence of TB is a global public health concern therefore World Health Organization (WHO) has launched new end-TB strategy in 2014. Despite the attempts to control tuberculosis by vaccination, sanitation and several other measures (Leung, 2012) still 10 million new TB cases have been reported worldwide during the year 2015 (Tatar *et al*., 2016; Beiranvand, 2016). Prevalence of TB in many areas of Pakistan is enormous depending on the availability of health care facilities, social and economic status. Pakistan is ranked at the 5th position in world among the countries bearing high burden of tuberculosis (Ahmad *et al*., 2018; WHO, 2015). There is no proper disease surveillance program in Pakistan, especially with respect to Quetta, Balochistan. The availability of data regarding prevalence, morbidity, mortality is limited to understand the disease pattern. Lower literacy rate and no awareness campaign have further aggravated the situation. Because of the mounted morbidity and mortality in many areas of country, it becomes necessary to at least evaluate the prevalence of tuberculosis in high-risk groups (Banu *et al*., 2010).

To date, there is very limited data available on the prevalence of tuberculosis in Balochistan province of Pakistan. Among marginalized settings in the country like inmates, in whom the prevalence of the disease seems to be higher and may be at high risk of contracting tuberculosis. Prisoners are grouped in high risk category to acquire tuberculosis infection and may lead to pulmonary tuberculosis compared to the general population, due to overcrowding, closed living condition, insufficient ventilation, generally low socioeconomic status, malnutrition and poor health (Coninx *et al*., 2000). Currently, very limited empirical data is available on the prevalence of pulmonary tuberculosis in jail inmates in Pakistan (Akhtar *et al*., 2007). The prevalence of tuberculosis in Karachi juvenile inmates was reported to be 3.9% (Hussain *et al*., 2003), while 4.8% in Khyber Pakhtunkhwa province prisons and in Karachi central prison the prevalence of TB was 3.75% (Rao, 2004).

* Corresponding author: tauseefcasvab@gmail.com
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Moreover, several studies have previously reported the prevalence of infectious diseases among prisoners and have suggested the comprehensive and standardized administration system to prevent and control those infectious diseases (Zhang *et al*., 2016; Shafee *et al*., 2014; Kakar *et al*., 2017). This study was designed to assess the prevalence of tuberculosis among inmates in district jail Quetta, Pakistan.

MATERIALS AND METHODS

Study design and area

A cross-sectional study was designed to estimate the prevalence and risk factors of tuberculosis among inmates of Central District Jail Quetta, Pakistan. The jail comprises of 11 barracks in total, 09 for male, 1 for juvenile and 1 for female prisoners. The total numbers of male prisoners were (n=450) of different duration of incarceration. In each barrack the numbers of prisoners were around 45-55. A pre-structured questionnaire was designed to collect the demographic data of age, educational level, profession, marital status, occupation, duration of incarceration, nutrition, current smoking status, family history of tuberculosis, vaccine (BCG), mean accommodation area (ft^2) per prisoner in a barrack, number of prisoners per toilet in barrack, sharing of blankets, utensils and towels etc.

A random sampling technique was used for male prisoners with 18-60 years of age and those were investigated for sputum acid fast bacilli (AFB) smear and culture technique and further confirmed by specific PCR. The inmates whose prison term was ≤ 3 months from the date of interview and those did not give consent to participate were excluded from the study.

Sample collection and processing for TB

Sputum samples were collected in 50ml tube and transported to laboratory in cold chain. All the samples were subjected to Ziehl-Neelsen (ZN) staining technique for the detection of acid fast bacilli (AFB) by microscopy and sputum culture for inoculation techniques. Briefly, loop-full sputum was spread evenly on slide and fixed by passing rapidly over flame. The slide was then covered with carbolfuchsin (1%) and heated to enable the dye to bind the cell wall mycolic acids of the bacterium. The smear was then washed with clean water and decolorized with H_2SO_4. Slide was then counter stained with methylene blue for 1min and immediately washed with clean water (Shafee *et al*., 2014; Moosazadeh *et al*., 2015).

For culture analysis, samples were added with an equal amount of 4% NaOH solution and were vortexed. Samples were further incubated for 15 min at room temperature for decontamination and centrifuged to concentrate the pellet. About 2-3 drops of the samples were placed on slopes of Ogawa medium and incubated at 37°C for extended period of 8 weeks.

DNA extraction and PCR amplification

Clinically suspected patients were reconfirmed through conventional PCR amplification by targeting IS 6110 gene with specific primer (Forward 5' CCTGCGAGCGTAGGCGTCGG 3', Reverse 5' CTCGTCCAGCGCCGCTTCGG 3'). Sputum samples of suspected patients were subjected to DNA extraction using thermolysis at 80°C for 60 min. The PCR reaction was carried out in 25 µl volume and PCR conditions was adopted as proposed by other researchers (Eisenach *et al*., 1990). Amplified product of 123 bp was visualized using gel doc system (Dolphin). *Mycobacterium tuberculosis* strain *H37Rv* was used as a positive control and was kindly provided by Provincial TB Reference Lab, Quetta while PCR grade water was used as a negative control.

RESULTS

During the study period, a total of 450 prisoners were detained in the central jail Quetta. Among these inmates 186 volunteered to take part in study. Those volunteers were interviewed and samples were collected. All the prisoners participated in this study were only male with the mean age of 39 (18-60 years) as permission was denied by Jail authorities to include the female prisoners. Majority of the inmates 128/186 (68.88 %) were with age of 18-30 years and only 14.51% (27/186) were 31-40 years old. While 21/186 (11.29%) were in age group of 41-50 years and 10 (5.37%) jail inmates were above 50 years of age (Table I).

The literacy rate was only 48/186 (25.89%) among the jail inmates. 34 (18.27%) were unemployed and 113 (60.75%) were working on daily wages. BCG Vaccination scar was evident only in 06 cases (3.27%). The number of prisoners with the internment duration of >4 years were 15 (8.06%) and 29 (15.59%) were for the duration of 2-3 years while 68 (36.59%) were incarcerated for 1-2 years and 74 (39.78%) were jailed for less than one year. The mean area per inmates was almost 20 ft^2 in barracks. The 44 (23.65%) inmates were reported as smoker of 1-5 cigarettes/day, 18 (9.67%) smoked 6-10 cigarettes/day and 66 (35.48%) smoked >10 cigarettes/day. Three (1.61%) cases reported with the family history of tuberculosis. Prisoners sharing blankets, towels and routine use utensils during their period of incarceration were 158 (84.49%), 152 (81.72%) and 165 (88.70%), respectively (Table I).

Table I.- Socio-demographic features of inmates tested for tuberculosis.

Variables	No of prisoners	Total %age
Age (years)		
18-30	128	68.88
31-40	27	14.51
41-50	21	11.29
> 50	10	5.37
Education		
Illiterate	138	74.11
Literate	48	25.98
Previous occupation		
Employed	39	20.96
Unemployed	34	18.27
Daily wages	113	60.75
Duration of incarceration		
<1 years	74	39.78
1-2 years	68	36.59
2-3 years	29	15.59
>4years	15	8.06
Smoking status		
Non smoker	58	31.18
1-5 cigarettes/day	44	23.65
6-10 cigarettes/day	18	9.67
≥ 10 cigarettes/day	66	35.48
Family history of tuberculosis		
Yes	03	1.61
No	183	98.38
H/O BCG vaccination		
Yes	06	3.27
No	180	96.77
Sharing of blankets		
Yes	158	84.49
No	28	15.05
Sharing of towels		
Yes	152	81.72
No	34	18.27
Sharing of utensil		
Yes	165	88.70
No	21	11.92
Married		
Yes	80	43.01
No	106	56.98
Drug addiction		
Yes	35	18.8
No	151	81

Microscopically, study revealed different grade of infection (Table II). Sixteen (8.60%) sample revealed acute (Polymorph nuclear leucocytes) inflammation, 20 (10.75%) chronic (mono nuclear cells) while 52 (27.92%) cases were of acute and chronic infection as shown in Table II. All of the sputum smear and culture were negative for acid fast bacilli (AFB) as shown in Table II but three were found positive by PCR as shown in Table III. The samples were further investigated by PCR and results revealed that 03 prisoners were positive for TB. The subject diagnosed positive for TB with PCR were incarcerated in jail for more than 3 years and were having all positives for TB infection like smoking, family history of TB and microscopic examination of their blood samples also revealed the abundance of PMN leucocytes and mononuclear cells.

Table II.- Microscopic examination of smears.

Characteristics	No	%age
Acute PMN leucocytes	16	8.60
Chronic mononuclear cells	20	10.75
Acute and chronic PMN leucocytes + mononuclear cells	52	27.92
Normal smear	98	52.68
Total	186	100%

Table III.- Prevalence of tuberculosis in jail inmates in Quetta, Pakistan.

No. of samples	Positive samples for TB by AFB method	Positive samples for TB by PCR
186	00	03

DISCUSSION

Direct smear microscopy is the most important and economical mean of diagnosis and control of tuberculosis in the community (Hajia *et al*., 2005). Culture technique was also included in this study to avoid false negative results that may occur due to paucibacillary, dead bacilli, and poor quality of specimen, staining or microscopy. Culture technique is known as standard in diagnostic studies, with its relative specificity of 99% and sensitivity around 80%. Previous study had identified substantial number of negative smear but culture positive TB cases, which demonstrate that sputum culture is more sensitive, specific and useful in these settings (Banu *et al*., 2010; Cheesbrough, 2005). The current study further provides the evidence that PCR based diagnosis could be helpful to diagnosis the tuberculosis at early stage of infection. Since PCR can amplify billions copies of targeted genes from tiny amounts of DNA so it's more efficient compared to any other diagnostic method including AFB. Furthermore, this study demonstrated that more than half (52.68%) of the prisoners smears were of normal cellular morphology, while 8.6% and 10.75% prisoners acquired acute and chronic infection, respectively (Table III). Tuberculosis

rate in prisons in some Asian countries is several times higher compared to their general population (Maclntyre *et al*., 1997). In Bangladesh, 2227/100000, Thailand, 568/100000, and in Pakistan, 657/100000 showing many times higher infection than general population (Banu *et al*., 2010). While in Karachi central prison prevalence was reported 3.75 times more than in general population (Cheesbrough, 2005).

Pakistan is high endemic country for tuberculosis and the situation is alarming in general population. The prevalence of latent TB infection in juvenile inmates in Karachi, Pakistan was 3.9 %, while in Khyber Pakhtunkhwa Province was much higher (48%) (Hussain *et al*., 2003). Our study revealed that 30 (56.60%) prisoners who were incarcerated for longer period (1-4 year) that may put them at a high risk of horizontal transmission or reactivation of past *M. tuberculosis* infection (Table II). As coughing, overcrowding and poor ventilation are the main factors that promote the transmission of TB infection (Cheesbrough, 2005).

Tobacco smoking also increases the risk of acquiring TB infection and plays a key role in the transmission of the disease by passive cigarette smoking in prison. Several studies have reported increased risk of transmission of TB infection due smoking. In our study 128 prisoners were cigarette smokers which highlight the risk of acquiring TB infection (Chiang *et al*., 2002; Nisar *et al*., 1993).

High proportions of the prisoners were poorly educated or illiterate and were with low socio-economic status. Similarly, Sharing utensils, towels and blankets was also higher among prisoners. These could also contribute in the dissemination of tuberculosis in jail inmates. Poverty, malnutrition and unemployment also contribute the associated risk factors for tuberculosis that might lead to a weakened immune system. In our study, 18.27% and 60.75% prisoners were unemployed or were working at daily wages, respectively.

Most importantly, majority 180/186 (96.77%) of the prisoners were non-vaccinated. In given living condition in prison, inmates are more exposed for acquiring TB infection. The possible explanation to be almost TB free prison could be well ventilated Barracks, improved hygienic conditions and regular inspection of prisoner's living conditions by higher authorities.

CONCLUSION

Early diagnosis, isolation and appropriate treatment of TB positive prisoners may help to reduce the transmission of tuberculosis. Special attention ought to be paid to those with history for tuberculosis in their families.

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Statement of conflict of interests

All the authors have declared no conflict of interest.

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