Original Article

The prevalence of pulmonary and extrapulmonary tuberculosis in forensic autopsies in a teaching hospital in South India

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Abstract

Introduction: An autopsy is a valuable tool for finding the cause of death, exploring the clinical diagnosis, documenting unexpected findings, and resolving diagnostic questions. However, this may subject the forensic pathologist and other workers to a wide variety of blood-borne and aerosolized pathogens. Tuberculosis (TB) is the most common cause of illness and death, resulting in infection transmission in the autopsy room. Our objective in this study was to estimate the prevalence of pulmonary and extrapulmonary tuberculosis among forensic autopsies in a tertiary care hospital in South India. **Material and Methods:** We identified positive TB cases from acid-fast bacteria staining and culture (Mycobacterium Growth Indicator Tube and Lowenstein–Jensen medium) out of 380 autopsy cases. **Results:** The prevalence of tuberculosis was 2.4% (n = 9), among which 2.1% of cases were positive for only pulmonary tuberculosis (n = 8), and 0.3% of cases had both pulmonary and extrapulmonary involvement (n = 1). In the bivariate analysis of TB cases, sex, occupation, family history of TB, habit of smoking, BCG vaccine scar, period of hospital stay, and cause of death were potentially significant. **Conclusion:** The prevalence of TB in forensic autopsy cases were similar to forensic autopsy-based studies, and it was less as compared to the prevalence of TB in the general population.

Keywords: Autopsy, forensic, infection, prevalence, tuberculosis

Introduction

Tuberculosis is caused by the bacteria *Mycobacterium tuberculosis,* which most commonly infects the lungs. Robert Koch first discovered *M. tuberculosis* in 1882. In 1962, Riley found the aerial dissemination of *M. tuberculosis* in a hospital ward. Tuberculosis is the leading cause of death as a single pathogen worldwide.[1] India is leading among the eight countries, which account for two-thirds of the global tuberculosis load.[2] An autopsy may subject the forensic pathologist and other workers to a wide variety of blood-borne and aerosolized pathogens. Tuberculosis is the most common cause of illness and death, resulting in infection transmission in the autopsy room.[3] There is a greater risk of infections at autopsy than in the clinical setting.[4] The aerosol can also be generated in other procedures, such as processing the tissue during an autopsy or embalming. In poorly ventilated

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spaces, the droplets generated may stay in the air even for hours and days. Some smaller droplets may reach another person's alveoli and may take the path of either latent or active tuberculosis infection.[5] The primary health care centers and teaching hospitals that are handling dead bodies are a hazardous source of infection for primary care physicians, forensic surgeons, and other workers coming in contact with dead bodies. Health care centers had difficulty in handling the dead bodies as these centers lacked hygienic mortuary, antiseptics, and trained manpower.

The post-mortem room is a potential source of infection for doctors and people handling the body after the autopsy.[6] Studies state that tuberculosis aerosols could be retrieved from glass slides hung 10 cm above cutting tissues during an autopsy. Tubercle bacilli have been recovered from various materials such as trays, towels, and tables 24 hours after autopsy.[7] In a study conducted in Sweden, only half of the cases diagnosed during autopsies were detected during life.[8] When applied over the tissue, the autopsy process generates infective aerosols by using oscillating saws, aspirators, and water hoses.[9] The increased incidence of tuberculosis in HIV patients and the emergence of Multi-Drug Resistant (MDR) TB reinforced the importance of the disease in autopsy safety.[10]

Mortuary staff may acquire infection through various routes such as sharp injuries, droplet inhalation, and direct inoculation.[11] There is also a risk of contracting infections such as human immunodeficiency virus, hepatitis B, hepatitis C, hepatitis D, and hepatitis G viruses, human T-cell lymphotropic virus type I, and diseases such as Creutzfeldt–Jakob disease, herpes, hantavirus pulmonary syndrome from the autopsy room.[12] There is also a high chance that tuberculosis infections that are not detected during life may be diagnosed only at autopsy.[13] There are very few prospective studies on estimating the prevalence of *M. tuberculosis* in forensic autopsies in India. Finding the disease's prevalence can help us assess the risk of occupational infections exposed by the mortuary staff and primary care physician. This study gives an idea of the prevalence of tuberculosis in forensic autopsies, the risk of undiagnosed tuberculosis, and the various laboratory techniques used to isolate *M. tuberculosis* in the autopsy.

Materials and Methods

It is a hospital-based cross-sectional descriptive study conducted in a teaching hospital in South India. We selected serially from the medico-legal autopsy cases performed from January 2019 to April 2021 in the Department of Forensic Medicine and Toxicology of our institute. During this period, we have conducted 1430 autopsies at our autopsy center. We studied 380 autopsy cases to estimate the prevalence of pulmonary and extrapulmonary tuberculosis in forensic autopsies. We obtained informed consent from the next of kin or concerned Police in unidentified cases. We collected case details and sociodemographic data from the relatives and medical records using an anonymous data collection proforma. Tissue samples of about one cubic cm were taken from the apex of both lungs [Figure 1a], the right lobe of the liver, kidneys, and spleen, after decontaminating the surface with a heated spatula. The samples were numbered and sent to the Department of Microbiology of our institute for processing. The lung tissue was processed for Ziehl–Neelsen (ZN) or acid-fast staining for TB bacilli [Figure 1b] and culture techniques such as Mycobacterium Growth Indicator Tube (MGIT 960) [Figure 1c] and Lowenstein–Jensen (LJ) [Figure 1d] medium. Other tissue samples from the liver, kidney, and spleen were processed only for acid-fast microscopy. The relevant data were entered into Microsoft Excel, and statistical analysis was performed using SPSS software version 20.0. We used bivariable and multivariable logistic regression to assess the factors associated with the prevalence of tuberculosis in forensic autopsies. The factors that were significant in bivariable analysis (P value < 0.2) were considered for multivariate analysis.

Results

We have collected samples from 380 medico-legal autopsies conducted during the study period. *M. tuberculosis,* which was detected through any of the techniques used, such as acid-fast bacteria (AFB) microscopy, MGIT culture, and LJ medium culture, was considered a positive tuberculosis case.

Among the 380 cases, 67.4% were males (n = 256) and 32.6% were females (n = 124). Most of the individuals belonged to the age group of 20 to 35 years (n = 127, 33.4%), followed by more than 50 years (n = 117, 30.7%), 107 cases belonged to 36 to 50 years of age (28.2%), and 29 cases were up to 19 years of age (7.6%).

Among the cases studied, 236 had received formal education (62.1%). One hundred forty-four cases were not educated (37.9%). Based on the monthly family income, 98 cases belonged to the group earning less than 10,000 rupees per month (25.8%), whereas 282 cases had a family income of more than 10,000 rupees per month (74.2%). Most of the individuals were non-agricultural laborers (n = 96, 25.3%), followed by agricultural laborers (n = 71, 18.6%), 64 homemakers (16.8%), 41 self-employed (10.7%), 37 individuals were students (9.7%), 33 were office employees (8.6%), 10 were unemployed (2.6%) and status of 3 cases were unknown. Out of the 380 cases tested, only 3 individuals had a positive family history of tuberculosis (0.8%). Among the cases tested, the smoking habit was present in 126 individuals (33.2%), and 254 individuals were non-smokers (66.9%). Significant past medical history of the deceased was analyzed, and it was found that 10 individuals had a history of tuberculosis, 56 cases had diabetes, 1 individual was under steroid therapy and 1 individual was HIV positive. The presence of BCG scars in autopsied bodies was recorded. Out of 380 cases, 336 cases had a BCG scar (88.4%) and 44 cases did not have a BCG scar (11.6%).

To analyze the chances of acquiring tuberculosis infection during the period of hospital stay, the number of days of admission

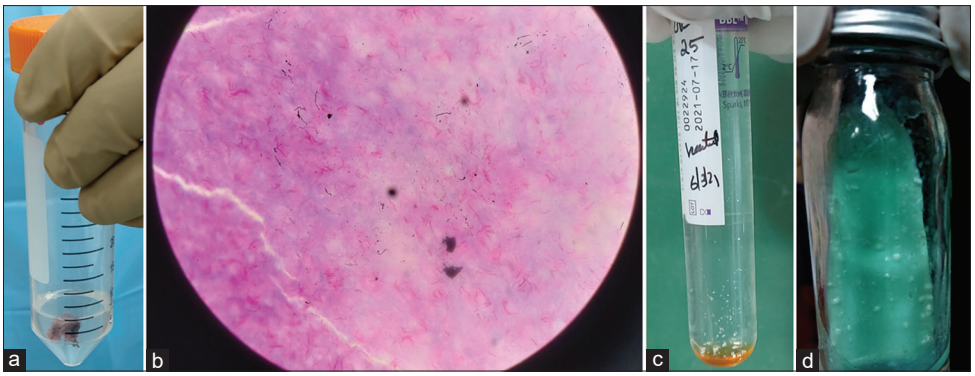

Figure 1: (a) Collection of a tissue sample in 5 ml of normal saline, (b) Ziehl-Neelsen staining shows clusters of acid-fast bacilli, (c) Mycobacteria Growth Indicator Tube (MGIT) showing white flakes indicating *M. tuberculosis* growth, (d) Lowenstein–Jensen culture medium showing positive *M. tuberculosis* growth appearing as non-pigmented, dry colonies

was considered. Out of 380 cases, 39 were brought dead to the hospital and 341 cases stayed for one or more days. On analyzing the time since death, the autopsy was conducted in 259 cases in less than 24 hours and in 121 cases more than 24 hours from the time of death.

The prevalence of tuberculosis age-wise is given in Table 1. The age group of up to 19 years did not have any positive cases. The prevalence of tuberculosis in the age group of 20 to 35 years was 3.1%. The prevalence in the age group of 36 to 50 years was 2.8%. The prevalence in the age group of more than 50 years was 1.7%. The association between age and the prevalence of tuberculosis was not found significant (*P* value > 0.2). In the bivariate analysis of TB cases sex, occupation, family history of TB, habit of smoking, BCG vaccine scar, period of hospital stay, and cause of death were potentially significant. On multivariate analysis, none of the factors were found to be significantly associated with the prevalence of tuberculosis in autopsies [Table 2].

The total number of positive cases was found to be 9. The prevalence of pulmonary tuberculosis among the autopsied cases was 8 (2.1%). One case was found to have both pulmonary and extrapulmonary involvement, making the prevalence of extrapulmonary tuberculosis 1 (0.3%). The total prevalence of tuberculosis among the autopsied cases was 2.4%. No cases were detected with only extrapulmonary involvement. The positive tuberculosis cases based on different diagnostic techniques are enumerated [Table 3]. Out of the positive TB cases, all nine cases were detected by the MGIT culture technique; three cases including one extrapulmonary, were detected through AFB microscopy, and two cases were detected through the LJ culture technique.

Males had more prevalence compared to females, with a total number of eight cases (88.9%). Whereas only one positive case was a female (11.1%) and male to female ratio was 8:1. On analyzing the family history, tuberculosis was more prevalent among families with a negative tuberculosis history. 88.9% of positive cases belonged to the group with a negative family history (n = 377). One case turned positive (11.1%) out of the three cases that had a positive family history of tuberculosis. Among the nine positive TB cases identified, only two subjects had a previous history of tuberculosis, and the remaining seven were newly identified only during autopsy. Among the BCG-vaccinated individuals, six cases tested positive for tuberculosis (66.7%), and three cases were positive among a non-vaccinated group (33.3%).

Table 1: Age-wise prevalence of tuberculosis in forensic autopsies

Age categories (years)	Status of Tuberculosis		Total
	Positive	Negative	
Up to 19	0 (0)	29	29
20-35	4 (3.1%)	123 (96.9%)	127
36-50	3 (2.8%)	104 (97.2%)	107
>50	2 (1.7%)	115 (98.3%)	117

Discussion

The comparison of the prevalence of tuberculosis in autopsies based on various studies is given in Table 4. Some studies' sample sizes were similar to our study like in Ozsoy *et al.* the sample size was 302, which is also a prospective study.[14] These types of studies had a high number of cases studied like in Rowińska-Zakrzewska *et al.*, 1500 autopsies were studied.[15] In Morales *et al.* 2180 cases were studied.[16] In Flavin *et al.* 4930 cases were analyzed.[13] Some studies had a small population for study such as Bavikar *et al.*, which is a prospective study that analyzed 112 consecutive cases as compared to 380 samples in our study.[17] In our study, most of the cases were males (67.4%). This was similar in studies like Ozsoy *et al.*[14] 76.8% were male, Bavikar *et al.*[17] 63% were men. In our study, most of the cases belonged to the age group of 20 to 35 years (33.4%). The age range studied by Ozsoy *et al.*[14] was 1 to 95 years with a mean age of 42. The median age of cases included in Bavikar *et al.*[17] was 32 years. Based on the time since death, most of the cases in our study were autopsied within 24 hours (n = 259;68.2%). This was similar to Ozsoy *et al.*[14] in which 94.2% of cases were autopsied within 24 hours. Our study concluded that the prevalence of tuberculosis among medico-legal autopsies was 9 (2.4%), which is higher when compared to Ozsoy *et al.*, who conducted a study on 302 cases of forensic autopsies.

Table 2: Factors associated with tuberculosis in forensic autopsies

Parameter	Total (*n*=380)	Number of cases with tuberculosis	Bivariate analysis		Multivariable analysis	
			cOR	*P*	aOR	*P*
Age categories (years)						
Up to 19	29	0 (0)	-			
20-35	127	4 (44.4%)	1.87 (0.34-10.4)	0.47		
36-50	107	3 (33.3%)	1.7 (0.27-10.1)	0.58		
>50	117	2 (22.2%)	1			
Sex						
Male	256	8 (88.9%)	3.97 (0.49-32.1)	0.19	2.2 (0.19-25.3)	0.52
Female	124	1 (11.1%)	1		1	
Education						
Literate	236	6 (66.7%)	1.22 (0.30-4.98)	0.77		
No formal education	144	3 (33.3%)	1			
Income (Rupees)						
<10,000	98	3 (33.3%)	1.45 (0.36-5.9)	0.6		
>10,000	282	6 (66.7%)				
Occupation						
Employed	367	7 (77.8%)	1		1	
Unemployed	13	2 (22.2%)	9.4 (1.74-50.3)	0.009	3.8 (0.30-47.7)	0.3
Family history of TB						
No	377	8 (88.9%)	1		1	
Yes	3	1 (11.1%)	23.1 (1.89-281.1)	0.01	8.37 (0.15-455.0)	0.29
History of smoking						
No	254	3 (33.3%)	1		1	
Yes	126	6 (66.7%)	4.2 (1.03-17.01)	0.05	2.88 (0.54-15.4)	0.21
Comorbidities						
No	312	7 (77.8%)	1			
Yes	68	2 (22.2%)	1.3 (0.27-6.50)	0.73		
BCG vaccine scar						
No	44	3 (33.3%)	4.02 (0.97-16.7)		2.13 (0.32-14.20)	
Yes	336	6 (66.7%)	1	0.05		0.43
Hospital stay						
Brought dead	39	3 (33.3%)	4.65 (1.12-19.4)	0.04	2.69 (0.41-17.56)	0.30
Stayed for sometime	341	6 (66.7%)	1			
Time since death						
<24 h	259	6 (66.7%)	1.07 (0.26-4.36)	0.9		
>24 h	121	3 (33.3%)	1			
Cause of death						
Natural	14	1 (11.1%)	3.44 (0.40-29.6)	0.26	1.14 (0.07-19.12))	0.9
Unnatural	366	8 (88.9%)	1			

cOR: Crude odds ratio, aOR: Adjusted odds ratio

Table 3: Positive tuberculosis cases based on various diagnostic techniques

Status	AFB microscopy		MGIT culture	LJ medium culture	Total tuberculosis cases
	Pulmonary	Extrapulmonary			
Positive	2	1	9	2	9
Negative	377		371	378	371
Total	380		380	380	380

AFB: Acid-fast bacteria, MGIT: Mycobacterium Growth Indicator Tube, Lowenstein–Jensen (LJ)

According to LJ medium culture results, 3 (1.0%) out of 302 cases detected the growth of *M. tuberculosis*.[14] In a retrospective study conducted by Morales *et al.* among the population of Spain, the prevalence of tuberculosis was found to be 4.2% which is higher than our study.[16] Sangma *et al.* conducted a retrospective study on the prevalence of tuberculosis in forensic autopsies in the Eastern Indian population. The study included 4415 autopsy cases from 2003 to 2012. The prevalence of tuberculosis was found to be 1.7%. This study was mainly based on the histopathological findings, in which granulomatous inflammation with caseous necrosis was considered active tuberculosis.[18]

Punia *et al.* concluded that the prevalence of TB was 39 cases (5.07%). Out of the 39 positive cases, 36 cases were

Table 4: Comparison of prevalence of tuberculosis in various autopsy-based studies

Study	Prevalence of *M. tuberculosis* (%)
Our study	2.4
Ozsoy *et al.*[14]	1.0
Rowińska-Zakrzewska *et al.*[15]	7.9
Morales *et al.*[16]	4.2
Flavin *et al.*[13]	0.3
Bavikar *et al.*[17]	21.0
Sangma *et al.*[18]	1.7
Punia *et al.*[19]	5.07
Theegarten *et al.*[20]	1.39

diagnosed only during the autopsy.[19] Most of the Indian studies on the prevalence of tuberculosis in autopsies are retrospective, based on case document analysis. In our research, we have conducted a prospective cross-sectional study emphasizing the microbiological techniques that can be used to isolate *M. tuberculosis* from dead bodies.

In analyzing the positive tuberculosis cases, most of the cases belonged to the age group of 20 to 35 years (n = 4; 44.4%). This was found to be similar to the study conducted by Punia *et al.*[19] in which the positive cases were commonly found in the age group of 20 to 40 years. In a retrospective study conducted by Sangma *et al.*[18] tuberculosis cases were commonly found in the age group of 40 to 50 years. In the study conducted by Rowińska-Zakrzewska *et al.*[15] the positive cases were distributed in a wide age range of 18 to 83 years. Active forms of tuberculosis were more frequent in the age groups of 30 to 59 years and 80 to 99 years in the study by Theegarten *et al.*[20] The mean age of positive tuberculosis cases among the autopsies conducted was found to be high in studies such as Morales *et al.*[16] which was 64 years and Flavin *et al.*, which was 63.2 years.[13] In our study, the male outnumbered the female in the number of tuberculosis cases detected. The total number of male cases was 8 (88.9%), and female cases were 1 (11.1%). This was similar to Rowińska-Zakrzewska *et al.*[15] in which 83 were male and 36 were female, Morales *et al.*[16] in which 57% of positive cases were males, Flavin *et al.* 13 were male and 2 were female.[13] In Punia *et al.*[19] it was 34 males and 5 females. This was found in contrast to Ozsoy *et al.*[14] in which there were two positive female cases and one male case. On analyzing the history of tuberculosis infection among the positive cases, we found that out of the nine cases detected, only two cases had a history of tuberculosis. The remaining seven cases (77.8%) were newly detected, and they did not bear any risk factors such as diabetes, HIV, or steroid therapy. In Rowińska-Zakrzewska *et al.*[15] 119 positive TB cases were detected out of 1500 autopsies conducted, out of which 75 cases (63%) were diagnosed during life and 44 cases (37%) were diagnosed only after death through autopsies. In a study conducted by Morales *et al.*[16] only 46% of positive cases were suspected to have tuberculosis. Flavin *et al.*[13] conducted a retrospective study on the prevalence of tuberculosis among autopsies. They recorded that out of the 15 cases detected, 10 cases were unsuspected (67% of total TB cases). Among the 10 cases, 5 cases had abundant TB bacilli in ZN staining (+2, +3 grades). Bavikar *et al.*[17] stated that among the 24 TB cases detected during the autopsy, 6 cases (34%) did not have any clinical suspicion of tuberculosis during their treatment. Punia *et al.*[19] conducted a North India-based study in the city of Chandigarh which detected 39 cases out of which 33 cases (84.6%) did not have the possibility of premortem diagnosis of tuberculosis. This has a high percentage of undetected cases compared to our study. This indicates the invisible risk in medico-legal autopsies and body handling. There is a need for proper utilization of personal protective equipment by primary care physicians and staff in the mortuary. This also emphasizes the need for pathological autopsies on suspected tuberculosis cases to explore the actual prevalence of the disease.

In our study, out of the nine positive TB cases detected 1 case (11.1%) had both pulmonary and extrapulmonary involvement. It involved various organs such as the intestine, heart, liver, and spleen. Multiple tuberculous nodules were found disseminated in organs. The prevalence of disseminated/extrapulmonary tuberculosis was high in studies such as Flavin *et al.*[13] (46.7%), Rowińska-Zakrzewska *et al.*[15] (39.5%), Bavikar *et al.*[17] (66.7%), Punia *et al.*[19] (43.5%). However, the prevalence was low in a study conducted by Sangma *et al.* (5.4%).[18] Bobrowitz *et al.* reported that 21 autopsy cases had tuberculosis as the primary cause of death. Eleven cases had pulmonary tuberculosis and 10 to miliary tuberculosis.[21] Savic *et al.* noticed 35 cases (0.56%) of unrecognized military tuberculosis of all autopsies; the mean age was 62.2 years and male to female ratio was 2:3.[22] Madadin *et al.* observed no cases of undiagnosed pulmonary tuberculosis detected for the first time at autopsy.[23] Mucheleng'anga *et al.* observed incidental tuberculosis in 52 cases (45 male and 7 female), and age range was 14-66. Thirty-nine out of 52 (75%) deaths were attributable specifically to tuberculosis only.[24]

National TB Elimination Programme (NTEP) in India recorded notification of 24.2 lakh cases in 2022. It was 13% more as compared to 2021. The case notification rate was 172 cases per lakh population.[25] The prevalence of positive pulmonary tuberculosis was 295.9 per 100,000 population. It was higher in males compared to females and common in rural areas. They reviewed culture-positive pulmonary tuberculosis (277.8/100,000 population), smear-positive pulmonary tuberculosis (196.6/100,000 population), and bacteriologically positive pulmonary tuberculosis (186.6/100,000 population).[26] Identification of this prevalence of TB cases in the general population depends on the RNTCP program and NTEP. Large samples are needed in autopsy-based to get the prevalence of the TB cases that were previously unidentified.

Conclusion

The prevalence of TB in forensic autopsy cases was similar to forensic autopsy-based studies, and it was less as compared to the prevalence of TB in the general population. Our study focuses

on AFB staining and the use of culture media to identify TB cases in the autopsies. Our study shows that *M. tuberculosis* is a hidden risk factor in medico-legal autopsies and the handling of dead bodies by primary care physicians and autopsy personnel.

Suggestions

Minimal handling of organs should be conducted in high-risk cases. Tuberculosis is a health hazard in live patients and the dead, which indicates the necessity of proper infrastructure of the autopsy hall, mortuary, and the regular health and vaccination monitoring of all staff involved in the autopsy. Even universal precautions are recommended for hospital staff, relatives, and primary health care professionals.

Ethical clearance

A prior approval was obtained from the Institutional Ethics (JIP/IEC/2018/429).

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Conflicts of interest

There are no conflicts of interest.

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