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Prevalence of pulmonary tuberculosis in Wardha district of Maharashtra, Central India

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Abstract A house based survey was conducted during 2007–2009 in a representative sample of population in Wardha district implementing Directly Observed Treatment Short Course strategy for tuberculosis (TB) control since 2001. The objective was to estimate prevalence of bacillary pulmonary TB (PTB) in individuals aged 15 years or above, and to estimate trends in prevalence when compared to a previous survey carried out in mid 1980's. Two sputum samples (one spot, one early morning) collected from individuals having symptoms suggestive of PTB, history of previous anti-TB treatment (ATT) or abnormal pulmonary shadow on Mass Miniature Radiography (MMR) consistent with possibly or probably active tuberculosis were subjected to Ziehl–Neelsen microscopy and culture on Lowenstein–Jensen medium.

Of 55,096 individuals registered into the survey, 50,332 (91.4%) were screened by interview for symptoms and history of ATT and/or by MMR. Of them, 4805 were

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eligible for sputum collection; both specimens were collected in 4285 (89.2%) and only one specimen in 27 (0.6%).

A total of 86 bacillary cases were detected during the survey. Prevalence of bacillary PTB was estimated at 188.7 (140.3–236.9) per 100,000 populations. There was a decline of 61% in the prevalence of PTB over a period of 22 years.

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1. Introduction

Tuberculosis continues to be a major public health problem in India with an estimated 2.1 million incidence cases and about 2,40,000 TB deaths annually [1]. A nation-wide survey carried out during 1955–1958 revealed a prevalence of bacillary pulmonary TB (PTB) at 400 per 100,000 population [2]. Consequently, the National TB Programme (NTP) was implemented all over the country from 1962. Serial surveys carried out thereafter in selected geographically defined areas revealed no significant change in prevalence [3–5]. Following the review of NTP in 1992, the Revised National Tuberculosis Control Program (RNTCP) adopting the Directly Observed Treatment Short Course (DOTS) strategy was introduced from 1997 and expanded in a phased manner to all the districts in the country by 2006 [6]. Three rounds of surveys in a rural area of Tamil Nadu state implementing RNTCP from 1999 revealed a decline of 50% in the prevalence of PTB from 1999 to 2006 [7]. Since, this observation pertained to a single geographical site, Government of India identified six institutions to undertake independent surveys in seven other sites (districts/sub-districts) using a generic protocol, with the objective to find out the point prevalence of bacillary PTB among adults, which would provide baseline data to measure trends of prevalence in future. The district level survey in Wardha, Maharashtra state, had the additional advantage of comparing the trends from a previous survey carried out in 1982–88 [8].

2. Material and methods

Prior approval of the Institutional Research Ethics Committee of Mahatma Gandhi Institute of Medical Sciences, Sevagram, Wardha was obtained. Each family was counseled by field staff and explained about their participation in the survey. Required written consent for participation (printed in local language) was obtained from each individual after counseling.

All smear positive or culture confirmed TB cases detected during the survey were referred to the

nearest RNTCP center for anti-TB treatment (ATT); details of the patients referred were sent to the District TB Officer for further necessary action. Individuals with symptoms but not having TB were advised to seek treatment at the local health center.

2.1. Setting

The survey in Wardha district, having a population of 1.3 million in 2007 was carried out during 2007–2009. About 70% of the district population resided in rural areas and was engaged in agrarian occupations. This district located in central India has a warm climate with maximum temperatures between 46 and 48 °C during summer and 15–30 °C in winter, with limited rainfall. RNTCP is being implemented from 2002. Between 2002 and 2006, notification rate of new smear positive cases (NSP) and all TB cases per 100,000 population increased from 25 to 58 and 45–143 respectively; and thereafter gradually decreased to 38 and 106 by 2009 [6]. Treatment success rate in NSP cases registered during 2002–2008 varied between 86% and 89%. For cases registered in 2009, 7% were HIV reactive; treatment success was 83% in NSP and 62% in previously treated cases [6].

2.2. Sampling

Sample size was calculated at 47,828 to estimate the prevalence within 20% of true value at 5% significance level with a design effect of 2 to account for cluster sampling while arbitrarily considering expected prevalence of bacillary PTB (positive for AFB on microscopy and/or culture) at 400 per 100,000 populations.

This sample size was allocated to urban and rural strata in ratio of population size. The villages were grouped into three sub-strata depending upon village population (<1000, 1000–1999 and ≥2000). Within each sub-stratum, 5% of the clusters (villages) were selected by Population Proportional to Size (PPS) sampling method. Thus 45 clusters were selected for the survey out of 1004 villages in the district. In the urban stratum, 9 clusters

(urban wards) were selected similarly out of 146 wards in the district.

2.3. Field procedures

Field and laboratory staff was trained in survey procedures at National Institute of Research in Tuberculosis, Chennai (NIRT).

Planning visit was undertaken to each cluster to sensitize the community and local leaders about the purpose of the survey and field procedures. In each cluster, a sketch of lanes and hamlets showing approximate number of houses was drawn. Survey in each cluster began on a mutually agreed date. Enumerators visited every household and recorded the age, sex and residential status of each individual. Each individual in the age-group of 15 years and above and residing in the district for ≥ 6 months was registered into an individual survey card. Subsequently, a symptom elicitor interviewed each of them for presence of symptoms suggestive of PTB (persistent cough for ≥ 2 weeks, fever or chest pain for ≥ 1 month, and history of haemoptysis in last 6 months) and history of ATT if any either in the past or currently, irrespective of its duration. For quality control of symptom screening, 10% of randomly selected subjects were re-interviewed by a supervisor.

Each registered individual, irrespective of presence or absence of symptoms and history of ATT, was also subjected to radiological screening by Mobile Mass Miniature Radiography (MMR). In each cluster, the MMR unit was stationed at a suitable place and the registered individuals were directed to the site where chest X-ray was done by a trained technician.

Prior to the survey, three professors of the Mahatma Gandhi Institute of Medical Sciences, Wardha (MGIMS) – two radiologists and one physician, had been imparted practical training in reading of MMR films in survey situations, by vastly experienced X-ray readers at the National Institute of Research in Tuberculosis (NIRT), Chennai. On any given day, each MMR roll was assigned for independent reading to any two of the three X-ray readers as per their availability.

Each reader classified individual film as under:-

- (A) Lung pathology other than tuberculosis.
- (B) Probably tuberculous but inactive.
- (C) Tuberculosis possibly active.
- (D) Tuberculosis probably active.

The detailed procedure of classifying MMR film is given in the [Appendix](#).

Individuals having symptoms suggestive of PTB, history of ATT or MMR reading as 'C' or 'D' by any

of the two readers were eligible for sputum examination. All such individuals were given pre-numbered sterilized screw capped bottles for collecting one spot and one early morning sputum specimen which were brought to the accredited laboratory of MGIMS on the day of collection, refrigerated and processed the next day. Where the clusters were at far off distance, specimens were preserved in refrigerator and transported to MGIMS twice or thrice a week and processed on the next day of arrival.

2.4. Laboratory procedures

A direct smear was prepared from each specimen on a new labeled slide under aseptic conditions in a bio-safety cabinet, stained by Ziehl–Neelsen (ZN) staining method and examined using Light Binocular Microscope. The remaining quantum of specimen after preparation of smear was concentrated by Modified Petroff's Method and inoculated on two slopes of Lowenstein–Jensen medium (LJ). The slopes were incubated at 37 °C and examined every day for first week and once every week for 8 weeks for the presence of mycobacterial colonies. In case of contamination or no growth, the cultures were discarded. If growth was observed, it was subjected to Niacin test and further incubated on LJ medium containing Para-nitro Benzoic acid (PNB) in a concentration of 500 µg/ml. If the Niacin test was found to be positive and no growth was observed on PNB containing medium, the isolate was labeled as *Mycobacterium tuberculosis* (*M. tuberculosis*).

2.5. Definitions

An individual was considered to be

- Smear positive case if the smear of either of the sputum specimen showed one or more acid fast bacilli (AFB), irrespective of culture result.
- Culture positive case if either of the specimens exhibited one or more colonies of *M. tuberculosis*.
- Bacillary case if at least one of the two specimens was smear and/or culture positive.

2.6. Statistical methods

The data were digitalized by two independent data entry operators after checking and correcting the data collected on cards. The data so entered were matched and the errors rectified by re-entry of the records by two independent operators. This procedure of checking, matching and re-entry of records was repeated three times to set right all errors.

Crude prevalence was estimated by dividing the total number of smear, culture or bacillary cases detected during the survey by the total number (n) of individuals screened for symptoms and history of ATT as well as by MMR and whose results of microscopy as well culture of both sputum specimen were available. Standard error (SE) was estimated as Standard deviation (SD)/ \sqrt{n} where $SD = \sqrt{P \cdot (1 - P)}$. Confidence intervals (95%) of the estimates were calculated as mean of the binomial exact $\pm 2SE$. To compare with the prevalence estimate during the earlier survey in the district, crude prevalence of bacillary TB ignoring screening for history of ATT and X-ray was also estimated.

Individual level analysis was performed to correct for the bias introduced by incompleteness of data, using logistic regression model with robust standard error [9]. To include all eligible individuals in analysis, missing value imputation was undertaken for individuals: (a) without symptom screening, (b) symptoms present but the result of one or both sputum specimen not available. This method accounted for clustering in survey design, variation in number of individuals tested in each cluster, between-cluster variability and uncertainty in estimating SE, under the assumption that within groups of individuals belonging to same sex, age-group, TB symptoms and X-ray results, data are missing at random. For imputation of missing values for each variable, starting values were assigned to missing data, which in turn were obtained from a random sample of values from individuals whose data were available. Model was then fitted with this particular variable as outcome variable and other variables as explanatory variables. This was done sequentially in the order of proportion of data that were missing starting with variables with least missing data. Finally, a logistic regression model with smear/culture/bacillary TB as the outcome variable and sex, age-group, TB symptoms and X-ray result as explanatory variables were fitted. Newly imputed values were used as starting values for next iteration of the process which was undertaken in ten cycles in order to obtain one imputed data set. Five such data sets were imputed and the average of their prevalence was taken as final prevalence.

3. Results

A total of 55,096 individuals (Males – 28,065, Females – 27,031) were registered into the survey. Of 50,332 (91.4%) screened for symptoms and ATT,

3219 (6.4%) were found to have symptom suggestive of TB and 988 (1.97%) had history of ATT. The number eligible for sputum collection based on history of ATT in addition to symptoms was 620. The agreement between the field workers and the supervisor for eliciting the presence of symptoms or history of ATT was satisfactory (Kappa = 0.81). Number of registered individuals screened by MMR was 47,663 (86.5%). Of them, MMR results were labeled as 'C' or 'D' for 1456 (3%). Number eligible for sputum collection based on MMR in addition to those eligible on the basis of symptom or history of ATT was 966. Of 4805 individuals eligible for sputum collection, sputum was collected from 4312 (89.7%); both specimens were collected from 4285 and only the spot specimen could be collected from 27. The distribution of study participants by sex, age-group and stratum is shown in Table 1.

About 1% of spot and 1.8% of overnight specimen were found to be contaminated on culture.

A total of 55 individuals were found to be smear positive and 66 culture positive. There were a total of 86 bacillary cases; their distribution by smear and culture status is shown in Table 2. Of them, 48 were picked up through screening for presence of symptoms, additional 11 through history of ATT and further 27 through MMR. Among cases with symptoms, 41 (85.4%) had cough with or without other symptoms while 7 (14.6%) had one or more symptoms other than cough.

Distribution of bacillary cases by age-group, sex and stratum is given in Table 3.

Prevalence among individuals aged 15 years and above estimated by individual level analysis, of smear positive, culture positive and bacillary PTB per 100,000 population was 121.1, 149.4 and 188.7 respectively (Table 4).

The estimated crude prevalence of smear positive, culture positive and bacillary PTB per 100,000 population was 110.1, 132.8 and 173.3 respectively (Table 3). Crude prevalence of bacillary PTB ignoring screening for history of ATT and X-ray was 104.1 (77.7, 136.4) per 100,000 populations. It was 100 (97.0, 103.0) per 100,000 populations when age-standardized to the participants in the previous survey during 1982–88. Comparing this estimate with the crude prevalence of 253 per 1,00,000 population in the age-group of 15 years and above during the previous survey using symptom screening followed by sputum examination [8], there was a decline of 61% over a period of 22 years considering 1986 and 2008 as the mid-points of the two surveys.

		Table 1 Study population — No. registered, eligible by symptom interview and X-ray, eligible for sputum examination and collected.									
		No. registered	No. screened by interview	No. symptomatic	No. with history of ATT	No. screened by MMR	No. with X-ray abnormality (e)	No. eligible for sputum examination	No. sputum examined/collected		
	No. (%)							Both specimens	Only one (spot) specimen		
Total	55,096	50,332 (91.4)	3219 (6.4)	988 (2.0)	47,663 (86.5)	1456 (3.1)	4805 (9.5)	4285 (89.2)	27 (0.1)		
Sex											
Female	27,031	25,204 (93.2)	1320 (5.2)	352 (0.7)	23,473 (86.8)	556 (2.1)	195 (7.7)	1746 (89.5)	10 (0.5)		
Male	28,065	25,128 (89.5)	1899 (7.6)	636 (1.3)	24,190 (86.2)	900 (3.2)	2854 (11.4)	2539 (89.0)	17 (0.6)		
Age group											
15–24	13,671	12,178 (89.1)	432 (3.5)	70 (0.1)	11,339 (82.9)	108 (1.0)	564 (4.6)	506 (89.7)	3 (0.5)		
25–34	11,980	10,809 (90.2)	575 (5.3)	156 (0.3)	10,171 (84.9)	190 (1.9)	808 (7.5)	710 (87.9)	5 (0.6)		
35–44	10,771	9989 (92.7)	703 (7.0)	245 (0.5)	9613 (89.2)	252 (2.6)	1008 (10.1)	906 (89.9)	7 (0.7)		
45–54	7703	7187 (93.3)	497 (6.9)	187 (0.4)	6913 (89.7)	261 (3.8)	787 (11.0)	694 (88.2)	3 (0.4)		
55–64	5301	4901 (92.5)	432 (8.8)	153 (0.3)	4705 (88.8)	252 (5.4)	683 (13.9)	615 (90.0)	6 (0.9)		
65+	5670	5268 (92.9)	580 (11.0)	177 (0.4)	4922 (86.8)	393(8.0)	955 (18.1)	854 (89.4)	3 (0.3)		
Stratum											
Rural	39,233	36,302 (92.5)	2666 (7.3)	714 (1.4)	34,560 (88.1)	1129 (3.3)	3838 (10.6)	3448 (89.8)	19 (0.5)		
Urban	15,863	14,030 (88.4)	553 (3.9)	274 (0.5)	13,103 (82.6)	327 (2.5)	967 (6.9)	837 (86.6)	8 (0.8)		

Table 2 Frequency distribution of bacillary cases by smears and culture status.

Smear/culture status	No. (%)
Smear+ culture+	35 (40.7)
Smear+ culture–	18 (20.9)
Smear– culture+	31 (36.1)
Smear+ culture contamination	02 (2.3)
Total	86 (100)

4. Discussion

The prevalence of bacillary pulmonary TB during the present survey in Wardha district among individuals aged 15 years and above was estimated at 189 per 100,000 population, considering the estimate by individual level analysis as the best estimate.

To find out the trends in prevalence, we compared the results of the present survey with the previous survey carried out in the district during 1982–88. Both the surveys were district level surveys, involved similar sampling techniques and were implemented by the same set of principal investigators who happened to be the first and the second authors of the present manuscript. While the first survey was carried out among persons aged ≥ 5 years of age, second survey was among ≥ 15 years of age. Similar set of symptoms and their duration were used for symptom screening during the two surveys. Only symptom screening was used for identifying individuals eligible for sputum examination at the previous survey. During the present survey, screening for history of ATT and screening by MMR were also employed to identify individuals eligible for sputum examination. In both the surveys, sputum specimens were stained by Ziehl–Neelsen method and examined under light microscope, and by culture using LJ Media. While crude prevalence was estimated at the first survey, analysis for second survey involved multiple imputations for missing values. In view of these differences, the crude prevalence of bacillary TB at the second survey ignoring screening for ATT and by MMR was compared with crude prevalence estimated at the first survey in individuals ≥ 15 years of age. The decline of 61% in prevalence thus observed may be attributable to RNTCP and might have occurred after its implementation from 2002, as serial surveys in other areas had not revealed any significant decline in prevalence during the pre-RNTCP era [3–5].

A decline of 56% has been observed in rural Bangalore between two surveys carried out there

Table 3 Age, sex and stratum wise distribution of bacillary cases.

Age group in years	Urban			Rural			Total No.
	Female	Male	Sub-total	Female	Male	Sub-Total	
	No.	No.	No.	No.	No.	No.	
15–24	2	4	6	2	6	8	14
25–34	0	1	1	4	5	9	10
35–44	1	3	4	5	5	10	14
45–54	1	4	5	2	8	10	15
55–64	0	1	1	2	13	15	16
65+	0	2	2	6	9	15	17
Total	4	15	19	21	46	67	86

Table 4 Prevalence of PTB per 100,000 population, by statistical method of estimation.

Type of PTB	Method of analysis	
	Crude prevalence	Individual level analysis
Smear positive PTB	110.1 (83.2–143.7) <i>N</i> = 49,812 [^]	121.1 (84.8–157.3) <i>N</i> = 55,096
Culture positive PTB	132.8 (102.7–168.9) <i>N</i> = 49,718 [^]	149.4 (102.1–196.7) <i>N</i> = 55,096
Bacillary PTB	173.0 (138.6–213.9) <i>N</i> = 49,720 [^]	188.7 (140.3–236.9) <i>N</i> = 55,096

(): 95% confidence intervals.

N: Numbers included in analysis.

[^] Reason for difference in numbers analyzed by method: for crude prevalence, only those individuals from whom all actual data are available were considered for analysis; individuals missed from screening for symptoms, history of ATT or X-ray and those who were eligible for sputum collection but one or both sputum specimen not collected or found contaminated on culture were excluded.

with the mid-points in 1975 and 2008 [10]. The prevalence of bacillary PTB in rural Bangalore in 2008 was 254 per 100,000 populations. In Thiruvallur, after implementation of RNTCP, prevalence of culture positive PTB declined by 50% from 609 in 1999 to 309 in 2006; but was found to have increased to 388 during a later survey in 2008 [7]. The trends in prevalence, after implementation of RNTCP, are not available from any other area.

The estimated prevalence in Wardha was lower than the prevalence estimated from surveys carried out simultaneously in rural Bangalore and Thiruvallur where MMR screening was also employed in addition to screening for symptoms and history of ATT [7,10]. Only screening for symptom and ATT was undertaken during the simultaneously carried out surveys in other five sites.

The screening methodology in Wardha had a limitation wherein only the individuals whose

MMR picture were labeled as 'C' or 'D' were considered eligible for sputum examination. However, its influence on prevalence estimate would be minimal considering that during the recent survey in rural Bangalore, the additional yield of cases by collecting sputum from individuals whose MMR shadows were labeled as A or B was 2.1% (unpublished data).

In our survey, 18 (21%) of 86 bacillary cases were smear positive but culture negative. This could be attributed to Modified Petroff's Method, used for digestion and decontamination of sputum specimens, which is harsher than the NALC-NaOH method and can result in loss of mycobacteria to an extent that pauci-bacillary specimen showed no growth [11]. Eight of these cases were scanty positive on sputum microscopy. Additional four cases had just completed ATT of 6 months and may be harboring non-viable bacilli; however, these cases have not been excluded from prevalence estimate.

5. Conclusion

There has been a significant decline in the prevalence of pulmonary TB in the district when compared to an earlier survey in mid-1980s. Nevertheless, TB is still a major problem in the district and requires further strengthening of TB control efforts.

Conflict of interest

None.

Author contributions

Protocol development: P.N., D.K.M. Implementation of survey procedures: P.N., D.K.M., R.N., U.N.J., A.T.T., P.H.P. Data management and analysis: N.K.T., M.A.S., V.K.C., D.K.M. Paper writing: P.N., D.K.M., V.K.C., M.A.S. Final approval: P.N.

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Appendix

Classification of individual MMR films was based on physical appearance of the lesion, if present, extent of the disease and impressions regarding etiology. Details are as under:-

Physical appearance of the lesion

Wherever, an abnormal pulmonary shadow was present, only one of the following was recorded:-

- (a) Infiltrate with cavity.
- (b) Infiltrate with doubtful cavity.
- (c) Infiltrate without cavity.
- (d) Pleural effusion.
- (e) *Hilar Adenitis* – Only dense shadows with distinct contours in the hilar regions were to be considered abnormal.

- (f) *Pulmonary scar* – Fibrotic strands or bands in the lung fields.
- (g) *Pleural scar* – Thickening of pleura or obliteration of the costo-phrenic angle.
- (h) Calcification.
- (i) Pneumothorax or Pneumoperitoneum.

Impression regarding etiology

For all abnormalities, the reader gave a judgment regarding the etiology of the lesion. For classification of lesion, probability of the finding bacilli in the sputum of examinee, nature of the lesion (presence of cavity, location of lesion) and its extent (number of zones involved and size of the lesion, uni- or bilateral distribution) is taken into account as under:-

- (A) Lung pathology other than tuberculosis.

All lesions which are considered of non-tuberculous origin. The probability of finding tubercle bacilli judged to be near zero.

- (B) Probably tuberculous but inactive.

All scars and calcification may be classified as inactive. Probability of finding tubercle bacilli is judged to be small.

- (C) Probably tuberculous, possibly active.

Lesions appearing to be of tuberculous nature but without a definite cavity and not extensive. The probability of finding tubercle bacilli is judged to be high.

- (D) Probably tuberculous and active.

The lesion appears to be of tuberculous nature, may be extensive, may be bilateral, or a definite cavity is present. The probability of finding tubercle bacilli is judged to be high.

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