

1 **Epidemiology of smear-positive pulmonary tuberculosis: A retrospective cross-sectional registry-based study**

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25 **Abstract**

26 **Introduction:** Tuberculosis (TB) remains one of the world's deadliest infectious diseases, and smear-positive pulmonary tuberculosis (SPPTB) is
27 the most contagious form. The Iranshahr province, Iran, bordering high TB-burden countries such as Pakistan and Afghanistan, has long been
28 considered an endemic area. The aim of this study was to determine the epidemiology and incidence of SPPTB in this endemic region.

29 **Materials and Methods:** A retrospective cross-sectional study was conducted using registry-based data of all suspected and confirmed TB patients
30 in Iranshahr city from 2021 to 2024. Information was collected using a standard checklist including demographic and clinical information features
31 and World Health Organization (WHO) TB patients' classification. Descriptive analyses for the study were performed using SPSS software.

32 **Results:** Among 3,078 persons evaluated for TB, 298 (9.7%) were smear-positive, 287 (96.3%) were confirmed as smear positive pulmonary TB
33 (SPPTB) by GeneXpert testing, and 283 (95.0%) patients were detected as SPPTB by culture. The mean $\pm$ SD age of SPPTB patients was 49.20
34 $\pm$ 19.43 years (range 14-106 years) with 154 males (53.7%) compared to 133 females (46.3%). The highest proportion of SPPTB cases occurred
35 in the 31–60-year age group, followed by the $\geq$ 61-year age group. Most patients were Iranian (279, 97.2%), resided in urban areas (195 patients,
36 67.9%), and were married (249 patients, 86.8%).

37 **Conclusion:** SPPTB continues to be a significant public health concern in southeastern Iran, predominantly affecting middle-aged adults, men,
38 and urban residents. Our results emphasize the impact of demographic factors and cross-border migration on TB transmission.

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40 **Keywords:** Epidemiology, GeneXpert, Infectious diseases, Smear-positive pulmonary tuberculosis, Tuberculosis

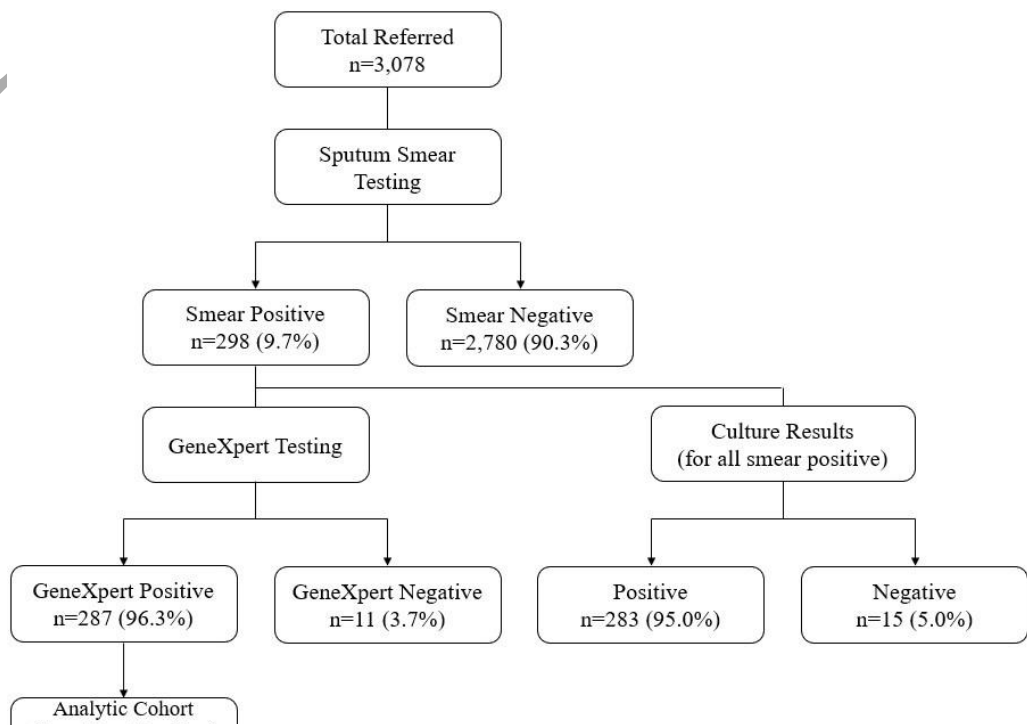
53 Tuberculosis (TB) is an airborne disease caused by *Mycobacterium tuberculosis* (MTB) that continues to be a major clinical problem in the world
54 (1, 2) . The bacterium mostly infects the lungs, but many other tissues or organs can be affected. MTB is mostly transmitted through cough or
55 sneeze droplets (3). Smear-positive pulmonary tuberculosis (SPPTB) is a highly concerning type of TB as it is more infectious than other forms
56 of TB and can be easily transmitted (4). TB with an annual incidence rate of 8.4 million cases kills approximately three people every minute (5,
57 6). According to the World Health Organization (WHO) 2022 report, there were 10.6 million new TB cases, 410,000 multidrug-resistant TB, and
58 1.6 million TB deaths (6). Over 80% of TB cases and deaths occur in low- and middle-income countries. Poverty and poor socioeconomic status
59 can weaken the immune system and increase the risk of TB (7, 8). According to the Global TB Report 2024, the prevalence of TB in Iran was
60 estimated to be 12 cases per 100 000 people (9). Early TB diagnosis and appropriate treatment are essential for disease management (10). Delayed
61 diagnosis of TB leads to missed opportunities for early intervention, more severe disease, increased risk of mortality, and higher disease
62 transmission (11). Different approaches to diagnosing TB include direct smear microscopy, culture, fluorochrome staining, interferon-gamma
63 release assays (IGRA), molecular tests, tuberculin skin testing (PPD), and chest X-rays (12). Currently, TB diagnosis primarily relies on direct
64 observation of sputum smear, which is a simple and commonly used technique. However, culture diagnosis is more sensitive and allows for
65 simultaneous determination of drug susceptibility. The effectiveness of smear microscopy for TB diagnosis depends on the examiner's expertise
66 and the quality of the sputum sample (13).The smear method detects approximately 50% of active TB cases. This method is limited by its low
67 sensitivity (60%), requires 5000 to 10,000 bacilli per mL of specimen to detect MTB on stained smears (14). Among molecular tests, Commercial
68 PCR-based molecular assays provide a rapid detection of TB and assessing its susceptibility to both first-line and second-line drugs shortly after
69 sample collection (15). Due to the significance of TB in Iranshahr city, Iran and high incidence rate in the eastern neighbors of the province, this
70 study was conducted to determine the incidence and epidemiology of TB in this province.

71 2. Materials and Methods

72 In this study, data were collected from all suspected and confirmed TB patients in Iranshahr during 2021-2024. The data were obtained from
73 patients' profiles in health care centers with the contribution of the TB center in Iranshahr city. All registered patients meeting the inclusion criteria
74 of a confirmed SPPTB diagnosis and residency in Iranshahr city were included in the study, resulting in a total of 287 patients. According to the
75 current criteria, a patient is classified as having SPPTB if they have two positive smears or one positive smear result plus more complex
76 confirmatory evidence. Data were extracted using a standardized checklist including demographic and clinical information, age, marital status,
77 group of disease record, nationality, area of residence, imprisonment history, GeneXpert result and degree of smear positivity prior to treatment .
78 The sputum smear microscopy grading system criteria classified results as follows: scanty = only 1-9 bacillus in 100 fields, grade 1+ = 10-99
79 bacilli per 100 fields, grade 2+ = 1-10 bacilli per one field, grade 3+ = more than 10 bacilli per one field. Descriptive analyses for the study were
80 performed using SPSS software, version 27 (IBM Corp., Armonk, NY, USA). Ethical approval for this study was obtained from the Ilam University
81 of Medical Sciences Research Ethics Committee (IR.MEDILAM.REC.1403.254).

82 3. Results

83 During the study, a total of 3,078 individuals were referred for TB evaluation in Iranshahr city, Sistan and Balouchestan province, southeastern
84 Iran. Of these, 2,448 (79.5%) were Iranian and



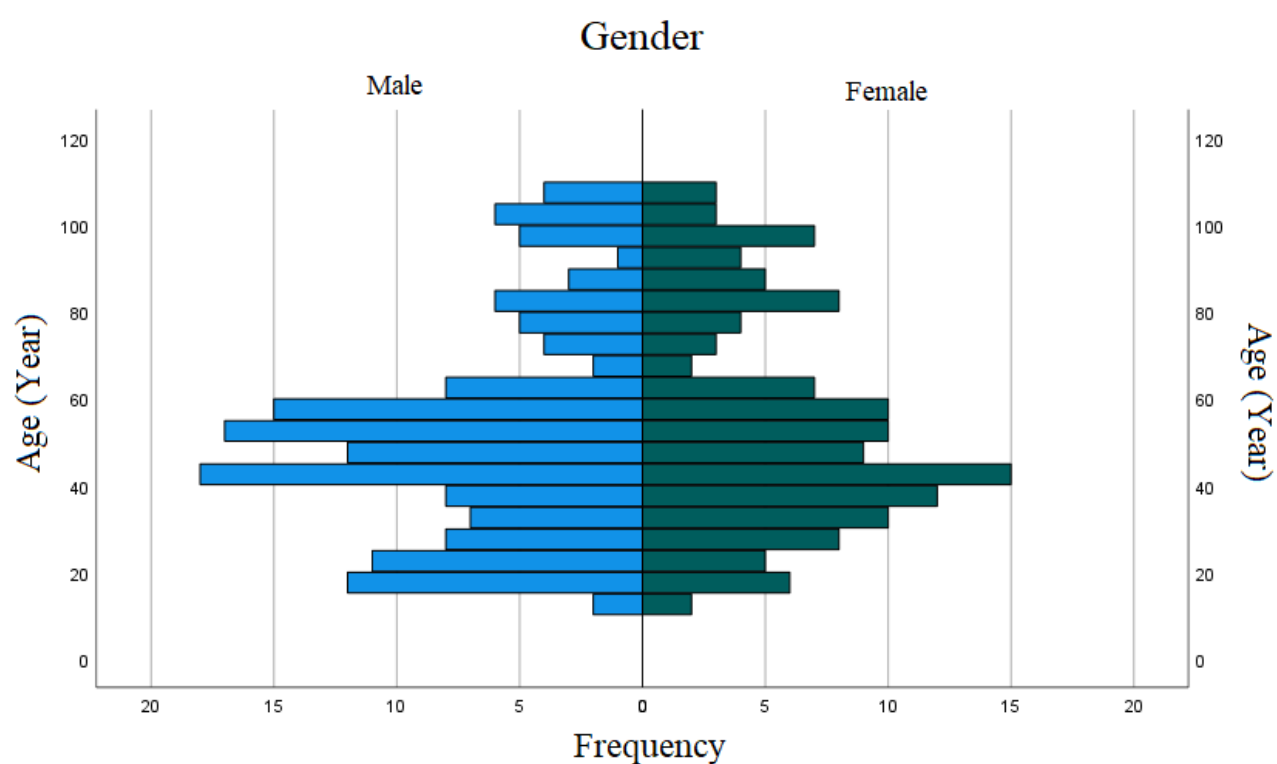


Figure 2. Age and gender distribution of smear-positive pulmonary tuberculosis (SPPTB) patients (n=287); blue bars represent male cases, and green bars represent female cases.

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86 Among the 298 smear-positive pulmonary TB cases, results from GeneXpert and culture testing were compared to assess diagnostic concordance.
 87 Overall agreement between the two methods was high, with concordant results (both positive or both negative), observed in 294/298 cases (98.7%).
 88 Specifically, 283 cases (95.0%) were positive by both GeneXpert and culture, and 11 cases (3.7%) were negative by both methods. Discordant
 89 results were observed in 4 cases (1.3%), all of which were GeneXpert-positive but culture-negative; no cases were GeneXpert-negative while
 90 culture-positive. These findings indicate substantial agreement between GeneXpert and culture as confirmatory diagnostic modalities in this smear-
 91 positive cohort (Table 1).

92 Table 1. Concordance between GeneXpert and Culture results among smear-positive TB cases (n = 298)

Culture Result GeneXpert Result	Culture Result		Total
	Culture Positive	Culture Negative	
GeneXpert Positive	283 (95.0%)	4 (1.3%)	287*
GeneXpert Negative	0 (0.0%)	11 (3.7%)	11
Total	283	15	298

93 *The analytic cohort

94 Among the GeneXpert-positive cases, 287 patients met the inclusion criteria for detailed analysis. The mean $\pm$ SD age of these patients was 49.20
 95 $\pm$ 19.43 years (range 14-106 years) and the age and gender distribution of SPPTB patients revealed a slight male predominance, with 154 males
 96 (53.7%) compared to 133 females (46.3%). The age and gender distribution of SPPTB patients is shown in Figure 2. The age distribution showed
 97 a peak in the 31-60 years age group, accounting for 151 patients (52.6%), followed by 82 patients (28.6%) over 61 years and 54 patients (18.8%)
 98 under 30 years. Ninety-two patients (32.1%) resided in rural areas and 195 (67.9%) in urban areas. The vast majority of patients were Iranian
 99 (279/287, 97.2%); 6 patients were from Afghanistan (2.1%) and 2 were from other nationalities (0.7%). Baseline smear microscopy grading
 100 revealed a high bacillary load in many patients, with grade 3+ being the most common (125/287, 43.6%), followed by grade 1+ (66/287, 23.0%),

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102 scanty (58/287, 20.2%), and grade 2+ (38/287, 13.2%). The detailed distribution of demographic and clinical characteristics is presented in Table
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Table 2. Distribution of demographic and clinical characteristics of SPPTB patients

Variable	Frequency	Percentage (%)
Gender		

Male	154	53.7
Female	133	46.3
Age (Year)		
<30	54	18.8
31-60	151	52.6
>61	82	28.6
Area of Residence		
Rural	92	32.1
Urban	195	67.9
Marital Status		
Married	249	86.8
Single	38	13.2
Prisoner		
Yes	4	1.4
No	283	98.6
Referral Center		
Health Network System	172	59.9
Self-introduce	92	32.1
Prison	4	1.4
Others	19	6.6
Degree of Smear Positivity Before Treatment		
Scanty	58	20.2
1+	66	23.0
2+	38	13.2
3+	125	43.6
Nationality		
Iranian	279	97.2
Afghan	6	2.1
Others	2	0.7
RIF Resistance		
RIF Resistance Not Detected	287	100.0
RIF Resistance Detected	0	0.0
GeneXpert Result (MTB detection level)		
MTB Detected High	154	53.7
MTB Detected Medium	38	13.2
MTB Detected Low	78	27.2
MTB Detected Very Low	17	5.9

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4. Discussion

The incidence rate of TB varies across different provinces in Iran, but Iranshahr city has reported the highest rate. This province shares borders with both Afghanistan and Pakistan and 14% of Afghan migrants live in this province. TB is an endemic disease in Pakistan and Afghanistan, which account for 71% and 9% of regional TB incidence, respectively (16, 17). In our study, 2.1% of TB-infected individuals were Afghan migrants. A survey conducted by Soleimanpour et al., in Arak, Iran showed that 6.5% of TB-infected individuals were Afghan migrants (18). In our survey, the mean $\pm$ SD age of patients was 49.20 ± 19.43 years (range 14-106 years). A study conducted by Moosazadeh et al., between 2005-2015 in Iran 34,012 SPPTB cases were recorded, with a mean monthly incidence of 404.9 (SD = 54.7). This study provided valuable insights about patterns of SPPTB across the country (19). In our study, slight discrepancies were observed between smear microscopy, GeneXpert, and culture results, with GeneXpert detecting a few additional cases compared to culture. Similar results have been reported in other studies, where the GeneXpert assay demonstrated higher sensitivity and specificity compared with sputum smear microscopy (20). In addition, Momeni et al., suggested using GeneXpert assay to avoid a missed diagnosis of smear-negative pulmonary TB (21). This variation may be attributed to differences in test sensitivity, sample quality, and bacterial load. The GeneXpert can detect MTB DNA even at low concentrations, whereas culture requires viable bacilli for growth. In a study by Pourostadi et al., 329 TB-confirmed patients were evaluated. Among them 254 cases (77.2%) were detected

as SPPTB and 75 (22.8%) were negative. This study revealed that about one-quarter of TB cases in the northwest of Iran are smear- negative which is a problem for diagnosis and treatment (22). In our study, the highest proportion of SPPTB cases occurred among individuals aged 31–60 years old. In contrast, a study conducted by Rastegar et al. reported that the highest proportion of SPPTB cases was among patients aged over 64 years old (13). Other study conducted by Alaei et al., the highest rate was observed among age between 61 and 70 years old (23). In contrast, in a study conducted by Farchi et al., in Italy, the highest proportion of SPPTB cases occurred among population aged 10-24 years old (24). This trend of incidence in old ages can be because of decreased lung function, immunosuppression, low tolerance to anti-TB drugs and age-associated comorbidities (25). Meanwhile, older people have spent more years in potential contact with infectious cases and have had more opportunity for latent infection to reactivate (25). In our study, like study conducted by Saghafipour et al. (26), incidence rate was higher among male than female. The global and international reports often reported a greater incidence of TB among male than among female. In this study, the number of patients from urban areas was higher than rural areas. This can be explained because of high population density and better diagnostic coverage in urban areas.

5. Conclusion

This study revealed that SPPTB continues to be a public health concern in Iranshahr city, where most patients were middle-aged adults, male, and residents of urban areas. The higher incidence rate among male may be attributed to lifestyle factors such as smoking, biological and immunological factors and occupational exposure. Although Afghan and other non-Iranian nationals represented a small minority (2.8%) of SPPTB cases in our study, their presence in this high-mobility border region underscores the importance of cross-border collaboration in TB surveillance and control. Molecular genotyping studies would be needed to elucidate transmission dynamics between migrant and host populations. Additional epidemiological studies are crucial to identify the underlying factors influencing TB transmission in this region, and educational programs are necessary to improve public awareness and understanding of the disease.

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7. Authors' contributions

H.R and M.Y conceived and designed the study, led data collection and curation, performed primary data processing, drafted the manuscript, and coordinated project activities. A.P, M.B and R.P performed the formal statistical analyses, prepared tables and figures, and reviewed analytical results. H.K and M.M supervised the study, contributed to study design and interpretation of results. Z.M and N.N assisted with data collection, case verification, and manuscript revision. FS and K.N supported data acquisition and contributed to manuscript editing.

8. Ethics

This study was approved by the Ilam University of Medical Sciences Research Ethics Committee IR.MEDILAM.REC.1403.254. All procedures were conducted in accordance with the Declaration of Helsinki. Patient consent was waived due to the retrospective nature of the study and anonymized registry data.

9. Funding

No Funding.

10. Conflict of Interest

The authors declare no conflicts of interest.

11. Consent to participate

This study used anonymized registry data and involved no direct interaction with patients.

12. Data availability

Data supporting this study's findings are available upon reasonable request from the corresponding author.

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