Journal of Chemical Health Risks

www.jchr.org

JCHR (2025) 15(5), 1349-1354 | ISSN:2251-6727

Interferon Gamma as a Biomarker in Tuberculosis Diagnosis in a Tertiary Care Centre

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Maternal Health, Maternal Near Miss, Obstetric Morbidity, Tertiary Care, WHO Criteria for Near Miss.

(Received: 16 July 2025 Revised: 20 August 2025 Accepted: 20 September 2025)

KEYWORDS

Tuberculosis, Interferon-Gamma, Biomarker, Diagnosis, Cytokine.

ABSTRACT:

Introduction: Tuberculosis (TB) is a major global health concern, with India accounting for a significant proportion of cases. Interferon-gamma (IFN-γ), a Th1 cytokine, is a potential biomarker for TB diagnosis. Currently, Interferon-gamma for Tuberculosis diagnosis is primarily used for research purposes only and is not commercially available for routine clinical use. The role of interferon gamma in TB patients has been evaluated in previous studies, however due to inconclusive results, this current study has been undertaken to compare blood IFN-γ levels in Tuberculosis patients versus healthy controls to evaluate its utility as a diagnostic biomarker for TB.

Objectives: To compareblood IFN-γ levels between Tuberculosis patients and healthy controls at a tertiary care centre to assess the utility of IFN-γ as a biomarker for TB diagnosis.

Methods: A cross-sectional study was conducted over a period of four months from November 2024 to February 2025 at the Department of Respiratory Medicine, Karpaga Vinayaga Institute of Medical Sciences, Chengalpattu. 60 participants were enrolled, including 30 newly diagnosed patients with TB (sputum-positive pulmonary TB, smear-negative pulmonary TB, and extrapulmonary TB) versus 30 healthy controls. Demographic and clinical data were also recorded. Venous blood (5 ml) was analysed for IFN-γ using a quantitative sandwich enzyme immunoassay.

Results: The mean age was 44.00±14.00 years in the TB group and 42.27±10.22 years in the control group ($p = 0.586$). The mean BMI was identical at 24.57±1.92 in both groups ($p = 1.000$). Females comprised 50% of TB patients and 60% of Healthy individuals ($p = 0.436$). Smoking was reported in 16.7% of TB patients and 6.7% of Healthy individuals ($p = 0.228$), whereas alcohol use was observed in 30% of TB patients and 16.7% of Healthy individuals ($p = 0.222$). Median IFN-γ was significantly higher in TB patients (203.0 pg/ml; IQR:18.7–485.0) compared to Healthy individuals (11.9 pg/ml; IQR:8.2–16.0) ($p < 0.0001$). At a cut-off of 17.1 pg/ml, IFN-γ yielded an AUC of 0.995, with sensitivity, specificity, PPV, NPV, and accuracy all at 96.67%.

Conclusions: Serum IFN-γ levels were significantly higher in active TB patients than in healthy controls. These results support IFN-γ as a reliable biomarker for TB diagnosis, especially in high-burden settings.

Journal of Chemical Health Risks

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JCHR (2025) 15(5), 1349-1354 | ISSN:2251-6727

1. Introduction

Tuberculosis (TB) remains a major public health concern worldwide, particularly in high-burden regions like India. In 2023, World Health Organization (WHO) reported 10.8 million new TB cases globally, with 26% reported from India, highlighting the need for better diagnostic strategies.[1] Interferon-gamma (IFN-γ), a cytokine mainly produced by T helper-1 (Th1) cells and natural killer cells, is essential in activating macrophages to destroy intracellular mycobacteria.[2] Due to this critical role in immune response, IFN-γ has been considered a promising biomarker for TB detection.

IFN-γ release assays (IGRAs), including QuantiFERON-TB Gold and T-SPOT.TB, detect latent tuberculosis infection (LTBI) by measuring IFN-γ production in response to specific antigens. These assays achieve high specificity by using Mycobacterium tuberculosis–specific antigens, such as ESAT-6 and CFP-10, which reduce cross-reactivity with BCG vaccination and most non-tuberculous mycobacteria.[3,4] Although IGRAs are widely used for identifying latent infection, their capacity to differentiate LTBI from active TB remains limited.[5,6]

Intensity of IFN-γ response may be linked to disease activity and the likelihood of progression. Elevated IFN-γ levels are associated with a higher probability of transition from LTBI to active TB. Despite this, most commercial assays dichotomise results (positive/negative), potentially overlooking valuable gradations in immune response.[7] Further research has indicated that IFN-γ levels vary across different clinical forms of TB. Patients with pleural tuberculosis exhibited significantly elevated IFN-γ in both blood and pleural fluid compared to non-tuberculous controls.[5] Moreover, IFN-γ alone may be insufficient to categorise active versus latent disease, prompting exploration of complementary biomarkers such as IP-10 (IFN-γ-inducible protein 10), IL-2, and TNF-α.[8]

In Indian, data on the comparative levels of IFN-γ in Tuberculosis and healthy individuals are sparse. Understanding such immunological differences could support the development of more refined diagnostic approaches.[10] Thus, this study aimed to compare blood IFN-γ levels of Tuberculosis patients and healthy controls at a tertiary care centre to assess the utility of IFN-γ as a biomarker for TB diagnosis.

2. Objectives

To compareblood IFN-γ levels between Tuberculosis patients and healthy controls at a tertiary care centre to assess the utility of IFN-γ as a biomarker for TB diagnosis.

3. Methodology

3.1. Study design and setting

This cross-sectional study included 60 patients and was conducted in the Department of Respiratory Medicine at Karpaga Vinayaga Institute of Medical Sciences and Research Centre, Chengalpattu District, over four months from November 2024 to February 2025. Approval was obtained from the Institutional Ethics Committee, and written informed consent was obtained from all participants before enrolment.

3.2. Inclusion criteria

Adults over 18 years of age, of all gender, who were newly diagnosed with sputum-positive pulmonary TB, smear-negative pulmonary TB, or extrapulmonary TB were included. Healthy adults without any comorbid conditions were also recruited as controls.

3.3. Exclusion criteria

Patients already receiving anti-tuberculosis therapy or those at higher risk for infection, such as healthcare workers and household contacts of TB patients, were excluded.

3.4. Methods

Purposive sampling was used to recruit all eligible participants until the required sample size was reached. All tuberculosis cases, including sputum-positive PTB, smear-negative PTB, and extrapulmonary TB, were combined into a single “case” group for analysis and compared with the healthy control group. The cited study by Hussain et al.[11] included a minimum of 40 participants in each group; however, in the present study, 30 participants were included in each group. The study population comprised 30 newly diagnosed TB patients (cases) and 30 healthy adults (controls), totalling 60 participants.

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JCHR (2025) 15(5), 1349-1354 | ISSN:2251-6727

Tuberculosis was diagnosed using a combination of clinical evaluation, sputum acid-fast bacillus (AFB) smear, GeneXpert testing, chest radiography, and, for extrapulmonary TB cases, additional imaging, fine needle aspiration cytology (FNAC), or biopsy. A 5 ml sample of venous blood was drawn from each participant using aseptic technique. The collected blood was centrifuged at 3000 rpm for 10 minutes in a swing-rotor centrifuge to separate the serum. The serum was preserved at −80 °C until further testing. Measurement of IFN-γ concentration was carried out using a quantitative sandwich enzyme immunoassay kit (ELK Biotechnology, USA), which had an analytical sensitivity of 5.9 pg/ml.. All procedures were performed according to the manufacturer's guidelines. Demographic and clinical variables, including age, gender, and body mass index (BMI), were documented in a predesigned proforma. All laboratory results were compiled in a database for analysis.

4. Statistical analysis

The data were processed using SPSS software version 20. Continuous variables are presented as mean values with standard deviations, while categorical variables are shown as frequencies and percentages. Group comparisons were carried out using the independent t-test for continuous variables and the chi-square test for categorical variables. A p-value <0.05 is considered significant.

5. Results

In this study, 30 tuberculosis cases comprising sputum-positive PTB, smear-negative PTB, and extrapulmonary TB were analysed together as a single "case" group and compared with the 30 healthy controls. The mean age of the participants was 44.00 ± 14.00 years in the TB Cases and 42.27 ± 10.22 years in the Healthy controls, with no significant difference (p = 0.586). The mean BMI was identical in both groups at 24.57 ± 1.92, showing no significant difference (p = 1.000). Of 60 patients, the Healthy controls included 18 (60%) females and 12 (40%) males, compared to 15 (50%) females and 15 (50%) males in the TB Cases (p = 0.436). Regarding smoking status, 28 (93.3%) Healthy controls were non-smokers and 2 (6.7%) were smokers, whereas 25 (83.3%) TB Cases were non-smokers and 5 (16.7%) were smokers (p = 0.228). For alcohol consumption, 25 (83.3%) Healthy controls reported no use and 5 (16.7%) reported use, while 21 (70%) TB Cases reported no use and 9 (30%) reported alcohol use (p = 0.222) (Table 1).

Table 1: Comparison of IFN-γlevels and demographic factors between groups

Parameter		Group N (%)		P value
		Healthy Controls	TB Cases	
Age		42.27 ± 10.22	44.00 ± 14.00	0.586
BMI		24.57 ± 1.92	24.57 ± 1.92	1
Sex	Female	18 (60%)	15 (50%)	0.436
	Male	12 (40%)	15 (50%)	
Smoking	No	28 (93.3%)	25 (83.3%)	0.228
	Yes	2 (6.7%)	5 (16.7%)	
Alcohol	No	25 (83.3%)	21 (70%)	0.222
	Yes	5 (16.7%)	9 (30%)	

The median IFN-γ level in the control group was 11.9 (IQR: 8.2–16.0), whereas in the case group, it was markedly higher at 203.0 (IQR: 18.7–485.0), and this difference was significant (p < 0.0001) (Table 2).

Table 2: Comparison of interferon gamma levels between groups

	Group						P value
	Healthy Control			TB Cases			
	Median	Percentile 25	Percentile 75	Median	Percentile 25	Percentile 75	
IFN-γ level	11.9	8.2	16	203	18.7	485	**<0.0001**

In IFN-γ Levels Between Groups, 29 (96.7%) controls had interferon gamma levels < 17.1 pg/mL, while 1 (3.3%) control had levels > 17.2 pg/mL. In contrast, 29 (96.7%) cases had interferon gamma levels > 17.2 pg/mL, and 1 (3.3%) case had levels < 17.1 pg/mL (Table 3).

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JCHR (2025) 15(5), 1349-1354 | ISSN:2251-6727

Table 3: Comparison of IFN-γ levels between groups

Parameter	Category	Group N (%)	
		Healthy Control	TB Cases
IIFN-γ level	>17.2	1	29
	<17.1	29	1

At a cutoff value of 17.1, IFN-γ level showed an AUC of 0.995, which was significant ($p < 0.0001$). The test demonstrated a sensitivity of 96.67%, specificity of 96.67%, positive predictive value of 96.67%, negative predictive value of 96.67%, and overall accuracy of 96.67% (Figure 1 and Table 4).

Table 4: Diagnostic performance of IFN-γ **level for differentiating groups**

Cut-off value	17.1
AUC	0.995
P value	<0.0001
Sensitivity	96.67%
Specificity	96.67%
PPV	96.67%
NPV	96.67%
Accuracy	96.67%

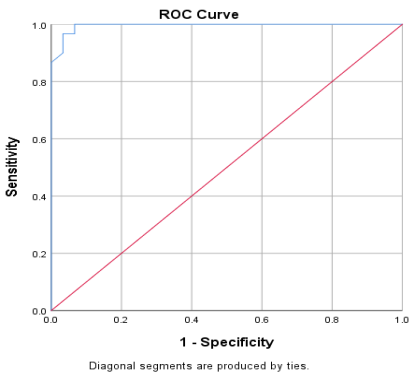

Figure 1: ROC curve for interferon gamma level in differentiating groups.

6. Discussion

In our study, there was no significant difference in age or BMI between the TB case and Healthy control groups, showing that they were comparable in these parameters. Similarly, **Jung et al.** reported a median age of 39 years (IQR 30–54) among 335 participants, with males accounting for 38.2%. The active TB group had a higher median age of 53 years, and 57.5% were male.[12]

Our study showed a balanced sex distribution with a mean age of about 44 years, with no significant difference between the TB case and Healthy control groups. **Goyal et al.** observed a slight female predominance (52.2%) among 69 patients with EPTB, most of whom were aged 18–30 years (55.1%).[10] Similarly, **Dar et al.** reported no significant difference in mean age between cases and controls (33.02 vs. 30.30 years), which is in line with our results (44.00 vs. 42.27 years).[13]

Our study observed that interferon gamma levels were markedly higher in the TB case group compared to the Healthy control group. **Chen et al.** reported median IFN-γ concentrations of 68.1 pg/mL in the non-TB group, 365.5 pg/mL in the clinically diagnosed TB group, and 382.1 pg/mL in the microbiologically confirmed TB group, with significantly elevated values in TB patients compared to controls.[14] Our findings showed median IFN-γ levels of 203.0 pg/mL (IQR: 18.7–485.0) in cases and 11.9 pg/mL (IQR: 8.2–16.0) in controls, with $p < 0.0001$.[14]**Jung et al.** assessed IFN-γ release assays (QFT-Plus and TB-Feron) and found IGRA-positive rates of about 50%, demonstrating strong agreement between tests and high sensitivity (~88–90%) along with specificity (~95–100%) for active TB detection.[12]

Metcalfeet al. found significantly higher median IFN-γ levels in tuberculosis cases than in non-cases, with levels linked to increased odds of active TB.[15]**Cattamanchi et al.** reported higher median IFN-γ spot counts after antigen stimulation in active TB patients than in non-TB patients.[16] These findings align with our results, which show significantly elevated IFN-γ levels in cases compared to controls.

Liang et al. reported significantly higher baseline IFN-γ levels in TB patients compared to healthy controls (1.83 ± 1.37 IU/ml vs. 0.14 ± 0.25 IU/ml; $p < 0.001$). Our study likewise found markedly elevated median IFN-γ levels in cases versus controls (203.0 vs. 11.9, $p < 0.0001$), though differences in units and assays may

Journal of Chemical Health Risks
www.jchr.org
JCHR (2025) 15(5), 1349-1354 | ISSN:2251-6727

account for numerical discrepancies.[17] **Dar et al.** reported significantly higher serum IFN-γ levels in cases at baseline (41.47 ± 43.25 pg/ml) compared to controls (1.81 ± 1.6 pg/ml; $p < 0.003$), with levels decreasing significantly after 2 and 6 months of treatment. This dynamic decline parallels our observation of significantly elevated IFN-γ levels in cases versus controls (median 203.0 vs. 11.9, $p < 0.0001$).[13] Our findings of significantly elevated IFN-γ levels in TB cases are consistent with multiple studies, reinforcing IFN-γ's role as a reliable biomarker for active tuberculosis diagnosis.

In our study, 96% participants in the TB case group had greater IFN-γ threshold, whereas most in the Healthy control group had levels below it. Similarly, **Goyal et al.** demonstrated that IFN-γ/IL-2 levels were significantly higher in patients positive by conventional diagnostic methods (AFB staining or culture) than in negative patients ($p < 0.05$), highlighting IFN-γ's discriminative capacity.[10] Our study similarly showed a clear separation of IFN-γ levels > and below a cutoff of 17.1, with optimal sensitivity and specificity.

Metcalfeet al. used the cutoff (0.35 IU/ml) and reported a sensitivity of 72% and specificity of 47% for active TB detection, with 85% of cases > IFN-γ quintiles. Our study, with a higher cutoff (17.1), demonstrated improved sensitivity and specificity (both 96.67%).[15] Similarly, **Cattamanchi et al.** reported an IGRA sensitivity of 73% and a specificity of 54%[16] .Our study demonstrated superior diagnostic accuracy of IFN-γ levels using a higher cutoff, outperforming previously reported sensitivities and specificities, and reinforcing its strong discriminative ability for active TB diagnosis.

In our study, at a cutoff of 17.1 pg/mL, IFN-γ demonstrated excellent diagnostic accuracy, with high sensitivity, specificity, and predictive values distinguishing between cases and controls. **Chen et al.** reported that IFN-γ and/or IL-2 positivity yielded a sensitivity of 91.04%, specificity of 65.34%, and accuracy of 86.85%, whereas requiring dual positivity increased specificity to 87.50% but reduced sensitivity to 65.82%. The ROC AUC for the combined detection was 0.782.[14]

Metcalfeet al. reported that adding quantitative IFN-γ improved the prediction accuracy from an AUC of 0.71 to 0.78 ($p < 0.001$) with moderate sensitivity and specificity. Our study showed superior performance with IFN-γ alone, achieving an AUC of 0.995 and sensitivity and specificity of 96.67%.[15] Our study demonstrated that IFN-γ at a 17.1 pg/mL cutoff offers outstanding diagnostic accuracy, surpassing previous reports in terms of sensitivity, specificity, and AUC, highlighting its strong potential as a standalone biomarker for active TB detection.

7. Limitations

This study was limited by its single-centre design with a small sample size, which may restrict the generalisability of the findings. As the study was cross-sectional with relatively short study duration, longitudinal changes in IFN-γ levels throughout the disease or treatment could not be performed.

8. Conclusion

Serum IFN-γ levels were significantly elevated in patients with active tuberculosis compared to healthy controls. The test demonstrated reliable diagnostic performance, achieving high sensitivity, specificity, and overall accuracy. These findings support IFN-γ as a robust and reliable biomarker for active TB diagnosis in clinical practice, particularly in high-burden settings. Further multicentre and longitudinal studies are warranted to validate these results and explore their role in monitoring treatment response.

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