

## Pre-treatment Interferon-Gamma Inducible Protein 10 (IP-10) is Associated with Recurrent Drug-Resistant Tuberculosis

### Interferon-Gamma Inducible Protein 10 (IP-10) Sebelum Pengobatan Berhubungan dengan Kekambuhan Pada Tuberkulosis Resisten Obat

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#### Abstract

**Background:** IP-10, a chemokine, is found in high levels in drug-resistant tuberculosis (DR-TB) patients. This study analyzed the pre-treatment IP-10 level and its association among recurrent DR-TB patients. **Method:** A case-control study was conducted among DR-TB patients, newly diagnosed as the control group and the recurrent as the case group. IP-10 level was measured using the sandwich ELISA method. We used Mann-Whitney to analyze the differences in IP-10 levels between the two groups. **Results:** 72 DR-TB patients enrolled in this study and 45 (62.5%) were recurrent cases. The median (IQR) of IP-10 was higher in recurrent than in newly diagnosed DR-TB, 1876.4 (625.2-8313) vs 1304 (590.4-2001) pg/ml, respectively, with a P-value of 0.001. Among recurrent DR-TB, those with lung cavities had higher IP-10 levels than those without, P-value = 0.001. According to the ROC curve, the optimal cut-off value of IP-10 was 1555.6 pg/mL, with a sensitivity and specificity of 66.7% in predicting recurrent cases. The pre-treatment of IP-10 level  $\geq 1555.6$  pg/ml was significantly associated with recurrent DR-TB (OR = 3.625; 95% CI: 1.325-9.917), and IP-10 level  $\geq 2012.4$  pg/ml was significantly associated with lung cavity among recurrent DR-TB patients (OR = 7.429; 95% CI: 1.939-28.466). IP-10 level correlates with sputum smear grading (P-value=0.010; Spearman rho=0.506) among recurrent DR-TB patients. **Conclusion:** IP-10 level was significantly high among recurrent DR-TB patients and those with lung cavities. An IP-10 level  $\geq 1555.6$  pg/ml is associated with recurrent DR-TB in this case-control study. IP-10 level  $\geq 2012.4$  pg/ml is significantly associated with lung cavities among recurrent DR-TB patients.

**Keywords:** DR-TB; Recurrent, IP-10 level.

#### Abstrak

**Latar Belakang:** IP-10 ditemukan pada kadar tinggi pada pasien tuberkulosis resisten obat (TB-RO). Studi ini menganalisis kadar IP-10 sebelum pengobatan dan hubungannya pada pasien TB-RO yang kambuh. **Metode:** Penelitian ini menggunakan desain kasus-kontrol. Pasien TB-RO dibagi menjadi kelompok kontrol yang terdiri dari pasien yang baru dan kelompok kasus yang terdiri dari pasien yang kambuh. Kadar IP-10 diukur menggunakan metode sandwich ELISA. Uji Mann-Whitney digunakan untuk menganalisis perbedaan kadar IP-10 antara kedua kelompok. **Hasil:** 72 pasien TB-RO ikut serta dalam studi ini, dan 45 (62,5%) di antaranya adalah kasus kambuh. Median (IQR) IP-10 lebih tinggi pada kasus kambuh dibandingkan dengan kasus baru, yaitu 1876,4 (625,2-8313) vs 1304 (590,4-2001) pg/ml, dengan P-value < 0,001. Di antara kasus kambuh, pasien dengan kavitas paru memiliki kadar IP-10 yang lebih tinggi dibandingkan dengan yang tanpa kavitas, P-value < 0,001. Berdasarkan kurva ROC, nilai ambang optimal IP-10 adalah 1555,6 pg/mL, dengan sensitivitas dan spesifisitas 66,7% dalam memprediksi kasus kambuh. Kadar IP-10 sebelum pengobatan  $\geq 1555,6$  pg/ml secara signifikan berhubungan dengan kasus kambuh (OR = 3,625; 95% CI: 1,325-9,917), dan tingkat IP-10  $\geq 2012,4$  pg/ml secara signifikan berhubungan dengan kavitas paru pada pasien DR-TB yang kambuh (OR = 7,429; 95% CI: 1,939-28,466). Kadar IP-10 berkorelasi dengan tingkat positif BTA (P-value=0,010; Spearman rho=0,506) pada pasien TB-RO yang kambuh. **Kesimpulan:** Kadar IP-10 secara signifikan lebih tinggi pada pasien TB-RO yang kambuh dan yang memiliki kavitas paru. Kadar IP-10  $\geq 1555,6$  pg/ml berhubungan dengan terjadinya kasus kambuh. Kadar IP-10  $\geq 2012,4$  pg/ml secara signifikan berhubungan dengan kavitas paru pada kasus kambuh.

**Kata Kunci:** Kadar IP-10, TB resisten obat, Kambuh.



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## Introduction

Tuberculosis (TB) is one of the most infectious diseases with a high transmission rate worldwide. Indonesia is one of the world's highest-burden countries of drug-resistant tuberculosis (DR-TB). The successful treatment of DR-TB or rifampicin-resistant TB in Indonesia is lower than 57%. [1] Those previously treated with antitubercular drugs or who had lung cavities have a high risk of DR-TB. [2] The term recurrent is associated with reinfection or reactivation. Several studies demonstrated that the time until six years is a significant risk factor for the reactivation of DR-TB. [3,4] The inflammatory response in DR-TB patients is thought to be higher than in those with drug-sensitive TB (DS-TB). A recent study reported that levels of IFN- $\gamma$ , TNF- $\alpha$ , IL-10, and IL-10 were significantly higher in DR-TB patients than in DS-TB patients. [5,6].

Although several studies have reported risk factors for the recurrence of MDR-TB cases, host immunological factors that contribute to recurrence have not been investigated. Currently, the host response for the available studies is measured using the interferon-gamma release assay (IGRA). [7] The neutrophil-lymphocyte ratio (NLR), a simple and inexpensive marker, may be used to predict disease severity and is associated with recurrent TB. However, none of these clinical risk factors reliably predict recurrence at an individual level, highlighting the need for a host biomarker that reflects the underlying immunological state. [8]

One of the cytokines or chemokines that can be considered alternative markers of the host response to TB infection is interferon-gamma inducible protein 10 (IP-10). IP-10 is a pro-inflammatory chemokine released by monocytes, dendritic cells, and TH1 cells, which activates macrophages to release TNF- $\alpha$  and IFN- $\gamma$ , a process involved in the elimination of TB bacteria. [9] However, excessive release of IP-10 may exacerbate inflammation and promote cell necrosis through the induction of mitochondrial reactive oxygen species (ROS). Unlike IFN- $\gamma$ , IP-10 levels are less affected by factors such as age, low CD4+ cell count, and immunosuppressive therapy. In addition, IP-10 is expressed at levels up to 100-fold higher than IFN- $\gamma$ , and elevated IP-10 levels have been associated with TB reactivation. [7] The IP-10 level was higher in TB patients who relapsed after treatment, and it can identify patients at high risk with lung cavities and treatment failures characterized by positive sputum. [10]

A previous study reported that the IP-10 level was higher in DR-TB patients than in DS-TB patients. [11] Therefore, in a limited-resource setting for sputum culture or for patients who can't provide sputum, measuring IP-10 levels can be a promising alternative for diagnosing TB, monitoring TB treatment, and predicting disease severity. [7] To date, no study has evaluated IP-10 levels in patients with new and recurrent DR-TB or examined their association with lung cavities and sputum bacterial burden. We hypothesized that pre-treatment IP-10 levels would be higher in recurrent DR-TB patients compared to newly diagnosed ones, and that higher levels would correlate with lung cavities and bacterial burden

Based on this background, we analyzed the pre-treatment IP-10 level among new and recurrent DR-TB patients. A good understanding of the factors associated with recurrent DR-TB helps to identify those at risk of recurrent and appropriate measures can be taken to reduce this risk.

## Material and Methods

### Study Design and Participants

The study employed a case-control design. Based on chest radiography, we enrolled newly diagnosed and recurrent DR-TB patients with or without lung cavities. This study aimed to analyze the pre-treatment IP-10 level among DR-TB patients and its association with lung cavity and sputum smear grading. The study was conducted at the MDR-TB outpatient department of Dr. Soetomo Hospital, Surabaya, from September 2022 to March 2023. This study received ethical approval from the Ethics Committee of Dr. Soetomo Hospital, with the number 0048/KEPK/VII/2022. All DR-TB patients confirmed through GeneXpert and receiving treatment were eligible to enroll in this study. The study populations included all the cases and controls accessible for sampling during the study period.

We divided DR-TB patients into two groups: recurrent or new DR-TB. Retreated cases were DR-TB patients previously treated in category I or II tuberculosis or DOTs with outcome treatment as a cure, failure, incomplete treatment, or loss to follow-up. Similarly, the new cases were DR-TB patients who never received antitubercular drugs. All eligible patients were required to complete and sign a written informed consent form before enrollment. The inclusion criteria for this study were (a) patients demonstrated resistance to rifampicin by GeneXpert, (b) aged  $\geq 18$  years, and (c) willing to sign informed consent. We interviewed all patients to collect a history of comorbid diseases and their associated medications. Exclusion criteria were (a) pregnant women, (b) those with HIV positive or other systemic inflammatory and infectious diseases, and (c) incomplete smear or culture sputum and chest radiography at baseline. We used consecutive sampling to gather study participants. DR-TB patients with no history of DM; however, the level of fasting blood glucose (FBG) was  $> 126$  mg/dL, 2-hour postprandial blood glucose (2hPPBG)  $> 200$  mg/dL, and HbA1c  $> 6.5\%$  at baseline screening; then, the patients were considered to have DM.

### Laboratory Examinations

IP-10 levels were measured using the sandwich enzyme-linked immunosorbent assay (ELISA) method. The assay procedure was performed according to the manufacturer's instructions using the Human IP-10 ELISA Kit (FineTest; Wuhan Fine Biotech Co., Ltd., China). The lower limit of detection for IP-10 was 7.813 pg/mL. Three milliliters of venous blood were collected from each patient into a plain Vacutainer tube and centrifuged at 3000 rpm for 15 minutes to obtain serum samples. The serum was then stored at  $-80^{\circ}\text{C}$  for up to three months before IP-10 measurement. Sputum smear microscopy and culture examinations were performed using Ziehl-Neelsen staining and Lowenstein-Jensen medium, respectively. Sputum smear grading was used to assess bacterial burdens and was classified as negative, scanty, 1+, 2+, and 3+. Chest radiography was used to determine whether the lung cavity was either unilateral or bilateral. Laboratory examinations were conducted at the clinical pathology laboratory of Dr. Soetomo Hospital.

### Statistical Analysis

Categorical variables, including sex, type of recurrent TB, type of DR-TB, diabetes mellitus, sputum smear and culture results, and presence of lung cavities, were presented as frequencies and percentages. Continuous variables, such as age, BMI, and IP-10 levels, were expressed as median and interquartile range (IQR) in pg/mL. The Mann-Whitney U test was used to compare IP-10 levels between patients with new and recurrent DR-TB, as well as between recurrent DR-TB patients with and without lung cavities. Categorical variables were analyzed using the Chi-square test or Fisher's exact test, as appropriate. Receiver operating characteristic (ROC) curve analysis was performed to determine the optimal cut-off value of IP-10 levels for differentiating between new and recurrent DR-TB and for identifying the presence of lung cavities. Bivariate and multivariate analyses were conducted to assess risk factors associated with recurrent DR-TB. Spearman's rank correlation test was used to evaluate the correlation between IP-10 levels and sputum smear grading. Statistical analyses were performed using SPSS version 20.0 (SPSS Inc., Chicago, IL, USA). A P-value of  $< 0.05$  was considered statistically significant.

### Results and Discussion

Seventy-two patients with DR-TB were enrolled in the present study, with males accounting for half of the participants. The median BMI was  $< 18.5$  kg/m<sup>2</sup>, indicating that patients in both groups were underweight. More than 60% of the patients had previously received antitubercular treatment, and approximately half of them were relapse cases. MDR-TB was the most prevalent form of DR-TB in both groups. The rates of positive sputum smear results were similar between the two groups. Patients in the recurrent group had a higher

prevalence of lung cavities than those in the new group, although the difference was not statistically significant. The baseline demographic characteristics of the two groups are presented in **Table 1**.

**Table 1.** Demographic data among DR-TB patients

| Variables                                    | Recurrent (n=45) | New (n=27)       | P-value |
|----------------------------------------------|------------------|------------------|---------|
| <b>Sex</b>                                   |                  |                  |         |
| Male                                         | 23 (51.1)        | 16 (59.2)        | 0.140   |
| Age (years)                                  | 48 (18-74)       | 49 (18-69)       | 0.635   |
| BMI (kg/m <sup>2</sup> )                     | 17.4 (8.0-26.7)  | 16.1 (11.7-25.4) | 0.431   |
| Underweight (<18.5kg/m <sup>2</sup> )        | 28 (62.2)        | 18 (66.6)        |         |
| Normal weight (18.5-24.9 kg/m <sup>2</sup> ) | 15 (33.3)        | 8 (29.6)         |         |
| Overweight (25.0-29.9 kg/m <sup>2</sup> )    | 2 (4.5)          | 1 (3.8)          |         |
| <b>Type of recurrent</b>                     |                  |                  |         |
| Relapse                                      | 31 (68.8)        | -                | NA      |
| Treatment failure                            | 11 (24.4)        | -                |         |
| LTFU                                         | 3 (6.8)          | -                |         |
| <b>Type of DR-TB</b>                         |                  |                  |         |
| MDR-TB                                       | 42 (93.3)        | 27 (100)         | NA      |
| Pre XDR-TB                                   | 2 (4.4)          | -                |         |
| XDR-TB                                       | 1 (2.3)          | -                |         |
| <b>Diabetes Mellitus</b>                     |                  |                  |         |
| Yes                                          | 22 (48.8)        | 14 (51.8)        | 0.214   |
| AFB                                          |                  |                  |         |
| Negative                                     | 20 (44.4)        | 10 (37.0)        | 0.537   |
| Positive                                     | 25 (55.6)        | 17 (63.0)        |         |
| 1+                                           | 8 (32.0)         | 2 (11.8)         |         |
| 2+                                           | 5 (20.0)         | 5 (29.4)         |         |
| 3+                                           | 12 (48.0)        | 10 (58.8)        |         |
| <b>Lung Cavity</b>                           |                  |                  |         |
| Yes                                          | 17 (37.7)        | 6 (22.3)         | 0.171   |
| Unilateral                                   | 10 (58.8)        | 6 (100.0)        |         |
| Bilateral                                    | 7 (41.2)         | 0 (0)            |         |
| No                                           | 28 (62.3)        | 21 (77.7)        |         |

Abbreviations: BMI: body mass index; LTFU: loss to follow-up; MDR-TB: multidrug-resistant tuberculosis; pre-XDR-TB: pre extensively drug-resistant tuberculosis; XDR-TB: extensively drug-resistant tuberculosis; AFB: acid fast bacilli

Among 72 DR-TB patients, 45 (62.5%) were recurrent cases previously treated with DOTs in category I or II. Among recurrent DR-TB patients in our study, 43% of 72 DR-TB patients were relapse cases after patients were declared cured or treatment completed in DOTs. A previous study in Indonesia reported that among DR-TB patients, 66.0% were secondary or recurrent cases.[12] The higher rates of recurrent cases in our study might be because of incomplete treatment, which leads to treatment failure, increasing the risk of developing DR-TB cases. Rifampicin is the main drug in the treatment of TB. The resistance of rifampicin is associated with its concentration in plasma. For TB patients who have previously used rifampicin, subclinical concentrations of rifampicin may increase the risk of mutations in the *rpoB* gene, resulting in decreased rifampicin activity. A study by Khan *et al*, reported that drug resistance was significantly associated with recurrent TB.[13] We found that the median BMI in both groups was underweight, and less than 40% of the participants were classified as normal weight. Our previous study demonstrated that more than 50% of DR-TB patients were underweight.[14] Being underweight is associated with malnutrition and represents a deficiency of protein and calories that lowers the efficiency of the cell-mediated immune system and several innate host defense mechanisms against TB infection. As a result, a shortage of protein and calories induces a secondary immunodeficiency state that increases the risk of developing TB. Patients with underweight were more likely to develop active TB, and this was significantly associated with unsuccessful treatment outcomes among DR-TB.[15] Furthermore, being male was a risk factor for DR-TB in underweight individuals(16), as demonstrated in our study, where more than 50% of DR-TB patients were male.

Our study demonstrated a high prevalence of diabetes among DR-TB patients. It was similar to the study by Araia *et al.*, reporting that more than 30% of TB patients had DM.[17] Patients with TB aged ≥ 45 were

more likely to develop DM. In addition, patients with TB-DM were six times more likely to develop MDR-TB and four times more likely not to have sputum conversion during treatment. Based on sputum smear examination, more than 50% of patients had positive smear results at baseline, with a high bacillary load (3+), and the distribution was similar between the two groups. It infers that approximately 50% of DR-TB patients in our study can transmit the drug-resistant *M. tuberculosis* strain to their community. The high bacillary load in our study was similar to the study by Kassa *et al*, who reported that one-third of DR-TB patients had a sputum smear grade level of 3+.[18] The probability of infectiousness increases with increased sputum smear grading. Our previous study also demonstrated a high positivity of sputum smear grading among DR-TB patients.[14] The bacillary load in our study was greater than that of DS-TB patients.

One of the main problems in DR-TB patients is the lung cavity, which can inhibit sputum culture conversion, increase the transmission of TB bacilli and antibiotic resistance, and be a risk factor for recurrence after treatment. Studies reported that the lung cavity was an independent risk factor for developing DR-TB.[19,20] Bacterial proliferation promotes replication-induced mutations at drug resistance-determining loci, resulting in a high likelihood of mutants acquiring drug resistance. We found that recurrent DR-TB patients had more lung cavities than new DR-TB patients. It was most likely due to MMP activity. Lung cavity formation in patients with TB is significantly associated with matrix metalloproteinase (MMP) activity. MMPs are proteolytic enzymes capable of degrading the extracellular matrix, and their activity is tightly regulated at the transcriptional level by tissue inhibitors of metalloproteinases (TIMPs).[8] Hypoxia in pulmonary TB lesions induces MMP-1 expression in respiratory tract epithelial cells through the activation of NF- $\kappa$ B and HIF-1 $\alpha$ . The association between high IP-10 and lung cavities may be explained by persistent antigen stimulation. Hypoxia within the cavity upregulates HIF-1 $\alpha$ , which in turn induces IP-10 expression. Sustained high IP-10 levels could contribute to excessive inflammation and tissue necrosis, creating a favorable niche for bacterial persistence and subsequent recurrence after treatment completion.[21] The median pre-treatment IP-10 level between the two groups, as well as the levels in those with and without lung cavities among recurrent DR-TB patients, are shown in **Figure 1**.

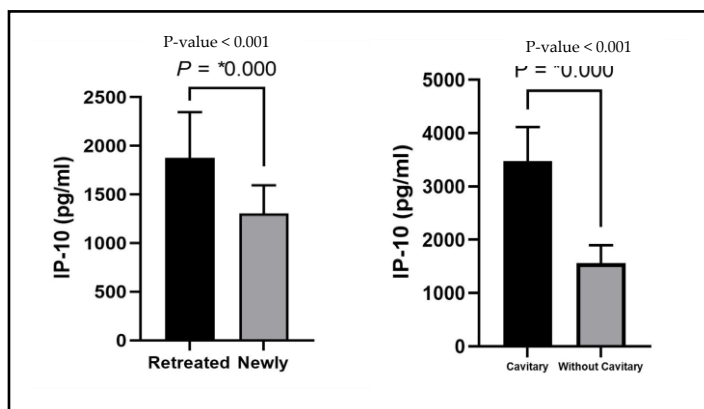


**Figure 1a.** Pre-treatment IP-10 level between the two groups. **Figure 1b.** Pre-treatment IP-10 level in the presence of lung cavity

The median (IQR) of pre-treatment IP-10 levels was significantly higher in recurrent than in newly diagnosed DR-TB patients, 1876.4 (625.2-8313) vs 1304 (590.4-2001) pg/ml, P-value < 0.000. Furthermore, among recurrent DR-TB patients, those with lung cavities had significantly higher pre-treatment IP-10 levels than those without lung cavities, 3474.6 (1270.2-7315) vs. 1560.6 (625.2-6554) pg/ml, P-value < 0.000. We found that the IP-10 level was significantly higher in recurrent DR-TB patients than in new DR-TB. IP-10 specifically activates the CXCR3 receptor, which is predominantly expressed on activated T and B lymphocytes, natural killer (NK) cells, dendritic cells, and macrophages. IP-10 induces chemotaxis and apoptosis, inhibits cell proliferation, and promotes angiostasis. It is produced by antigen-presenting cells (APCs) and activates macrophages during infection, thereby facilitating chemotaxis, leukocyte migration, regulation of cell growth, and angiogenesis. Recent evidence also suggests that IP-10 may contribute to limiting *Mycobacterium tuberculosis* (MTB) replication.[9] Compared to studies in drug-sensitive TB, where the median IP-10 was around 384 pg/mL, our DR-TB patients had substantially higher levels (1876 pg/mL), suggesting that drug resistance is associated with a hyperinflammatory state.[22]

According to the receiver operating characteristic (ROC) curve, the optimal cut-off value of pre-treatment IP-10 was 1555.6 pg/ml, with a sensitivity and specificity of 66.7% in predicting recurrent cases, as shown in **Figure 2a**. However, the sensitivity and specificity of 66.7% indicate modest diagnostic accuracy, and this cut-off value requires external validation before clinical application. Furthermore, among recurrent DR-TB patients, the best cut-off value of pre-treatment IP-10 level  $\geq 2012.4$  pg/ml, with a sensitivity of 72.2% and a specificity of 74.1%, was found to differentiate those with lung cavities, as shown in **Figure 2b**.

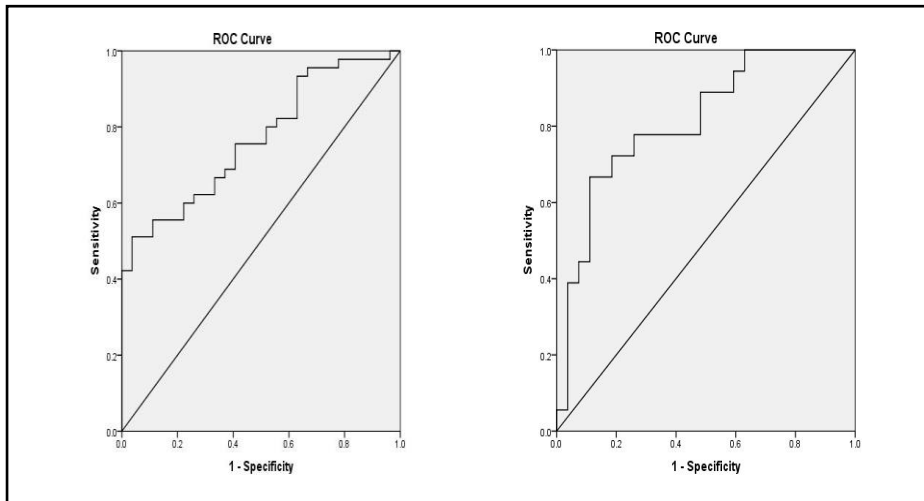


**Figure 2a.** ROC curve of pre-treatment IP-10 between newly and recurrent cases. **Figure 2b.** ROC curve of pre-treatment IP-10 among recurrent case with and without lung cavity

**Table 2.** Bivariate analysis of risk factors for recurrent DR-TB

| Parameter                     | Recurrent (n=45) | New (n=27) | Unadjusted odds ratio<br>(95% CI) | P-value |
|-------------------------------|------------------|------------|-----------------------------------|---------|
| <b>Sex</b>                    |                  |            |                                   |         |
| Male                          | 23 (51.1)        | 16 (59.2)  | 0.719 (0.274-1.886)               | 0.502   |
| Female                        | 22 (48.9)        | 11 (40.8)  |                                   |         |
| <b>Age (years)</b>            |                  |            |                                   |         |
| $\geq 45$                     | 29 (64.4)        | 18 (66.6)  | 0.906 (0.331-2.479)               | 0.848   |
| $< 45$                        | 16 (35.6)        | 9 (33.4)   |                                   |         |
| <b>BMI (kg/m<sup>2</sup>)</b> |                  |            |                                   |         |
| $< 18.5$                      | 28 (62.2)        | 18 (66.6)  | 0.824 (0.302-2.242)               | 0.704   |
| $\geq 18.5$                   | 17 (37.8)        | 9 (33.4)   |                                   |         |
| <b>History of smoking</b>     |                  |            |                                   |         |
| Yes                           | 10 (22.2)        | 8 (29.6)   | 0.679 (0.229-2.007)               | 0.842   |
| No                            | 35 (77.8)        | 17 (70.4)  |                                   |         |
| <b>DM</b>                     |                  |            |                                   |         |
| Yes                           | 22 (48.8)        | 14 (51.8)  | 0.888 (0.342-2.308)               | 0.808   |
| No                            | 23 (51.2)        | 13 (48.2)  |                                   |         |
| <b>Lung Cavity</b>            |                  |            |                                   |         |
| Yes                           | 17 (37.7)        | 6 (22.2)   | 2.125 (0.715-6.315)               | 0.271   |
| No                            | 28 (62.3)        | 21 (77.8)  |                                   |         |
| <b>Smear Positive</b>         |                  |            |                                   |         |
| Yes                           | 25 (55.5)        | 17 (63.0)  | 0.735 (0.277-1.955)               | 0.537   |
| No                            | 20 (44.5)        | 10 (37.0)  |                                   |         |
| <b>IP-10 (pg/ml)</b>          |                  |            |                                   |         |
| $\geq 1555.6$                 | 29 (64.4)        | 9 (33.3)   | 3.625 (1.325-9.917)               | 0.021*  |
| $< 1555.6$                    | 16 (35.6)        | 18 (66.7)  |                                   |         |

\*Significant, P-value  $< 0.05$

A study analyzing serum biomarkers before and after treatment for TB found that three markers, including IP-10, sIL-6R, and Apo-CIII, can predict relapse in TB patients at the end of treatment with a sensitivity of 71% and specificity of 74%.[23] However, the modest sensitivity and specificity of the IP-10 cut-off value in this study limit its potential as a standalone diagnostic or predictive test. Future prospective cohort

studies with larger sample sizes are needed to validate this finding. Baseline IP-10 also demonstrated predictive potential for relapse before treatment initiation. The analysis of risk factors associated with recurrent DR-TB is presented in **Table 2**. Multivariate analysis was not performed due to the limited sample size ( $n=72$ ), which would result in an unstable model given the number of potential confounders. Therefore, residual confounding cannot be excluded.

According to the bivariate analysis, only the pre-treatment IP-10 level was associated with recurrent DR-TB ( $P$ -value  $< 0.05$ ); therefore, we cannot further analyze it with multivariate analysis. In DR-TB patients, high baseline IP-10 levels are associated with delayed sputum culture conversion and poorer treatment outcomes. Studies measuring immunity markers have found that IP-10 is a baseline marker that predicts delayed sputum culture conversion in patients with DR-TB.[22] IP-10 is a chemokine induced by IFN- $\gamma$ , and its levels reflect the activation of the Th1 immune response, often associated with a high bacterial load and tissue inflammation. Very high levels may indicate more severe disease, thereby increasing the risk of treatment failure or recurrence.[24] Currently, IP-10 is still limited to research outcomes, and there are no established recommendations to change the management of DR-TB patients based on IP-10 levels alone. However, high results may indicate the need for more rigorous monitoring (e.g., more frequent culture examinations, therapy evaluations). In an observational cohort study of active pulmonary TB (including several MDR patients), the median serum IP-10 level before therapy was 384.1 pg/mL, then decreased to 251.85 pg/mL at 2 months after treatment ( $P$ -value  $< 0.001$ ).[7] Although it does not directly assess relapse, high IP-10 levels during treatment indicate a suboptimal response to therapy, which may ultimately be associated with a higher risk of relapse. Furthermore, although specific evidence for DR-TB is limited, TB studies suggest that combining IP-10 with other markers provides a better prediction of relapse than any single marker alone.[25] IP-10 may be considered for incorporation into prospective biomarkers to predict delayed conversion and relapse in DR-TB using a longitudinal design and external validation. The correlation between pre-treatment IP-10 level and smear sputum grading is shown in **Figure 3**.

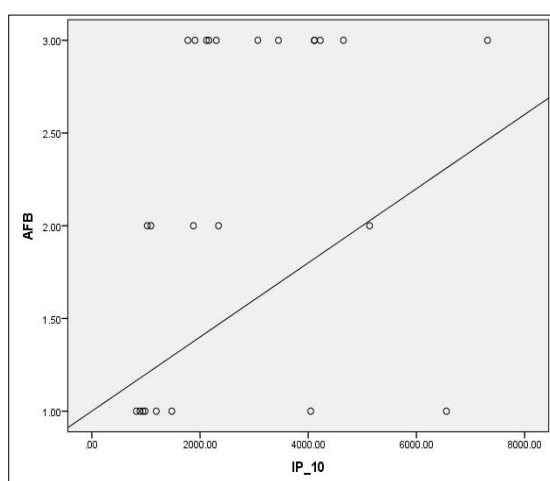


**Figure 3.** Correlation between pre-treatment IP-10 level with smear sputum grading

**Figure 3** shows a correlation between the pre-treatment IP-10 level and smear sputum grading ( $P$ -value = 0.010, Spearman's rho = 0.511). It indicated that the higher the pre-treatment IP-10 level, the greater the smear sputum grading among recurrent DR-TB patients. A higher *Mycobacterium tuberculosis* load stimulates a greater inflammatory response by releasing IFN- $\gamma$  and IP-10 mediators. Therefore, IP-10 often reflects the level of immunological activity against mycobacterial antigens associated with the bacterial load in the lungs, which is characterized by high sputum smear positivity.[26] Nevertheless, studies in countries with a high TB burden reported no association between IP-10 and sputum grading.[27] This suggests heterogeneity among studies depending on the population, measurement methods, and sampling time. HIV coinfection, malnutrition, and other diseases may affect IP-10 and immune response, thereby distorting the correlation between IP-10 levels and AFB. Until evidence is widely validated, IP-10 cannot replace smear sputum or culture sputum for diagnosis or therapy monitoring; therefore, it is more suitable as an additional marker.

This study has several additional limitations. First, we did not perform genotyping or whole-genome sequencing to distinguish between true relapse and reinfection, which have different immunological implications. Second, medication adherence data were not systematically collected, although all patients were treated under direct observation. Third, the wide confidence intervals reflect the limited sample size and

should be interpreted cautiously. Finally, the lack of long-term follow-up after treatment completion prevents us from assessing whether pre-treatment IP-10 predicts future recurrence prospectively

## Conclusions

The pre-treatment of IP-10 level was significantly high among recurrent DR-TB patients and those with lung cavities. Pre-treatment IP-10 level  $\geq 1555.6$  pg/ml is associated with recurrent DR-TB. Pre-treatment IP-10 level  $\geq 1012.4$  pg/ml was significantly associated with lung cavities among recurrent DR-TB patients. This study suggests measuring IP-10 levels after patients have been successfully treated with DOTs to determine those at risk of developing recurrent DR-TB.

## Conflict of Interest

All authors declare that there are no conflicts of interest related to this study.

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## Supplementary Materials

Not available.

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