

ORIGINAL ARTICLE

Detection of Serum IL-1 β and IL-10 as Biomarkers for Anti-Tuberculosis Drug Resistance

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ABSTRACT

Background: To investigate the levels of cytokines (IL-1 β , IL-2R, IL-6, IL-8, IL-10, TNF- α , IFN- γ) in tuberculosis (TB) patients with different clinical characteristics (sputum smear status, cavity presence, lesion extent, *Mycobacterium* type, and drug resistance) and explore their clinical significance.

Methods: A total of 258 active TB patients (divided into drug-resistant and non-drug-resistant groups) and 62 quiescent TB controls were enrolled. Serum cytokine levels were detected by ELISA.

Results: Serum IL-1 β , sIL-2R, IL-6, IL-10, TNF- α , and IFN- γ were significantly higher in active TB patients than in controls (all $p < 0.01$), while IL-8 showed no difference. IL-1 β levels were notably higher in drug-resistant vs. non-drug-resistant patients ($p < 0.01$). In subgroups with positive sputum smear, extensive lesions, cavity formation, or bovine TB infection, IL-1 β and IL-10 levels increased with disease severity. IL-1 β was significantly elevated in streptomycin (SM)-resistant, rifampicin (RFP)-resistant, and related combined drug-resistant subgroups ($p < 0.01/0.05$).

Conclusions: Serum IL-1 β in combination with IL-10 may serve as a marker for evaluating TB activity, lesion progression, and bacillary type, while IL-1 β alone could indicate partial drug resistance (e.g., to SM, RFP, and related combinations).

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KEYWORDS

tuberculosis, drug resistance, serum, IL-1 β , IL-10

INTRODUCTION

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTB), was largely brought under control after 1943 with the introduction of drugs such as streptomycin (SM) [1,2]. However, drug-resistant TB remains a global health menace. Traditional drug resistance testing methods, such as MTB culture and drug sensitivity tests, are time-consuming, can be inaccurate, and lack standardization. Alternative methods like bacteriophage amplification, DNA sequencing, polymerase chain reaction (PCR), gene chip technology, and rapid culture systems face issues like high costs, technical complexity, and demanding requirements, restricting their widespread use [3-5].

Previous studies have explored serum cytokines in pulmonary TB [6], but the relationships between these cytokines and factors such as smear status, cavity presence, lesion extent, MTB type, or drug resistance remain unclear. This study used enzyme-linked immunosorbent assay (ELISA) to measure cytokine levels (IL-1 β , IL-2R, IL-6, IL-8, IL-10, TNF- α , IFN- γ) in patients with drug-resistant and non-drug-resistant secondary pulmonary TB patients, as well as in a control group with old TB lesions. The aims were to understand the role of cytokines in the development of TB, to identify a rapid way to detect drug-resistant TB, and explore the molecular mechanisms of resistance.

MATERIALS AND METHODS

Materials: The experimental flowchart is shown in Figure 1.

Sample collection

Between June 2020 and January 2025, a total of 258 patients diagnosed with active pulmonary TB were recruited from the Yancheng First/Second/Third People's Hospitals, as well as the Yancheng Center for Disease Control and Prevention. Inclusion criteria required patients to be diagnosed according to World Health Organization (WHO) guidelines for TB and drug-resistant TB [7], with confirmation by both clinical assessment and laboratory sputum culture. Participants were aged 16 years or older and had not recently undergone surgery or received treatment with immunosuppressants or antibacterial drugs. Exclusion criteria included the presence of other lung diseases, infectious diseases, malignant tumors, drug allergies, diseases of vital organs, or blood or immune system disorders, as well as pregnancy, lactation, or extensive drug resistance. Additionally, 62 patients with quiescent TB were enrolled as a control group during the same period, characterized by three consecutive negative sputum cultures and no changes on imaging examinations over six months. The study was approved by the Ethics Committee of the Yancheng Third People's Hospital (02-02-2018), with informed consent obtained from all patients or their families.

Patient grouping

Patients with active pulmonary TB were assigned to the experimental group (Ex-Gr, n = 258), while those with quiescent TB comprised the control group (Con-Gr, n = 62). Based on laboratory drug susceptibility testing results, the Ex-Gr was further subdivided into Experimental Group 1 (Ex-1, n = 156), constituting the drug-resistant group, and Experimental Group 2 (Ex-2, n = 102), constituting the drug-sensitive group. Table 1 outlines the distribution of gender, age, place of residence, occupation, history of TB, sputum smear results, lesion extent, and bacterial type [MTB, *Mycobacterium bovis* (*M. bovis*)].

Antimicrobial susceptibility test (AST)

The procedure followed the "Tuberculosis Laboratory Biosafety Manual" by the WHO, adhering to the standards established in 2012 [8]. The drugs evaluated included two first-line agents (SM: 4 μ g/mL, Ethambutol: 2 μ g/mL), as well as two second-line agents (Kanamycin: 30 μ g/mL, Ofloxacin: 4 μ g/mL). Drug resistance was classified according to WHO guidelines as follows [7]: 1) Monoresistant TB: resistance to one first-line drug; 2) Polyresistant TB: resistance to multiple first-line drugs, excluding simultaneous resistance to both isoniazid (INH) and rifampicin (RFP); 3) Multiple-drug-resistant tuberculosis (MDR-TB): resistance to both INH and RFP; 4) Pre-XDR-TB: resistance to either a fluoroquinolone or a second-line injectable drug; 5) XDR-TB: resistance to both a fluoroquinolone and a second-line injectable drug; 6) Rifampicin-resistant tuberculosis: resistance to at least RFP. All laboratory personnel were trained in biosafety protocols, and quality control was ensured through the use of standard strains for drug sensitivity testing and in-reagent controls for gene chip assays.

Sputum sample handling

According to the "Guidelines for the Treatment of Latent Tuberculosis Infection" [9], sputum samples should consist of deep lung secretions, which may include blood-tinged sputum, mucus, cheese-like material, or brown blood sputum. These samples must be stored at -80°C. Prior to analysis, samples should be thawed in a refrigerator set at 4°C and subsequently transferred to a biosafety cabinet. Slides should be labeled with the specimen number using a grease pencil. To minimize the risk of aerosol generation or spillage, the container should be opened with caution. Using a broken-end toothpick, approximately 0.05 - 0.1 mL of the sample's characteristic components - such as cheese-like material, brown blood sputum, or purulent areas - should be selected and spread evenly on a slide to form a 10 $\times$ 20 mm oval film. The film is then air-dried for approximately 30 minutes before performing Ehrlich-Ziehl-Neelsen (EZN) stain and examining it under a microscope. The grading for EZN staining results is as follows: "Acid-fast negative" indicates no acid-fast bacteria observed in 300 fields. The acid-fast bacilli count is

Table 1. Comparison of general information between the groups.

Items	Ex-1	Ex-2	t, F values / χ	p	Con-Gr
n	156	102			62
Age (years)	45.84 ± 22.16	42.12 ± 21.51	1.33	0.183511	44.26 ± 17.71
Gender					
Male	127	78	0.92	0.336962	47
Female	29	24			15
Cavity					
Have	47	28	0.21	0.643349	0
None	109	74			62
Sputum smear					
0	28	22	27.28	0.000002	62
1+ - 2+	52	62			0
3+ - 4+	76	18			0
Lesion range					
1 - 2 Lung fields	57	36	0.67	0.716010	-
3 - 4 Lung fields	60	44			-
5 - 6 Lung field	39	22			-
Mycobacterium tuberculosis type					
Human type	131	80	1.42	0.491585	-
M. bovis	15	12			-
Human type/M. bovis	10	10			-
Career					
Student	15	10	0.73	0.947818	6
Farmers	42	28			13
Workers	36	20			20
Business	26	16			16
Others	37	28			7
Household registration					
City	55	30	0.95	0.328788	21
Rural	101	72			41

Control group (Con-Gr): patients with quiescent tuberculosis, Experimental group 1 (Ex-1): patients with active pulmonary tuberculosis and drug-resistant, Experimental group 2 (Ex-2): patients with active pulmonary tuberculosis and drug-sensitive.

reported as follows: 1 to 8 per 300 fields (1+), 3 to 9 per 100 fields (2+), 1 to 9 per field (3+), and ≥ 10 per field (4+). For a 1+ result, observe at least 300 fields; for 2+, at least 100 fields; and for 3+ and 4+, at least 50 fields.

Serum cytokine assay

On the second day following admission and diagnosis, 3 mL of fasting venous blood was collected in the morning. The blood samples were centrifuged at 3000 rpm for 10 minutes to obtain serum, which was subsequently stored at -80°C. Prior to analysis, the serum was thawed in a 4°C refrigerator and allowed to equilibrate to room temperature before processing in a biosafety cabinet. The concentrations of IL-1 β , IL-2R, IL-6, IL-8,

IL-10, TNF- α , and IFN- γ were quantified using an ELISA kit (BIORAD). Absorbance readings at 450 nm were measured using a microplate reader. A standard curve was generated from the standard concentrations to determine the actual concentration of each sample based on its optical density (OD) value.

Statistical treatment

Quantitative data are expressed as mean $\pm$ standard deviation. The Wilcoxon Rank Sum test was utilized for group comparisons, with statistical significance set at $p < 0.05$.

Table 2. Serum cytokine levels and TB cavity in Ex-Gr ($\bar{x} \pm s$, ng/L).

Items	Ex-Gr		t	p	Ex-1		t	p	EX-2		t	p
	non-cavity	cavity			non-cavity	cavity			non-cavity	cavity		
n	183	75			113	43			70	32		
IL-1 β	15.0 $\pm$ 1.87	20.5 $\pm$ 1.40	25.78	0.000, 000	15.70 $\pm$ 1.448	20.76 $\pm$ 1.376	20.23	0.000, 000	13.93 $\pm$ 1.959	20.16 $\pm$ 1.381	16.22	0.000, 000
sIL-2R	8,752 $\pm$ 1,138	8,761 $\pm$ 1,164	0.06	0.954, 782	8,843 $\pm$ 1,152	8,747 $\pm$ 1,157	0.46	0.642, 923	8,651 $\pm$ 1,189	8,760 $\pm$ 1,115	0.44	0.662, 427
IL-6	67.1 $\pm$ 1.498	67.1 $\pm$ 1.497	0.19	0.845, 665	67.1 $\pm$ 1.489	67.12 $\pm$ 1.495	0.22	0.822, 556	67.14 $\pm$ 1.497	67.19 $\pm$ 1.556	0.15	0.877, 443
IL-8	117.6 $\pm$ 2.189	117. $\pm$ 2.160	0.34	0.736, 876	117.8 $\pm$ 2.117	117.9 $\pm$ 2.183	0.26	0.794, 149	117.6 $\pm$ 2.260	117.9 $\pm$ 2.214	0.63	0.532, 749
IL-10	10.4 $\pm$ 0.8724	14.4 $\pm$ 0.9509	31.49	0.000, 000	10.90 $\pm$ 0.7969	15.11 $\pm$ 0.5505	31.84	0.000, 000	9.663 $\pm$ 0.1457	13.54 $\pm$ 0.5326	56.73	0.000, 000
TNF- α	77.0 $\pm$ 1.399	76.8 $\pm$ 1.375	0.74	0.460, 658	77.0 $\pm$ 1.364	76.91 $\pm$ 1.346	0.37	0.712, 210	76.83 $\pm$ 1.433	77.02 $\pm$ 1.463	0.62	0.538, 435
IFN- γ	47.1 $\pm$ 1.366	47.1 $\pm$ 1.366	0.11	0.915, 040	47.1 $\pm$ 1.350	47.11 $\pm$ 1.387	0.04	0.967, 324	47.09 $\pm$ 1.359	47.11 $\pm$ 1.400	0.07	0.945, 667

Experimental group (Ex-Gr): patients with active pulmonary tuberculosis, Control group (Con-Gr): patients with quiescent tuberculosis, Experimental group 1 (Ex-1): drug-resistant active pulmonary tuberculosis patients, Experimental group 2 (Ex-2): drug-sensitive active pulmonary tuberculosis patients.

Table 3. Serum cytokine levels and TB tuberculous cavity Ex-1 VS Ex- 2 ($\bar{x} \pm s$, ng/L).

Items	Cavity				Non-cavity			
	Ex-1	Ex-2	t	p	Ex-1	Ex-2	t	p
n	43	32			113	70		
IL-1 β	20.89 $\pm$ 1.424	20.02 $\pm$ 1.381	2.65	0.009, 760	15.70 $\pm$ 1.448	13.93 $\pm$ 1.959	7.00	0.000, 000
sIL-2R	8,843 $\pm$ 1152	8,651 $\pm$ 1189	0.70	0.483, 554	8,760 $\pm$ 1,115	8,747 $\pm$ 1,157	0.08	0.939, 856
IL-6	67.19 $\pm$ 1.489	67.18 $\pm$ 1.532	0.03	0.977, 409	67.18 $\pm$ 1.513	67.12 $\pm$ 1.495	0.26	0.793, 693
IL-8	117.8 $\pm$ 2.106	117.6 $\pm$ 2.260	0.39	0.694, 525	117.9 $\pm$ 2.214	117.8 $\pm$ 2.183	0.30	0.765, 635
IL-10	15.11 $\pm$ 0.5505	13.54 $\pm$ 0.5326	12.39	0.000, 000	10.90 $\pm$ 0.7969	9.663 $\pm$ 0.1457	12.84	0.000, 000
TNF- α	76.91 $\pm$ 1.346	76.90 $\pm$ 1.385	0.03	0.975, 010	77.02 $\pm$ 1.463	77.00 $\pm$ 1.364	0.09	0.926, 638
IFN- γ	47.11 $\pm$ 1.387	47.09 $\pm$ 1.359	0.06	0.950, 499	47.12 $\pm$ 1.350	47.11 $\pm$ 1.400	0.05	0.961, 758

Control group (Con-Gr): patients with quiescent tuberculosis, Experimental group 1 (Ex-1): patients with drug-resistant active pulmonary tuberculosis, Experimental group 2 (Ex-2): patients with drug-sensitive active pulmonary tuberculosis.

RESULTS

General information about the study subjects

An analysis of the baseline characteristics between the two study groups indicated no statistically significant differences in age, gender, cavity formation, sputum smear status, lesion extent, MTB type, occupation, or place of residence ($p > 0.05$), as detailed in Table 1.

Analysis of AST results in Ex-Gr

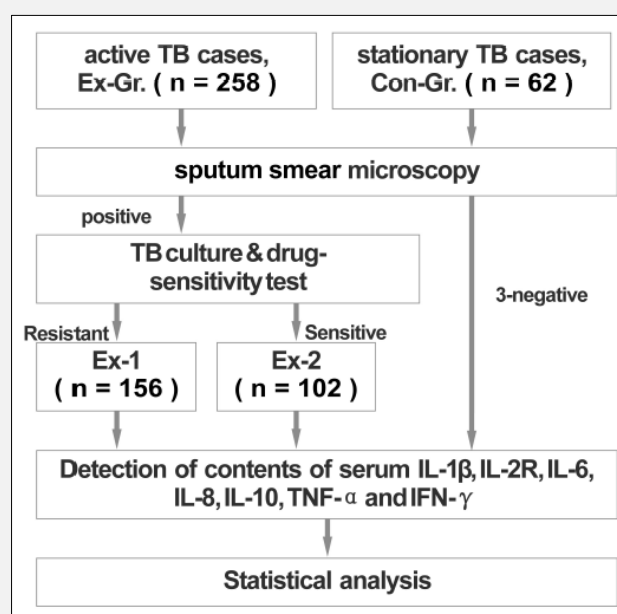
The AST results for EX-1 are depicted in Figure 2, monoresistance rates to SM, INH, and RFP ranged from 21.79% to 55.13%, dual-drug resistance rates from 7.69% to 15.74%, and triple-drug resistance rates were 5.13%. Furthermore, AST conducted on mycobacterial cultures from EX-2 indicated an absence of drug resistance.

Table 4. The resistance and the levels of serum cytokine ($\bar{x} \pm s$, ng/L).

	Ex-2	Ex-1						
		I	II	III	IV	V	VI	VII
n	102	86	34	36	20	23	12	8
IL-1 β	14.29 $\pm$ 1.77	20.07 $\pm$ 1.77 *	20.37 $\pm$ 1.40 *	19.73 $\pm$ 1.68 *	20.30 $\pm$ 1.37 *	18.04 $\pm$ 1.76 *	16.24 $\pm$ 1.31 *	20.72 $\pm$ 1.46 *
sIL-2R	8,725 $\pm$ 1,105	8,690 $\pm$ 1,168	8,530 $\pm$ 1,129	8,589 $\pm$ 1,135	8,953 $\pm$ 1,271	8,472 $\pm$ 1,255	8,792 $\pm$ 1,252	9,129 $\pm$ 1,053
IL-6	67.16 $\pm$ 1.52	67.23 $\pm$ 1.48	67.20 $\pm$ 1.52	67.11 $\pm$ 1.50	67.25 $\pm$ 1.54	67.27 $\pm$ 1.55	66.86 $\pm$ 1.44	67.63 $\pm$ 1.44
IL-8	117.85 $\pm$ 2.21	117.86 $\pm$ 2.16	117.91 $\pm$ 2.17	117.78 $\pm$ 2.18	117.54 $\pm$ 2.21	117.82 $\pm$ 2.19	117.41 $\pm$ 2.29	118.38 $\pm$ 1.95
IL-10	9.74 $\pm$ 0.28	13.84 $\pm$ 1.29 *	12.57 $\pm$ 0.72 *	12.70 $\pm$ 0.80 *	13.61 $\pm$ 0.49 *	14.78 $\pm$ 0.14	15.25 $\pm$ 0.49	14.76 $\pm$ 0.10
TNF- α	76.97 $\pm$ 1.47	76.97 $\pm$ 1.37	77.18 $\pm$ 1.29	77.07 $\pm$ 1.32	77.05 $\pm$ 1.36	76.97 $\pm$ 1.40 *	77.31 $\pm$ 1.50 *	76.37 $\pm$ 1.37 *
IFN- γ	47.09 $\pm$ 1.40	47.10 $\pm$ 1.35	47.16 $\pm$ 1.36	47.04 $\pm$ 1.39	46.95 $\pm$ 1.45	47.04 $\pm$ 1.36	46.98 $\pm$ 1.41	47.21 $\pm$ 1.39

Group I: Streptomycin (SM) resistance, Group II: Isoniazid (INH) resistance, Group III: Rifampicin (RFP) resistance, Group IV: SM + INH resistance, Group V: SM + RFP resistance, Group VI: INH + RFP resistance, Group VII: SM + INH + RFP resistance.

Experimental group 1 (Ex-1): patients with drug-resistant active pulmonary tuberculosis, Experimental group 2 (Ex-2): patients with drug-sensitive active pulmonary tuberculosis. * Statistical analysis compared with experimental group 2 showed significant difference ($p < 0.001$).

**Figure 1.** The experimental flowchart.

Analysis in serum cytokine contents

Figure 3 illustrates the serum cytokine profiles. Levels of IL-1 β , IL-10 levels were significantly elevated in EX-1 compared to EX-2 ($p < 0.001$), whereas levels of sIL-2R, IL-6, IL-8, TNF- α , and IFN- γ levels didn't exhibit significant differences ($p > 0.05$). Both EX-1 and 2 demonstrated significantly higher levels of IL-1 β , sIL-2R, IL-6, IL-10, TNF- α , and IFN- γ compared to

Con-Gr ($p < 0.001$), while IL-8 levels showed no significant difference ($p > 0.05$).

Correlation between sputum smear status vs. serum cytokine profiles in EX-Gr, EX-1, EX-2 cohorts

Figure 4 illustrates the sputum smear results and serum cytokine levels in the Ex-Gr. In Ex-Gr, Ex-1, and Ex-2, serum levels of IL-1 β and IL-10 progressively increased

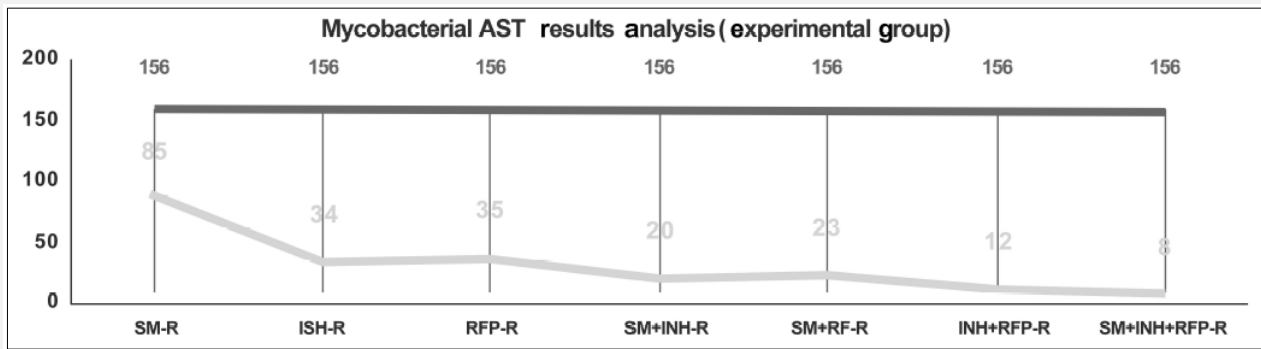


Figure 2. Mycobacterial AST Results Analysis (Ex-Gr).

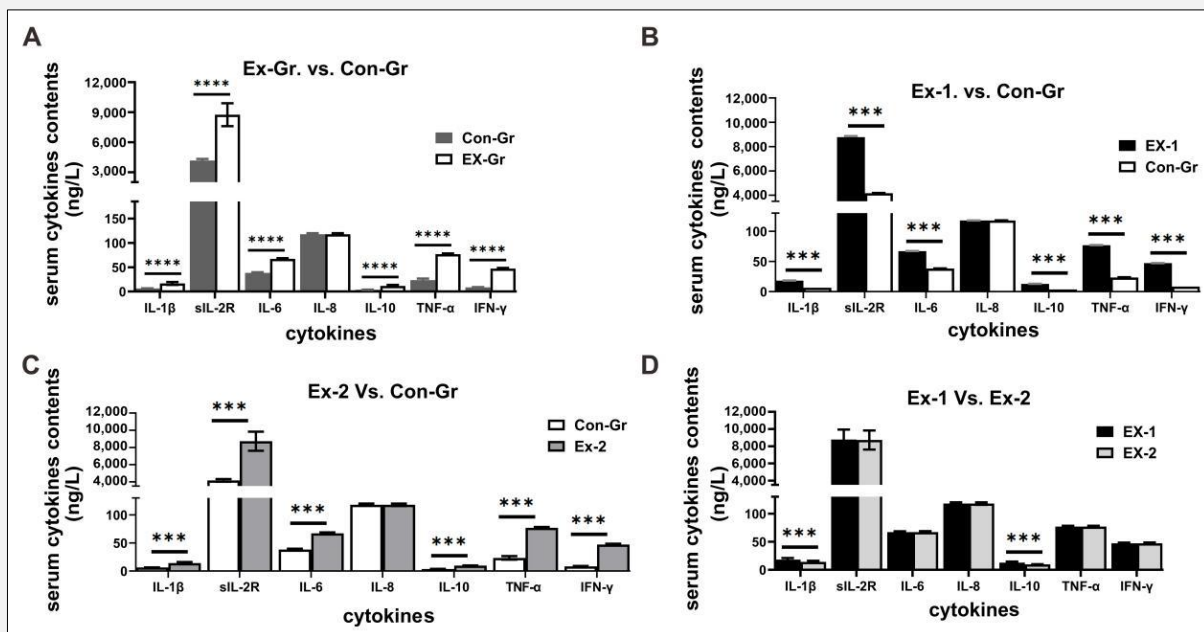


Figure 3. Serum cytokine dynamics: Comparative analysis across study groups.

Group designations: Experimental group (Ex-Gr): patients with active pulmonary TB, Control group (Con-Gr): patients with stable pulmonary TB, Experimental subgroup 1 (Ex-1): patients with active, drug-resistant TB, Experimental subgroup 2 (Ex-2): patients with active, drug-sensitive TB.

A: Ex-Gr vs. Con-Gr, B: Ex-1. vs. Con-Gr, C: Ex-2 vs. Con-Gr, D: Ex-1 vs. Ex-2. **** $p < 0.001$.

from sputum smear (-) to (1+ and 2+) and then to (3+ and 4+) groups, with statistically significant differences ($p < 0.01/0.05$). However, no significant differences were observed in sIL-2R, IL-6, IL-8, TNF- α , and IFN- γ

levels between Ex-Gr and Con-Gr ($p > 0.05$). For Ex-2 specifically, only IL-1 β levels showed a significant stepwise increase from sputum smear (-) to (1+ - 2+) and then to (3+ - 4+) groups ($p < 0.01$), while the levels

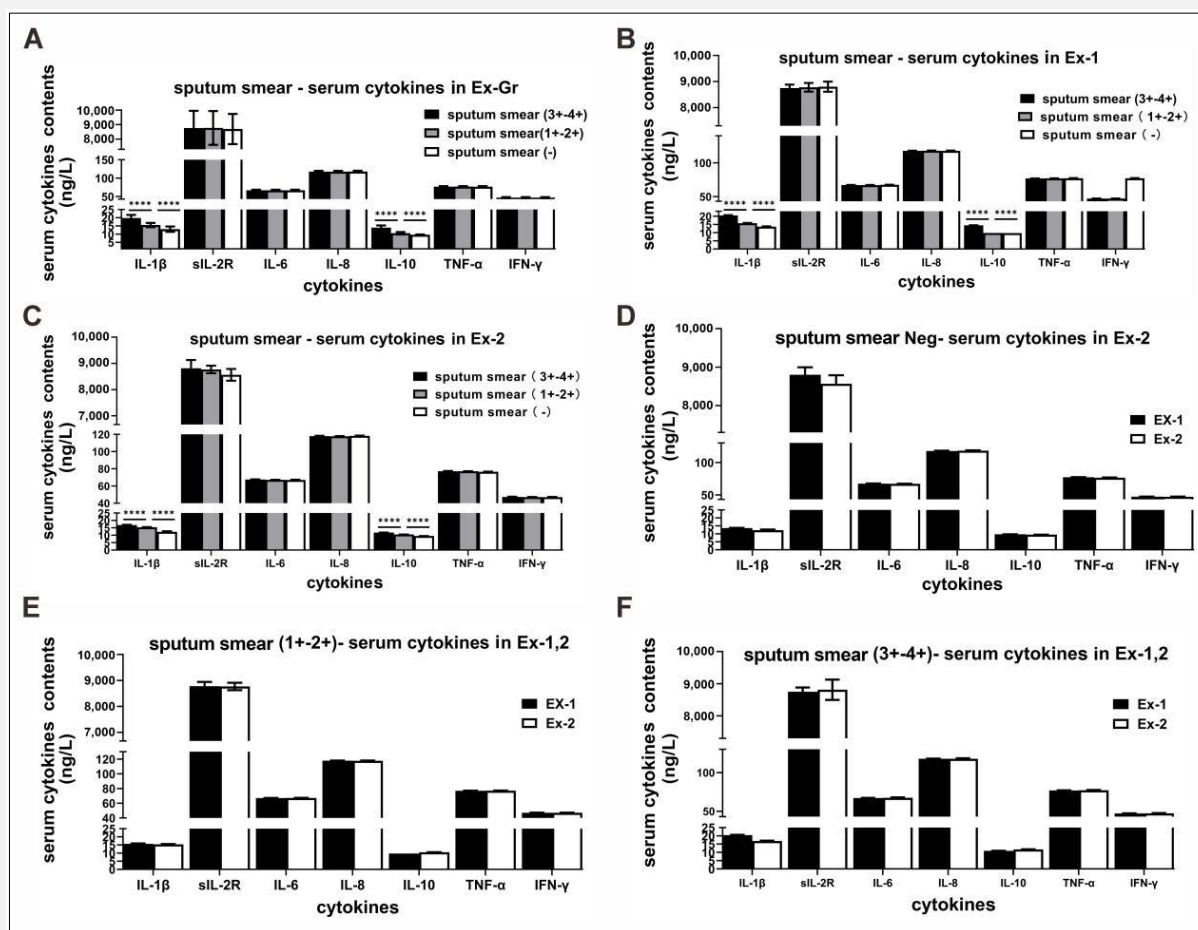


Figure 4. Serum cytokine levels with sputum smear positivity/negativity in EX.

Group designations: Experimental group (Ex-Gr): patients with active pulmonary TB, Control group (Con-Gr): patients with stable pulmonary TB, Experimental subgroup 1 (Ex-1): patients with active, drug-resistant TB, Experimental subgroup 2 (Ex-2): patients with active, drug-sensitive TB.

A: Ex-Gr vs. Con-Gr, B: In Ex-1, C: In Ex-2, D: Sputum smear neg: EX-1 vs. Ex-2, E: sputum smear (1+2+) EX-1 vs. Ex-2, F: sputum smear (1+2+) Ex-2; EX-1 vs. Ex-2. **** $p < 0.001$.

of other cytokines showed no significant differences compared to the Con-Gr ($p > 0.05$).

Correlation between lesion range vs. serum cytokine profiles in EX-Gr, EX-1, EX-2

In EX-Gr, Ex-1 and Ex-2, the serum levels of IL-1 β and IL-10 in the 3 - 4 lung fields group were significantly higher than those in 1 - 2 lung field group, and levels in the 5 - 6 lung field group were higher than those in the 3 - 4 lung field group, with statistically significant differences ($p < 0.01/0.05$). However, there were no significant changes in the serum levels of sIL-2R, IL-6, IL-8, TNF- α and IFN- γ ($p > 0.05$). When comparing Ex-1 and Ex-2, the serum IL-1 β level in Ex-1 was significantly higher than that in Ex-2 ($p < 0.01/0.05$), while

there were no significant differences in the levels of other cytokines between the two groups ($p > 0.05$) (Figure 5).

Lesion cavity of pulmonary TB vs. serum cytokines in Ex-Gr

As shown in Table 2 and 3, the relationships between TB cavity status and serum cytokine levels in EX-Gr, Ex-1, and EX-2 are as follows: serum IL-1 β and IL-10 levels were significantly higher in the cavity subgroup than in the non-cavity subgroup ($p < 0.01$ or $p < 0.05$). Levels of sIL-2R, IL-6, IL-8, TNF- α , and IFN- γ levels showed no significant differences between the Ex-Gr and the Con-Gr ($p > 0.05$).

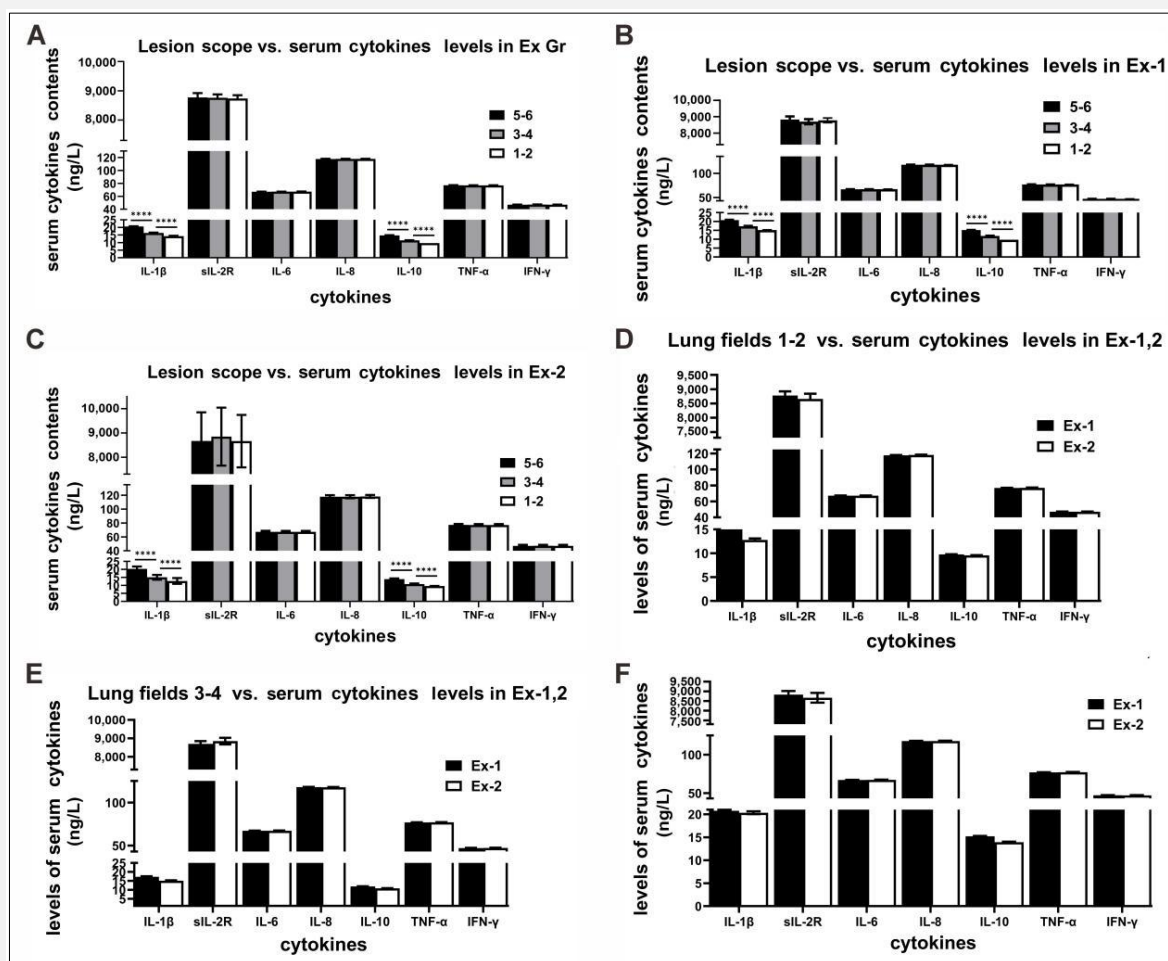


Figure 5. Lesion range and serum cytokine profiles in EX.

Group designations: Experimental group (Ex-Gr): patients with active pulmonary TB, Control group (Con-Gr): patients with stable pulmonary TB, Experimental subgroup 1 (Ex-1): patients with active, drug-resistant TB, Experimental subgroup 2 (Ex-2): patients with active, drug-sensitive TB.

A: Ex-Gr, B: In Ex-1, C: In Ex-2, D: Sputum smear neg: EX-1 vs. Ex-2, E: Sputum smear (1+ -2+) EX-1 vs. Ex-2, F: Sputum smear (1+ -2+) Ex-2, EX-1 vs. Ex-2. **** $p < 0.001$.

Relationship between MTB types and serum cytokine levels

Figure 6 shows the relationship between MTB types and serum cytokine levels. In Ex-Gr, serum levels of IL-1β, IL-6, and IL-10 were significantly higher in the bovine MTB subgroup compared to the human MTB subgroup, and higher in the human MTB subgroup than in the human/bovine mixed MTB subgroup ($p < 0.01$ or $p < 0.05$). Conversely, levels of sIL-2R, IL-8, TNF-α, and IFN-γ showed no significant differences between Ex-Gr and Con-Gr ($p > 0.05$).

In Ex-1, serum levels of IL-1β and IL-10 were significantly higher in the bovine MTB subgroup than in the human MTB subgroup, and higher in the human MTB

subgroup than in the human/bovine mixed MTB subgroup ($p < 0.01$). However, levels of sIL-2R, IL-6, IL-8, TNF-α, and IFN-γ did not differ significantly between the Ex-Gr and Con-Gr ($p > 0.05$).

Only the human MTB subgroup in Ex-1 showed significantly higher levels of IL-1β and IL-10 compared to Ex-2 ($p < 0.01$). There were no significant differences in the levels of sIL-2R, IL-6, IL-8, IL-10, TNF-α, and IFN-γ levels between the two groups ($p > 0.05$). Additionally, no significant differences in cytokine levels were observed between Ex-1 and Ex-2 for the bovine MTB and human/bovine mixed MTB subgroups ($p > 0.05$).

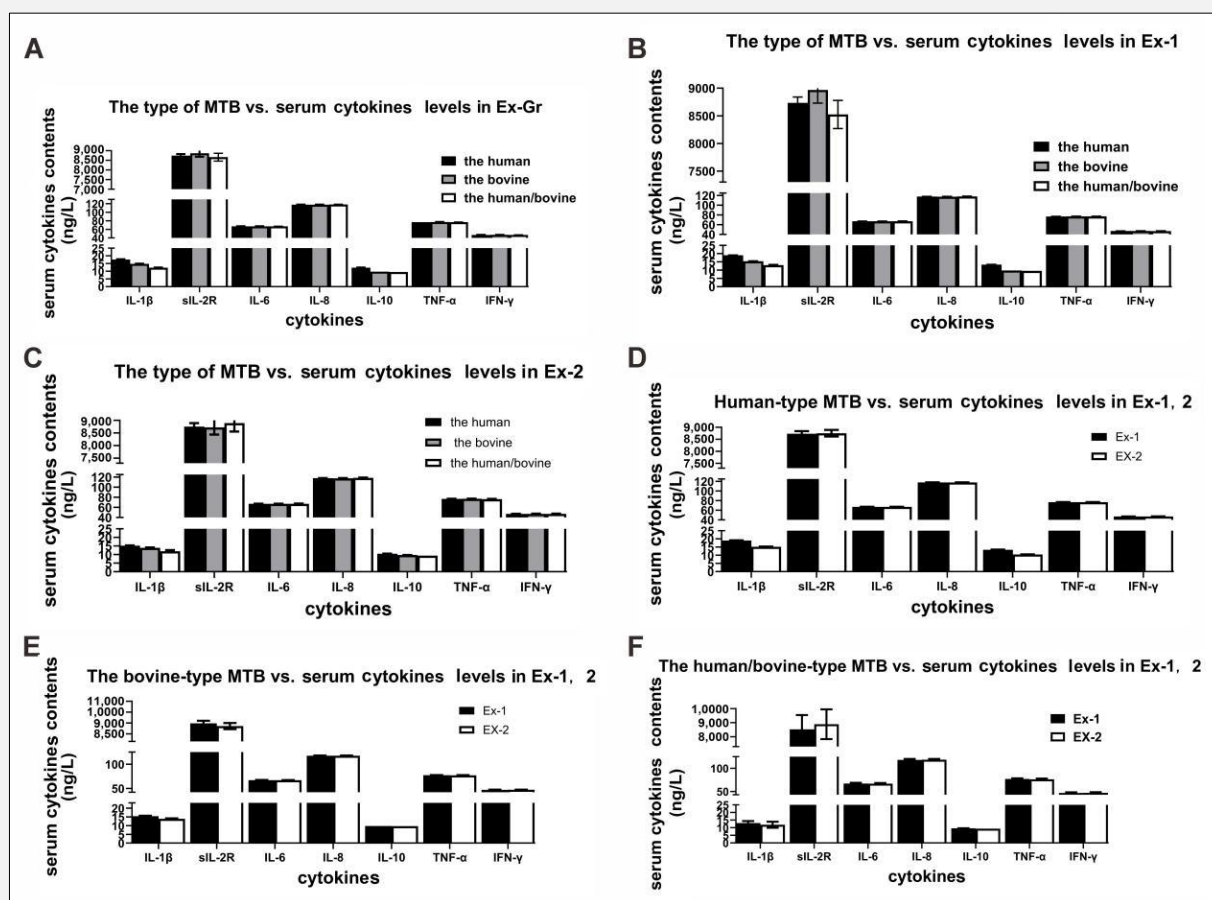


Figure 6. Types of MTB and serum cytokine profiles in EX.

Group designations: Experimental group (Ex-Gr): patients with active pulmonary TB, Control group (Con-Gr): patients with stable pulmonary TB, Experimental subgroup 1 (Ex-1): patients with active, drug-resistant TB, Experimental subgroup 2 (Ex-2): patients with active, drug-sensitive TB.

A: In Ex-Gr, B: In Ex-1, C: In Ex-2, D: the human/bovine mixed MTB, EX-1 vs. Ex-2, E: the bovine mixed MTB, EX-1 vs. Ex-2, F: the human/bovine mixed MTB, EX-1 vs. Ex-2. **** $p < 0.001$.

Relationship between drug resistance and serum cytokine levels

Individual drugs [SM (Group I), INH (Group II), RFP (Group III)] and combined drug resistance patterns [SM-INH (Group IV), SM-RFP (Group V), INH-RFP (Group VI), SM-INH-RFP (Group VII)] are shown in Figure 7. Table 4 illustrates the relationship between drug resistance and serum cytokine levels in Ex-Gr. Compared to Ex-2, the subgroups with resistance to SM (Group I), RFP (Group III), SM-INH (Group IV), SM-RFP (Group V), and SM-INH-RFP (Group VII), showed significantly higher levels of IL-1 β and IL-10 levels ($p < 0.01$ or $p < 0.05$). However, levels of sIL-2R, IL-6, IL-8, TNF- α , and IFN- γ did not differ significantly ($p > 0.05$). No significant differences were observed in any cytokine levels between the INH-resistant

(Group II) and INH + RFP-resistant (Group VI) subgroups and Ex-2 ($p > 0.05$).

DISCUSSION

MTB is first phagocytized by macrophages upon alveolar inhalation. If the bacillus is highly virulent or present in large quantities, it causes macrophage rupture, releasing bacilli to reinfect tissues and other phagocytes. Released antigens sensitize T lymphocytes, inducing cell-mediated immunity. Naive CD4 $^{+}$ T cells differentiate into Th1 and Th2 cells, whose balance regulates TB progression. Th1 cells secrete IL-1 β , IL-2, IFN- γ , TNF- α to enhance anti-intracellular pathogen immunity, while Th2 cells produces IL-4/5/6/10/13, negatively

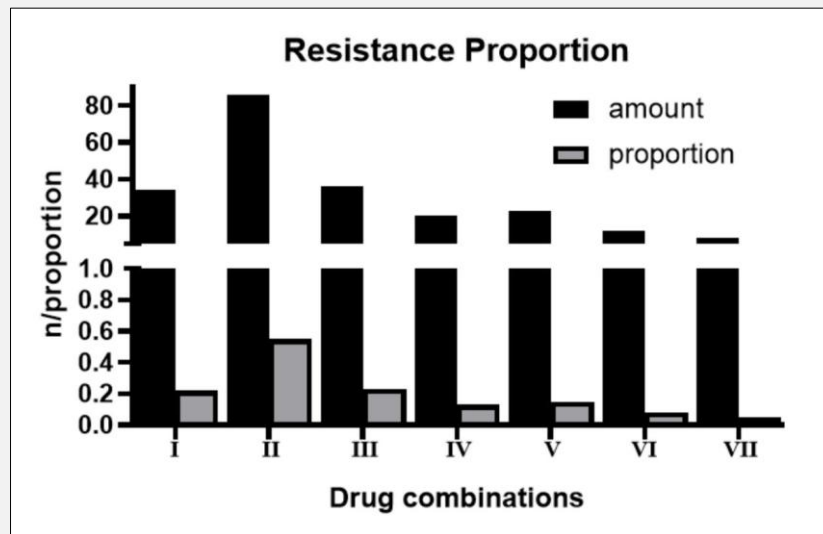


Figure 7. TB drug resistance proportion chart in EX-1.

I: SM resistance, II: INH resistance, III: RFP resistance, IV: SM + INH resistance, V: SM + RFP resistance, VI: INH + RFP resistance, VII: SM + INH + RFP resistance.

regulating TB chronic transformation [10,11]. For IL detection, ELISA and FACS (fluorescence-activated cell sorting) are commonly used. ELISA offers advantages of high equipment availability, low cost, and simple operation, making it ideal for batch sample processing. FACS boasts higher sensitivity for detecting trace inflammatory factors in serum but requires costly equipment.

SM, INH, and RFP are first-line antitubercular drugs. SM, an aminoglycoside, inhibits bacterial protein synthesis by blocking 70S initiation complex, inducing mRNA-tRNA mismatching via 30S subunit binding, and preventing ribosome dissociation, while disrupting cell membranes to cause vital substance leakage. INH enters MTB passively, where it is decomposed to inhibit mycolic acid synthesis, compromising cell wall integrity and acid-fastness. RFP binds to mycobacterial RNA polymerase β -subunit, inhibiting RNA transcription to block protein synthesis [12,13].

Drug resistance in MTB predominantly stems from gene mutations. Specifically, RFP resistance is closely associated with mutations in the *rpoB* gene, whereas INH resistance is intricately linked to alterations in *ahpc*, *inhA*, and *katG* genes. TB strains that exhibit resistance to at least two key drugs, namely INH and RFP, are classified as MDR-TB [13].

Encoded by the *IL-1 β* gene, IL-1 β is a protein cytokine produced by monocytes, macrophages, and other immune cells. As a pivotal inflammatory mediator, it regu-

lates cell proliferation and apoptosis. Recognized as the most potent pro-inflammatory cytokine, IL-1 β safeguards against infections by recruiting neutrophils, activating adhesion molecules, inducing cytokines, and triggering fever. Upon MTB infection, macrophages up-regulate TNF- α , IL-6, and IL-1 β , with studies demonstrating abundant IL-1 β secretion [14-17]. This study found serum IL-1 β levels were higher in Ex-Gr compared to Con-Gr, and in drug-resistant subgroups compared to non-drug-resistant subgroups. Levels correlated with sputum smear results: (3+ - 4+) > (1+ - 2+) > negative. Regarding lesion extent, levels in involvement of 5 - 6 lung fields were higher than in 3 - 4 fields, which were in turn higher than in 1 - 2 fields; levels were also higher in cavitary cases than in non-cavitary cases. Infection with bovine MTB strains showed the highest levels, followed by human strains and hybrid strains. Ex-1 had higher levels across drug resistance groups ($p < 0.01/0.05$). IL-1 β was associated with sputum smear, lesion severity, cavity formation, and drug resistance, likely reflecting the body's innate immune response, corroborating previous research by Rungelrath V [18].

A pro-inflammatory cytokine produced by macrophages, T cells, epithelial cells, and fibroblasts, IL-6 enhances immunity by amplifying cytokine production and inflammation [18,19]. As an anti-inflammatory cytokine produced by Th2 cells, Th0 cells, monocytes/macrophages, and B cells, IL-10 suppresses CD4/CD8 T-

cell activity and downregulates co-stimulatory molecules on macrophages [20-22]. In this study, IL-10 levels were elevated in the Ex-Gr, cavity-positive subgroups, and bovine/human MTB subgroups ($p < 0.01/0.05$), suggesting an association of IL-10 with cavity formation and MTB type, but not with sputum smear status, lesion extent, or drug resistance.

A soluble IL-2 receptor - complex mucoprotein - released by activated T cells, sIL-2R inhibits immunity by binding IL-2, reducing T-cell proliferation [23]. A key cytokine for controlling TB, IFN- γ is expressed by CD4⁺/CD8⁺ T cells and NK cells, activating macrophages to kill MTB [24-26]. Secreted by macrophages and T cells, TNF- α promotes protective immunity by inducing inflammatory cytokines, containing infection via granuloma formation, and preventing MTB dissemination [27-29].

In the present study, no statistically significant differences in the levels of sIL-2R, IL-6, TNF- α , and IFN- γ were detected across subgroups stratified by sputum smear status (positive/negative), presence or absence of cavitary lesions, lesion extent, MTB species type, and drug resistance status. These results indicate that sIL-2R, IL-6, TNF- α , and IFN- γ are not associated with the formation of sputum smear positivity, cavitary lesion development, MTB species classification, or drug resistance in TB. This finding is consistent with the results reported by Chen Xingnian and Vankayalapati R [29-30].

CONCLUSION

1. The combination of serum IL-1 β and IL-10 may serve as a significant biomarker profile for sputum smear-positive pulmonary TB, lesion extent, tuberculosis cavitation, and MTB type.
2. Serum IL-1 β levels may serve as key indicators for resistance to SM, RFP, SM + INH, SM + RFP, and SM + INH + RFP in TB.

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Data Available:

Data is available from the corresponding author on request.

Ethics Statement:

The present study was approved by the Ethics Committee of Yancheng Third People's Hospital (02-02-2018) and written informed consent was provided by all patients prior to the study start. All procedures were performed in accordance with the ethical standards of the Institutional Review Board and The Declaration of Helsinki, and its later amendments or comparable ethical standards.

Declaration of Interest:

The authors have no conflicts of interest to declare.

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