Research article

Correlation between Gene polymorphism levels of serum matrix metalloproteinases with cavitary features and pulmonary fibrosis of the Patient tuberculosis multi-drug resistance using high-resolution computerized tomography of the Thorax

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ABSTRACT

Matrix metalloproteinases (MMPs) are proteins that play a role in the inflammatory and remodeling processes caused by infections, including pulmonary tuberculosis (TB), especially multidrug resistance. This study aims to investigate the relationship between variations in MMP-1 and MMP-9 blood levels, cavity features such as number, diameter, and wall thickness, and the location of fibrosis in multidrug-resistant (MDR) and drug-sensitive (DS) tuberculosis patients. This study used a comparative cross-sectional study design. The subjects, who were outpatients at Abdoel Moelok Hospital, Lampung, Indonesia, had passed the ethical test. We divided the subjects into two groups: 34 in the MDR-TB group and 36 in the DS-TB group. An ELISA test determined the levels of MMP-1 and MMP-9, while the PCR-sequencing method determined the genotypes of MMP-1 and MMP-9. Additionally, we measured cavities and fibrosis using thoracic high-resolution computerized tomography (HRCT) imaging. In MDR-TB patients, there was a significant difference in the number of cavities larger than 6.6 mm in diameter, as well as cavity thickness, compared to DS-TB patients. The distribution of fibrosis in lung segments was also significantly different in MDR-TB compared to DS-TB. Although MMP-9 levels in the MDR-TB group were higher than in the DS-TB group, there was no statistically significant difference. Based on HRCT measurements, this study found a link between MDR-TB and DS-TB in terms of the number of cavities, the diameter of the cavities, the thickness of the cavity walls, and the location of fibrosis in the affected lung segments. There was no link between the MMP-1 (−1607G) and MMP-9 (C1562T) genotypes and the levels of MMP-1 and MMP-9 in the blood. The MMP-1 genotype in the two study groups was very different and was linked to twice as many cases of MDR-TB. In addition, there was a substantial difference in cavity wall thickness between the G/G MMP-1 1607 genotype and the T/T MMP-9 genotype in the two study groups.

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1. Introduction

Mycobacterium tuberculosis (MTB), an infectious illness that causes pulmonary tuberculosis (TB), is still an issue around the world, particularly in Asian nations like Indonesia. Antibiotic therapy is still an option for treating TB. However, prolonged and ineffective treatment can cause one or perhaps more anti-tuberculosis medications to become resistant to treatment. Multi-drug-resistant (MDR) TB was identified in an estimated 500,000 cases in 2019, and 1.4 million people died from the disease, making it the infectious disease with the highest mortality rate. Antimicrobial resistance is also a factor in the deaths caused by pulmonary tuberculosis. The rise in drug-resistant tuberculosis (DR-TB) cases poses one of the major risks to global efforts to treat and prevent the disease, with 480,000 cases of TB being multi-resistant to at least two first-line treatments or resistant to at least one OAT (isoniazid and rifampicin) [1].

Using thoracic radiography and computed tomography, MDR-TB patients frequently have lesions such as cavities and fibrosis (CT). The first imaging modality to diagnose patients with suspected pulmonary tuberculosis, screen them for the disease, assess OAT, and look for suspicious masses is thoracic radiography. However, it has limitations because it cannot detect active lesions in the distal respiratory tract or diagnose patients with advanced disease progression. A thoracic CT scan is an imaging technique that can detect both active and inactive lesions, small lung lesions, distal (endobronchial) respiratory system involvement, and significant lung parenchymal damage. Thoracic CT showed a sensitivity and specificity of 98 %, which was greater than thoracic radiography's sensitivity and specificity of approximately 19–58 % [2,3].

The literature reports that 50.8 % of MDR-TB patients had cavities, but the appearance of thoracic high-resolution computerized tomography (HRCT) in MDR-TB patients varies from patient to patient [4]. Park et al. [5] observed that 75 % of cavities were from MDR-TB patients, and these cavities were highly contaminated with MTB germs. Another study in Peru discovered that 66.6 % of patients had bilateral lung abnormalities, with MDR-TB patients having numerous cavities more frequently than drug-sensitive-TB (DS-TB) drug patients [6,7]. These findings are similar to those of Joshi et al. [8]. who reported that 52 % of patients had extensive lung damage and 88 % of patients had multiple cavities.

An inflammatory reaction from the host is a hallmark of MTB lesions. According to Parasa et al. [9], granulomas liquefy into necrotic tissue that is ejected to create voids. The cavity is believed to be responsible for the subsequent transmission of infection in advanced pulmonary TB, or MDR-TB, where even small lesions can cause irreversible harm to lung structures like bronchiectasis, bronchovascular distortion, or fibrosis, leading to emphysema. Increased extracellular matrix (ECM) turnover and tissue healing are frequently linked to this disease process. The lungs' extracellular matrix contains the majority of type I collagen and elastin. For mycobacteria to spread from the lung parenchyma to the airways and form cavities, proteolytic enzymes must break down the extracellular matrix (ECM) [10]. The only enzymes that can break down ECM are matrix metalloproteinases (MMPs) but type I collagen is extremely hard to break down by proteolytic enzymes. Therefore, it appears likely that MMPs have a significant impact on how cavities and fibrosis develop [11].

The MMP protein is one of the enzymes responsible for damage to the lung parenchyma. Most MMPs were not expressed under normal circumstances; however, subsequent analysis revealed that overexpression in the inflammatory process was present. Anti-inflammatory cytokines and bacterial lipopolysaccharides control how much MMP is expressed [12–15]. Tissue inhibitors of metalloproteinases (TIMPs) and 2-macroglobulin regulate proteases, which contribute to the protein's post-translational activation following its initial production as a pro-enzyme. The majority of the connective tissue in the lungs, which is the primary structural protein of the lungs, is destroyed in part as a result of MMP molecules [16,17]. Tumor necrosis factor and interleukin beta, among other cytokines, control the release of several MMPs by monocytes in ECM7, including MMP-1 (interstitial collagenase), MMP-9 (gelatinase B), and MMP-12 (macrophage metalloelastase), which break down collagen fibrils. Monocytic cells infected with MTB secreted more MMP-1, MMP-9, MMP-3, MMP-10, and MMP-11 than MMP-2 and MMP-8. Patients with DS-TB had greater MMP-1 and MMP-9 levels than those with congestive heart failure [18].

The airway epithelium next to the TB granuloma also showed increased MMP-1 expression in addition to monocytic cells [9]. As a result of the ECM's degradation brought on by the elevated MMP-1 expression, the cavity ruptures spread to the surrounding tissue and cause extensive tissue damage [19]. According to various study, the MMP-1-1607G polymorphism raises the incidence of tracheobronchial stenosis [20], initiates the breakdown of type I collagen [21], and is crucial for fibrosis [22]. Additionally, different metalloproteinase types (MMP-1, -3, -8, and -9) were discovered, with the amounts of each fluctuating according to the severity of lung parenchymal damage [21]. MMP-9 activity is directly correlated with the degree of the substantial lung parenchymal damage process, while MMP-8 is a neutrophilic component that affects chemokine activity [23–26].

In a study on DS-TB patients, Wang et al. [26] evaluated genetic variables in the form of MMP-1, MMP-9, and MMP-12 gene polymorphisms. In that study, TB patients with moderate to advanced pulmonary fibrosis had a considerably greater frequency of the MMP-1 polymorphism (−1607G) than did TB patients with mild pulmonary fibrosis. There was a 5.0 (95 % CI 1.25–20.30) and 9.87 (95 % CI 2.39–0.88) fold increase in the probability of moderate to severe fibrosis in individuals with at least one −1607G MMP-1 polymorphism. MMP-9 (−1562T) and MMP-12 (Asn357Ser) polymorphisms did not correlate with pulmonary fibrosis. In comparison to those with the 2G/2G genotype, subjects with the 1G allele genotype produced more MMP-1 from monocytes treated with interleukin 1 beta. MMP-9 (C1562T) and MMP-12 (Asn357Ser) were not linked to pulmonary fibrosis susceptibility, whereas MMP-9 (C1562T) production was directly proportionate to severe lung damage. Another study by Rius et al. [27] and Belton et al. [28] demonstrates the relationship between MMP-1 and MMP-9 levels and the cavity, which is a hypoxic region with various gradations in the periphery. Both contribute to inflammation and are mediators of inflammatory cell migration and collagen and elastin breakdown in the immunological response to tuberculosis infection. Another study utilizing transgenic mice as experimental animals revealed that MMP-1 was involved in the development of caseous cavities and severe lung damage as compared to mice of the wild type [19,29,30]

and that MMP-1 and MMP3 may cause lung parenchymal damage [9,11,12].

The aim of this study was to evaluate genetic polymorphisms and levels of MMP-1 and MMP-9, whose activity has implications for MDR-TB and DS-TB, and their correlation with cavity characteristics (number, diameter, and thickness) and fibrosis by thoracic HRCT examination.

2. Material and methods

This study was approved by the Health Research Ethical Committee of the Faculty of Medicine, Universitas Lampung, Indonesia, with protocol number 997/UN26.18/PP.05.02.00/2021. The study adhered to relevant guidelines and regulations in all its methods. Prospective study participants receive an explanation of the study's objectives and procedures. Prospective study subjects have the right to agree or not to agree to become study subjects after understanding the explanation. The identity of the patients is kept confidential, and written informed consent was obtained from all enrolled patients. This comparative cross-sectional study between the DS-TB and MDR-TB groups examines the relationship between gene polymorphisms and protein levels in relation to cavity characteristics and fibrotic distribution patterns using thoracic HRCT.

The selection of study subjects was based on polyclinic data and medical records of DS-TB and TB-MDR patients from the Lung Polyclinic of the Hospital in Lampung Province from April 2021 to August 2021. This study included a total sample size of 70, comprising 36 DS-TB samples and 34 MDR-TB samples, collected through consecutive sampling from all study subjects who met the inclusion criteria until the sample count reached the required number. The inclusion criteria in this study were as follows: Patients have MDR-TB or DS-TB, according to their medical records. They are either male or female, between the ages of 18 and 70, and have expressed their willingness to participate in the study through written informed consent after learning about it.

Blood samples were collected from the study subjects. We centrifuged 1 cc of blood at 3000 rpm to obtain serum for ELISA and immediately stored it at 4 °C in the laboratory for immediate molecular processing. The leftover samples were then stored at −20 °C for later use.

2.1. Determination of serum matrix Metalloproteinase-1 and matrix Metalloproteinase-9 levels by ELISA

A quantitative analysis of serum MMP-1 and MMP-9 was done by using a commercial human MMP-1 and MMP-9 ELISA Kit from Elabscience® (Cat. Number: E-EL-H6075, USA). according to the manufacturer's instructions. Briefly, all the kits are based on the two-site ELISA sandwich format [31,32]. We read the absorbance at 450 nm using the Allsheng AMR-100 (Huangzou Allsheng Instrument, China). The corresponding standard curves and run for each plate separately.

2.2. Determination of alleles of MMP-1 and MMP-9 genes

We used the Genomic DNA Mini Kit (Blood/Cultured Cell) (Geneaid) to extract blood and obtain pure DNA. The primers used in the detection PCR test for MMP-1 and MMP-9 are shown in Table 1. The PCR process was done using the KAPA2G Fast Hotstart Readymix PCR Kit (Merck) according to the instructions. The final reaction volume was 50 μl, and the annealing temperature was set to 56 °C for 40 cycles. After the sample was amplified, it was electrophoresed on a 1.5 % agarose gel (1st base) and stained with 0.5 μg/ml ethidium bromide (Vivantis). We used a marker of 100 bp (VC 100 bp Plus DNA Ladder Vivantis) as a standard measure. All the samples that showed the bands of MMP-1 and MMP-9 were sequenced using Sanger's method. We used MEGA X and Bioedit software to compare the genetics and mutations of −1607 and −1562 in MMP-1 and MMP-9, respectively.

2.3. The HRCT examination

Low-dose HRCT analysis using MSCT 128 slices, Hitachi brand SCENARIA with parameters of 100 kV, data collection speed: 2880/sec, minimum slice thickness of 1 mm, FOV (field of view): 20–500 mm. The maximum scan range (in/mm) is 79/2000. The process involves scanning and reconstructing images with a beam width of 0.625–10 mm and a volume scan pitch ranging from 0.578 to 1578. We reconstructed the raw data into axial images, each with a 5 mm slice thickness at 5 mm intervals. We obtained axial images for thoracic HRCT using a slice thickness of 0.625 mm at 1 mm intervals and a high spatial frequency algorithm. Images of the mediastinal window (window width, 400 H; window level, 20 H) and lung (window width, 1500 H; window level, 700 H) appeared on the monitor and were available for analysis. We transfer the reconstructed image data to a CD-ROM or external disk. The resulting image will be expertly analyzed by two radiology specialists from the Thoracic Division of the Radiology Department at the Faculty of Medicine, Universitas Indonesia. We counted the number of cavities by observing every lung segment on the axial, coronal, and sagittal planes of

Table 1
Primer for detection of MMP-1 and MMP-9 genes.

Target Genes	Primer	Sequence (5′–3′)	Amplicon (bp)
MMP-1	Forward	CTTCAGTGGCAAGTGTTCTTTG	304
	Reverse	CCCACCTTTCCCACTGTATC	
MMP-9	Forward	TCTCCATCTCACAGTCTCATTTATT	462
	Reverse	CATCGGGCAGGGTCTATATTC	

the lungs. By observing the axial plane, we measured the anteroposterior and lateral diameters of the cavities. We measured the cavity wall thickness by observing the axial plane and measuring the distance between the innermost edge and the outermost edge of the cavity wall. Lastly, the fibrosis distribution was observed from the axial plane by counting the segments affected.

2.4. Data analysis

The present study used a comparative cross-sectional design in two groups, namely the MDR-TB group and the DS-TB group. We processed the data obtained in this study using the SPSS for Windows version 20.0 program. The data in this study are not normally distributed, so the Mann-Whitney test is used, which is a non-parametric test used to determine the difference in the median of two groups if the dependent variable data scale is ordinal or interval/ratio but not normally distributed.

3. Results

3.1. HCRT examination outcomes

The sample thoracic HRCT examination is shown in Fig. 1A–D. The results of the thoracic HRCT examination consist of two parts: Table 2, which shows the distribution of subjects according to the description of the thoracic HRCT in the study group, and Table 3, which shows the distribution of the thoracic HRCT in the study group. There were significant differences in the characteristics of the cavity, fibrosis, consolidation, and bronchiectasis. Patients with both MDR-TB and DS-TB often exhibit tree-in-bud and bronchiectasis, followed by emphysema, calcification, and, infrequently, pleural effusion. It was found that there were more cavities in the MDR-TB group than in the DS-TB group. The walls of the cavities were also thicker in the MDR-TB group than in the DS-TB group, and fibrosis was more common in the MDR-TB group than in the DS-TB group. Statistical tests show significant differences in HRCT parameters between the two study groups.

3.2. Serum MMP-1 and MMP-9 level

The results showed that there was no significant difference in the MMP-1 and MMP-9 ELISA results (Table 4) in MDR-TB and DS-TB, although there was a difference in the quantity of MMP-9 ELISA results in MDR-TB and DS-TB. Conversely, the MMP-1 and MMP-9 ELISA results exhibit a negative correlation with HRCT parameters (number of cavities, diameter, cavity thickness, and fibrosis distribution), implying an inverse relationship between these HRCT parameters and MMP-1 and MMP-9 levels (Table 5). Statistical analysis showed that there was no significant difference between the two study groups.

3.3. MMP-1 and MMP-9 genotype and allele frequency

After obtaining the PCR products, we performed electrophoresis to confirm the correct amplified MMP-1 and MMP-9 (Fig. 2A and
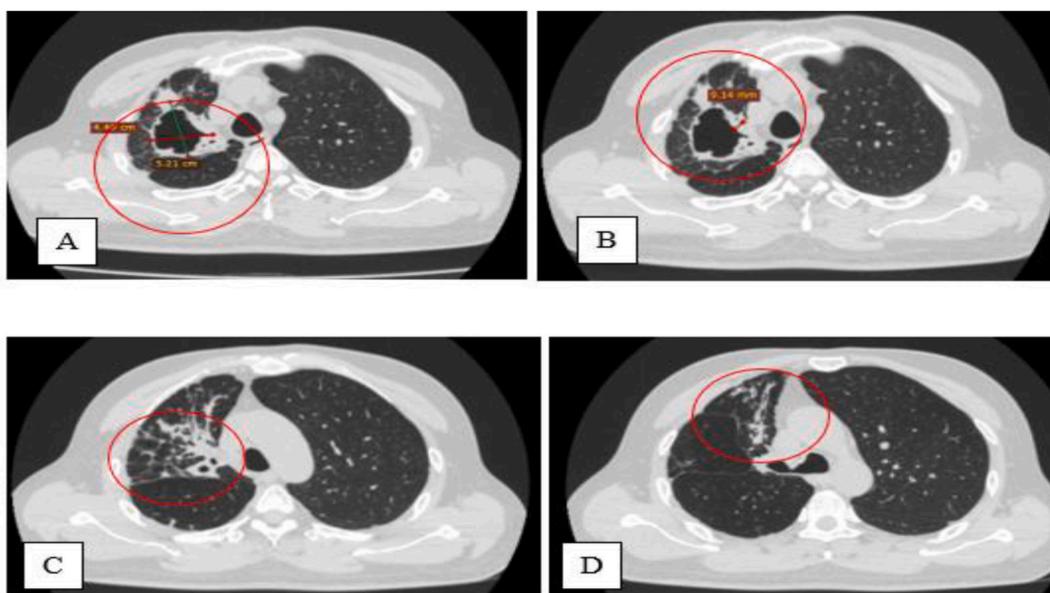

Fig. 1. Thoracic HRCT image. A) Cavity diameter measurement, B) Cavity wall thickness measurement, C) and D) Fibrosis distribution in right lung segment.

Table 2
The description of the thoracic HRCT in the study group.

Thoracic HRCT	Group		P Value
	MDR (n = 34)	DS(n = 36)	
Number of Cavities	6.5(0–45)	1(0–15)	0.002
No cavity	1	0	
<3	8	25	
>3	25	11	
Median range diameter of the cavity	38(0–75)	17.5(0–75)	0.004
The median range wall thickness of the cavity	6(0–18)	4.5(0–18)	0.017
Fibrosis distribution	9.5(3–18)	7(0–18)	0.051
<3 segment	18	23	
>3 segment	16	13	
Consolidation			
Yes	31	26	0.042
Non	3	10	
Ground Glass Opacity			
Yes	9	13	0.385
Non	25	23	
Calcification			
Yes	9	12	0.531
No	25	24	
Tree bud			
Yes	32	32	0.674[a])
No	2	4	
Pleural effusion			
Yes	4	1	0.192[a])
No	30	35	
Emphysema			
Yes	12	8	0.226
No	22	28	
Bronchiectasis			
Yes	30	21	0.005
No	4	15	

[a] Fisher Exact Test.

Table 3
Variable on thoracic HRCT examination.

Thoracic HRCT	Group				P Value
	MDR (n = 34)		DS (n = 36)		
	Median	Range	Median	Range	
Number of cavities	6.5	0–45	1.0	0–15	0.002
Diameter of cavity (mm)	38.0	0–75	17.5	0–75	0.004
Thickness of cavity (mm)	6.0	0–18	4.5	0–18	0.017
Fibrosis distribution	9.5	3–18	7.0	0–18	0.051

Note: Fibrosis distribution is measured in segments observed from axial plane. Multiple adjacent fibrosis is counted as one segment.

Table 4
MMP-1 and MMP-9 ELISA results.

| ELISA	Group				P value
	MDR (n = 34)		DS (n = 36)		
	Median	Range	Median	Range	
MMP-1 (ng/µl)	3.6	1.2–7.1	3.8	1.5–8.9	0.321
MMP-9 (pg/µl)	411,9	11.7–4986	347.5	11.7–1288	0.514

B). We sequenced the MMP-1 polymorphism at position 1607 and discovered three genotypes: 1G2G (heterozygous), 1G1G (homozygous), and 2G2G (homozygous) (Fig. 3A–C). The frequencies of these genotypes in each study group are shown in Table 6. Heterozygous 1G2G at position −1607 was the most common MMP-1 genotype in both MDR-TB and DS-TB. The frequency of the G allele is 44.11 % and the GG allele is 55.89 % in MDR-TB, while in DS-TB G allele frequency is 31.94 % and the GG allele frequency is 68.06 %. Chi-Square statistical analysis showed there is a significant difference between MDR-TB with G/GG genotype and DS-TB with the same genotype ($p < 0.05$). The GG allele showed preventive odds to develop MDR-TB (OR = 0.5, CI 95 % 0.19–1.30). Besides the MMP-1

Table 5
Correlation between parameter thoracic HCRT and levels of protein serum of MMP-1 and MMP-9.

Thoracic HRCT	MDR (n = 34)		DS(n = 36)		P-value
	R-value	P-value	R-value	P-value	
MMP-1					
Number of cavities	−0.02	0.909	−0.13	0.442	0.442
Diameter of cavity (mm)	0.17	0.318	−0.05	0.773	0.743
Thickness of cavity (mm)	0.07	0.691	−0.23	0.184	0.161
Fibrosis distribution	0.20	0.249	−0.15	0.399	0.399
MMP-9					
Number of cavities	0.24	0.159	−0.14	0.415	0.415
Diameter of cavity (mm)	−0.01	0.973	−0.05	0.758	0.413
Thickness of cavity (mm)	0.02	0.910	−0.08	0.632	0.839
Fibrosis distribution	−0.06	0.733	−0.08	0.669	0.669

Note: Spearman rank correlation test. Fibrosis distribution is measured in segments observed from axial plane.
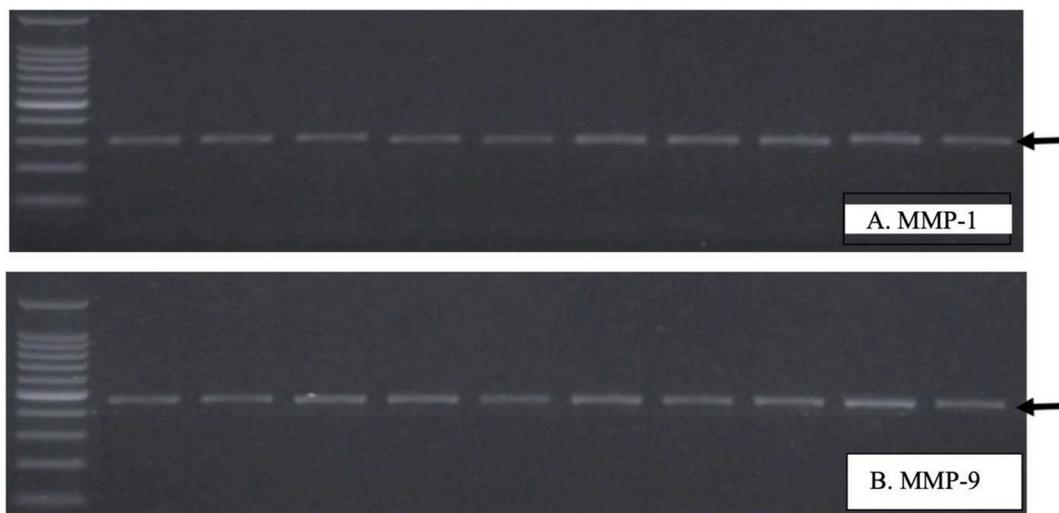
Fig. 2. Electrophoresis of PCR product from sample 1–10.
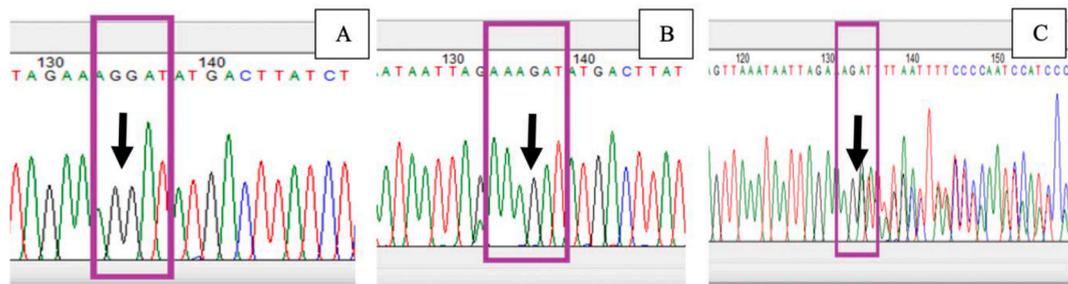
Fig. 3. Sequencing chromatograms of MMP-1 polymorphisms. A) Homozygous GG, B) Homozygous G, C) Heterozygous GG/G.

polymorphism at position −1607G, we also detected point mutations at positions G1578A, T1694G, A1835T, A1677G, T1692A, and A1683T. Both the MDR-TB and DS-TB groups had point mutations, with G1578A (47.06 %) being the most common in the MDR-TB group and A1835T in the DS-TB group.

We also sequenced the MMP-9 gene (Fig. 4A–C). The most common MMP-9 genotype at position −1562 in both MDR-TB and DS-TB was homozygous CC. The frequency of the C allele in MDR-TB is 76.5 %, and the T allele is 23.5 %. The C allele in DS-TB was 79.2 %, and the T allele was 20.8 %. The TT genotype has OR = 2.20 (CI 95 % 0.19–26.16). Chi-Square statistical analysis showed no significant difference between MDR-TB and DS-TB (Table 6). The sequencing results revealed the presence of mutation points at positions

Table 6
Genotype and allele of MMP-1-1607G and MMP-9 C1562T.

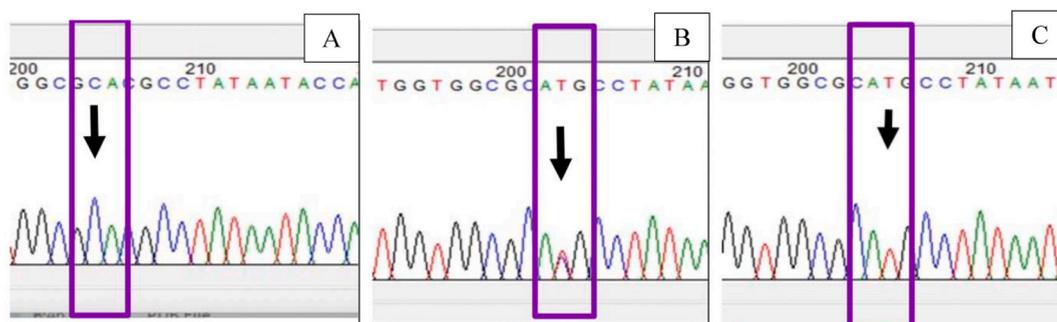| Genotype	Groups		Odd Ratio (OR)	CI95 %	p-value
	MDR	DS			
	(n = 34)	(n = 36)			
	n (%)	n (%)			
MMP-1 (−1607 GG)					
G/G	5 (14.7)	1 (2.8)	1	–	0.00
G/GG	20 (58.3)	21 (58.3)	0.19	(0.02–1.78)	
GG/GG	9 (26.5)	14 (38.9)	0.13	(0.01–1.29)	
Allele G	19 (55.9)	14 (38.9)	1	–	0.045
Allele GG	15 (44.1)	22 (61.1)	0.5	(0.19–1.30)	
MMP-9 (−1562 C/T)					
CC	20 (58.8)	22 (61.1)	1	–	0.53
CT	12 (35.3)	13 (36.1)	0.17	(0.45–3.12)	
TT	2 (5.9)	1 (2.8)	2.20	(0.19–26.16)	
Allele C	20 (58.8)	22 (61.1)	1	–	0.18
Allele T	14 (41.2)	14 (38.9)	1.10	(0.42–2.86)	

Fig. 4. Sequencing chromatograms of MMP-9 polymorphisms. A) Homozygous CC, B) Heterozygous CT, C) Homozygous TT.

A1825T and C1590T, in addition to the MMP-1 polymorphism at position C1562T. At these two points, the most common mutation positions were MDR-TB at position A1825T and DS-TB at position C1590T.

The relationship between MMP-1-1607 and MMP-9-1562 genotypes with the degree of lung damage (number of cavities, cavity diameter, cavity wall thickness, and fibrosis distribution) is shown in Table 7. The results showed a significant difference between the 2G MMP-1 genotype and the TT genotype of MMP-9 in terms of cavity wall thickness.

4. Discussion

We examined how serum levels and genetic differences of MMP-1 and MMP-9 were related to the shape of cavities and fibrosis distribution in a small group of multidrug-resistant (MDR) and drug-sensitive (DS) tuberculosis patients. Age grouping was based on the WHO classification, which categorizes individuals into young adults and elderly groups. The WHO reported a higher incidence of tuberculosis, particularly MDR-TB and DS-TB, among young adults, showing a notable disparity between the two groups [1]. Individuals with greater mobility who work outside their homes were more susceptible to infection compared to those with lower mobility who stayed indoors. While employment status appeared to influence infection rates, statistical analysis did not indicate a significant difference [8].

Smoking behaviors, both active and passive, did not exhibit significant variances between the research groups, contrary to Anasufalah's study indicating a link between smoking and higher mortality rates in pulmonary tuberculosis patients [33]. Smokers and ex-smokers were identified as a high-risk population for tuberculosis, underscoring the necessity to address smoking cessation for TB prevention in regions with high prevalence. Additionally, a meta-analysis by Burusie et al. highlighted the strong association between smoking and suboptimal tuberculosis treatment outcomes [34].

The most prevalent location of lesions in this study is the left lung in the MDR-TB group and the right lung in the DS-TB group; nevertheless, statistical analysis reveals no statistically significant difference between the two groups. Joshi et al. [8] discovered that involvement of both lungs is more prevalent in MDR-TB. Cavities are one of the anomalies observed in TB patients, particularly in MDR patients. According to a recent study by Kahkouee et al., fewer than three and more than three cavities were analyzed in this investigation [35]. Patients with MDR-TB frequently report having more than six cavities. These findings are identical to those of Lee

Table 7
Correlation genotype with characteristic cavity and fibrosis distribution.

Genotype	P Value			
	Number of cavities	Diameter of cavity (mm)	Thickness of cavity (mm)	Fibrosis Distribution
MMP-1				
G/GG	0.565	0.549	0.245	0.674
GG/GG	0.967	0.513	0.02	0.442
G/G	0.843	0.903	0.565	0.100
MMP-9				
CC	0.565	0.549	0.245	0.674
CT	0.421	0.495	0.191	0.795
TT	0.967	0.513	0.02	0.442

Note: Kruskal Wallis rank test. Fibrosis distribution is measured in segments observed from axial plane.

et al. [36]. A statistical analysis revealed a substantial difference between the two study groups in terms of the number of cavities.

The characteristics of the cavity's diameter and thickness in MDR-TB were thicker and wider than those in the DS-TB group. Statistical analysis showed that there were significant differences between the two research groups. These results are similar to those of Cheon et al. [37], Chuchottaworn et al. [38] Research from Belton et al. [28] found that the cavity is a highly hypoxic area, and in hypoxic conditions, there is an increase in MMP-1 expression in primary respiratory epithelial cells through the intercellular network regulated by TB. Furthermore, MTB infection promotes HIF-1α accumulation even under normoxia. The study concluded that under tissue and cavity hypoxia conditions, MTB would promote the accumulation of HIF-1α, synergistically increasing collagenase activity, which would lead to lung and cavity destruction.

This study revealed that MDR-TB patients were more likely to have fibrosis than DS-TB patients. Fibrosis distribution in the lung parenchyma is one of the most prevalent illnesses and sequelae identified in patients with pulmonary tuberculosis. Fibrosis is an excessive accumulation of fibroblasts caused by the inflammatory process, and MMPs play a significant role in the occurrence of fibrosis, as demonstrated by Wang et al. [26] who discovered that MMP-1 played a significant role in the occurrence of fibrosis in the Taiwanese population after OAT treatment. In addition to the characteristic features and distribution of fibrosis, other abnormalities, such as consolidation, GGO, tree in bud, calcified pleural effusion, and BE, were identified. There were significant differences in consolidation and BE abnormalities between the two study groups, consistent with the findings of Kim [4,7,39,40].

In tuberculosis (TB), MMPs are frequently associated with tissue injury and matrix remodeling. This research utilized serum samples. We used ELISA to determine the serum concentrations of MMP-1 and MMP-9. According to the serum level results, people with MDR-TB and TB-DS had the same amount of MMP-1 in their blood. There were also no statistically significant differences between the two study groups. There was a difference in the amount of MMP-9 protein in the serum of MDR-TB and TB-DS patients. The levels of MMP-9 protein in the serum of MDR-TB patients were higher than those of TB-DS patients, but this difference was not statistically significant.

We performed a correlation analysis on the serum levels of MMP-1 and MMP-9 in the systemic circulation of both study groups to determine the association between the systemic circulation of these molecules and the severity of lung parenchymal damage. The table demonstrates a negative correlation between MMP-1 and MMP-9 blood levels, cavity features, and fibrosis distribution. This indicates that the correlation between the two research groups is unequal. In this investigation, serum protein levels did not influence cavity features or fibrosis distribution. While serum protein levels may not directly reflect enzyme activity, it's possible that other inflammatory mediators, in addition to serum MMP levels, also contribute to the development of cavity and fibrosis characteristics. There are other substances, like transcription factors, cytokines, and chemokines, that can affect and become immunological mediators of lung tissue remodeling and decreased lung function in TB [41]. These substances can make tissue-degrading enzymes more active. Setijawan et al. discovered that MMP-9 had a moderate connection with lung parenchymal damage in TB-DS patients in Surabaya using chest radiography [42].

Sequencing of the MMP-1 revealed G/GG, G/G, and GG/GG genotypes at position −1607. The genotype for MDR-TB and DS-TB is G/GG. There was no statistically significant difference between the two research groups. This research contradicts the findings of Wang et al., who concluded that genotype G and female gender were independent predictors of the development of endobronchial TB. Patients with endobronchial TB and genotype G had a 9.86-fold increased risk of developing tracheobronchial stenosis [43]. Moreover, tuberculosis patients with genotype G's peripheral blood monocytes showed IL-1beta-influenced serum protein levels of MMP-1, and endobronchial tuberculous granulomas also showed its activity. Wang's study concludes that MMP-1 polymorphism with genotype G is connected with an increased risk of developing tracheobronchial constriction via MMP-1 activity upregulation [26]. This contradiction may occur because of the small number of samples in this study. The levels of MMP-1 measured in this study also did not directly tell MMP-1 activity. Thus, it is recommended to measure the enzyme activity as well.

High serum MMP-9 concentrations were independently related to the MMP-9 C1562T rs3918242-TT genotype, younger age, current smoking status, increased fasting plasma glucose, and fibrinogen concentrations in Taiwanese persons [44]. The cavity thickness of MMP-9 C1562T homozygous TT was significantly different, while the fibrosis number, diameter, and distribution were not. The sequencing of the MMP-9 rs3918242 gene at position −1562 revealed the presence of two alleles, C and T, with the C allele predominating in both study groups. This is the same as previous research, which showed that the −1562 C/C genotype (allele C) was

more likely to produce significant lung damage or multilobes and had implications for the origin of infection but did not discuss cavity characteristics such as cavity wall thickness in detail [25,36].

5. Conclusion

Patients with MDR-TB have different characteristics of cavities and fibrosis compared to those with DS-TB. The MMP-1 and MMP-9 serum levels in people with MDR-TB and DS-TB were about the same, but they did not correlate with the MMP-1 ($-1607G$) and MMP-9 (C1562T) genotypes. There was a substantial difference in cavity wall thickness between the G/G MMP-1 1607 genotype and the T/T MMP-9 genotype in the two study group, but not with the number of cavities, diameters, and fibrosis distribution. However, due to the small number of samples, there is a limitation to this study, as it might overestimate its significance. Adding more samples is advantageous for future studies. We also recommend evaluating the MMPs' activity instead of just their levels.

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its supplementary materials. The complete HRCT images and electrophoresis gel images are also available upon request.

CRediT authorship contribution statement

Anse Diana Valentiene Messah: Conceptualization. **Sawitri Darmiati:** Investigation, Formal analysis. **Cleopas Martin Rumende:** Writing – original draft. **Retno Ariza Soemarwoto:** Writing – review & editing, Data curation. **Joedo Prihartono:** Writing – review & editing, Data curation. **Asmarinah Asmarinah:** Supervision, Formal analysis, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:Asmarinah reports financial support was provided by Universitas Indonesia, Indonesia. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e33671.

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