

Alpha-1 antitrypsin in COVID-19 patients: a dual-center screening study in Malaysia

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BACKGROUND: Alpha-1 antitrypsin (A1AT) deficiency has been recognized as an adverse prognostic determinant in severe instances of COVID-19.

OBJECTIVE: To determine the A1AT phenotypes and levels in individuals at various clinical stages of COVID-19 compared to healthy controls.

DESIGN: Case-control study

SETTINGS: Hospital Raja Perempuan Zainab II (HRPZ II) and Hospital Ampang, Malaysia

PATIENTS AND METHODS: The analysis included a total of 282 patients. We categorized 188 COVID-19 patients from two centres in Malaysia into two groups: mild to moderate (stages 1-3) and severe to critical (stages 4-5) and compared them with 94 healthy controls.

MAIN OUTCOME MEASURES: A1AT phenotypes and levels in different COVID-19 stages compared to healthy controls

SAMPLE SIZE: 282 subjects

RESULTS: The frequency (n) and percentage (%) in the control group, 88 (93.6) exhibited PiMM phenotypes, whereas 6 (6.4) displayed PiXM/PiYM phenotypes. Within the mild to moderate COVID-19 group, 88 (93.6) had PiMM phenotypes, 3 (3.2) featured PiXM/PiYM, and 1 presented PiBM phenotypes. Among severe to critical COVID-19 patients, the PiMM phenotype was identified in 61 (64.9) with 16 (17) having PiBM phenotypes, 4 (4.5) displaying PiCM, 2 (2.1) featuring PiXM/PiYM, and 1 (1.1) presenting PiEM phenotypes. Variants such as MS, MZ, S, and Z were undetected. However, 12 COVID-19 patient samples yielded inconclusive results. Median (IQR: 25th to 75th percentile) A1AT concentrations for controls were 1.8 (1.3-2.3) g/L, for mild to moderate cases 1.9 (1.2-2.6) g/L, and for severe to critical COVID-19 cases 2.1 (1.4-2.8) g/L.

CONCLUSION: This research identifies the PiMM phenotype as the predominant phenotype expression within the studied population. This prevalence underscores the potential role of genetic factors in determining the biological response to SARS-CoV-2 infection. The presence of another phenotype variant across the study population suggests a

nuanced genetic landscape that warrants further exploration.

LIMITATION: The absence of follow-up A1AT quantification and baseline measurements limits the assessment of disease progression. The isoelectric focusing phenotyping technique used might have missed specific A1ATD variants.

CONFLICT OF INTEREST: None

KEYWORDS: COVID-19, Alpha-Antitrypsin, screening, protein

The COVID-19 public health emergency persisted for over three years, concluding when the World Health Organization (WHO) officially declared it over on May 5, 2023.¹ COVID-19 has infected approximately 777 million individuals, resulting in around 7.0 million fatalities globally.¹ In COVID-19, biomarkers exhibited various alterations indicative of the disease's severity and progression. These biomarkers are essential for diagnosing the condition, predicting its course, and evaluating patient outcomes. Among the primary biomarkers associated with COVID-19 are various pro-inflammatory cytokines, neuron-specific enolase (NSE), lactate dehydrogenase (LDH), aspartate transaminase (AST), neutrophil count, troponins, creatine kinase (MB), myoglobin, D-dimer, brain natriuretic peptide (BNP), and its N-terminal prohormone (NT-proBNP).²

The alpha-1 antitrypsin (A1AT) protein, primarily secreted by hepatocytes, is crucial in inhibiting inflammation by neutralising proteolytic enzymes like neutrophil elastase, impedes proteinase³ and proteinase G.³ A1AT is a multifunctional protein that protects tissues from lung tissue from the harmful effects of serine proteases⁴ aids tissue repair and has anti-inflammatory and immunomodulatory properties.⁵ Recent studies suggest that A1AT plays a role in impeding SARS-CoV-2 infection by targeting two crucial proteases involved in the pathogenesis of COVID-19; inhibiting TMPRSS2 S protein priming⁶ and disintegrin and metalloproteinase 17 (ADAM17).⁷ Consequently, it is plausible to hypothesise that A1AT deficiency may contribute to developing severe manifestations of COVID-19.

PiM is the most common and normally functioning A1AT allelic form, so the healthy human phenotype is PiMM.⁸ There are over 100 genetic A1AT variants, the most common deficient variant and clinically significant of which are PiZ and PiS. Among individuals with PiMM, A1AT serum levels typically range from 0.9 to 2.0 g/L, corresponding to 20 to 48 μ M.⁹ Additionally, carriers of the PiMS, PiMZ, PiZ, and PiS alleles show significantly lower serum A1AT levels than those with normal allele expression. Specifically, the average reductions are as

follows: PiMS exhibits an 80% decrease, PiMZ shows a 60% decrease, PiZ demonstrates a 15% decrease, and PiS displays a 30% decrease.¹⁰

A1AT Deficiency (A1ATD) is inherited as an autosomal codominant condition that causes the defective production of A1AT protein, resulting in the deterioration of lung function.¹¹ A1ATD is characterised by reduced plasma levels or the abnormal functioning of A1AT, a human blood serine protease inhibitor encoded by the serine protein inhibitor-A1 (SERPINA1) gene.¹² A1ATD manifests clinically in individuals with mutations in both gene Pi alleles, particularly the PiZZ variant. In contrast, in the heterozygous state, the defect is partially compensated by the normal allele found in individuals with PiMZ and PiMS phenotypes.¹³ Recent findings suggest that patients with A1ATD may be more susceptible to severe COVID-19 outcomes. A geographic overlap between A1ATD prevalence and regions with high rates of severe COVID-19 cases in Italy further supports a potential link.¹⁴ Regions exhibiting a high prevalence of A1ATD demonstrated an elevated incidence of infection and mortality. Conversely, low-prevalence areas are associated with a reduced mortality rate.¹⁵ This highlights the importance of considering A1ATD as a potential risk factor for severe COVID-19 progression. Furthermore, the number of infections and deaths due to COVID-19 were correlated with the prevalence of A1ATD for variant PiS and PiZ.¹⁴ Reduced levels of A1AT, especially in individuals with the PiZZ genotype, are linked to an increased susceptibility to COVID-19 infection.¹⁶

The evaluation of A1ATD requires a combination of biochemical tests and/or genetic tests, which include A1AT protein quantification, determining A1AT phenotype, and/or A1AT genotyping test to identify specific A1AT alleles.¹⁷ However, A1AT is a positive acute-phase reactant that increases 75-100%¹⁸ in the bloodstream as an inflammatory response caused by infection or inflammatory disease, and this surge is thought to be an attempt to counteract pro-inflammatory activities.¹⁹ Thus, identifying deficiency phenotypes may be hindered if

systemic inflammation occurs during sample collection. Patients with an intermediate deficiency may go unrecognised since their A1AT levels fall within the normal range, which prevents further phenotypic evaluation.²⁰ Individuals with rare null alleles are frequently identified via genotype testing because null alleles do not produce a protein that a band can identify in the isoelectric focusing electrophoresis (IEF) analysis.²¹ Moreover, in some cases, whole exon or whole gene sequencing may be required to identify rare and novel variants.

This study aimed to compare the A1AT levels and phenotypes of healthy controls at two centres in Malaysia with those of persons infected with COVID-19 at different clinical stages. This preliminary study offers an overview of COVID-19 with A1ATD, empowering clinicians and patients and paving the way for future comparisons.

PATIENTS AND METHODS

Study design, participants, data collection

A case-control study was conducted among adult COVID-19 patients aged 18 to 80 years admitted to Hospital Raja Perempuan Zainab II (HRPZ II) and Hospital Ampang, Malaysia, from January 2021 to June 2023. This study was approved by The Human Research Ethics Committee of USM (JEPeM-USM) protocol code USM/JEPeM/21100691 and Ministry of Health Malaysia protocol code NMRR-21-762-58458 (IIR).

COVID-19 diagnosis was confirmed using either an Antigen Rapid Test Kit (RTK-Ag) or Reverse Transcription-Polymerase Chain Reaction (RT-PCR) from the nasopharyngeal swab. A total of 188 adult patients who tested positive for COVID-19 were included in this study, regardless of their comorbidities, medication use, and lifestyle factors. This inclusive approach ensures a comprehensive understanding of the disease's impact across diverse patient profiles. The patients were classified into five clinical stages as per Malaysia's Ministry of Health guidelines (Annex 2e) and further categorised by disease severity into mild to moderate (Stages 1-3) and severe to critical (Stages 4-5). The investigation utilised residual blood samples from plain gel separator tubes of hospitalised COVID-19-positive patients. Patients were classified as survivors if they were discharged alive from the hospital, while those who died during hospitalisation due to COVID-19-related complications were classified as non-survivors. Blood samples were collected from patients while they were still alive during their hospitalisation. Demographic data, clinical presentation and laboratory parameters of the patient were

obtained from the hospital's electronic medical system. All samples were collected in three-layer packaging and placed inside a container with absorbent material to prevent moisture. Subsequently, they were placed inside a primary watertight polystyrene box, which was filled with ice to maintain optimal low-temperature conditions and then sealed securely to ensure integrity. The transportation, storage, and processing of samples were conducted by established standard operating procedures aligned with Laboratory Biosafety guidelines for COVID-19, adhering to the protocols outlined by the World Health Organization (WHO).²² Samples deemed insufficient or hemolysed were excluded from the study to ensure the integrity of the results.

During the same period, 94 healthy controls were recruited from volunteers aged between 18 and 80 years. Participants received detailed information about the study, and informed consent was obtained from those who agreed to participate. The exclusion criteria for the healthy controls included any history of acute or chronic infections within the past month, as well as autoimmune diseases, neoplastic or endocrine disorders, immunocompromised or immunosuppressed conditions, the use of long-term oral steroids or cytotoxic drugs, chronic obstructive lung disease, history of transient transaminitis and liver disease.²³ Additionally, 2 ml of peripheral blood samples were collected in plain tubes from healthy controls to analyse A1AT phenotype and serum A1AT levels. All samples were stored at -80°C and were subsequently analysed in batches after completed sample collection, using the same assay methods to ensure consistency and minimise inter-assay variability.

Sample analysis

A1AT Phenotype

A1AT phenotyping was conducted through isoelectric focusing (IEF) on agarose gel performed on semiautomated Hydrasys 2 system SEBIA (Hydrasys 2 system, Sebia, Lisses, France). The analysis was carried out as per the instructions in the manual. Samples underwent the isoelectric focusing migration, immunofixation with anti-A1AT peroxidase-labeled antiserum, and subsequent staining to obtain A1AT profiles. The phenotypes of serum A1AT samples were determined by visual inspection comparing their migration patterns with control samples, allowing the identification of the most common A1AT alleles: the common M alleles and the two variant S and Z alleles associated with severe A1AT deficiency. **Figure 1** depicts electrophoretic patterns of the main A1AT isoform migration by IEF using a quality control sample.

A1AT concentration

According to the manufacturer's instructions, serum A1AT was measured using an immunoturbidimetric assay on an Abbott Architect c8000 biochemistry analyser (Abbott Laboratories, Abbott Park, Illinois, United States of America) in the Chemical Pathology Laboratory, Hospital Pakar Universiti Sains Malaysia. The standard reference range for this test falls between 0.9 to 2.0 g/L.

Sample size calculation

The sample size was determined using the formula for estimating a single proportion: $n = (Z_{\alpha/2})^2 P(1-P)$ for genetic mutation of AATD via DNA sequencing. Referring to a study by Maltais F et al where the prevalence is 31.8%,²⁴ then P is equal to 0.3 of patients were identified as AATD through exon sequencing, the re-

quired sample size for estimating this proportion with a 95% confidence level ($Z_{\alpha/2}=1.96$) and a 10% margin of error (Δ) was calculated as 84 patients. Considering a 10% anticipated dropout due to pre-analytical error, the adjusted sample size became 94 patients. Therefore, the study included 282 participants, distributed into three groups, each comprising 94 subjects representing mild to moderate cases, severe to critical cases, and healthy controls.

Statistical analysis

The data were analysed using SPSS version 27 (IBM Corporation, Armonk, New York, USA). All categorical variables were presented as frequencies (n) and percentages (%) and were compared using the Chi-square test. Descriptive statistics were used to tabulate the characteristics of A1AT phenotype among the mild to moderate cases, severe to critical cases, and healthy controls. The comparison of A1AT levels among these groups was conducted using the Kruskal-Wallis test. We compared the laboratory parameters of the groups using an independent T-test or a Mann-Whitney U test, depending on whether the distribution was normal or not. Numerical data with a normal distribution were presented as mean and standard deviation (SD), while non-parametric numerical variables were expressed as the median and interquartile range (IQR). Differences between groups were considered significant at a P value of $<.05$.

RESULTS

The analysis encompassed 282 subjects, with 94 individuals in each of the mild to moderate and severe to critical COVID-19 cohorts, alongside 94 individuals in the healthy control group. **Table 1** provides an overview of the sociodemographic and clinical characteristics of the study participants. The participants from the COVID-19 groups and control group differ significantly in age ($P<.001$). Multiple pairwise comparisons with Bonferroni correction revealed a significant difference of age between control vs. mild to moderate COVID-19 ($P=.001$), control vs. severe to critical COVID-19 ($P<.001$) and mild to moderate vs. severe to critical COVID-19 ($P<.001$). Moreover, there was a significant difference in gender between control and COVID-19 groups ($P=.005$). Multiple pairwise comparisons with Bonferroni correction showed that gender significantly differs between mild to moderate vs. severe to critical COVID-19 ($P<.006$). However, there is no significant difference between control vs. mild to moderate COVID-19 ($P=.053$) and control vs. severe to critical COVID-19 ($P=1.000$). The severe to critical

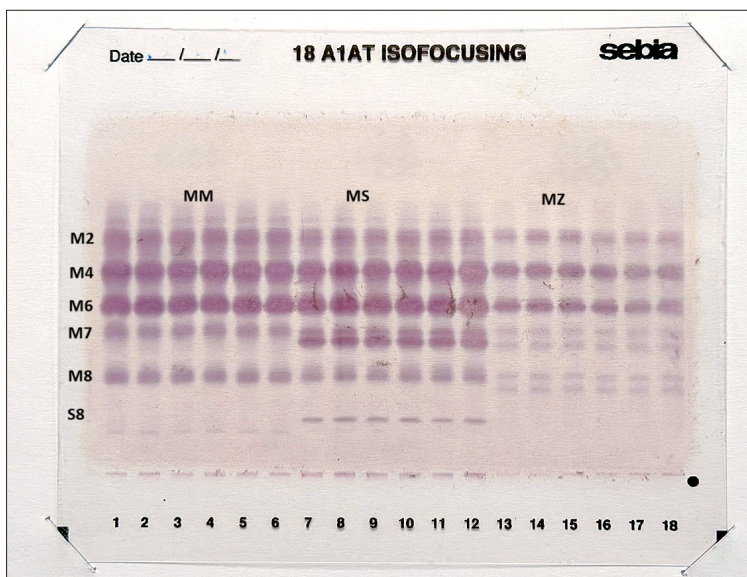


Figure 1. Examples of A1AT phenotype patterns of QC samples, obtained by IEF. Electrophoretic A1AT protein variants are denoted by letters according to their migration patterns. The MM variant typically appears as a well-defined band that migrates at a moderate rate, often positioned centrally of the gel as seen in lanes 1 to 6. The resolution consists of five separate bands, labelled M2, M4, M6, M7, and M8. M2 is the band located furthest towards the positive electrode (anodal), whereas M8 is the band located furthest towards the negative electrode (cathodal). The MM variant's migration pattern serves as a reference point for comparing other A1AT variants. The S variant is a deficiency variant that produces moderately low levels of A1AT. The major bands associated with the S variant, such as S4 and S6, migrate towards the cathodal end of the gel and align with the migration locations of M6 and M7. The S8 minor band is visible at the cathodal region of the gel as seen in heterozygote MS in lanes 7 to 12. The Z variant exhibits significantly reduced activity, resulting in minimal to absent AAT production, which may obscure the visualization of minor bands. The major bands are visible, although occasionally faint, and migrate just cathodally to the M bands as seen in heterozygous MZ at lanes 13 to 18. QC: Quality control. A1AT: alpha-1-antitrypsin. IEF: isoelectric focusing.

Table 1. Characteristics of COVID-19 patient and healthy controls (n=282).

	Mild to moderate (n=94)	Severe to critical (n=94)	Healthy control (n=94)	P value ^a
Age in years, Median (25th ,75th percentile)*	44 (29-59)	59 (50-68)	36 (31-40)	<.001 ^c
Gender				
Male	26 (27.7)	47 (50)	42 (45)	.005 ^a
Female	68 (72.3)	47 (50)	52 (55)	
Smoking status				
Smoker	0 (0)	2 (2.1)	0 (0)	.497 ^b
Non-smoker	94 (100)	92 (97.9)	94 (100)	
				P value ^f
Days of admission Median (25th ,75th percentile)*	9 (6-12)	14 (9-19)	N/A	<.001 ^d
Pulmonary disease				
COPD	7 (7.4)	3 (3.2)	N/A	.194 ^a
Pneumonia	0 (0)	1 (1.1)	N/A	1.000 ^b
ARDS	6 (6.4)	1 (1.1)	N/A	.118 ^b
Lung malignancy	3 (3.2)	1 (1.1)	N/A	.120 ^a
Comorbidities				
Diabetes mellitus	26 (27.7)	53 (56.4)	N/A	<.001 ^a
Hypertension	19 (20.2)	27 (28.7)	N/A	.175 ^a
Chronic kidney disease	4 (4.3)	6 (6.4)	N/A	.516 ^a
Cerebellar vascular accident	0 (0)	1(1.1)	N/A	1.000 ^b
Liver disease	2 (2.1)	1 (1.1)	N/A	1.000
Heart disease	10 (10.7)	7 (7.4)	N/A	.446 ^a
Malignancy	4 (4.3)	2 (2.1)	N/A	.682 ^b
Mortality				
Survivors	89 (94.7)	75 (79.8)	N/A	.002 ^a
Non survivors	5 (5.3)	19 (20.2)		

The data has been represented as n (%) and *denotes median (25th, 75th percentile). N/A; not available.

Test statistics: ^aChi-square, ^bFisher's exact, ^cKruskal Wallis, and ^dMann-Whitney

^eComparison between mild to moderate, severe to critical COVID-19 patient, and healthy control groups; significant level at P value <.05.

^fComparison between mild to moderate, severe to critical groups COVID-19 patients; significant level at P value <.05.

group had higher rates of diabetes mellitus than mild to moderate COVID-19 [$n=53$ patients (56.4%) vs. $n=26$ patients (27.7%), $P<.001$]. At the same time, there was no significant difference in other associated comorbidities such as hypertension, chronic kidney disease (CKD), ischaemic heart disease, liver disease, and pre-existing lung disease between severe to critical and mild to moderate COVID-19 group. The mortality rate was significantly higher in the severe to critical group than mild to moderate group [$n=19$ patients (20.2%) vs $n=5$ patients (5.3%), $P=.002$].

A comparison of the A1AT phenotype between patients and the control group can be seen in **Table**

2. Other phenotypes, such as MS, MZ, S, and Z, were undetected in both COVID-19 and healthy control groups. However, inconclusive results were obtained from 12 COVID-19 patients—two with mild to moderate disease and ten with severe to critical illness.

Figures 2 to 5 showed rare A1AT phenotypes: PiBM, PiXM/PiYM, PiCM, and PiEM²⁵ as determined by IEF assay in serum samples. The rare X and Y variants demonstrated comparable migration patterns. Distinguishing between variants prove to be challenging without the application of gene sequencing techniques.²⁶

The median A1AT concentration was higher in the

Table 2. Characteristic of A1AT phenotype and concentration among mild to moderate, severe to critically ill COVID-19 patient and control.

A1AT phenotype	Mild to moderate COVID-19 (n=94)	A1AT Level (g/L) Mean (SD)	Severe to critical COVID-19 (n=94)	A1AT Level (g/L) Mean (SD)	Control (n=94)	A1AT Level (g/L) Mean (SD)
PiMM	88 (93.6)	2.2 (0.9)	61 (64.9)	2.4 (1.1)	88 (93.6)	2.0 (0.7)
PiBM	1 (1.1)	3.5 (0)	16 (17.0)	2.6 (1.2)	N/A	N/A
PiCM	N/A	N/A	4 (4.3)	1.9 (0.3)	N/A	N/A
PiEM	N/A	N/A	1 (1.1)	2.9 (0)	N/A	N/A
PiXM/PiYM	3 (3.2)	3.4 (1.7)	2 (2.1)	1.8 (0.5)	6 (6.4)	1.6 (0.4)
Unknown/inconclusive	2 (2.1)	1.4 (0.2)	10 (10.6)	2.6 (0.8)	N/A	N/A

The data has been represented as n (%) and mean (SD). N/A; not available.

Table 3. The comparison median of A1AT level between control, mild to moderate and severe to critical COVID-19.

Variable	Healthy control (n=94)	Mild to moderate (n=94)	Severe to critical (n=94)	Z-Statistics	P value ^a
Median (25th, 75th percentile)					
A1AT (g/L)	1.8 (1.3-2.3)	1.9 (1.2-2.6)	2.1 (1.4-2.8)	9.117 (2)	<.01

^aKruskal Wallis test was done; significant at $P<.05$.

Table 4. Characteristics of A1AT phenotype and concentration among survivors and non-survivor of COVID-19 patient.

A1AT phenotype	Survivor (n=164)	A1AT level (g/L) Mean (SD)	Non-survivor (n=24)	A1AT level (g/L) Mean (SD)
PiMM	136	2.2 (0.9)	13	3.2 (1.5)
PiBM	13	2.4 (0.3)	4	3.5 (2.1)
PiCM	3	1.9 (0.4)	1	1.9 (0)
PiEM	N/A	N/A	1	2.9 (0)
PiXM/PiYM	5	2.8 (1.5)	N/A	N/A
Inconclusive	7	2.4 (1.0)	5	2.4 (1.0)

The data has been represented as mean (SD). N/A; not available.

severe COVID-19 group. As shown in **Table 3**, there was a significant difference in A1AT concentration between the three groups ($P < .05$). To assess differences in A1AT levels between groups, Dunn's pairwise tests were performed for all three group comparisons. A significant difference was observed between the control group and the severe to critical COVID-19 group (Bonferroni-adjusted $P = .008$). There was no difference in A1AT levels between other pairs.

The median A1AT levels, irrespective of phenotype, were significantly higher in the non-survivor group than the survivor group [2.9 (2.0-3.8) for non-survivors and 1.9 (1.3-2.6) for survivors, ($P = .003$)]. **Table 4** compares

the A1AT phenotypes between the survivor and non-survivor groups.

DISCUSSION

Studies have shown that having mutations or variants in the *SERPINA1* gene, known to impact the activity or expression of the A1AT protein, and reduced A1AT levels were associated with an increased likelihood of experiencing severe cases of COVID-19.²⁷ Based on our findings, the most prevalent phenotype among the study subjects was the PiMM phenotype regardless of the stages of COVID-19 patients or healthy control. Most of our study subjects had a normal A1AT level

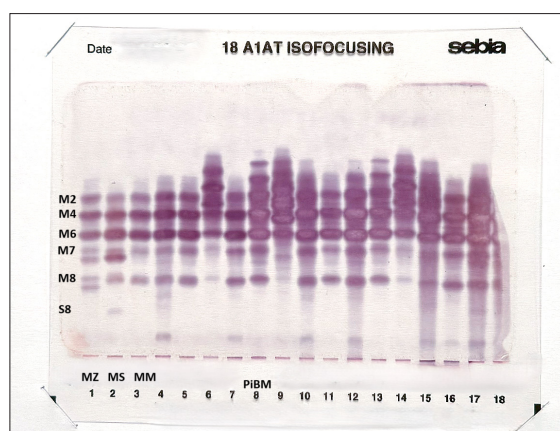


Figure 2. Examples of A1AT phenotype patterns of PiBM, obtained by IEF. The variant with the highest anodal migration is PiB (lane 8). The B2 minor band moves towards the anodal point of the gel. The B4 and B6 major bands migrate towards the anode about the M2 minor band. A1AT: alpha-1-antitrypsin. IEF: isoelectric focusing.

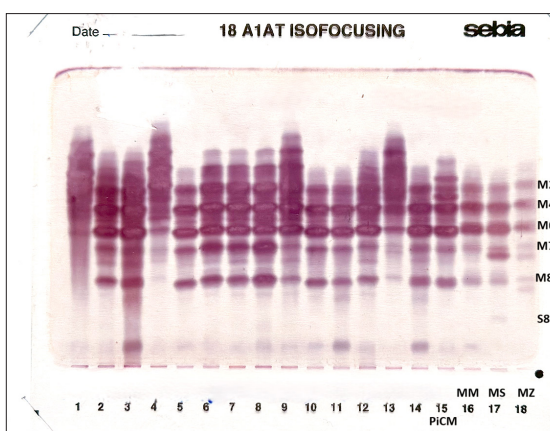


Figure 4. Examples of A1AT phenotype patterns of PiCM, obtained by IEF. The PiC variation can be distinguished by the presence of the C4 and C6 major bands located on either side of the M2 band (lane 15). The PiC phenotype exhibits a small C2 band that typically displays a strong dark staining intensity located anodally to the C4 band.

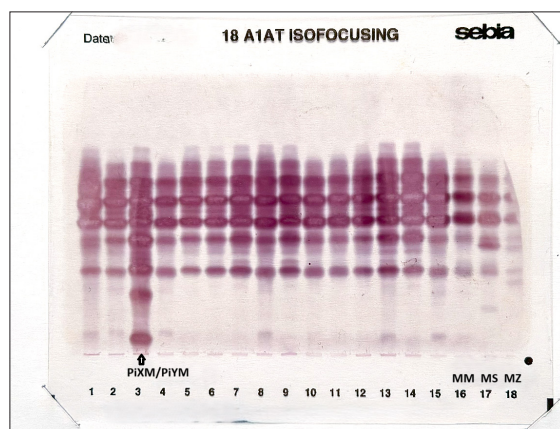


Figure 3. Examples of A1AT phenotype patterns of PiXM or PiYM, obtained by IEF. The migration pattern of the rare X or Y variation in (lane 3), showed similar migration pattern which revealed that X8 or Y8 appeared cathodally to S8 as slow-moving bands on the gel.

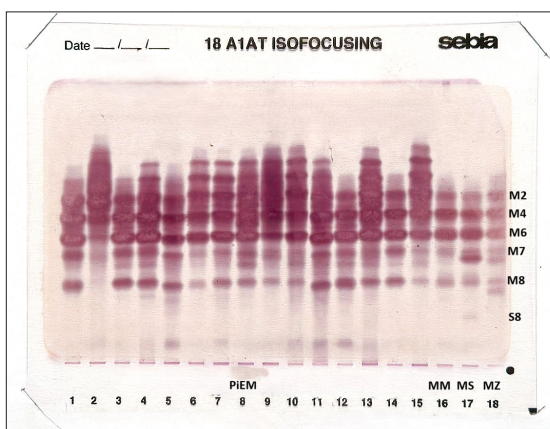


Figure 5. Examples of A1AT phenotype patterns of PiEM, obtained by IEF. PiEM, the significant bands E4 and E6 migrate slightly cathodal to M2 and M4, respectively (lane 8).

of more than 0.9 g/L. Indeed, PiMM subtypes are regarded as the most common normal A1AT variants in the Malaysian population. These variants are associated with normal A1AT levels and are generally considered non-deficient. Moreover, the less frequently encountered variants linked to A1AT deficiency (PiS and PiZ) were undetected in our study subjects. Thus, this evidence suggests that the A1ATD in Malaysia may not be as frequent as observed in Western countries. A review of the global prevalence of A1ATD also revealed that the PIZZ genotype was rarely found in Malaysia.²⁸ A separate study involving the analysis of 950 samples determined that only 10 samples exhibited the Z homozygous phenotype.²⁹ Moreover, our study also found no evidence linking A1ATD to an increased risk of COVID-19 infection or severe illness development, consistent with other studies.^{30,31} In contrast, previous studies in Spain have detected the frequency of PiS was 176/1000 and PiZ 25/1000 in COVID-19 patients, with the frequency of the PiZ being more frequently seen in the severe compared to non-severe COVID-19 group.¹² Another observational study in Italy found a higher frequency of COVID-19 with severe A1ATD (PiZZ).³² Similarly, a study in Portugal identified that The PiZZ genotype showed a significant association with a higher incidence of COVID-19 (33.3%, $P=.012$), followed by PiMS (14.3%) and PiSZ (10.0%).¹⁶ Their findings align with the estimated prevalence of A1ATD in their respective countries, as Spain, Italy, the United Kingdom, and France demonstrate the highest prevalence of PIZZ, PISZ, and PIMZ genotypes.³³ The mortality rate of these countries from severe COVID-19 cases was also the highest among other countries.³⁴ While these findings are significant, their interpretation is complicated by numerous confounding factors, including comorbidities and ethnic variations, thus needing further research. Interestingly, our study discovered some rare normal phenotypes, such as PiBM, PiCM, PiEM and PiXM/PiYM. These phenotypes were classified as non-deficient phenotypes exhibiting a normal level of A1AT levels.²⁵ The scarcity of data on the prevalence of A1AT phenotypes in Malaysia hinders the comparison of these uncommon variants within the Malaysian population, particularly their impact on the prevalence and severity of COVID-19. Further research and collaborations with local research institutions and healthcare professionals are required to collect and analyse data on A1AT phenotypes in Malaysia, allowing for a more comprehensive understanding of the population's genetic diversity and health implications.

We observed higher levels of A1AT among patients with COVID-19 than healthy controls. This contra-

dicts other study findings, which observed that individuals with lower A1AT levels are more susceptible to COVID-19.^{12,16,35} A possible explanation is that A1AT is an acute-phase plasma protein that increases 3-5 folds during infection or inflammation.³⁶ As a result, the observed high A1AT levels may be attributed to inflammation, increasing from a normal baseline. Nevertheless, A1AT phenotypes M and Z had distinct responses during the acute period. Specifically, the M phenotype exhibited a robust and pronounced reaction, with a significant elevation in A1AT levels and a quick response to inflammatory stimuli. In contrast, the Z phenotype manifested a markedly diminished reaction, with a slower response and lower levels of AAT production. This indicates a potential impairment in its ability to respond effectively to acute inflammation.¹⁸ Most of our study subjects were M phenotypes, which contribute to a higher level of A1AT in COVID-19 infection than healthy individuals. Several other studies found similar results. McElvaney et al found high A1AT levels in both groups in a cohort study of 40 patients with COVID-19, 20 stable and 20 requiring intensive care support.³⁷ The study also discovered a direct correlation between the rise in A1AT and the rise in IL-6, which supports the anti-inflammatory function. In addition, elevated A1AT was found to be a marker for severe disease in clinical trials that linked A1AT levels to the severity of COVID-19 illness.³⁸ Another study in Turkiye also found significantly higher levels of A1AT in the COVID-19 group as compared to the control group.³⁹

Ideally, the combination of A1AT quantification and phenotyping effectively identifies approximately 95% of A1AT abnormalities, primarily focusing on ZZ, SZ, and SS phenotypes.⁴⁰ In case the A1AT level is unexpectedly low, or the IEF pattern is atypical with clinical history, further investigation involves using whole *SERPINA1* gene sequencing to confirm the A1AT variant. Our study encountered 12 inconclusive results regarding the A1AT phenotype, needing further DNA analysis to identify the specific pathogenic variants responsible. Repeat phenotyping for these samples was impossible due to the limited quantity of available serum and the compromised quality of the samples. This highlights the significance of whole genome sequencing, which should not be limited to the identification of specific Z and S variants. Instead, it should involve the simultaneous testing of a broad range of genetic variants across numerous genes.

Our study has limitations. Firstly, the findings may not be generalizable to the broader population since the study was conducted at two-centers. Additionally,

the IEF phenotyping technique employed in the study may not have identified specific A1ATD variants, requiring further research incorporating A1AT genotyping and whole *SERPINA1* genome sequencing for a more comprehensive evaluation of A1ATD. Furthermore, our inability to follow up with A1AT quantification and baseline measurements limits our ability to consider disease progression variations.

In conclusion, our study identifies the PiMM phenotype as the predominant phenotype expression within the studied population. It demonstrates the absence of

A1ATD across varying degrees of COVID-19 severity, which might indicate its negligible role in the disease's genetic predisposition framework or its rarity within the population studied. This research thus provides a foundational step towards understanding the A1AT genetic predisposition of COVID-19 severity, paving the way for personalized medicine approaches in managing and treating this disease. Further studies are necessary to expand on these findings, potentially leading to the development of targeted therapies and preventive strategies based on genetic profiling.

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