Research Article

Alpha 1-antitrypsin as a potent biomarker for monitoring of disease severity in patients with Covid-19 and its correlation with Liver Enzymes and Lactate Dehydrogenase

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ABSTRACT

Objectives: Alpha 1-antitrypsin (A1AT) is a single-chain glycoprotein containing 394 amino acids. It is primarily synthesized in the liver as an acute phase protein. According to recent studies, the COVID-19 virus can infect host cells by binding to the ACE2 receptor via a membrane protein. On the other hand, A1AT has the potential to inhibit neutrophil elastase and prevent the entry of the virus into host cells. Consequently, A1AT can reduce the severity and duration of COVID-19 disease

Methods: Thirty-one hospitalized COVID-19 patients with a positive PCR test and thirty healthy volunteers with a negative test as the control group were selected. Upon hospitalization, demographic and biochemical tests were conducted for both patients and controls. Serum A1AT levels in both groups were measured using nephelometry as the reference method. Liver enzymes and total protein were also determined using commercially available kits.

Results: Serum A1AT levels in the patients were increased compared to the control group, and this increase was inversely proportional to the duration of hospitalization and the relative improvement for discharge. Additionally, this elevation was correlated with qualitative C-reactive protein (CRP) levels. Serum liver enzymes, ALP, and LDH in patients were significantly higher than in the controls ($P<0.05$), while serum total protein in patients was significantly lower than in the controls ($P<0.05$).

Conclusion: These data belong to a homologous group and show a correlation between serum A1AT levels and the duration of hospitalization, as well as with qualitative CRP levels. Furthermore, the increase in A1AT is proportional to the levels of serum AST, ALT, total protein, ALP, and LDH, which may serve as an alarm for potential liver involvement in such a disease. Thus, monitoring the liver condition is warranted.

Keywords: Alpha 1-antitrypsin, Covid-19, Serum proteins, AST, ALT, Total protein, ALP

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Introduction

In December 2019, an acute respiratory syndrome coronavirus emerged in Wuhan, Hubei province, China, and rapidly became a global pandemic within a few months (1). Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a single-stranded RNA virus with a length of approximately 29.9 kb, making it larger than any other RNA virus (2). This virus possesses four structural proteins: nucleocapsid (N) protein, spike (S) protein, envelope (E) protein, and membrane (M) protein. Based on its molecular characteristics, SARS-CoV-2 is classified as a new beta-coronavirus belonging to the Sarbecovirus subfamily (3).

The S protein mediates the entry of the coronavirus into host cells. This homotrimeric glycoprotein protrudes from the viral surface, and its presence is essential for viral penetration into host cells. The S protein is composed of two functional subunits, S1 and S2. The S1 subunit is responsible for binding to the host cell receptor, while the S2 subunit facilitates the fusion of the viral membrane with the host cell membrane (2). The S1 subunit binds to the angiotensin-converting enzyme 2 (ACE2) receptor on the host cell surface through its receptor-binding domain (RBD). Following this binding, a host cell protease called TMPRSS2 cleaves the S protein at a specific site, severing the connection between S1 and S2. This cleavage allows the virus to enter the host cell (4).

Alpha-1 antitrypsin (A1AT) is a single-chain glycoprotein consisting of 394 amino acids with a total molecular weight of 52 kDa (6-9). A1AT is also an acute-phase protein, constituting over 90% of the alpha-1-globulin band in serum proteins (10). Recent studies have shown that A1AT inhibits SARS-CoV-2 infection by targeting two crucial proteases involved in the pathophysiology of COVID-19: transmembrane serine protease 2 (TMPRSS2) and disintegrin and metalloproteinase 17 (ADAM17). Additionally, A1AT functions as an anti-inflammatory protein, inhibiting molecules such as interleukin-8 (IL-8), tumor necrosis factor-alpha (TNF-α), and neutrophil elastase. Other potential protective mechanisms of A1AT include the inhibition of thrombin, delayed thrombus formation, and decreased oxidative stress, inflammation, and cell wall deterioration (11).

The liver, a vital organ, is also affected by COVID-19. The presence of ACE2 receptors in liver cells, cytokine storms, side effects of medications, underlying liver diseases, hypoxia caused by COVID-19, and blood clot formation can all contribute to liver function damage (12, 13).

This study aimed to evaluate the serum levels of A1AT, a protein with protective, anti-inflammatory, and anti-protease activities, in patients with COVID-19 and its potential impact on early recovery and hospital discharge. To gain a better understanding of liver disease progression in COVID-infected individuals, liver enzymes, total protein, alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were also measured in both patient and control groups.

Materials and Methods

Patients

This study enrolled thirty-one patients hospitalized with COVID-19 at Alghadir Hospital, Abhar, Zanjan, Iran, between October and November 2021. All patients had a positive test for COVID-19 via PCR on the first day of hospitalization. Inclusion criteria were a positive PCR test, no history of smoking, and non-pregnancy. Patients with any comorbid diseases were excluded. Additionally, thirty healthy volunteers participated as a control group.

Table 1 summarizes demographic information (gender, age), some biochemical markers, and hematological parameters for both patients and controls. All patients received respiratory oxygen therapy, adjusted based on their individual respiratory needs. Thankfully, none of the patients in this study succumbed to the disease.

Ethical Approval and Funding Disclosure

This study was supported by Tarbiat Modares University. Ethical approval was obtained from the National Ethics Committee of the Ministry of Health, Islamic Republic of Iran (reference number: IR.MODARES.REC.1401.126).

Blood Sampling

Four milliliters of whole blood were collected from each patient and control subject into tubes without anticoagulant. The samples were centrifuged at 4000 rpm for 10 minutes. Serum was then separated, aliquoted into microtubes, and coded to ensure confidentiality. The coded serum samples were stored at -20°C until analysis.

A1AT Analysis

Serum alpha-1-antitrypsin levels were measured using a nephelometric reference method. Nephelometry is a rapid and accurate technique that measures A1AT concentration based on antigen-antibody (Ag-Ab) interaction. In this study, a Cobas c311 auto analyzer and an alpha-1-antitrypsin kit from Roche Company were used. The nephelometer measured the amount of light scattered at a 90° angle by antigen-antibody complexes formed between A1AT and its specific antibody

C - reactive protein (CRP) Measurement

C-reactive protein (CRP) levels in patients and controls were measured using a commercially available kit (Enison Company). In this kit, a specific IgG type antibody is

Table 1: Clinical and Biochemical parameters in the study groups

Parameters	Control (N=31)	Patients (N=31)	P value
Gender (Male/Female)	13/17	13/18	NS
Age (Years)	37±9	56±21	0.01
FBS (mg/dL)	97±8	98±8	NS
WBC(*10^3)	6.1±0.9	6.8±3.4	NS
RBC(*10^6)	4.65±0.37	4.60±0.6	NS
Hb(g/dl)	14.7±0.9	13.3±2.0	NS
Plt(*10^3)	224±42	194±80	NS
Neutrophil (%)	63.3±4.2	76.5±13.1	0.02
Lymphocyte (%)	29.4±4.1	17.7±10.7	0.02
INR	1.0±0.1	1.1±0.1	NS
PTT	30±4	31±4	NS
D-Dimer (Negative/ Positive)	0/30	5/26	0.001

NS: Non significant

attached to latex particles. If there is at least 4 mg/liter of CRP in the serum, agglutination of latex particles is observed, and the results are reported qualitatively.

Liver Enzyme Activities

Activities of aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), and lactate dehydrogenase (LDH) were measured in serum using commercially available kits from Bionic Company to assess liver function. Briefly, for AST, ALT, and LDH, the decrease in NADH+H concentration was monitored at 340 nm. For ALP, the increase in p-nitrophenol concentration was measured at 405 nm

Total Protein Estimation

Serum total protein, another marker of liver function, was measured using the Biuret method with a commercially available kit. The color intensity developed at 546 nm is directly proportional to the amount of protein present in the sample.

Statistical Analysis

Statistical analyses were performed using SPSS software version 24. The Kolmogorov-Smirnov test was used to assess the normality of data distribution. Subsequently, the t-test was applied for normally distributed variables. Based on the Kolmogorov-Smirnov test results, all data were found to be normally distributed.

Results

Serum A1AT Levels

Serum A1AT levels were significantly elevated in patients with COVID-19 compared to healthy controls ($P < 0.0001$) (Figure 1A). In 38.7% of COVID-19 patients, A1AT levels exceeded the upper limit of normal (>200 mg/dL). Interestingly, no significant difference in A1AT levels was observed between men and women with COVID-19 ($P > 0.05$) (Figure 1B).

Correlation between A1AT Levels and Hospitalization Duration

Analysis of hospital records revealed a positive correlation between serum A1AT levels and hospitalization duration in COVID-19 patients. This suggests that patients with higher A1AT levels may have experienced faster recovery and earlier hospital discharge (Figure 2).

Relationship between A1AT Levels and C - reactive protein (CRP)

Our data also demonstrate a trend of increasing A1AT levels with increasing CRP levels. The mean A1AT levels for patients with CRP scores of 1+, 2+, and 3+ were 172.75 mg/dL (29.6), 189.75 mg/dL (44.8), and 221.3 mg/dL (57.8), respectively (Table 3).

Liver Function Tests

Liver function tests revealed significant increases in serum levels of AST and ALT ($P < 0.05$ and $P < 0.0001$, respectively) in COVID-19 patients compared to controls (Figures 3A and 3B). Notably, 32.3% and 29.1% of patients exhibited ALT and AST levels exceeding the upper limit of normal, although total protein significantly decreased compared to the controls ($P < 0.05$) (Figure 3C). Additionally, serum levels of ALP and LDH were also significantly elevated in COVID-19 patients compared to the control group ($P < 0.05$) (Figures 4A and 4B).

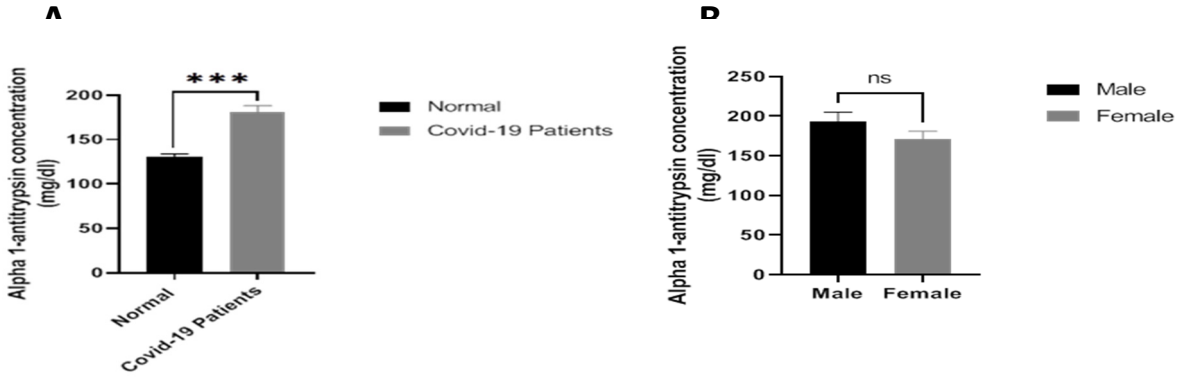

Figure 1: (A) Comparison of serum alpha 1-antitrypsin level between patients with Covid-19 and healthy people (P<0.05). (B) Comparison of serum alpha 1-antitrypsin level between men and women with Covid-19 (P>0.05).

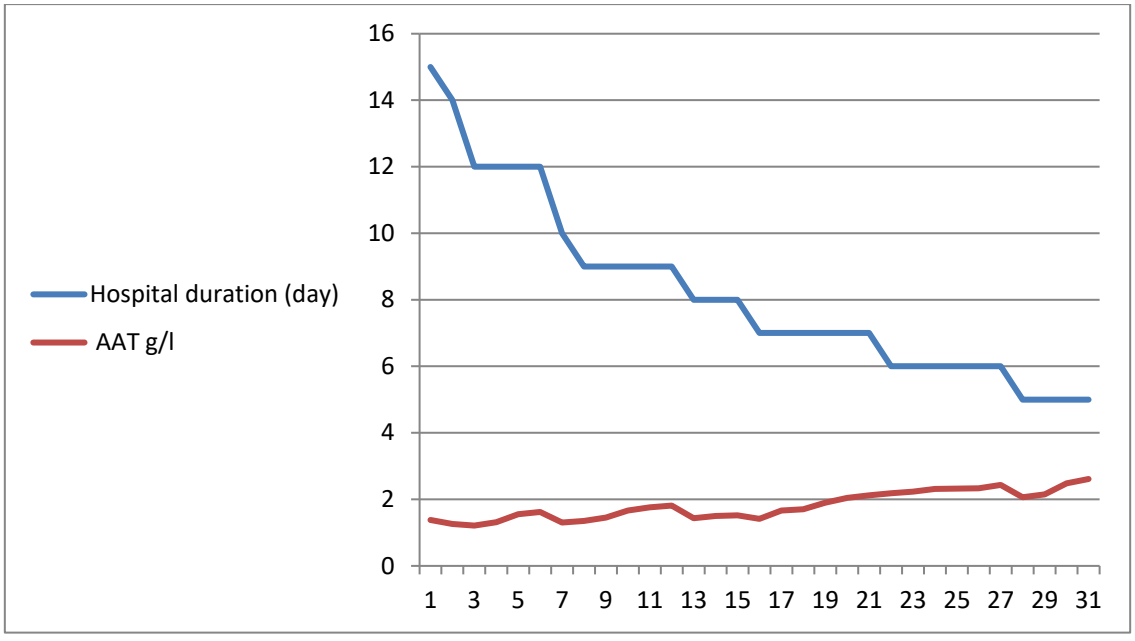

Figure 2: Association between hospitalization duration and serum level of A1AT.

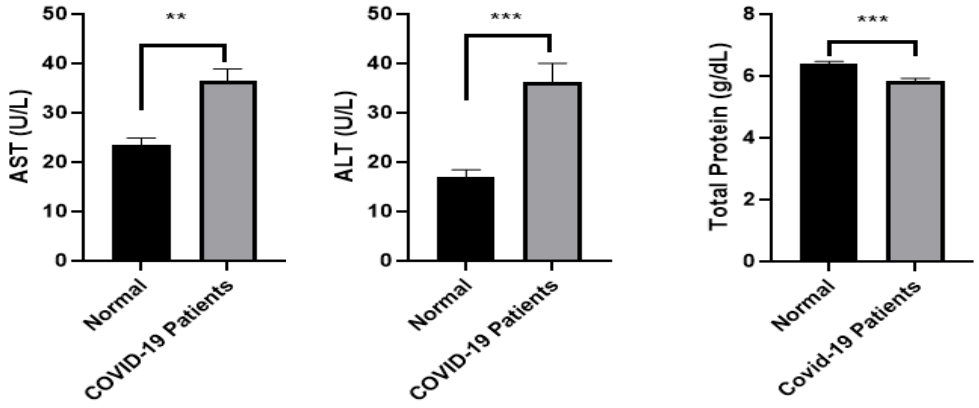

Figure 3: Comparison of AST, ALT and total Protein levels in normal and COVID-19 patients.

Table 2: Results of AAT and CRP in control group and patients

Parameters	Control (N=30)	Patients (N=30)	P value
AAT (mg/dL)	131±16	181±42	0.001
CRP (Normal)	30	4	0.000
CRP (1+)	0	12	
CRP (2+)	0	12	
CRP (3+)	0	3	

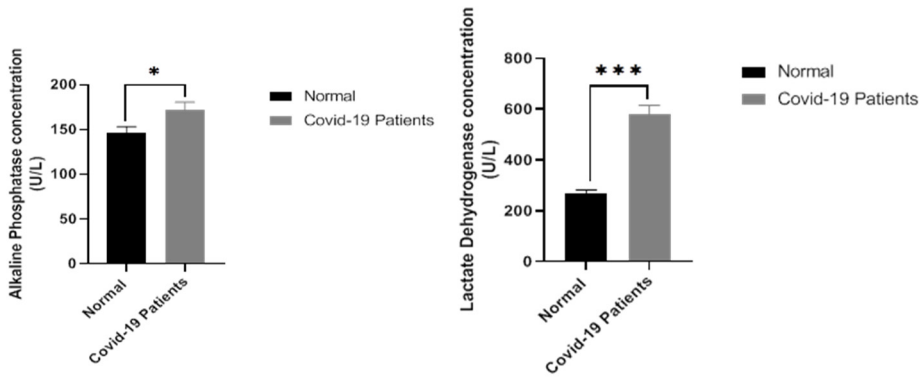

Figure 4: Comparison of ALP and LDH levels in normal and COVID-19 patients.

Discussion

A1AT is a major human anti-protease, and recent studies suggest that it may also possess anti-inflammatory and immune-regulatory properties (14, 15). During acute inflammation or infection, serum A1AT levels can increase four to five-fold above the normal range (16). Elevated A1AT levels can also occur during pregnancy, tissue damage, after surgery, and in older adults, remaining elevated for 7 to 15 days (16-19). A1AT's immunomodulatory properties include inhibiting pro-inflammatory cytokines and enhancing anti-inflammatory mediators (16). In vitro and in vivo studies suggest a potential antiviral role for A1AT (20-22). Additionally, A1AT may play a protective role against various viral infections, including rhinovirus, HIV, and hepatitis B and C (11). Pérez et al. observed elevated A1AT levels in 45 COVID-19 patients, suggesting A1AT as a potential marker for reduced disease severity and patient deterioration (14). The IL-6/A1AT ratio may be a valuable indicator of the balance between pro-inflammatory and anti-inflammatory factors in COVID-19 patients. Higher ratios are observed in patients requiring intensive care compared to those with milder illness, potentially signifying a worse prognosis (23). Notably, recent studies have reported a correlation between the COVID-19 pandemic and the prevalence of alpha-1 antitrypsin deficiency (AATD) in specific geographic regions (11). Vianello et al. observed that approximately 38% of COVID-19 deaths occurred in regions with a high prevalence (47%) of individuals with AATD, suggesting a possible link between AATD and COVID-19 morbidity and mortality rates (24).

This study investigated A1AT as a potential inhibitor of coronavirus entry into host cells. We observed a significantly higher average serum A1AT level in patients with COVID-19 compared to the control group. Interestingly, there was no significant difference in A1AT levels between male and female patients. Additionally, a positive correlation was found between A1AT levels and CRP levels.

It is important to note that all participants in this study were from Abhar, Zanjan, Iran, a homogenous population with similar lifestyles, nutritional habits, and environmental conditions. This homogeneity strengthens the reliability of the observed correlation between serum A1AT levels, hospitalization duration, and qualitative CRP levels.

This study also revealed elevated serum levels of AST, ALT, ALP, and LDH in COVID-19 patients compared to the healthy control group. These observations are consistent with previous reports by Lei et al., who documented increased serum ALT and AST levels alongside decreased albumin levels in COVID-19 patients (25). Notably, AST levels were found to have the strongest correlation with mortality compared to other liver function markers (25). Przekop et al. suggested that abnormalities in liver function tests, particularly the AST/ALT ratio, could serve as a valuable marker for assessing mortality risk in COVID-19 patients (26). Dinvari et al. (2022) conducted a study on 1017 COVID-19 patients and found that those with high AST

and ALT levels during admission and hospitalization were more susceptible to complications. The observed association between elevated liver enzymes and poor outcomes in these patients may be partially attributed to increased inflammatory factors (27). LDH is recognized as a powerful predictor for early diagnosis of lung injury and severe COVID-19 cases (28). A study by Henry et al. evaluated the relationship between high initial LDH levels and patient outcomes in COVID-19. They found that elevated LDH levels were associated with a 6-fold increase in the likelihood of severe disease and a 16-fold increase in the risk of death (29).

Conclusion

This study identified a correlation between serum A1AT levels and hospitalization duration, as well as qualitative CRP levels, in COVID-19 patients. Furthermore, the observed increase in A1AT levels alongside elevated serum AST, ALT, ALP, and LDH and decrease in total protein suggests potential liver involvement in COVID-19. These findings warrant further investigation and close monitoring of liver function in COVID-19 patients.

Conflict of Interests

The authors have nothing to declare.

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