Can Alpha 1 Antitrypsin (AAT) be Considered in the Treatment of Covid-19 (SARS-CoV-2)?

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Running head: Alpha 1 Antitrypsin (AAT) and Covid-19 (SARS-CoV-2) treatment

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Summary

Background

Alpha 1 Antitrypsin (AAT) plays a crucial role in protecting the lungs from the damage caused by excessive inflammation.It is known that intense inflammation manifests in COVID-19 (SARS-CoV-2) pneumonia. The aim of this study was to measure AAT levels in COVID-19 pneumonia patients and to predict AAT treatment in these patients.

Methods

This cross-sectional, clinical study included 154(61 females, 93 males) COVID-19 pneumonia patients. The age, sex, biochemical parameters and survival information of the patients were recorded. The independent variables affecting the AAT level were examined by linear regression analysis, and the regression model was found to be statistically significant($F=3.051$; $p=0.001$).

Results

There was no correlation between AAT mean values and survival ($p=0.133$). While the AAT level decreased by 0.003 units as the CRP value increased by 1 unit ($p=0.003$), AAT level decreased by 0.027 units as the lymphocyte value increased by 1 unit ($p=0.001$). As the neutrophil value increased by 1 unit, the AAT level decreased by 0.019 units ($p=0.014$). There was no statistically significant effect of other independent variables ($p>0.050$).

Conclusion

The negative correlation between rising inflammatory indicators and AAT levels in pneumonia patients shows that defense mechanisms got weakened. In patients with a viral infection, AAT may have an antiviral effect due to its regulatory and anti-replication effects on both inflammation and the immune system. Although there is no significant relationship between AAT level and survival, the negative correlation between AAT and inflammation indicators may suggest that AAT treatment may be effective in these patients.

Keywords: **Alpha 1-antitrypsin, COVID-19, Alpha 1-antitrypsin-leukocyte elastase complex,**

Treatment

INTRODUCTION

Alpha 1 Antitrypsin (AAT) is a glycoprotein synthesized by liver cells. Its main task is to prevent the destructive effects of proteases such as proteinase 3, elastin, and cathepsin G released from activated neutrophils. It plays an important role in protecting our lungs from damage caused by excessive inflammation. AAT is also considered an acute phase reactant since there is an increase in serum AAT levels during inflammation, [1], especially in neutrophilic inflammation[2]. COVID-19 is a novel, viral-induced respiratory disease that progresses to acute respiratory distress syndrome (ARDS) triggered by a cytokine storm in 10-15% of patients. Intense inflammation is observed in COVID-19 pneumonia [3]. Moreover, the increase in the amount of free radicals released from leukocytes reduces AAT activity [4]. In patients with a viral infection, AAT may have an antiviral effect due to its regulatory and anti-replication effects on both inflammation and the immune system [5]. In vitro and in vivo clinical

studies show that AAT has immunomodulation, anti-inflammation, antiprotease, and anticoagulation effects and provides protection against cell death [6]. The aim of the study is to measure the AAT levels of patients with COVID-19 pneumonia and to create a prediction about AAT treatment.

Materials and Methods

Patients with a previous diagnosis of AAT deficiency, hypertension, heart failure, chronic renal failure, or chronic obstructive pulmonary disease, and smokers were excluded from the study. Patients with low alpha 1 antitrypsin levels and other disease were excluded from the study because these patients were likely to have severe covid 19. This could have affected our results. From the patients, 5 cc of blood was taken on the 48th hour of hospitalization. The serum liquids, the supernatant of which were separated by centrifugation at 2500 rpm for 15 minutes, were stored in a deep freezer at -80 C until the study day. AAT level, Abbott brand on the Architect C16000 analyzer from Abbott.It was measured by immunoturbidimetric method using Quntia A-1 Antitrypsin kit. Alpha -1 antitrypsin the expected range in adults is 90-200 mg/dl (0.9-2.0 g/L). Samples taken without waiting were studied or the samples were stored at -80 C and kept until the time to be studied Samples were thawed at room temperature before running. After 2 days at room temperature, the diameters of the circles formed on the plate were measured. Samples after thawing, mixing was done. The Quantia A1-AT reagent is a goat serum anti-human alpha 1 – antitrypsin which reacts specifically with the alpha 1 – antitrypsin of the sample to yield an insoluble aggregate which can be measured by turbidimetry. Results are expressed in mg/dL or g/L based on the International Reference Material CRM470.The age, gender, biochemical parameters (CRP, D dimer, ferritin, lymphocyte, and neutrophil levels), and survival information of the patients were recorded.

Statistical Method

The data were analyzed with IBM SPSS V23. Kolmogorov–Smirnov, and Shapiro–Wilk tests were applied to data to check whether it has a normal distribution. Independent two-sample t-tests were used to compare normally distributed data in binary groups. Independent risk factors affecting mortality were examined by binary logistic regression analysis in univariate and multivariate models. Multiple linear regression analysis was used to examine the independent variables affecting the alpha 1 antitrypsin level, and the Enter method, including all independent variables in the model at once, was employed in the model construction step. Spearman's rho correlation coefficient was used to examine the relationship between nonnormally distributed data, and the Pearson correlation coefficient was used to examine the relationship between normally distributed data. Analysis results are presented as the mean ± standard deviation and median (and *minimum* – *maximum* in parenthesis) for quantitative data. The level of significance was taken as $p < 0.05$.

Results

This cross-sectional, clinical study included 154 patients (61 females, 93 males) with COVID-19 pneumonia. The age, sex, biochemical parameters (CRP, D dimer, ferritin, lymphocyte, and neutrophil levels), and survival information of the patients were recorded. The relationship between AAT level and sex, survival, and other quantitative data was examined (Table 1, Table 2). The independent variables affecting the alpha 1 antitrypsin level were examined by linear regression analysis, and the regression model was found to be statistically significant ($F=3.051$; $p=0.001$) (Table 3). In the predictor selection step for the regression model, the enter method was used, and 13.1% of the variation in the dependent variable was explained with the

independent variables. There was no correlation between alpha 1 antitrypsin mean values and survival (p=0.133). While the alpha 1 antitrypsin level decreased by 0.003 units as the crp value increased by 1 unit (p=0.003), the alpha 1 antitrypsin level decreased by 0.027 units as the lymphocyte value increased by 1 unit (p=0.001). As the neutrophil value increased by 1 unit, the alpha 1 antitrypsin level decreased by 0.019 units (p=0.014). There was no statistically significant effect of other independent variables (p>0.050). According to the data presented in Table 3, the univariate analysis revealed that sex was an independent risk factor affecting mortality status. When women are taken as a reference, the mortality risks of men are 9.75 times higher (p=0.030). As creatine values increase, the risk of mortality increases 6.703 times (p=0.013). As the crp values increase, the risk of mortality increases 1.014 times (p=0.003). As ferritin values increase, the risk of mortality increases 1.004 times (p<0.001). As a result of the multivariate analysis, ferritin values were risk factors affecting mortality status. As ferritin values increase, the risk of mortality increases 1.003 times (p=0.002). Other independent risk factors were not found to be statistically significant (p>0.050) (Table 3).

Discussion

The COVID-19 outbreak caused by SARS-CoV-2 has emerged as the largest medical problem of our century. Although its pathogenesis has been defined, studies on the treatment of COVID-19, which has no known treatment, are still a work in progress. Alpha 1 Antitrypsin (AAT) plays an important role in protecting our lungs from damage caused by excessive inflammation. Due to the increase in serum AAT levels during inflammation, AAT is also considered an acute phase reactant [1], especially in neutrophilic inflammation [2]. In patients with a viral infection, AAT may have an antiviral effect due to its regulatory and anti-replication effects on both inflammation and the immune system [5]. In vitro and in vivo clinical studies show that AAT has immunomodulation, anti-inflammation, antiprotease, and anticoagulation effects, as well as provides protection against cell death [6]. Recent evidence suggests that alpha1-antitrypsin (AAT) may inhibit the infection by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) [6]. AAT can heal or alleviate COVID-19 through at least seven different mechanisms [7]. It acts primarily as a serine protease inhibitor and thus prevents the virus from binding to the ACE-2 receptor [8,9,10].

TMPRSS2 is a cell membrane-bound protease that facilitates the entry of viruses (including SARS-CoV-2) into host cells by proteolytically degrading and activating viral envelope glycoproteins. Preliminary evidence suggests that alpha $_1$ proteinase inhibitors may inhibit SARS-CoV-2 infection by inhibiting TMPRSS2 activity [10,11]. Second, AAT has antiviral activity. Its effectiveness in HIV, influenza, and MERS-CoV patients are already known [12,13,14]. In one study, AAT levels were observed to be low in people with HRV-16 infection. When the patients were given AAT treatment, the viral load decreased on average 29 times [15] . AAT has a third anti-inflammatory activity as nuclear factor inhibits kappa B activation [16,17]. Fourth, by inhibiting neutrophil elastase, neutrophils prevent the formation of extracellular traps and prevent acute lung injury [18]. Fifth, AAT also has inhibitory effects on thrombin and plasmin, and AAT levels should be high to be able to protect the patients who are prone to thrombus due to COVID-19 [19,20]. Sixth, AAT prevents multiple organ failures by preventing endothelial damage [21]. AAT appears to be an attractive drug that is administered intravenously once, in nasal spray and nebulizer forms, and has a high safety profile. Nasal

sprays can be used in patients with mild symptoms, nebulizer forms in patients with a moderate clinical course, and intravenous forms in patients with a severe clinical course. In countries with the highest number of cases of COVID-19 (Spain, Italy, France, and the United Kingdom), AAT levels are low in the general population [22,23]. Studies show that AAT levels are low and mortality is high in patients who develop cytokine storms in patients admitted to intensive care units [24]. People with low AAT levels have disruptive conditions such as emphysema and bronchiectasis in the lungs, and these patients benefit from alpha 1 protease inhibitor treatment [25]. In patients with low AAT levels before COVID-19, additional comorbidities (hypertension, chronic kidney disease, chronic obstructive pulmonary disease (COPD), and diabetes) can determine COVID-19 predisposition and prognosis [26]. Although patients diagnosed with AAT deficiency were not included in the study, it may not be possible to determine the carrier patients only with blood AAT levels. Further genetic studies may be needed, which was missing in this study. As the COVID-19 pandemic continues, the presence of multicenter studies involving a large number of patients on AAT levels may bring AAT treatment to the agenda in these patients at an early stage. An alpha $_1$ proteinase inhibitor is being tested as a treatment for COVID-19 patients in four clinical trials: Saudi Arabia (NCT04385836), Spain (NCT04495101), and the USA (NCT04547140), and Ireland (EudraCT 2020-001391-15). Although such studies are ongoing, the evidence for the effectiveness of AAT treatment has not yet been established.

Limitation

In this study, blood AAT levels were measured. Serious deficiencies can be detected in this way. However, carriers with AAT values close to the normal range may be overlooked. Although the absence of a genetic study was a limitation of our study, looking at the AAT levels of the patients on the first day and the last day could have provided information on how they changed during this process.

In conclusion

The negative correlation of AAT, which is an acute phase reactant, with inflammation parameters in COVID-19 patients suggests that AAT deficiency may play a role in clinical deterioration in these patients. AAT supplementation may be therapeutic, especially in patients who deteriorate clinically and biochemically. There should be a sufficient number of clinical studies to confirm this.

Author Contributions

All authors contributed to the conception and design of the study, analysis and interpretation of the data, and critical revision of the manuscript.

Declaration of interest

The author(s) declare that there are no conflicts of interest.

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Table 1. Comparison of alpha 1 antitrypsin levels by sex and survival status

	Alpha 1 Antitrypsin Level(g/dl)		Test statistics	p
	Ave. ± S. Deviation	Median (Min. -Max.)		
Gender				
Female	1.58 ± 0.45	1.47 (0.83 - 2.85)	t=1.112	0.268
Male	1.50 ± 0.49	1.41 (0.36 - 3.2)		
Survival				
Alive	1.55 ± 0.46	1.45 (0.36 - 3.2)	t=1.510	0.133
Ex	1.35 ± 0.56	1.21 (0.36 - 2.55)		

t: Independent two-sample t test

Table 2. Investigation of factors affecting Alpha 1 antitrypsin level by linear regression (Linear regression)

	β^0 (95% CI)	Standard Error	β^1	t	p	R^1	R^2	VIF
Gender	-0.101 (-0.251 - 0.048)	0.076	,106	-1,341	0.182	-0,100	.113	1.075
Female	Reference							
Male	-0.003 (-0.008 - 0.003)	0,003	-0.087	-1.029	0.305	0,039	-0.087	1.227
Age	0,201 (-0,04 - 0,442)	0.122	.158	1.647	0.102	0,078	0.138	1.579
Creatine(mg/dL)	0 (-0.001 - 0.001)	0,000	0.047	0.606	.546	-0,050	.051	1.024

D-Dimer(µg/mL)	0.002 (0 - 0.004)	0,001	0,194	2,203	**0,029**	0.184	0.184	1,341
C-reactive protein(mg/dL)	-0.003 (-0.005 - -0.001)	0,001	295	-3.077	**0,003**	0,086	0.253	1,583
Ferritine(µg/L)	0.062 (-0.065 - 0.19)	0,064	0,127	,966	0.335	-0,030	0.082	2.975
Ly(mcL)	-0.027 (-0.043 - -0.011)	0,008	653	-3,391	**0,001**	-0.178	0.276	6,407
NE(mcL)	-0.019 (-0.034 - -0.004)	0,008	-.594	-2.482	**0,014**	0,012	-0.206	9.875
Ly(%)	-0.007 (-0.018 - 0.004)	0,005	219	-1.297	.197	0,149	0.109	4,934
Ne(%)	0.019 (0 - 0.039)	0,010	0.209	1.932	0.055	0.183	0.162	2,016
Leukocyte(WBC)	-0.003 (-0.008 - 0.003)	0,003	-0.087	-1.029	305	0,039	-0.087	1.227

F=3.051; p=0.001; R^2=19.4%; corrected R^2=13.1%; β^0: unstandardized beta coefficient; β^1: standardized beta coefficient; r^1: zero-order correlation; r^2: partial correlation; *Ferritin values were too high to be included in the study.

Table 3. Examination of risk factors affecting mortality

	Univariate		Multivariate	
	OR (95% CI)	p	OR (95% CI)	p
AAT Level(g/dl)	0.34 (0.08 - 1.39)	0,133	0.374 (0.043 - 3.274)	.374
Gender				
Female	Reference		Reference	
Male	9.750 (1.241 - 76.598)	**0,030**	4.841 (0.414 - 56.568)	0.209
Age	1.002 (0.965 - 1.041)	0.908	0.936 (0.861 - 1.017)	0.118
Creatine(mg/dl)	6,703 (1,498 - 29,998)	**0,013**	11.036 (0.353 - 345.249)	-0.172
D-Dimer (µg/ml)	0.999 (0.986 - 1.012)	0,830	1.002 (0.981 - 1.024)	0.854
C-reactive protein(mg/dl)	1.014 (1.005 - 1.023)	**0,003**	1.003 (0.986 - 1.02)	0.724
Ferritine(µg/l)	1.004 (1.002 - 1.006)	**<0.001**	1.003 (1.001 - 1.006)	**0,002**
lymphocyte (mcL)	0.629 (0.32 - 1.235)	,178	0.719 (0.114 - 4.546)	0,726
neutrophil (mcL)	0.935 (0.86 - 1.015)	0,110	0.997 (0.753 - 1.32)	0.982

Lymphocyte(%)	1.004 (0.968 - 1.042)	0,817	1.087 (0.875 - 1.351)	0,449
Neutrophil(%)	1.027 (0.988 - 1.068)	0.171	1.079 (0.901 - 1.292)	0.406
Leukocyte(WBC)	1.021 (0.924 - 1.128)	0,681	1.042 (0.818 - 1.327)	0,739