Study of ATPase and GTPase levels in Fibrotic Lung Disease with and without COVID-19 Vaccination

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Abstract:

Background: In eukaryotic cells, the acidification of intracellular compartments is the responsibility of vacuolar H+ ATPase, a family of proton pumps, sometimes known as V-ATPases. Small GTPases are signaling molecules that regulate important cellular processes as well as subcellular activities making them essential players, particularly in a wide variety of coronavirus infection processes.

Objectives: The purpose of this research was to assess the levels of ATPase and GTPase in fibrotic lung disease patients who had received or had not received the COVID-19 vaccination, and then to compare these levels with those of the control group.

Methods: A total of 150 individuals took part in this study, which were divided into three groups. The first group was the control group (N=50). In the second group (N=50) patients with fibrotic lung disease did not get the COVID-19 vaccination. A total of fifty patients who had received the COVID-19 vaccination made up the third group(N=50). Enzyme-linked immunosorbent assay was the method that was used to determine the amounts of ATPase and GTPase. The P- P-value of 0.05 or less is considered statistically significant. ROC tests were examined for ATPase and GTPase.

Results: The data analysis reported that there was a significant rise in alkaline phosphatase, Alanine aminotransferase, and Aspartate-aminotransferase () among the three groups. Both ATPase and GTPase levels were shown to have significantly increased in Groups 3 and 2 as compared to Group 1 levels. Moreover, a substantial rise was discovered in Group 3 in comparison to Group 2 which was detected.

Conclusion: ATPase and GTPase levels are increased in patients with fibrotic lung disease regardless of the COVID-19 vaccination state.

Keywords: ATPase; GTPase; COVID-19 Vaccination; Fibrotic Lung Disease.

Introduction

Fibrotic lung disease (FLD) is a chronic lung ailment that is characterized by an inflammation that interferes with the lung's ability to receive sufficient oxygen (1). SARS-COV-2 is a virus that is transmitted to humans. It has been shown that, even within the same SAAS-COV-2 strain, several genomic alterations have been found which suggests that mutations were acquired by a process of consensus (2,3). Research demonstrated that lung dysfunction is a consequence of COVID-19 caused by prolonged ventilation acute respiratory distress syndrome or direct harm from the virus (4,5).

vacuolar H+ ATPase, the proton pumps are reliant on ATP. They are important for several cellular functions that require vacuolar compartment acidification. These processes include the trafficking of membranes, proteolysis of lysosomes, the elimination of invading pathogens by phagosomes,

and the secondary transport of metabolites (6). Two subunits make up the V-ATPase: a V0 complex that is trans-membrane and a V1 complex that is cytosolic (7). This V-ATPase subunit was examined in lung damage and fibrosis model in a recent work, which showed a function that had not been known about before. Within the tiny airway, the concentration of proton is responsible for regulating location (8).

The GTPases, also known as Rho, are enzymes that play the role of a molecular switch. They are regulated by guanine nucleotide exchange agents and are responsible for catalyzing the conversion of guanidine diphosphate to guanosine triphosphate (GTP). The GTPase-activating proteins, which are responsible for stimulating GTP hydrolysis, are responsible for inactivating the enzyme when it is in its active state, which occurs when the enzyme is phosphorylated (9).

The Rho/ROCK signaling system is implicated in smooth muscle contraction sensitization via its contribution. This is a significant aspect of the

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system, by demonstrating that the Rho/ROCK signaling system is accountable for airway smooth muscle contraction. Myosin phosphatase, which is an essential part of the process, is inhibited to achieve this goal (10).

The purpose of this research was to assess the levels of ATPase and GTPase in FLD patients who had, or had not, received the COVID-19 vaccination, and then to compare these levels with those of the control group Additionally, ROC studies were conducted to test for ATPase and GTPase, both of which have the potential to be regarded significant prognostic factors in the area of COVID-19 vaccination.

Materials and Methods:
For this investigation, samples were obtained from Ibn Al-Nafis Teaching Hospital and the Baquba Teaching Hospital/Internal Medicine and Chest Consultant between March 2022 and January 2023. The current study involved 150 individuals who were divided into three groups. The first group (G1) comprised fifty individuals and acted as the control group without contracting COVID-19. Fifty individuals with FLD who had not received the COVID-19 vaccination made up the second group (G2). fifty FLD patients who had received the COVID-19 vaccination (Pfizer) made up the third group (G3), ll patients have COVID-19. The age group of participants was 25-55 years. Every patient was subjected to a personal interview conducted using a well-crafted questionnaire. This interview included a comprehensive history. Most patients infected with COVID-19 developed FLD more than 4 months after they were diagnosed with a CT scan and by doctors in the hospital. Following an infection with COVID-19, it has been shown that several individuals have developed new instances of emphysema, cysts, and mosaic attenuation, (11, 12). It was determined that five milliliters of venous blood should be taken from each participant. The serum was utilized to determine the levels of ATPase and GTPase using the ELISA method (My bio source in the United States, America, manual procedures used in ALP, ALT, and AST determination).

Statistics: The SPSS was used for statistical analysis. The results are expressed as mean ± SD. Independent t-tests were utilized to compare the groups in the study. To compare the differences between the two groups, the Pearson Chi-square test was used. The P- P-value of 0.05 or less is considered statistically significant. ROC tests were examined for ATPase and GTPase.

Results:
It is our understanding that this is the first research that has assessed the levels of ATPase and GTPase in fibrosing lung disease, both with and without the COVID-19 vaccine.

Data in Table (1) represented a significant increase in serum ALP level in G2 compared to G1. A highly significant increase was found in G3As compared to G1, and G3As compared to G2.

However, ALT levels showed a non-significant increase in G2 in contrast to G1. A highly significant increase in $G3_3$ compared to G1 and G3 compared to G2.

Data in Table (1) revealed a highly significant increase in G2 and G3 match to G1 for AST levels. Highly-significant increase was seen in G3 compared to G2.

Table (2) shows an ANOVA analysis of GTPase and ATPase levels for G1, G2, and G3. Results of GTPase and ATPase display a highly significant increase in G2 and G3 compared to G1. A highly-significant rise in G3 compared to G2.

Analysis of the Receiver's Operating Characteristics (ROC)

1. (ROC) for GTPase:
The Receiver Operating Characteristic (ROC) curve analysis of GTPase to three distinct groups reveals an impressive area under the curve (AUC) of 0.90, which is significant at 95%, with a P-value of 0.0027, less than 0.01t threshold. The sensitivity and specificity were 88% and 94%, respectively, as shown in Table (3) and Figure (1). Results demonstrated high accuracy levels in discrimination between the three groups and efficiency of the GTPase test among the three groups.

2. (ROC) for ATPase
ROC curve analysis of ATPase illustrated a 0.91 AUC which is a significant amount in 94% levels with a 0.0023 P-value, less than the threshold of 0.001. The ATPase optimal cut–off value was 4.48. Sensitivity and specificity were 91% and 94%, respectively. Data display a high accuracy among the three groups and efficacy, as shown in Table (5) and Figure(2).

Table (1): ANOVA test of ALP, ALT, and AST for G1, G2, and G3.

Group		Mean	Std. Deviation	Std. Error	Sig.
ALP(U/L)	G_1	103.62	9.92	3.14	
	G_2	113.09	8.49	1.90	HS
	G_3	130.81	10.34	2.31	
ALT(U/L)	G_1	29.21	2.03	0.64	
	G_2	31.32	2.35	0.52	HS
	G_3	43.21	8.84	1.98	
AST(U/L)	G_1	24.15	4.19	1.32	
	G_2	29.48	3.18	0.71	HS
	G_3	39.31	6.75	1.51	

HS: Highly significant correlation between parameters (P- value ≤ 0.01)

Table (2): ANOVA test of GTPase and ATPase for G1, G2, and G3

Group		Mean	Std. Deviation	Std. Error	Maximum
GTPase(ng/ml)	G_1	17.72	0.46	0.14	
	G_2	24.00	3.53	0.79	HS
	G_3	27.27	0.65	0.15	
ATPase(ng/ml)	G_1	3.01	0.09	0.03	
	G_2	4.98	0.71	0.16	HS
	G_3	7.31	1.80	0.40	

Table (3): Difference between sensitivity and specificity of GTPase

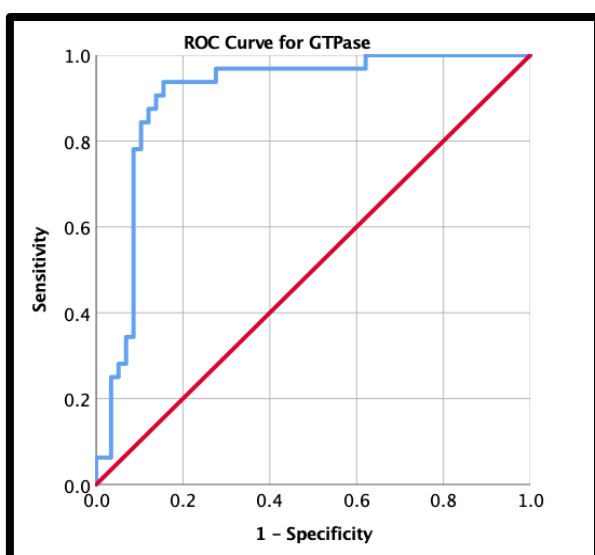| Variable	Sensitivity	Specificity	The area under the curve	Accuracy		Cut off value
				L.B.	U.B.	
GTPase	0.88	0.94	0.90	0.83	0.97	26.20

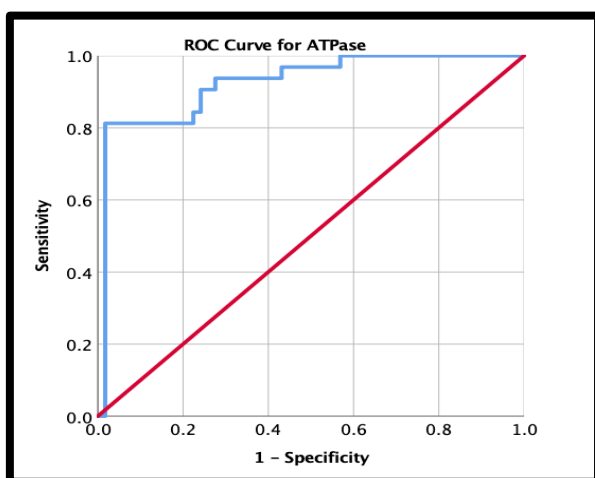

Figure (1) curve of the GTPase.

Figure (2): ROC curve of ATPase.

Discussion

There is a possibility that COVID-19 might result in several adverse effects, including lung fibrosis, pneumonia, respiratory failure, and syndrome of cytokine release (13,14). An infection caused by COVID-19 cannot be treated with any of the drugs that are presently on the market and are effective. The data will likely make it simpler to carry out clinical experiments on COVID-19 (15).

The results of analytical investigations indicated that the COVID-19 vaccination has a substantial influence on hearing thresholds for a variety of frequencies that are distributed and distinct from one another (irregular distribution). The research proposed using a larger study sample size, a novel.

Design, or researching a more extended age range to accomplish the goal of validating the results (16,17). Recent research demonstrated that a strong intelligence swarm algorithm is important in addition to chest X-ray classifiers in differentiating COVID-19 patients from conventional chest X-ray pictures (18).

Another recent research concluded that mRNA sequences that were involved in the study have a length of 107 bases, and the deterioration rates scored in the first 68 bases of the sequence. The actual COVID-19 mRNA vaccine should be longer, which displays that additional study should be conducted to investigate the predicting longer sequences of algorithms reliability (19).

ALP, ALT, and AST signs of liver damage, which are used as biomarkers in sepsis, viral pneumonia, and obstructive pulmonary diseases, have been reported to increase in patients with COVID-19 [20,21]. The results of the current study agreed with the findings of another study, which demonstrated that the levels of AST, ALT, and ALP in the blood of the patients who had recovered were all high compared to the control. Researchers concluded that certain biochemical markers are relevant (22-24). The analysis of GTPase and ATPase levels for G1, G2, and G3 display a highly significant increase in G_2 and G_3 compared to G_1. A highly-significant rise in G_3 compared to G_2. To determine the levels of GTPase and ATPase for G1, G2, and G3, ANOVA test was carried out. When compared to G_1, the

results of the GTPase and ATPase tests suggested that there was a considerable increase in the levels of G2 and G3 in comparison to G1. Furthermore, in contrast to G2, there has been a very significant increase in G3, which is a trend that should be seen as beneficial.

An examination of the lung cDNA library led to the discovery that the isoform subunit of V-ATPase G1 has direct contact with the 3CLpro that is generated by the SARS coronaviruses. In addition, it was found that the G1 subunit has a 3CLpro cleavage site, and it was shown that the viral protease can cleave cell culture experiments. A drop in the intracellular pH was shown to be connected with cleavage of the G_1 subunit in cells that expressed 3CLpro.

A key component of mammalian V-ATPase, Ac45, is capable of interacting with a component of the SARS CoV-2 viral replicas/transcriptase complex. It seems that V-ATPase could have a role in the viral transmission process (25,26).

The results of a study that investigated single-cell RNA sequencing (bronchoalveolar lavage fluid), bulk-RNA sequencing, and proteomics revealed that the expressions of V-ATPase were found to be enhanced in SARS-CoV-2 infection. These results revealed that S protein boosted V-ATPase in COVID-19 infection resulting in the generation of a microenvironment that was more conducive to cleavage of S protein. Activation of inflammatory cells was likely to occur as a result of calprotectin enhancement in respiratory epithelium (27,28).

As time goes on, it is becoming more apparent that several viruses can form a broad range of connections with Rho GTPase signal lings and manipulate these interactions to have its aid. Rho GTPases, in particular, have a role in the pre-entry process and endocytosis when it comes to the infection that is induced by the COVID-19 virus. (29-31). Rho GTPases are the most potent signaling cells' molecules. They are present in eukaryotic creatures and govern cell polarity via their effect on the cytoskeleton, trafficking of membranes, and adhesion cells (32). It was discovered that small GTPase has the potential to be used as an adjuvant target in the process of developing a vaccine against CoV. Within the context of the hunt for new adjuvants for CoV vaccines, this might prove to be of aid (33). It was possible to examine the response to infection in nasal swabs obtained in persons who were positive for SARS-CoV-2 and those who were negative for the virus. This was accomplished by evaluating the expression of genes in the respective host cell retarget role with Rho GTPases (34).

Conclusions:

Many COVID-19 vaccinations were used in the protection from this virus. The current study showed that lung fibrosis, a side effect appears in many people diagnosed by CT scan and some laboratory parameters such as ALP, ALT, and AST serum levels. Moreover, elevated ATPase and GTPase levels in recipients of the coronavirus vaccine showed a relationship between these markers and the vaccination in these individuals. The Receiver Operating Characteristic (ROC) study for ATPase and GTPase revealed that these parameters could act as efficient tests across patient groups.

Author Contributions:
Study conception & design: (Raed Mahmoud Al-Azawee). Literature search: (Raed Mahmoud Al-Azawee & Zeinab M. Al-Rubaei). Data acquisition: (Raed Mahmoud Al-Azawee & Zeinab M. Al-Rubaei). Data analysis & interpretation: (Raed Mahmoud Al-Azawee & Zeinab M. Al-Rubaei). Manuscript preparation: (Raed Mahmoud Al-Azawee & Zeinab M. Al-Rubaei). Manuscript editing & review: (Raed Mahmoud Al-Azawee & Zeinab M. Al-Rubaei).

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دراسة مستويات ATPase و GTPase في مرض التليف الرئوي للملقحين وغير الملقحين لفايروس كورونا

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الخلفية : في الخلايا حقيقية النواة، تقع مسؤولية تحمض الأجزاء داخل الخلايا على عاتق عائلة من مضخات البروتون المعروفة باسم-+H ATPasesالفراغية، والمعروفة أحيانًا باسم V-ATPases. إن GTPases الصغيرة عبارة عن جزيئات إشارة تنظم العمليات الخلوية المهمة بالإضافة إلى الأنشطة دون الخلوية، خاصة في مجموعة واسعة من عمليات الإصابة بفيروس كورونا . **هدف الدراسة:** كان الغرض من هذا البحث هو تقييم مستويات ATPase وGTPase لدى مرضى مرض الرئة الليفي (FLD) الذين تلقوا أو لم يتلقوا تطعيم COVID-19، ومن ثم مقارنة هذه المستويات مع تلك الموجودة في المجموعة الضابطة . **المرضى وطرق العمل** : شارك في هذه الدراسة 150 شخصاً تم تقسيمهم إلى ثلاث مجموعات، المجموعة الأولى (G_1)، كانت بمثابة مجموعة ضابطة وتضمنت (50) شخصاً. المجموعة الثانية (G_2) تكونت من (50) مريضاً بمرض التليف الرئوي غير الحاصلين على لقاح كوفيد-19. المجموعة الثالثة (G_3) تكونت من (50) مريضً بالتليف الرئوي مع لقاح (فايزر) كوفيد-19. كانت الايلازا هي الطريقة التي تم استخدامها لتحديد كميات ATPase وGTPase.
النتائج: أظهر تحليل البيانات وجود ارتفاع معنوي في إنزيم الفوسفاتيز القلوي، الأنين أمينوترانسفيراز، الأسبارتات-أمينوترانسفيراز بين المجموعات الثلاث، كما أظهرت النتائج أن مستويات ATPase وGTPase قد زادت بشكل ملحوظ في المجموعتين 3 و2 مقارنة بمستويات المجموعة 1. كذلك، تم اكتشاف ارتفاع كبير في المجموعة الثالثة مقارنة بالمجموعة الثانية.
الاستنتاج: ارتفعت مستويات ATPase و GTPase لدى مرضى تليف الرئة بغض النظر عن حالة التطعيم ضد فايروس كورونا باستخدام لقاح (فايزر) المستخدم في العراق .
الكلمات المفتاحية : ATPase, GTPase , , , , لقاح (فايزر) كوفيد-19, تليف الرئوي .