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Correlation between Baseline Serum Levels of Vitamin A and Vitamin C and the Severity of COVID-19: A Retrospective Cohort Analysis

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ABSTRACT

Background: Vitamins A and C are crucial nutrients of the human immune system, present in micro amounts. They have important antioxidant properties. Different studies point out their importance in improving the individual immune system's performance.

Objective: To find out the relation between the serum concentrations of Vitamin A and Vitamin C and the severity of COVID-19.

Methodology: This retrospective cohort study examined the association between baseline serum levels of Vitamin A and Vitamin C and COVID-19 severity. A total of 94 individuals were enrolled, including 64 COVID-19 patients and 30 controls. Blood samples were collected from all participants to measure serum Vitamin A and C levels. CT imaging was conducted for COVID-19 patients to assess lung involvement, with CT Severity Scores (CT-SS) calculated for each patient. The length of hospitalization (LOH) was also recorded. Statistical analyses compared vitamin levels between groups and assessed correlations with CT-SS and LOH. The study followed ethical guidelines, with informed consent obtained from participants and approval from the institutional review board.

Results: Level of Vit. A (mean ± SD: 489±98 ng/ml) compared to healthy individuals (mean ± SD: 695±95 ng/ml; p<0.002) is significantly lower. Similarly, the level of C in COVID-19 patients (median [IQR]: 2958 [1992-31717] ng/ml) and in healthy controls was (median [IQR]: 3955 [1389-8781] ng/ml; p = 0.006). Additionally, correlation analysis revealed a negative association between the levels of Vitamin A and C to that of outcome measures. Top of Form

Conclusion: This study concluded that Vitamins A and C levels were significantly lower in COVID-19 patients compared to healthy individuals. Additionally, a negative correlation was observed between the levels of these vitamins and both the CT Severity Score (CT-SS) and the length of hospitalization (LOH), indicating that lower Vitamin A and C levels were associated with greater disease severity and longer hospital stays in COVID-19 patients.

Keywords: Vitamin A; COVID-19; Pulmonary Disease; Vitamin C

Introduction

Coronavirus disease 2019 (COVID-19) is an illness caused by a virus named coronavirus called Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2), which was first identified in China. While SARS-CoV-2 primarily targets the lungs, it is now well-established that the severity of COVID-19 is influenced by a range of host factors, including age, existing health conditions, and nutritional status.[1] Individuals with malignancies, cerebrovascular conditions, persistent kidney problems, chronic obstructive lung disease, diabetes mellitus (both type 1 and type 2), and cardiovascular issues are at increased risk for SARS-CoV-2 infection.[2] Most individuals infected with the coronavirus will experience mild to moderate respiratory symptoms and may recover without needing specialized care. However, some will develop severe symptoms that require medical treatment. The virus can be transmitted through tiny droplets released from the mouth or nose of an infected person when they speak, breathe, sneeze, or cough. The best way to protect yourself and others from this virus is to maintain a distance of 1 meter, wash hands properly, and wear a mask and gloves. Moreover, an adequate immune system is needed to protect against the coronavirus. With weak immune systems, individuals are more susceptible to severe infection due to the coronavirus. Nutritional factors play an important role in the immune system.[3]

Among the various nutritional factors, the role of micronutrients, particularly vitamins, has garnered significant attention. Vitamins A and C, known for their immunomodulatory properties, have emerged as potential modulators of COVID-19 severity. Vitamin A and Vitamin C are essential micronutrients in our body.[4] They have antioxidant properties. They remove harmful molecules from the body, which can cause a disease or may damage cells. They are essentially defensive molecules that shield the body from damage inflicted by free radicals. Many studies have suggested that micronutrient deficiencies may contribute to the severity of viral infections, including those caused by coronaviruses. For instance, a lack of Vitamin A has been linked to a higher risk of respiratory infections, and providing supplements has been demonstrated to lower both illness rates and death rates in patients with pneumonia. Similarly, vitamin C deficiency has been linked to increased susceptibility to infections and poorer clinical outcomes. However, despite the established roles of these vitamins in immune function, there is a paucity of data on their impact on COVID-19 severity. This gap in knowledge underscores the importance of investigating the baseline serum levels of Vitamin A and Vitamin C in COVID-19 patients and their potential association with disease severity.[5]

The latest COVID pandemic has highlighted the critical need for evidence-based nutritional strategies to support immune function and improve patient outcomes. Understanding the relationship between baseline serum levels of Vitamin A and Vitamin C and COVID-19 severity could have significant implications for clinical practice and public health. It could inform guidelines on nutritional supplementation for at-risk populations and help identify individuals who may benefit from targeted nutritional interventions. Furthermore, exploring the role of these vitamins in influencing immune responses to COVID-19 may support the creation of complementary treatments aimed at reducing the disease's severity.

So, this study was planned to explore the association between baseline serum levels of Vitamin A and Vitamin C and the severity of COVID-19. By analyzing the serum levels of these vitamins in patients with varying degrees of disease severity, we seek to provide insights into their potential role as biomarkers for predicting outcomes and guiding treatment strategies. Our findings could pave the way for further research into the role of micronutrients in COVID-19 and other viral infections, ultimately contributing to the global effort to mitigate the impact of the pandemic.

Objective

To find out the relation between the serum concentrations of Vitamin A and Vitamin Cand the severity of the COVID-19.

Methodology

This cross-sectional cohort study was conducted at Khyber Teaching Hospital, Peshawar, to investigate the relationship between baseline serum levels of Vitamin A and Vitamin C and the severity of COVID-19. The study enrolled adults aged 18 and older who were symptomatic and had a confirmed SARS-CoV-2 diagnosis via polymerase chain reaction (PCR) testing. All participants provided informed written consent before their inclusion in the study.

Data were sourced from the hospital's electronic health records, including information documented on the first day of admission. The primary outcome measures included the severity of COVID-19, categorized according to the Chinese Clinical Guidance for COVID-19 Pneumonia Diagnosis and Treatment (7th edition), along with length of ICU stay, duration of mechanical ventilation, time to discharge, and time of death. COVID-19 severity was classified into four categories: mild (mild symptoms or asymptomatic), moderate (fever and respiratory involvement), severe (dyspnea and more severe symptoms), and critical (respiratory failure requiring mechanical ventilation).

Various biomarkers were analyzed to assess disease severity in COVID-19 patients, including complete blood

Table 1. Demographic characteristics of study cases

Characteristics	Control group	COVID-19 group	P-value
Age, years; Mean ± SD	31.3 ± 6.1	32.5 ± 6.3	0.498
BMI, kg/m2; Mean ± SD	31.1 ± 2.1	32.2 ± 3.9	0 ± 879
Diabetes, n (%)	9 (30)	13 (20)	0.414
Hypertension, n (%)	8 (26)	16 (25)	0.888
Smoking, n (%)	8 (26)	18 (28)	0.964
COPD, n (%)	6 (20)	15 (23)	0.875
Vitamin A, ng/mL; Mean ± SD	695 ± 95	489 ± 98	<0.002
Vitamin C, ng/mL; Median (Range)	3958 [1989-31721]	2953 [1342-8456]	0.006

count, albumin levels, cytokines, sequential organ failure assessment score, erythrocyte sedimentation rate, C-reactive protein, and lactate dehydrogenase. Venous blood samples were collected from each participant twice—once on the first day of hospitalization and again on the fifth day, with an interval of 60 to 70 hours between collections. The samples were stored at -80°C in heparinized plasma tubes.

Serum and intracellular levels of Vitamin A and Vitamin C were measured using a commercial kit from Chrom Systems (Germany). The central diagnostic laboratory at Lady Reading Hospital performed the analysis. The procedure involved protein precipitation, Vitamin C stabilization, and quantification via high-performance liquid chromatography (HPLC) with UV detection. Intracellular Vitamin C levels were measured by extracting the vitamin from peripheral blood mononuclear cells (PBMCs), isolated from 8 mL of heparinized blood through density centrifugation.

Before hospitalization, patients underwent a thoracic CT scan, and a chest CT Severity Score (CT-SS) was calculated. Non-contrast CT was utilized to prevent nephrotoxicity, with patients scanned in a supine position during breath-holding. Lung involvement was assessed using a semi-quantitative scoring system ranging from 0 (no lung involvement) to 4 (75% lung involvement).

Statistical analyses were conducted using SPSS software version 26.0. The Shapiro-Wilk test was applied to assess the normality of variables. Descriptive statistics were reported for demographic data, with means and standard deviations (SD) for serum levels of Vitamins A and C. T-tests were used to compare COVID-19 patients with healthy controls for normally distributed variables, while the Mann-Whitney U test was applied for non-normally distributed variables.

The study was approved by the medical ethical committee at Khyber Teaching Hospital, Peshawar.

Result

A total of 64 individuals as COVID-19 patients with a mean age of 32.5±6.3 years and 30 individuals as controls with a mean age of 31.3± 6.1 years were enrolled in our study. Serum concentration of Vitamin A (ng/ml, 489±98 vs. 695±95; p<0.002) and serum concentration of Vitamin C (ng/ml, 2953 [1342-8456] vs. 3958 [1989-31721]; p=0.006) both were comparatively lower as compared to the healthy individuals.

In this present study, the mean length of hospitalization (LOH) in COVID-19 patients was 5.9±1.4 days, and the mean CT-SS was 15.3±3.9 points (Table 1).

Results of our study showed that levels of Vitamin A and C are comparatively lower in COVID-19 patients as compared to normal individuals. The rate of COVID-19 is higher in those with comorbidities such as hypertension and diabetes.

The study found significant negative correlations between vitamin A levels and both the length of hospitalization (LOH) ($r = -0.294$, $p = 0.010$) and the CT Severity Score (CT-SS) ($r = -0.479$, $p < 0.002$). Similarly, significant negative correlations were observed between Vitamin C levels and both LOH ($r = -0.290$, $p = 0.011$) and CT-SS ($r = -0.735$, $p < 0.002$).

Discussion

This study aims to investigate the relationship between serum levels of Vitamin A and Vitamin C and the severity of COVID-19 infection in patients diagnosed with the virus. Our findings indicate that the concentrations of

Table 2. Correlations of both vitamins with length of the hospital stay and the CT severity score

	Length of Hospital		CT severity score	
	R-value	P-value	R-value	P-value
Vit A	-0.294	0.010	-0.479	<0.002
Vit C	-0.290	0.011	-0.735	<0.002

both Vitamin A and Vitamin C are lower in COVID-19 patients compared to healthy individuals. Additionally, we observed that the levels of these vitamins are inversely correlated with the length of hospitalization and CT Severity Scores (CT-SS).

Several other studies have reported that serum levels of both vitamin A and vitamin C are significantly lower in COVID-19 patients compared to healthy control groups. A study by Tepasse et al. underscored the significance of 'Vitamin A', noting that its levels were lower in severe cases compared to moderate ones; however, this difference did not reach statistical significance. This suggests that while vitamin A depletion may correlate with disease severity, it may not be the sole determinant of clinical outcomes.[6] Another study by Tomasa-Irriguible et al. supported these results, showing that 71.1% of the patients examined had lower levels of Vitamin A with an average of 0.17 (SD 0.06) µg/ml.[7] Many research efforts have indicated that COVID-19 frequently triggers an inflammatory reaction known as a cytokine storm, which predominantly affects the lungs, liver, and kidneys. This response can heighten the risk of vitamin A deficiency. These studies underscore the importance of Vitamin A for maintaining lung health and bolstering the immune system.[8-10]

Vitamin C also plays an important role in the body's immune system, just like Vitamin A. Research consistently highlights the significance of vitamin C in combating various diseases. The findings of this study are consistent with others that have reported low Vitamin C levels in COVID-19 patients.[11] For example, one study observed that more than 90% of COVID-19 patients had reduced vitamin C levels. Additionally, two other studies found that up to 82% of critically ill adult COVID-19 patients experienced vitamin C deficiency. These results underscore the importance of monitoring and potentially supplementing Vitamin C in managing COVID-19 and supporting overall immune function.[12,13]

The beneficial effects of ascorbate during viral infections stem from various mechanisms. A major aspect of its protective action is likely due to its ability to reduce inflammation. For example, research has shown that high-dose ascorbate therapy in patients with severe COVID-19' leads to a significant reduction in IL-6 levels compared to those receiving a placebo. Additionally, the antioxidant properties of Vitamin C help mitigate oxidative stress, which in turn can alleviate the impact of cytokine storms on cellular balance.[14,15]

The findings of this study offer significant insights into medical strategies aimed at mitigating the impact of COVID-19. The results align with existing literature on the connection between vitamin C and SARS-CoV-2 infection. Vitamin C levels could play a dual role: preventive and supportive. On the preventive side, measuring plasma ascorbate levels in healthy individuals may help identify those at risk of developing severe COVID-19. Those with decreased plasma ascorbate levels are more likely to experience severe symptoms. On the supportive side, assessing plasma ascorbate levels in COVID-19 patients could be crucial for managing the disease. Regular monitoring and supplementation of Vitamin C could therefore be valuable both in preventing and treating the disease. Furthermore, integrating Vitamin C into broader therapeutic protocols may enhance overall patient outcomes and help manage the progression of COVID-19 more effectively.

Vitamin A is crucial for the proper functioning of our immune system. It is vital for preserving the health of epithelial tissues, such as those in the respiratory tract, which act as the primary barrier against microorganisms and other infections. It increases the activity of different types of defense cells in our body, such as T-cells and B-cells.[16] Different studies have investigated that this vitamin is lower in COVID-19 patients which makes it difficult for the body to fight against different types of infections. That's why COVID-19 infection is more severe in those patients who will have lower serum levels of Vitamin A.

On the other hand, Vitamin C acts as a free radical neutralizer.[17] It plays an essential role in the protection of the body against damage that can be caused by free radicals. During any type of infection, the quantity of reactive oxygen species rises, which is harmful to the body, Vitamin C helps to neutralize this increased concentration of reactive oxygen species. It also increases the activity of the immune response. The level of vitamin C also decreases in COVID-19 patients. So, the lower the concentration of vitamin C, the higher the severity of the disease.[18]

Although the current study did not confirm the fact about Vitamin C levels as definitive biomarkers for assessing

COVID-19 severity, we have observed in many cases that patients were critically ill who had lower levels of Vitamin C compared to those who had moderate levels of Vitamin C.18 Moreover, the trend of severity of disease increases in those having low serum Vitamin C levels. Low levels of Vitamin C have been recognized as a risk factor for disease severity, and this risk is affected by age.

Vitamins A and Vitamin C are often administered together because of their synergistic effects in strengthening the immune system. In this study, we discovered a significant deficiency of both vitamins C and Vitamin A in COVID-19 patients relative to those in the control group, with the degree of deficiency corresponding to the severity of the illness. Furthermore, this deficiency was notably more common among patients in the ICU.

Conclusion

The study highlights a significant deficiency in the level of Vitamins A and C among COVID-19 patients compared to healthy controls. Additionally, lower levels of these vitamins are correlated with higher CT severity scores and extended lengths of hospitalization. These findings suggest that inadequate levels of 'Vitamins A and C' may contribute to more severe disease outcomes and prolonged recovery in COVID-19 patients. Therefore, monitoring and potentially supplementing these vitamins could be beneficial in managing disease severity and improving patient outcomes.

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