Original Article

ISSN: 1735-0344 *Tanaffos 2024; 23(3): 238-245*

TANAFFOS

Potential Laboratory Markers in COVID-19 Patients with Gastrointestinal Impairments as Prognosticators of the Disease Severity

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Received: 2 December 2023
Accepted: 18 February 2024

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Background: The new coronavirus infectious disease, COVID-19, is constantly killing people around the world. The main clinical symptoms of COVID-19 include fever, cough, shortness of breath, weakness, fatigue, and sputum/discharge. Gastrointestinal (GI) impairments are often associated with respiratory manifestations in COVID-19 patients. The current study aimed to examine potential laboratory markers in COVID-19 patients with gastrointestinal impairments as prognosticators of disease severity.

Materials and Methods: In the present study, hospitalized COVID-19 patients with respiratory symptoms as the main clinical manifestations and gastrointestinal symptoms were studied to evaluate relevant blood biomarkers including vitamins B12, D, and K in addition to albumin, pre-albumin, calcium, and magnesium.

Results: Sixty individuals (29 males and 31 females) participated in the study aged between 29 and 72 years, with a mean of 45.87 years. Our results showed a significant increase in albumin, pre-albumin, transferrin, and vitamin K and a significant decrease in magnesium and vitamin B12. Decreases in calcium and vitamin D were modestly associated with the clinical picture of the patient group.

Conclusion: Our findings suggest that monitoring laboratory markers such as transferrin, serum albumin, vitamin K, and magnesium-to-calcium ratio could be valuable prognosticators for COVID-19 infection. They could assist in better patient management and disease control.

Keywords: SARS-CoV-2; COVID-19; Respiratory Symptom; Biomarker

INTRODUCTION

In December 2019, a new member of the SARS coronaviruses with an unprecedented efficiency to infect humans emerged from Wuhan city in China and spread among countries. Within three months, the World Health Organization (WHO) declared the COVID-19 contagious disease pandemic due to its alarming levels of spread and severity (1). As of November 2023, COVID-19 remains a significant public health concern, with over 721,000,000 cases diagnosed worldwide and over 7 million deaths reported, according to the Worldometer website statistics (2).

Research regarding the disease characterization showed that people affected by COVID-19 demonstrated various symptoms ranging from mild to severe illnesses, even though the majority of infected people generally

developed mild to moderate illnesses and did not need hospitalization (3). COVID-19 has proven to have less case-fatality compared to SARS-CoV and MERS but a higher transmission rate (4). Therefore, acquiring knowledge of the clinical picture of COVID-19 is necessary to facilitate the early and differential diagnosis of the disease and promote measures that contribute to disease transmission management and prevention. In terms of clinical manifestations, COVID-19 has been classified by the WHO to display the most common, less common, and serious symptoms (5). They are caused by the divergent SARS-CoV-2 variants that mainly infect the pulmonary system in the clinical manifestations of the disease. Nevertheless, based on systematic reviews and meta-analyses, the most observed clinical symptoms of COVID-19 include fever, dry cough, fatigue, sore throat, and dyspnea (6). Patients infected with SARS-CoV-2 could also suffer impairment of other organs such as the gastrointestinal tract (GI), heart, kidney, and nervous system (7-9).

Still, several disparities, such as those related to the liver, require explanation. In this sense, it is decisive to have tools such as biomarkers to rapidly predict the disease severity among patients with COVID-19 (10). Several biomarkers have been proven to predict severity and mortality in COVID-19 patients including neutrophil to lymphocyte ratio, procalcitonin, D-dimer, interleukin-6, ferritin, apolipoproteins, etc. (10). However, some of these may be inaccurate or expensive to test and thus limited in certain settings and some developing countries (11).

Prediction of mortality has further been related to liver failure, total bilirubin, platelet count, and serum albumin concentration (12). In a certain classification, disease manifestations can be grouped into enzymatic (ALT and AST), metabolic (hypoglycemia and hyperammonemia), secretory (hyperbilirubinemia), synthetic (hypoalbuminemia and prothrombin time), and functional (D-dimer) disorders (13).

The individual's nutritional status has been shown to have a critical impact on immune function as well as the pathogenesis of the infection (14, 15). Nutrients and nutraceuticals have proved to affect respiratory viral infections such as COVID-19 (16). Therefore, the level of certain serum factors such as albumin, pre-albumin, transferrin, calcium, potassium, magnesium, vitamin B12, and vitamin D could assist with the prognosis of COVID-19 as well as predicting the severity of the disease (17, 18).

In this study, we have examined the serum level of various factors in patients with non-severe COVID-19 in categories such as inflammatory, hematological, immunological, biochemical, etc., and their relationship with COVID-19 patients who show fewer symptoms. Data from a cohort of COVID-19 patients with complaints of respiratory problems combined with gastrointestinal problems are presented and discussed. This may potentially influence disease management, transmission, and more optimal patient care.

MATERIALS AND METHODS

Patient participation and sample collection

Sixty individuals participated in this study including 30 COVID-19 patients with GI symptoms and 30 control healthy persons (29 males and 31 females) admitted to the Ayatollah Kashani Hospital (Tehran-Iran) during August and September 2022. The participants enrolled in this case-control study were aged between 29 and 72 years, with a mean of 45.87 years. Clinical examinations were performed by medical specialists and the COVID-19 infection was initially diagnosed based on the clinical and para-clinical tests. The presence of SARS-CoV-2 was confirmed by Real-Time PCR (SANSURE Novel Coronavirus Nucleic Acid Diagnostic Kit), performed on pharyngeal swab specimens collected from all participants. Serum samples from patients with positive Real-Time PCR for SARS-CoV-2, as well as control individuals, were collected for further tests on the serum factors including Albumin (ALB), pre-albumin (PRE ALB), Transferrin, VIT D, VIT K, VIT B12, Ca^{++}, and Mg^{++}. All participants were informed about the study and voluntarily provided a signed consent form.

Quantification of VIT B12, VIT D, and VIT K

Vitamins B12 and D were quantitatively detected using the ELISA kit from Ideal Diagnostics (Tehran, Iran), conforming to the manufacturer's instructions. This work was done using the HPLC method to measure the vitamin K. After blood extraction and preparation of the serum sample, a 50 microliter of each sample was injected into a Super Pacpep-S reverse phase column with a flow rate of 1.5 ml/min, and a mobile phase composed of 95% methanol in water. The duration of the experiment in each run was 15 minutes; quantification was performed based on the UV absorbance.

Measurement of other blood Factors in Serum Samples

The levels of ALB, Pre ALB (immunoturbidometric method), calcium, and magnesium ions (colorimetric method), as well as transferrin, were determined using appropriate kits from Byrex Fars (Tehran, Iran). The test was carried out conforming to the manufacturer's instructions, and measurement was performed using a Chemistry Analyzer (Hitachi-911).

Statistical Analysis

All the data were initially analyzed for normal distribution using the Shapiro-Wilk test. The Mann-Whitney and Unpaired T-test were used for comparing two groups of non-parametric and parametric data, respectively. The correlations are studied using the Pearson 2-tailed test. The significance level for p-values less than 0.05 and 0.01 are marked with an asterisk and double-asterisk symbols, respectively. All the statistical analyses were performed using SPSS Statistics V 21.0 software. The graph is presented using GraphPad Prism V 6.01 software.

RESULTS

In this study, sixty individuals participated: 30 COVID-19 patients with respiratory and GI symptoms as the main clinical manifestations including fever, cough, dyspnea, malaise, fatigue, and sputum/secretion, and 30 control healthy persons. Participants were 29 males and 31 females aged between 29 and 72 years, with a mean of 45.87 years. The quantification and comparison of serum albumin and pre-albumin between patients and control groups revealed a significant increase in individuals with COVID-19, showing respiratory and gastrointestinal difficulties as the main clinical symptom. A similar difference was also observed in the level of serum transferrin. However, the transferrin level was in the standard range (200-400mg/dl) for both groups, as shown in Table 1.

The relationship between nutrition and defense against infections and its role in COVID-19 has been noticed. Quantifying the respiratory and nutritional markers in this study has shown a significant increase in vitamin K and, at the same time, a considerable decrease in vitamin B12 serum levels. In this study, the level of vitamin D sufficiency has been considered to be more than 20 ng per ml. Despite a decrease in the serum level of vitamin D in persons with COVID-19, the difference between patients and control individuals was not statistically significant. Calcium and magnesium are among the ions that play an essential role in maintaining fluid homeostasis. Maintaining the distribution and concentration of those ions in intracellular and extracellular fluids is highly important. Our results showed a significant difference between the patient and control group for the magnesium ion concentration. Although the magnesium ion concentration was significantly lower in the patient group compared to the control group, the average magnesium concentration was within the standard concentration range introduced for the serum magnesium (1.6-2.4 mEq/l) both in control and test groups, as illustrated in Figure 1 and Table 1. Similarly, the calcium ion concentration was in the standard range with no significant difference between the two groups.

Table 1. Comparison of the serum levels for laboratory markers between case and control groups. Data of Vit K, Vit B12, Mg, Ca, Vit D, Transferrin, Pre-albumin, and Albumin have been analyzed either with the Mann-Whitney test or Unpaired T-test, marked with # and * symbols, respectively

	Control	Case	Difference (95% CI)	P Value
VIT K# (0.2-3.2) ng/ml	2.1000±1.05	2.7000±1.72	-0.60 (-.81460 to .16126)	0.129
VIT B12# (200-900) ng/ml	336.0±166.75	242.0±203.50	94.00(2.78894 to 126.74440)	0.020
Mg* (1.6-2.4) mEq/l	2.0233±.43683	1.6367 ±.48029	0.38667(0.14940 to 0.62393)	0.002
Ca* (8.8-11) mg/dl	9.3333±.55232	9.1400±.62786	0.19333(-.11227 to .49894)	0.210
VIT D# (>20 ng/ml)	24.5000±10.25	19.0000±15.25	5.50(-1.12286 to 7.58952)	0.051
Trans# (200-400) mg/dl	306.5±79.50	343.5 ±161.75	-37.00(-108.40917 to -26.99083)	0.002
Pre Alb* (20-40) mg/dl	31.2000±6.22786	42.4000±10.2942	-11.20000(-15.61734 to -6.78266)	0.000
Alb* (3.5-5) gr/dl	4.4067±.72679	5.2433±.84187	-.83667(-1.24313 to -.43020)	0.000

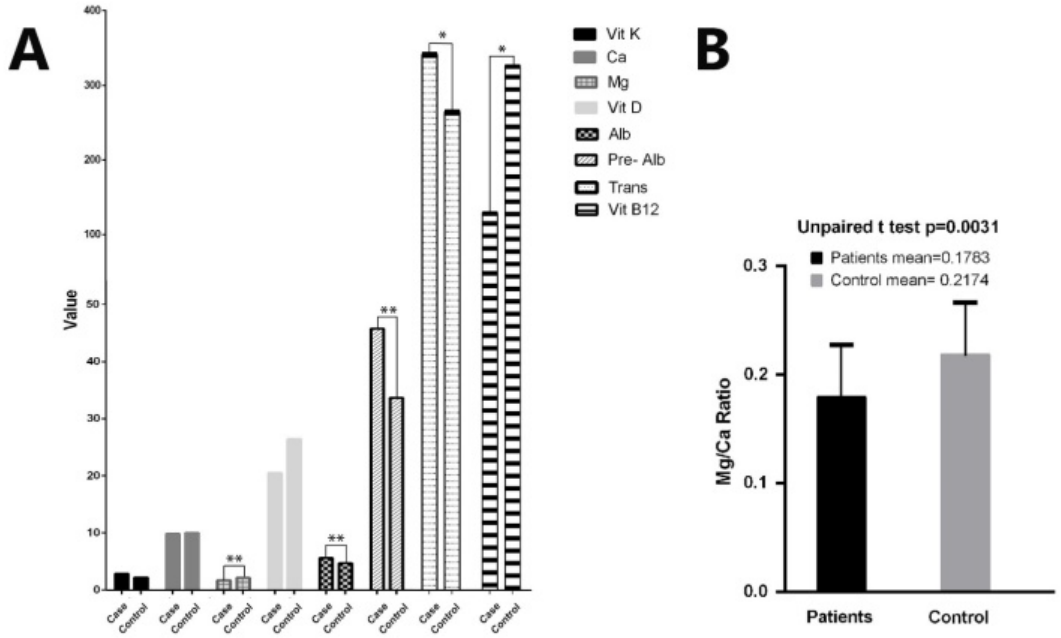

Figure 1. The graphical presentation of laboratory parameters studied in case and control groups. (A) The comparison of the serum levels between patient and control groups. The mean of each group is presented as a bar with a specific pattern. The significance level is shown with * and ** symbols for p<0.05 and p<0.01 values, respectively. (B) The magnesium-to-calcium ratio of the patient and control groups. The p-value (0.0031) demonstrates a significant difference between the two groups. The graphs are presented using GraphPad Prism software.

The correlations between different laboratory findings in patients' sera were evaluated. There was a statistically significant positive correlation between hyperalbuminemia with pre-albumin and transferrin levels. A decrease in serum vitamin D has shown a strong positive correlation with magnesium and calcium ions. Those ions also have a significant positive correlation, showing a magnesium-to-calcium ratio of 0.18, as demonstrated in Figure 1. As a consequence, there was a notable negative correlation between vitamin D and magnesium ions with pre-albumin and transferrin, and also between vitamin B12 and pre-Alb.

DISCUSSION

This study has shown that certain laboratory markers and clinical assessments could potentially provide objective information to COVID-19 clinical prognosis, namely in COVID-19 patients with respiratory and gastrointestinal impairments, which are among the least clinical manifestations associated with the infection. Our comparison of serum albumin and pre-albumin levels showed a statistically significant increase in individuals with COVID-19, showing respiratory and gastrointestinal

difficulties as the main clinical symptom. During hospitalization, nutritional status may also be assessed by measuring nutrition-related biomarkers. Visceral proteins such as albumin, pre-albumin, and transferrin, among the negative reactants of the acute phase of the inflammation, are particularly useful for detecting possible changes in the protein pool and for predicting the disease severity (17). Serum albumin concentrations are influenced by the rate of synthesis, distribution, and degradation, as well as factors such as TNF and Interleukin-1, which affect its synthesis by the liver (7, 19, 20).

The precise temporal association of hypoalbuminemia with COVID-19 has not yet been detected; however, in a meta-analysis of 11 studies, patients on admission with severe and non-severe COVID-19 had a mean albumin level of 35 and 40.5 grams per liter, respectively (21). Low albumin levels at hospitalization are predictive of the damaging outcomes of COVID-19, independently of other known indicators such as lymphocyte count or comorbidities (22, 23). On the other hand, hyperalbuminemia is generally the result of respiratory disorders, loss of body fluids, and dehydration due to severe diarrhea and vomiting. In our study, hyperalbuminemia was detected in the patient group significantly more than in the control group (with a mean serum albumin value of 52.433 g/L), which, as can be expected, showed no correlation with the patient group.

A significant increase in the levels of inflammatory factors such as CRP and IL-6 has been strongly attributed to the decrease in transferrin concentration (24). In addition, systematic reviews and meta-analyses have shown that lower serum pre-albumin levels were significantly associated with patients having severe disease and also with non-survivors (25). Interestingly, there was a high correlation between transferrin and pre-albumin in the patients participating in this study. In addition, both of them were significantly higher in the patient group than in the control subjects (p-value <0.001). Although this increase is somewhat consistent with mild symptoms of COVID-19, increased transferrin levels mean decreased blood iron, which may lead to iron malabsorption due to respiratory and gastrointestinal disturbances.

Ions play an essential role in maintaining homeostasis in the human body and are carefully regulated through various mechanisms, such as their distribution in intracellular and extracellular fluid (26). Calcium and magnesium are mostly found in extracellular (normal serum range: 8.8-10.7 mg/dL) and intracellular fluid (normal serum range: 1.46-2.68 mg/dL), respectively. In critically ill COVID-19 patients, respiratory and gastrointestinal disorders, as well as renal loss of magnesium, are the main causes of hypomagnesemia (27). They are also the main causes of secondary hypocalcemia and hypokalemia, which could lead to severe cardiovascular and neuromuscular clinical manifestations (Table 1) (28). Various reports concerning the contribution of hypomagnesemia attribute a higher mortality rate in patients with hypomagnesemia than in normomagnesemic individuals (29). Hypermagnesemia is associated with higher disease severity and increased mortality among hospitalized COVID-19 patients. However, it has been observed in 5% to 10% of critically ill patients (30). In our study, a considerably significant decrease was observed in the magnesium average concentration in patients when compared to the control group. Hypomagnesemia may also result in an exaggerated inflammatory response and aggravate the disease through the blockade of IL-6 disruptors (31). In the case of the calcium average concentration, studies have shown that a significant decrease in calcium levels was rather associated with severe-to-critical than mild-to-moderate cases in the early stage of the infection (32). Our results have shown an insignificant decrease in calcium concentration in the case of patients with non-severe COVID-19. However, calcium and magnesium ions have remained within the relevant standard ranges, which might explain their association with non-severe cases in this study. Given that hypermagnesemia in hospitalized patients is more associated with poor outcomes, there are several studies introducing iMg (ionized magnesium) as the only specific

marker to identify patients with dysmagnesemia (33). It has also been shown that 69- 85% of critically ill patients had low total magnesium with normal Mg levels (34). Nevertheless, this study has not measured the Mg due to certain technical containments. In a certain classification, a decrease in magnesium-to-calcium ratio with a cut-off point of 0.20 has been reported as a high mortality risk from COVID-19 (35). Several COVID-19 risk factors and certain co-morbidities are linked to magnesium deficiency (27, 36), nevertheless, no one has shown severe clinical manifestations in our study despite an average magnesium-to-calcium ratio of 0.178.

Vitamin B12, also known as cobalamin, is a micronutrient that is obtained mainly through the consumption of foods of animal origin and is absorbed through metabolic pathways involving substances such as hydrochloric acid, pepsin, and the intrinsic factor in the digestive system (37). In addition, studies have shown that vitamin B12 has an important role in the immune system by helping to balance the gut microbiota (38), rapid proliferation of B lymphocytes, and direct effect on the production of antibodies (39).

Interference of SARS-CoV-2 with vitamin B12 metabolism could favor activation of coagulation cascade and cytokine storm. Cohort studies assessing the effect of the combination of vitamins D and B12 plus magnesium on the severe outcomes of COVID-19 in older patients have shown less requirement for oxygen therapy and/or intensive care support in individuals receiving that combination (18). Considering the absence of severe cases in this study, one could suspect that the significant vitamin B12 decrease might be due to poor or malabsorption of the vitamin, which is consequential to gastrointestinal problems. Notably, the clinical symptoms of the individuals with COVID-19 infection in the present study are close to and could be misinterpreted as vitamin B12 deficiency (40). However, the presence of SARS-CoV-2 in all patient individuals in the current research has been confirmed by PCR.

Vitamin D has an important role in modulating the function of lymphocytes, induction of antimicrobial peptides at mucosal surfaces, and regulation of calcium balance. The relationship between the level of vitamin D and disease severity in COVID-19 has been discussed elsewhere (41). The present study showed an insignificant decrease in vitamin D levels in the patients compared to the healthy control group. These results also agree with the three meta-analyses performed for the relationship of vitamin D levels with COVID-19 infection, severity, and mortality (42). More recent results from two trials have concluded, although prudently, that vitamin D levels did not affect the incidence of COVID-19 (43).

Several studies have introduced vitamin K as a risk factor associated with severe COVID-19 (44, 45). Another study has demonstrated the effect of vitamin K deficiency on the severity of COVID-19 in association with IL-6 (46). The level of vitamin K in the patients' group in this study was significantly higher compared to the control group. It has been suggested that the vitamin K usage induced during the inflammation in COVID-19 may be the reason for vitamin K deficiency (47), which might be conceivable for the non-severe COVID-19 cases in this study. Increased vitamin K and an insignificant decrease in vitamin D levels could correspond to synergistic interplay previously suggested for these two vitamins in COVID-19 (45).

There were certain impediments in the present study, such as the lack of data on prescribed drugs that might interfere with magnesium homeostasis since magnesium supports the action of various anti-covid drugs. Decreased vitamin K and transferrin, along with the elevation of IL-6 and hyperinflammation, have been reported in severe COVID-19 (24, 46).

CONCLUSION

Our study showed that patients with COVID-19 who have respiratory symptoms and also suffer from digestive disorders experience changes in a series of body metabolic indicators that monitoring vitamin K, blood iron, and transferrin could be a valuable predictor of disease severity and progression in COVID-19.

Conflict of interest

The authors declare no conflict of interest.

Acknowledgements

The authors would like to thank Shahid Beheshti University of Medical Sciences for financial support of this study (Ethics code: IR.SBMU.RETECH.REC 1402.661).

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