Plasma Phospholipids: A Promising Simple Biochemical Parameter to Evaluate COVID-19 Infection Severity

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ABSTRACT

BACKGROUND: Coronavirus-19 (COVID-19) pandemic is a worldwide public health problem that has been known in China since December 25, 2019. Phospholipids are structural components of the mammalian cytoskeleton and cell membranes. They suppress viral attachment to the plasma membrane and subsequent replication in lung cells. In the virus-infected lung, phospholipids are highly prone to oxidation by reactive oxygen species, leading to the production of oxidized phospholipids (OxPLs).

OBJECTIVE: This study was carried out to explain the correlation between the level of plasma phospholipids in patients with COVID-19 infection and the levels of cytokine storms to assess the severity of the disease.

METHODS: Plasma samples from 34 enrolled patients with mild, moderate, and severe COVID-19 infection were collected. Complete blood count (CBC), plasma levels of D-dimer, ferritin, C-reactive protein (CRP), cholesterol, high-density lipoprotein cholesterol (HDL-C), low-density lipoprotein cholesterol (LDL-C), phospholipids, secretory phospholipase A2 (sPLA2)α2, and cytokine storms were estimated, and lung computed tomography (CT) imaging was detected.

RESULTS: The CBC picture showed the presence of leukopenia, lymphopenia, and eosinopenia in patients with COVID-19 infection. Furthermore, a significant increase was found in plasma levels of D-dimer, CRP, ferritin, tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-6, and IL-13 as well as sPLA2α2 activity compared to normal persons. However, plasma levels of phospholipids decreased in patients with moderate and severe COVID-19 infection, as well as significantly decreased in levels of triacylglycerols and HDL-C in plasma from patients with severe infection only, compared to normal persons. Furthermore, a lung CT scan showed the presence of inflammation in a patient with mild, moderate, and severe COVID-19 infection.

CONCLUSIONS: This study shows that there is a correlation between plasma phospholipid depletion and elevated cytokine storm in patients with COVID-19 infection. Depletion of plasma phospholipid levels in patients with COVID-19 infection is due to oxidative stress, induction of cytokine storm, and systemic inflammatory response after endothelial cell damage promote coagulation. According to current knowledge, patients with COVID-19 infection may need to administer surfactant replacement therapy and sPLA2 inhibitors to treat respiratory distress syndrome, which helps them to maintain the interconnected surfactant structures.

KEYWORDS: COVID-19 pandemic, phospholipids, OxPLs, pulmonary surfactant, sPA2, cytokine storm

Introduction

COVID-19 disease is an infectious disease caused by a newly discovered coronavirus.1 Most patients with COVID-19 infection will experience mild to moderate breathing problems and recover without specific treatment.2 In addition, COVID-19 represents a spectrum of clinical severity that ranged from asymptomatic to critical pneumonia, acute respiratory distress syndrome (ARDS), and even death.3 The COVID-19 spreads mainly by saliva droplets or nose discharge when an infected person coughs or sneezes4, so it is important that people also practice respiratory etiquette.5

Accumulating evidence suggests inflammatory mediators play a crucial role in COVID-19.6,7 Inflammatory responses caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) replication leading to cell destruction and macrophages as well as cytokine release.8

Multiple inflammatory markers identify and detect the specificity of diseases fatality.9 It has been documented that inflammatory markers such as procalcitonin (PCT), serum ferritin, erythrocyte sedimentation rate (ESR), C-reactive protein (CRP), and interleukin (IL)-6 have been documented to be
Phospholipids are the main components of pulmonary surfactants to reduce surface tension within the alveolar compartment at the air–tissue interface. It is the most abundant molecular species that suppresses inflammatory responses through direct interactions with certain cell receptors.

Phospholipids are highly prone to modification by reactive oxygen species (ROS) to form oxidized phospholipids (OxPLs) that induce inflammation, cytotoxicity, and apoptosis. Furthermore, OxPLs are involved in cytokine transcription and apoptotic mechanisms of modified phospholipids.

However, oxidative stress has been identified as a significant etiological factor in the pathogenesis of chronic inflammatory conditions, metabolism, and cancer.

Due to the production of ROS and oxidative stress in COVID-19 infected lungs, a wide range of structurally diverse OxPL species can be generated. OxPL formation in vivo has been demonstrated in a variety of disease settings with a prominent inflammatory component, including atherosclerosis, severe and chronic microbial infections, severe lung injury, and neurodegenerative disorders.

Recently, Hussein reported that the expression of cPLA2 caused by COVID-19 infection induces depletion of phosphatidylglycerol, inflammation, and lung damage. Also, secretory phospholipase A2 (sPLA2) is involved in eicosanoid synthesis and cytokine production by monocytes and macrophages.

In this case, COVID-19 infection induces many pro-inflammatory cytokines, including tumor necrosis factor (TNF), interferon-γ, IL-1, IL-6, IL-18, and IL-33, to secret unrestrained, causing a cytokine storm.

Here we hypothesize that the plasma level of phospholipids will change during COVID-19 infection and can be used to monitor the disease. In this study, we sought to elucidate the relationships between the plasma phospholipid level in patients with COVID-19 and the levels of cytokines to assess the disease severity.

Patients and Methods

Study population

In this prospective nonrandomized study, we enrolled 45 males with an average age 40 to 45 years, suspicion of COVID-19 at Shobra Health Insurance Hospital, Egypt.

According to the results of the biochemical and radiological examination, they are classified as follows:

Group 1 (negative COVID-19): 11 persons with normal blood pressure (BP; 120/80 mm Hg) and healthy hemodynamic and biochemical parameters were recruited into our study as a healthy control group.

Group 2: 15 patients with mild COVID-19 infection.

Group 3: 10 patients with moderate COVID-19 infection.

Group 4: 9 patients with severe COVID-19 infection.

On the contrary, the study protocol was approved by the local ethics committee of the Shobra Health Insurance Hospital, Egypt.

Patients should be considered for COVID-19 testing for a significant respiratory disease: fever with dry cough, fatigue, or difficulty breathing and were tested using the COVID-19 IgG/IgM Rapid Test Kit, Abbexa, Cambridge, United Kingdom) as well as lung CT using syngo. CT DE lung analysis—Siemens Healthineers Global, Germany.

Healthy participants were recruited under the conditions of a free routine health examination. All participants in this study met the following inclusion criteria: (1) Proven diagnosis of COVID-19 infection or benign disease by Prof. Dr. Noor Eldin Mohamed Ismail, a consultant on chest diseases, Shobra Health Insurance Hospital; (2) no adjuvant therapy or surgery prior to blood sample collection from people with a negative test of COVID-19 infection as well as patients with a positive test of COVID-19 infection; (3) Exclusion criteria included presence of autoimmune disease, severe kidney injury, or with the unsatisfactory vascular access or any other known condition that would alter cytokines and phospholipid levels. In addition, none of our patients had received antibiotics, anti-inflammatory, or corticosteroid medications during the study period. This study was reviewed and approved by the ethics committee of Shobra Health Insurance Hospital, Cairo, Egypt.

Clinical evaluations included taking a complete medical history to confirm the suitability of patients to the inclusion criteria.

Blood samples and biochemical analyses

Venous blood samples (4 mL) were obtained from all persons with a negative test of COVID-19 infection as well as patients with a positive test of COVID-19 infection and were divided into 2 aliquots: one aliquot was anticoagulated and divided into 2 test tubes; the first one for hematological examinations of total erythrocyte count (TEC), hemoglobin (Hb), mean cell volume (MCV), mean corpuscular hemoglobin concentration (MCHC) and total leukocyte, lymphocyte, eosinophil, and monocyte counts using a Hema Screen 18-Automated Hematology Analyzer (Hospitex Diagnostics, Sesto Fiorentino, Italy). The second test tube, plasma separated for D-dimer with a Sysmex® CA-7000 system coagulation analyzer (Sysmex, Kobe, Japan), and detection of hs-CRP with a Hitachi Model 7600 Series Automatic Analyzer (Hitachi High Technologies Corporation, Hitachi, Japan). The kits used in the experiments were D-dimer PLUS (Siemens Healthcare Diagnostics Products GmbH) and Reagent kit for the hs-CRP test (latex agglutination assay). Their reference values were 0.1417 (90% CL 0.00-0.55) µg/mL for D-dimer and < 6 mg/L for hs-CRP, respectively.
From other aliquots of blood, samples were allowed to clot and then sera were separated by centrifugation (3500 r/min, 20 min, 25°C) stored at −20°C for later biochemical determinations. Plasma levels of total cholesterol, HDL-C, triglycerides, and phospholipids were estimated using the Synchrocn cx5 autoanalyzer (Beckman, USA) and LDL cholesterol levels were calculated using the Friedewald formula. In addition, pro-inflammatory markers such as TNF-α, IL-1β, IL-6, and IL-13 were detected using an ultraviolet (UV) microplate reader (Thermo Electric Corp., Shanghai, China) according to the manufacturer’s instructions at 450 nm. Serum iron was determined using the bathophenanthroline method (American Monitor Corporation; Indianapolis, IN). Serum ferritin was quantitated using the enzyme-linked fluorescent assay technique (VIDAS® Ferritin, bioMérieux SA, France).

Plasma sPLA2 activity was assayed according to the method adopted by Petrovic using a spectrophotometer (Molecular Devices, Sunnyvale, California, USA) at an absorbance of 425 and 600 nm.

Statistical analysis

All data were expressed as mean ± SD. All analyses utilized SPSS 15.0 statistical package for Windows (SPSS Inc., Chicago, IL). A 1-way analysis of variance (ANOVA) was used for comparisons of means of the different groups. A P-value <.05 was accepted as statistically significant with the least significant difference (LSD) test as the post hoc test. The Spearman rank correlation test was used for the assessment of the correlation. The statistical significance was accepted as P-value <.05.

Results

Table 1 shows some hematological parameters of COVID-19 patients compared to negative persons. All patients in Groups (2-4) had normal CBC (normal Hb, RBCs, MCV, MCH, and MCHC) and non-significant change compared with COVID-19-negative persons (Group 1). In addition, the platelet count of patients with COVID-19 (Groups 2-4) was significantly decreased (P <.05) compared with persons with COVID-19-negative persons (Group 1). Also, patients with severe COVID-19 infection have mild thrombocytopenia (platelet count 140 × 10^9/L).

On the contrary, mild, moderate, and severe leukopenia was observed in patients with COVID-19 infection (Groups 2-4) with WBC (5.00 × 10^3/L, 4.00 × 10^3/L, and 2.00 × 10^3/L, respectively). However, mild, moderate, and severe lymphopenia was observed in patients with COVID-19 infection (Groups 2-4) with lymphocyte count (1.6 × 10^9/L, 1.2 × 10^9/L, and 1.0 × 10^9/L, respectively) compared with COVID-19-negative persons (Group 1). On the contrary, mild, moderate, and severe eosinopenia appeared in patients with COVID-19 infection (Groups 2-4) with eosinophil count (1.8 × 10^3/L, 1.7 × 10^3/L, and 1.5 × 10^3/L, respectively) compared with COVID-19-negative persons (Group 1).

Also, serum ferritin levels were significantly increased in all patients with COVID-19 infection Groups (2-4) compared with COVID-19-negative persons (Group 1; P <.05). The ferritin levels were more pronounced in patients with severe infection than in patients with mild and moderate COVID-19 infections. Also, normal levels of CRP and D-dimer in patients with mild infection. However, plasma D-dimer levels increased significantly in patients with moderate and severe COVID-19 infection, Groups (3 and 4) compared with COVID-19-negative persons (Group 1; P <.05). The D-dimer levels were more pronounced in patients with severe infection than in moderately infectious patients with COVID-19.

Table 2 shows phospholipids, total cholesterol, triglycerides, high-density lipoprotein cholesterol (HDL-C), and low-density lipoprotein–cholesterol (LDL-C) levels of patients with COVID-19 compared to COVID-19-negative persons. All patients in Groups 2 and 3 had normal levels of phospholipids, total cholesterol, triglycerides, HDL-C, and LDL-C levels of patients with COVID-19 compared to COVID-19-negative persons. However, plasma phospholipids, triglycerides, HDL-C, and LDL-C of patients with severe COVID-19 infection decreased significantly compared to COVID-19-negative persons. Also, plasma total cholesterol levels of patients with severe COVID-19 infection increased significantly compared to COVID-19-negative persons.

Table 3 shows plasma sPLA2 activity, as well as TNF-α, IL-1β, IL-6, and IL-13 levels of patients with COVID-19 compared to COVID-19-negative persons. All patients in Group 2 had normal levels of plasma sPLA2 activity as well as TNF-α, IL-1β, IL-6, and IL-13 in patients with mild COVID-19 infection when compared to COVID-19-negative persons. On the contrary, plasma sPLA2, TNF-α, IL-1β, IL-6, and IL-13 of patients with moderate and severe COVID-19 infection (Groups 3 and 4) increased significantly compared to COVID-19-negative persons.

Our data in Table 4 show a significant correlation between plasma phospholipids and sPLA2 activity, as well as TNF-α, IL-1β, IL-6, and IL-13 in patients.

sPLA2, secretory phospholipase A2; TNF, tumor necrosis factor; IL, interleukin.

All patients with mild COVID-19 infection had normal plasma phospholipid levels compared to negative persons. On the contrary, a negative correlation was observed between plasma phospholipids of the patients with moderate COVID-19 infection and plasma sPLA2 activity (P = .016) as well as TNF-α (P = .005), IL-1β (P = .023), IL-6 (P = .030), and IL-13 (P = .019).

Also, a negative association was observed between plasma phospholipids of patients with severe COVID-19 infection and plasma sPLA2 activity (P = .001) as well as TNF-α (P = .009), IL-1β (P = .015), IL-6 (P = .001), and IL-13 (P = .004).

Clinical characteristics of a normal person as well as patients with mild, moderate, and severe COVID-19 infection.
Figure 1 showed a normal CT study of the chest (Group 1, healthy person).

Figure 2 showed bilateral peripheral ground-glass opacities, suggested as mild COVID-19 infection (Group 2).

Figure 3 showed moderately bilateral ground-glass infection with COVID-19 (Group 3).

Figure 4 showed extensive bilateral ground-glass infiltration of viral bronchopneumonia. Most probably COVID-19 (Group 4).

Table 1. Hematological parameters and serum ferritin as well as plasma C-reactive protein and D-dimer levels of patients with COVID-19 compared to negative persons.

| PARAMETERS                  | GROUP 1 NEGATIVE COVID-19 INFECTION | GROUP 2 MILD COVID-19 INFECTION | GROUP 3 MODERATE COVID-19 INFECTION | GROUP 4 SEVERE COVID-19 INFECTION | NORMAL RANGE   |
|-----------------------------|-------------------------------------|----------------------------------|-------------------------------------|-----------------------------------|----------------|
| RBCs (10^6/µL)*             | 4.35 ± 0.04a                        | 4.55 ± 0.07a                     | 4.66 ± 0.21a                        | 4.40 ± 0.15a                      | 4.5-5.2        |
| Hb (g/dL)                   | 12.9 ± 1.4a                         | 13.65 ± 1.40a                    | 14.00 ± 0.38a                       | 13.2 ± 0.56a                      | 11.5-15.5      |
| MCV (fl)                    | 83.0 ± 4.00a                        | 85.0 ± 3.00a                     | 88.0 ± 5.00a                        | 89.00 ± 6.00b                     | 80-100         |
| MCHC (%)                    | 32.0 ± 2.00a                        | 28.00 ± 4.00a                    | 29.0 ± 2.00a                        | 29.00 ± 3.00a                     | 31%-37%        |
| MCH (%)                     | 28.00 ± 3.00a                       | 28.00 ± 3.00a                    | 27.0 ± 4.00a                        | 30.0 ± 2.00a                      | 27%-33%        |
| Platelet count (10^9/L)     | 320 ± 12.00d                        | 210.00 ± 10.00c                  | 180.00 ± 4.00b                      | 140 ± 7.00a                       | 150-450        |
| WBCs (10^3/µL)*             | 10.5 ± 2.00d                        | 5.00 ± 0.20c                     | 4.00 ± 0.10b                        | 2.00 ± 0.05a                      | 4-11           |
| Lymphocyte (10^9/L)*        | 3.87 ± 0.10a                        | 1.60 ± 0.03c                     | 1.20 ± 0.04b                        | 1.0 ± 0.03a                       | 1-4.8          |
| Neutrophil (10^3/µL)*       | 5.27 ± 0.70a                        | 4.33 ± 0.05c                     | 3.50 ± 0.04b                        | 3.50 ± 0.5b                       | 2.0-7.0        |
| Eosinophil (10^3/µL)*       | 2.00 ± 0.25d                        | 1.80 ± 0.40c                     | 1.70 ± 0.60c                        | 1.50 ± 0.50a                      | Up to 6        |
| Monocyte (10^3/µL)*         | 7.6 ± 0.80a                         | 6.50 ± 0.50a                     | 5.00 ± 0.30c                        | 4.00 ± 0.20c                      | 2-10           |
| Ferritin (ng/ml)            | 29.5 ± 2.00d                        | 186.90 ± 11.80c                  | 268.60 ± 17.68c                     | 540 ± 12.76c                      | 10-120         |
| C-reactive protein (IU/mL)  | 1.20 ± 0.2a                         | 1.60 ± 0.05b                     | 19.00 ± 0.70c                       | 26.40 ± 2.50c                     | Less than 6    |
| D-dimer (UgFEU/mL)          | 0.30 ± 0.02a                        | 0.31 ± 0.10a                     | 0.87 ± 0.040b                       | 1.32 ± 0.15c                      | Less than 0.55 |

abcdMeans with different superscripts within the same row are statistically different according to Duncan's multiple range test.

*Values are statistically significant at P < 0.05 were compared with group 1 (negative COVID-19 infection).

Table 2. Plasma phospholipids, total cholesterol, triacylglycerols, high-density lipoprotein-cholesterol (HDL-C), and low-density lipoprotein-cholesterol (LDL-C) levels of patients with COVID-19 compared to negative persons.

| PARAMETERS             | GROUP 1 NEGATIVE COVID-19 INFECTION | GROUP 2 MILD COVID-19 INFECTION | GROUP 3 MODERATE COVID-19 INFECTION | GROUP 4 SEVERE COVID-19 INFECTION |
|------------------------|-------------------------------------|----------------------------------|-------------------------------------|-----------------------------------|
| Phospholipids (mg/dL)  | 168.98 ± 7.60a                      | 170.80 ± 8.50b                   | 155.0 ± 9.00a                       | 94.30 ± 6.54b                     |
| Total cholesterol (mg/dL) | 175.8 ± 4.80a                      | 172.15 ± 8.70a                   | 177.48 ± 6.50a                      | 190.6 ± 11.25b                    |
| Triacylglycerols (mg/dL) | 97.65 ± 5.68a                       | 99.0 ± 8.14a                     | 98.5 ± 5.69a                       | 70.08 ± 10.33b                    |
| HDL-C (mg/dL)          | 47.87 ± 3.20a                       | 46.00 ± 3.10a                    | 45.3 ± 2.65a                       | 35.99 ± 4.35b                     |
| LDL-C (mg/dL)          | 108.4 ± 4.80a                       | 108.35 ± 5.98a                   | 112.48 ± 6.57a                     | 50.59 ± 4.87b                     |

abcdMeans with different superscripts within the same row are statistically different at level P < .05 according to Duncan's multiple range test. LDL-cholesterol levels were calculated by using the Friedewald formula (LDL-C = Cholesterol − HDL-C − [1/5 Triacylglycerols]).
Clinical characteristics of healthy normal persons and patients with COVID-19 pneumonia are listed in Table 5. Eleven healthy persons and 34 patients with COVID-19 infection were male and the mean age was 45 ± 6 years. Thirty-four (100%) patients had no underlying diseases, including diabetes mellitus, hypertension, renal, liver, and coronary heart disease.

For symptoms, 5 (45.4%) normal healthy persons, as well as 12 (80%) mild, 9 (90%) moderate, and 7 (77.7%) severe patients with COVID-19 infection had fever (ranging from 37.5°C to 39.3°C). Also, 2 (18.1%) normal healthy persons as well as 10 (66.6%) mild, 7 (70%) moderate, and 8 (80%) severe patients with COVID-19 infection had cough. However, 0 (0.0%) normal healthy persons as well as 6 (40%) mild, 4 (40%) moderate, and 5 (55.5%) severe patients with COVID-19 infection had diarrhea. Furthermore, 0 (0.0%) normal healthy persons as well as 4 (26.6%) mild, 6 (60%) moderate, and 9 (100%) severe patients with COVID-19 infection had chest tightness. On the contrary, 0 (0.0%) normal healthy persons as well as 12 (80%) mild, 3 (30%) moderate, and 3 (33.3%) severe patients with COVID-19 infection had dry mouth. Finally, 0 (0.0%) normal healthy persons as well as 14 (93.3%) mild, 9 (90%) moderate, and 9 (100%) severe patients with COVID-19 infection had fatigue.

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CT findings showed that 0 (0.0%) normal healthy persons as well as 15 (100%) mild, 10 (100%) moderate, and 9 (100%) severe patients with COVID-19 infection had ground glass opacity (GGO). Also, 0 (0.0%) normal healthy persons as well as 0 (0.0%) mild, 5 (50%) moderate, and 9 (100%) severe patients with COVID-19 infection had consolidation. However, 0 (0.0%) normal healthy persons as well as 0 (0.0%) mild, 2 (20%) moderate, and 4 (44.4%) severe patients with COVID-19 infection had a sign of the vascular perforator. In addition, 0 (0.0%) normal healthy persons as well as 10 (66.6%) mild, 5 (50%) moderate, and 5 (55.5%) severe patients with COVID-19 infection had a predominance of the right lung. On the contrary, 0 (0.0%) normal healthy persons as well as 1 (6.6%) mild, 2 (20%) moderate, and 1 (11.1%) severe patient with COVID-19 infection had a predominance of the left lung. The results of our study showed that 0 (0.0%) normal healthy persons as well as 15 (100%) mild, 3 (30%) moderate, and 3 (33.3%) severe patients with COVID-19 infection had upper lung predominance. However, 0 (0.0%) normal healthy persons as well as 10 (66.6%) mild, 7 (70%) moderate, and 6 (66.6%) severe patients with COVID-19 infection had a lower lung predominance. Also, 0 (0.0%) normal healthy persons as well as 15 (100%) mild, 10 (100%) moderate, and 9 (100%) severe patients with COVID-19 infection, had peripheral lung predominance. In addition, 0 (0.0%) normal healthy persons as
well as 2 (13.3%) mild, 1 (10%) moderate, and 0 (0.0%) severe patients with COVID-19 infection had unilateral predomi-
nance. Finally, 0 (0.0%) normal healthy persons as well as 13
(86.6%) mild, 9 (90%) moderate, and 9 (100%) severe patients
with COVID-19 infection had bilateral predominance.

Discussion
Coronaviruses are a large family of viruses that cause diseases
ranging from the common cold to more serious diseases, such
as Middle East Respiratory Syndrome (MERS-CoV) and
SARS-CoV. A novel coronavirus (nCoV) is a new strain that
was not previously discovered in humans. The COVID-19
genome contains 15 nsps, nsp1-nsp10, and nsp12-16, and 8
accessory proteins (3a, 3b, p6, 7a, 7b, 8b, 9b, and/or f14). All
those proteins play a functional role in viral replication. Our
results showed that the detection of COVID-19 infections
was concentrated between February 9 and July 13, 2020 and
peaked. The diagnosis of patients is according to 3
procedures:

1. The presence of significant respiratory illness: fever with
dry cough, fatigue, or difficulty breathing.
2. Measurement of COVID-19 IgG/IgM Rapid Test Kit, Abbexa, Cambridge, United Kingdom).
3. Lung CT diagnosis using syngo Dual Energy Lung
Nodules 1 - Siemens Healthineers, Germany. The patients
were classified according to the degree of severity into three
groups: mild, moderate, and severe COVID-19 infection
plus the first group of healthy persons with a negative test
for COVID-19 infection tests.

The present results showed that a significant difference in
hematology indices between COVID-19-positive and
COVID-19-negative persons. The hematology indices in this
study indicated that the proportion of leukopenia, lymphopen-
ia and eosinophil changes was higher among mild, moderate,
and severe patients with COVID-19 infection.

Our results are confirmed with the results of Zhang et al who reported that the patients infected with COVID-19

Figure 1. Normal CT study of the chest.
showed a decrease in leukocyte, lymphocyte, and eosinophil counts. However, leukopenia has been reported in other studies to be between 28.1% and 68.1% depending on the severity of the disease and the underlying conditions, suggesting a possible association between the severity of leukopenia and the severity of COVID-19.35,36

Although the mechanism of significant lymphocyte reduction in severe COVID-19 remains unclear, there are hypotheses other than lymphocyte infiltration and sequestration in the lungs, gastrointestinal tracts, and/or lymphoid tissues: (1) lymphocytes express the ACE2 receptor and maybe a direct target of SARS-CoV-2 infection,37 and (2) an increase in pro-inflammatory cytokines in COVID-19, especially IL-6, could induce further lymphocyte reduction.38

Human eosinophils express several endosomal Toll-like receptors (TLRs) that recognize single-stranded RNA viruses such as coronavirus and stimulating this receptor in human eosinophils triggers the production of eosinophil cytokine, degranulation, generation of superoxide and nitric oxide (NO), and prolonged cell survival.39

In addition, allergic lung responses to COVID-19 have increased severity by a thymic stromal lymphopoietin (TSLP)-dependent mechanism, and diseases associated with eosinophils such as eosinophilic esophagitis and hypereosinophilic diseases.40 Taken together, although current data are limited, there is little indication that eosinophils play a protective or exacerbating role during SARS-CoV-2 infection. However, eosinopenia may serve as a prognostic indicator of the severity of COVID-19.

On the contrary, our result showed a significant increase in ferritin, CRP, and D-dimer levels in patients who tested positive for COVID-19. Hyperferritinemia in this study as a known factor of inflammation was not confirmed by Connelly et al41 who investigated serum ferritin levels in patients at risk for and with ARDS and found serum ferritin to be a predictor of ARDS. In addition, patients critically ill with COVID-19 show hyper inflammation, and associated biomarkers (ferritin, CRP, and D-dimer) may be beneficial for risk stratification.42

This finding supports the hypothesis that SARS-CoV-2 infection could induce hemostatic system dysfunction, leading to
Recent evidence of lung pathology dissection has shown occlusion and micro thrombosis formation in small pulmonary vessels of patients critically ill with COVID-19. In addition, CRP is a severe phase inflammatory protein produced by the liver that may be elevated in several conditions, such as inflammation, cardiovascular disease, and infection.

The D-dimer might be a manifestation of an inflammatory reaction, as inflammatory cytokines could cause the imbalance of coagulation and fibrinolysis in the alveoli, which may activate the fibrinolysis system.

COVID-19 infection can develop into sepsis and induce coagulation dysfunction, which was common in inflammatory reaction then elevation of D-dimer plasma levels.

Our study suggested that elevation of plasma ferritin occurs when intracellular iron concentration augments and production of hepcidin increases. Elevation of ferritin is related to the inflammation process and could be a direct indicator of cellular damage.

Many publications suggested that the evaluation of ferritin as a syndrome and one of the main clinical parameters in COVID infection.

Our results have revealed a relationship between plasma phospholipid levels and the severity of COVID-19.

Pulmonary phospholipids as a surfactant form a film at the alveolar air–liquid interface and lower surface tension, thereby preventing atelectasis during breathing. Phospholipids are a complex mixture of lipids and proteins that are active tension-lowering agents and are not reactive to air oxidation.

Under oxidative stress, phospholipids can be altered, leading to OxPLs. Influenza viruses and SARS-CoV can induce accumulation of OxPLs in the lungs, which is associated with a pro-inflammatory response, acute injury, and organ damage. Our results suggested that the depletion of plasma phospholipids in patients with COVID-19 infection is due to oxidation of phospholipids by oxidative stress, ROS production, and cytokine storm induced by virus infection.

The plasma cholesterol elevation in patients with COVID-19 infection has confirmed the results reported by Li et al., who showed the cholesterol biosynthesis increases in patients with COVID-19 by SREBP-2 activity.

In addition, in COVID-19 infection and sepsis cases, SREBP-2 induces the production of IL-1β and TNF-α. When cells are

Figure 3. Showed multiple bilateral peripheral ground glassy appearance, suggested as moderate COVID-19 infection.
infected, they secrete lipids and cholesterol to inactivate the viruses.\(^{58,59}\) Also, SREBP2 acts as a signaling hub for inflammation and cholesterol metabolism activator.\(^{60,61}\) However, the activation of cholesterol biosynthesis is a result of decrease in cellular cholesterol level, and subsequent activation of SREBP-2 is involved in the exocytosis process of SARS-CoV2, which explains its role in virus budding and envelop formation.\(^{62,63}\)

Recently, Masuda et al\(^{64}\) reported that apolipoprotein A1 and apolipoprotein A2 were reduced during SARS-CoV2 infection, which has previously been associated with a variety of cardiovascular risk factors and mortality from pulmonary hypertension (PHT) and may have mechanistic significance with respect to blood hemodynamic and coagulation changes found in SARS-CoV-2 infections.

However, statistical analysis clearly showed that this is a reflection of systemic inflammation. There are different mechanisms explain the plasma level of phospholipid depletion. First, it has been suggested that phospholipids can bind to

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**Table 5.** Clinical characteristics of 11 healthy persons and 34 patients with COVID-19.

| SYMPTOMS       | NORMAL (11) | MILD (15) | MODERATE (10) | SEVERE (9) |
|----------------|-------------|-----------|---------------|------------|
| Fever          | 5 (45.4%)   | 12 (80%)  | 9 (90%)       | 7 (77.7%)  |
| Cough          | 2 (18.1%)   | 10 (66.6%)| 7 (70%)       | 8 (80%)    |
| Diarrhea       | 0 (0)       | 6 (40%)   | 4 (40%)       | 5 (55.5%)  |
| Chest tightness| 0 (0)       | 4 (26.6%) | 6 (60%)       | 9 (100%)   |
| Dry mouth      | 0 (0)       | 12 (80%)  | 3 (30%)       | 3 (33.3%)  |
| Fatigue        | 0 (0)       | 14 (93.3%)| 9 (90%)       | 9 (100%)   |

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![Figure 4. Diffuse lung parenchyma ground glass opacities involving almost of both lung lobes, suggested as severe COVID-19 infection.](image)
severe phase reactants, including CRP, which increased significantly in patients with severe COVID-19 infection. Also, the results of phospholipids, D-dimer, and CRP, as well as cytokine storm levels supported our suggestion. Second, phospholipids can be hydrolyzed by phospholipase A2 in patients with severe COVID-19 infection, releasing 1 fatty acid and lysophosphatidylcholine, which can then be hydrolyzed by lysophospholipase to produce another free fatty acid. Our study suggested that activation of cPLA2 by COVID-19 infection led to the overproduction of TNF-α, IL-1β, IL-6, and IL-13 that led to impairment of lung mechanics and the creation of infectious virus progeny (Figure 5). Furthermore, the significant alteration of plasma cholesterol, triacylglycerols, HDL-C, LDL-C, CRP, D-dimer, sPLA, and cytokines supports this suggestion.

In addition, Siddiqi and Mehra, who proposed the systemic hyper inflammation phase of COVID-19 via elevation of interleukin (IL)-2, IL-6, IL-7, granulocyte-colony-stimulating factor, macrophage inflammatory protein 1-α, TNF-α, CRP, ferritin, and D-dimer. This stage is the most severe manifestation of the cytokine storm, that excessive hyper inflammation can lead to cardiopulmonary collapse and multi-organ failure.

Hussein recently reported a schematic diagram to explain the correlation between COVID-19 infection and sPA2 enzyme

| CT FINDINGS                          | NORMAL (11) | MILD (15) | MODERATE (10) | SEVERE (9) |
|--------------------------------------|-------------|-----------|---------------|------------|
| Ground glass opacity (GGO)           | 0 (0)       | 15 (100%) | 10 (100%)     | 9 (100%)   |
| Consolidation                        | 0 (0)       | 0 (0)     | 5 (50%)       | 9 (100%)   |
| Mixed GGO and consolidation          | 0 (0)       | 0 (0)     | 5 (50%)       | 9 (100%)   |
| Bronchial wall thickening            | 0 (0)       | 0 (0)     | 1 (10%)       | 2 (22.2%)  |
| Crazy paving sign                    | 0 (0)       | 1 (1.5%)  | 2 (20%)       | 5 (55.5%)  |
| Air bronchogram                      | 0 (0)       | 1 (1.5%)  | 2 (20%)       | 6 (66.6%)  |
| Vascular perforator sign             | 0 (0)       | 0 (0)     | 2 (20%)       | 4 (44.4%)  |
| Intrathoracic lymph node enlargement | 0 (0)       | 0 (0)     | 0 (0)         | 0 (0)      |
| Pleural effusions                    | 0 (0)       | 0 (0)     | 0 (0)         | 0 (0)      |
| Right/left distribution              |             |           |               |            |
| Right lung predominant               | 0 (0)       | 10 (66.6%)| 5 (50%)       | 5 (55.5%)  |
| Left lung predominant                | 0 (0)       | 1 (6.6%)  | 2 (20%)       | 1 (11.1%)  |
| Equivalent in both lung              | 0 (0)       | 4 (66.6%) | 3 (30%)       | 3 (33.3%)  |
| Craniocaudal distribution            |             |           |               |            |
| Upper lung predominant               | 0 (0)       | 5 (33.3%) | 3 (30%)       | 3 (33.3%)  |
| Lower lung predominant               | 0 (0)       | 10 (66.6%)| 7 (70%)       | 6 (66.6%)  |
| Transverse distribution              |             |           |               |            |
| Central                              | 0 (0)       | 0 (0)     | 0 (0)         | 0 (0)      |
| Peripheral                           | 0 (0)       | 15 (100%) | 10 (100%)     | 9 (100%)   |
| Lung region distribution             |             |           |               |            |
| Unilateral                           | 0 (0)       | 2 (13.3%) | 1 (10%)       | 0 (0)      |
| Bilateral                            | 0 (0)       | 13 (86.6%)| 9 (90%)       | 9 (100%)   |
| Scattering distribution              |             |           |               |            |
| 1                                    | 0 (0)       | 1 (6.6%)  | 0 (0)         | 0          |
| 2                                    | 0 (0)       | 2 (13.3%) | 0 (0)         | 0          |
| > 3                                  | 0 (0)       | 12 (80%)  | 9 (100%)      | 9 (100%)   |

CT: computed tomography.
gene expression, which resulted in overproduction of cytokine storms and oxidation of cell membrane phospholipid to OxPLs.

The accumulation of OxPLs during COVID-19 infections plays a central role in the induction of inflammatory responses and the production of ROS. We suggest that surfactant phospholipids are oxidized with the massive formation of OxPLs during COVID-19 infections. Furthermore, an elevation of cytokine storm in this study has been observed in patients with COVID-19 infection, and single-cell transcriptomic analysis of peripheral blood in COVID-19-infected patients also shows an increase in IL-1β-producing monocytes. In addition, pulmonary thrombosis has been detected in autopsy from COVID-19 patients. However, all of these effects can be attributed to a harmful inflammatory response, which also drives the release of TNF-α, an initiator of the coagulation cascade. Therefore, OxPLs, as an inflammatory modulator, could elicit IL-1β, IL-6, and TNF-α, and coordinate inflammation as well as hemostasis during COVID-19 infection. Indeed, CD14, which regulates the inflammatory response in phagocytes in response to OxPLs, has been proposed as a possible therapeutic target against COVID-19.

CT imaging of COVID-19 pneumonia patients revealed that the pulmonary lesions involved bilaterally with multiple lung lobes, with a predominant distribution in the lower and peripheral part of the lungs, but this rarely was unilaterally involved. These lesions were mainly distributed in the bilateral subpleural and basilar lung regions because they were blocked by the pleural surface and progressed along the pleural surface. The main diameter of the lesions was approximately parallel to the pleura and perpendicular to the Broncho vascular bundles. Viruses in the same family share a similar pathogenesis and presentation; peripheral predominance lung involvement has also been observed in patients with SARS and MERS. The most common high-resolution computed tomography (HRCT) findings were pure GGO, GGO with interlobular septal thickening, and mixed GGO with consolidation, while no complete consolidation was found in our patients. Furthermore, we also found nodules, including solid nodules and solid nodules surrounded by a halo and linear opacities in some patients. Each patient may have 2 or more types of opacity lesions, but most of the lesions were GGO. Studies have reported that COVID-19 pneumonia is a viral interstitial pneumonia, and the early stage of pathogenesis is type II alveolar epithelial cell injury, edema, proteinaceous exudate, and focal hyperplasia of pneumocytes with only patchy inflammatory cellular infiltration, which were presented by GGO in HRCT results. With further thickening of the reticular and/or interlobular septa, the density of the GGO would increase, typical signs may appear, and swelling of the congestion could be observed in some lesions. However, Huang et al reported that pleural effusion may be an indication of
severe COVID-19 pneumonia, and further study is required to clarify the discrepancy.

Our study suggested that COVID-19 first seizes the intracellular membranes of host cell to create new compartments known as double-membrane vesicles (DMVs) needed for viral genome amplification. A different virus required a specific phospholipid composition to form the perfect repliative organelles that are suitable for their replication. DMVs are membranous structures that contain viral proteins and an array of confinced host factors, which jointly orchestrate an exclusive lipid micro-environment ideal for coronavirus replication. Cytosolic phospholipase A2α enzyme (cPLA2α) is critical for DMVs’ formation and coronaviruses’ replication.

Our study provides new data on mild, moderate, and severe patients with COVID-19 infection. We can confirm that phospholipids play a role as a diagnostic indicator. Specifically, changes in the phospholipid content of cellular membranes can reveal changes in biological, biochemical, and biophysical properties, as well as a proinflammatory environment and lung disorders.

Furthermore, our results are in line with studies of Yan et al., Yuan et al., and Alketbi et al. who reported that some lipids have been suggested as an indicator for certain human diseases, but we add here more patients and specifically, we suggest that phospholipids may be better than cholesterol because the alveolar surfactant is a phospholipid-based substance that is required for respiratory function. It also contains small amounts of other lipids and surfactant proteins. In addition, depletion in plasma phospholipid level can serve as early biomarkers foracute infections including acute pneumonia, but the alterations in plasma cholesterol level are late biomarkers for diagnosis of chronic diseases, diabetes mellitus, fatty liver, obesity, and cardiovascular disease.

On the contrary, evaluation of the plasma phospholipids level as a biochemical marker to estimate the severity of COVID-19 infection has not been reported earlier to our knowledge, and this study is perhaps the first observation of its kind.

Patients with severe COVID-19 infection showed significant alterations in their cytokine storm levels and exhibited an overall decrease in the availability of alveolar phospholipids that were directly correlated with decreased lung function.

Conclusions
This study clearly explains that depletion of plasma phospholipids as well as elevation of plasma sPLA2 activity and cytokine levels in patients with COVID-19 infection leads to alveolar epithelial cell injury and alveolar collapse at expiration. Oxidation of phospholipids to OxPLs as endogenous stressors that reprogram phagocyte metabolism and boost their pro-inflammatory responses, inducing a novel hyperinflammatory phenotype that sustains chronic inflammatory diseases. Under the effect of oxidative stress, phospholipids were oxidized to OxPLs, leading to hypercoagulopathy. The results of CT findings of patients with COVID-19 supported the biochemical and immunological results obtained. Consequently, the patients with COVID-19 infection and characterized by dramatic depletion of plasma phospholipids may need surfactant replacement therapy and sPLA2 inhibitors to treat respiratory distress syndrome due to COVID-19 and help them maintain interconnected surfactant structures.

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Acknowledgements
Thanks to Prof. Abdel-Kereem Qoutb, Vice Dean for Postgraduate Studies and Scientific Research, Faculty of Language and Translation, October 6 University, Egypt, for his aids in English editing and improving the article’s language.

Author Contributions
The experimental design of this study was carried out by all authors. All participants in this study satisfied the following inclusion criteria: Proven diagnosis of COVID-19 infection or benign disease by Prof. Dr. Noor Eldin Mohamed Ismail, a consultant on chest diseases, Shobra Health Insurance Hospital, and Dr. Yasser O. Mosaad, Faculty of Pharmacy, Department of Pharmacology, Toxicology, and Biochemistry, Future University. Biochemical analysis was carried out by Prof. Ali A. Ali, Prof. Rita M. Borik, and Prof. Mohammed Abdalla Hussein. The CT imaging and its reports were carried out by Dr. Ahmed H. Mohamed. The writing, protocol, first draft of the article, management study analyses, and the management of the literature searches were carried out in collaboration between all authors. All authors read and approved the final article.

Availability of Data and Materials
Supporting data will be made available as it contains CT images and analytical data of normal persons, and patients with mild, moderate, and severe COVID-19 to study the correlation between disease severity and plasma levels of phospholipids and sPLA2α, as well as storms of cytokine.

Consent for Publication
The authors gave their consent for their data to be used in the article.

Ethical Approval and Consent to Participate
Ethical approval for the data collection was granted by the Research Ethics Committee of Shobra Health Insurance Hospital. The present data were collected from routine biochemical analysis and CT imaging examination for patients with mild, moderate, and severe COVID-19 infection. In addition, this work was carried out to suggest a convenient mechanism for the severity of COVID-19 and explain its correlation with plasma levels of phospholipids and sPLA2α, as well as a cytokine storm.
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