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Original Article

Study of Microbial Infections and Some Immunological Parameters among Covid-19 in ICU Patients in Najaf Governorate, Iraq

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Abstract

The present study aimed to investigate some microbial infections and immunological parameters associated with Covid-19 patients admitted to the intensive care unit (ICU) of Al-Amal Specialized Hospital in AL-Najaf Governorate during February and March 2021. The study included 50 patients who were assigned to two groups: 20 patients aged ≤70 years and 30 patients aged ≥70 years. The method of microbial culture was adopted to isolate bacteria and yeasts by collecting sputum specimens and oral swabs from patients and cultivating them on diagnostic media and then confirming the diagnosis by Vitek. Moreover, serum samples were collected from patients' blood to diagnose fungal infections. Thereafter, some immunological criteria were assessed, including Covid-19 diagnosis by measuring Immunoglobulin M (IgM) and IgG, as well as examining the concentration of cytokines (Interleukin 6 (IL-6) and IF) using the enzyme-linked immunosorbent assay (ELISA) method. The results demonstrated that bacterial species *Streptococcus pneumonia* (n=5; 25%), *Haemophilus Influenzae* (n=7; 35%), and *Moraxella catarrhalis* (n=3; 15%) were isolated from the first group of patients (≤70 years). The recorded data pointed out that *Streptococcus pneumonia* (n=10; 33.3%), *Streptococcus pyogenes* (n=5; 16.6%), *Streptococcus viridans* (n=1; 3.3%), *Haemophilus Influenzae* (n=6; 20%), *Mycobacterium tuberculosis* (n=2; 6.6%), and *Pseudomonas aeruginosa* (n=2; 6.6%) were the isolated and identified microorganisms in the second age group (≥ 70 years). The results revealed that the isolated yeast from the first age group was *Candida albicans* (n=5; 25%) and *Candida glabrata* (n=3; 10%), while in the second age group, 1 (3.3%) *Candida albicans* was isolated. The results of this study proved that 30% and 10% of patients in the first and second age groups had invasive pulmonary *aspergillosis* co-infection by detecting Galactomannan (GM) in the blood serum (1.05±0.59, 1.25±0.38), respectively. The results indicated that IgM and IgG levels in the serum of patients in the first age group were 11.42±6.82 and 0.47±6.82, respectively. Moreover, the levels of IgM and IgG in the second age group were 14.84±9.21 and 0.12±0.11, respectively. Furthermore, IFɤ and IL6 levels were 98.37±65.70, and 146.12±46.35 in the first group, while IFɤ and IL6 were obtained at 110.69±47.60 and 133.28±116.94 in the second group, respectively. Elderly patients with severe COVID-19 are more frequently admitted to ICUs since the proportion of severe cases and comorbidities caused by a weakened immune system is higher among this age group. Secondary bacterial infections can also occur, especially Gram-negative bacteria which are among the most significant public health problems worldwide. Moroever, *aspergillosis* may infect patients hospitalized with COVID-19 and lead to death.

Keywords: Covid-19, ICU, IFɤ, IgG, IgM, IL-6, IPA

1. Introduction

Coronavirus disease (COVID-19) began in Wuhan, China, and the World Health Organization declared it a pandemic in 2020 (1). It is transmitted via droplets, sneezing, and contact, leading to an acute respiratory infection that can cause mild to severe symptoms;

moreover, in asymptomatic cases, there are no signs or symptoms. This disease may cause respiratory and organ failure, and as confirmed by research, the elderly are more vulnerable to this virus and run a higher risk of developing osteoporosis and other diseases that lead to death (2).

In severe cases of COVID-19, patients can develop acute respiratory distress syndrome (ARDS) (3). Hypoxemic respiratory failure is one of the most common symptoms that occur in the elderly, especially those over 65 years of age who suffer from elevated temperature (T>39°C), neutropenia, lymphocytopenia, as well as elevated signs of hepatic and renal failure (4). Secondary microbial infections in patients infected with the virus are associated with higher mortality rates. A related study reported that some COVID-19 patients were co-infection with *Chlamydia pneumonia* and *Mycoplasma pneumonia* (5). In the ongoing COVID-19 pandemic, viral, bacterial, and fungal co-infections have been reported among patients with COVID-19. Moreover, 7% of hospitalized patients with COVID-19 developed secondary infection with bacteria, which increased to 14% in studies that included intensive care unit (ICU) patients only. The collective possible co-pathogens recognized were Gram-negative bacteria, including *Klebsiella spp.*, *Escherichia*, *Aspergillus fumigatus*, and *Candida parapsilosis* (5).

The pathogenesis of COVID-19 is not yet known; nonetheless, some studies indicated that the severity of the disease is associated with increased inflammatory signs, such as cytokines (IL-6, IL-10, tumor necrosis factor (TNF)-α, and chemokines) (6). Cytokine release syndrome is one of the main pathophysiological processes involved in the rapid deterioration of COVID-19, causing ARDS and other multiple organ failures (7). In light of the aforementioned issues, the present study aimed to assess some microbial infections and immunological parameters associated with Covid-19 patients admitted to the ICU of Al-Amal Specialized Hospital in AL-Najaf Governorate during February and March 2021.

2. Materials and Methods

2.1. Collection of Specimens

A total of 50 specimens were collected from two groups of ICU patients suffering from Covid-19 at the Al-Amal Specialized Hospital in Najaf Governorate during February and March of 2021. The subjects in the first group were aged ≤ 70, while their counterparts in the second group were aged ≥ 70. Specimens taken from patients include sputum, oral swabs, and blood. Sputum and oral swaps were used for the identification of bacterial and yeast infection, while blood samples were employed for the identification of fungal infection and immunological tests.

2.2. Identification of Bacteria of Samples

Fresh clinical samples (oral swab and sputum) were Gram-stained to differentiate gram-positive and gram-negative bacteria and then cultivated on MacConkey, blood, and chocolate agar, incubated at 37°C for 18-24 h. The morphological features of the colonies, including size, shape, and color, were noted. The number of bacteria, including *Streptococcus pneumonia*, *Haemophilus Influenzae*, and *Moraxella catarrhalis*, was consequently recognized and measured by Vitek-2 Compact (Bio Mérieux, France).

2.3. Identification of Fungi in Samples

2.3.1. Invasive Pulmonary Aspergillosis Detection

Invasive pulmonary aspergillosis detection was carried out by Galactomannan Antigen Test in patients' Serum by using a Human *aspergillus* galactomannan Antigen (GM-Ag) ELISA kit (My BioSource, USA).

2.3.2. Yeasts and Yeast-Like Organisms Detection

Fresh medical samples (oral swab and sputum) were obtained from patients. Tests were attained from subcultures grown up 24-55 h on Sabouraud-gentamicin-chloramphenicol agar plates and identified by VITEK 2 system and ID-YST card (Bio Mérieux, France) which involved 64 wells with 47 fluorescent biochemical tests.

2.4. Immunological Profile

The evaluation of immunological criteria, which included diagnosing Covid-19 infection by measuring

IgM and IgG was performed using FAIDsSARS-CoV-2KIT (bioMérieux S.A). For the evaluation of cytokines (IL-6 and IF) levels, the commercially available ELISA kits (Elabscience, USA) and serum samples were utilized.

2.5. Statistical Analysis

The statistical analysis was performed in SPSS software (version 24) to find out the probability (*P-value*) and Chi-square (X^2). If the probability value was greater than 0.05, the results were considered statistically non-significant, while the *P*-value was statistically significant if it was lower than or equal to 0.05.

3. Results

A total of 50 specimens were collected from Covid-19 patients admitted to the ICU of Al-Amal Specialized Hospital in AL-Najaf Governorate. Based on the result, 20 patients aged ≤70 years, and 30 patients aged ≥70 years. Therefore, specimens taken from patients include sputum, oral swabs, and blood. The results demonstrated that bacterial species *Streptococcus pneumonia* (n=5; 25%), *Haemophilus Influenzae* (n=7; 35%), and *Moraxella catarrhalis* (n=3; 15%) were isolated from the first age group (≤ 70 years). The recorded data suggested that *Streptococcus pneumonia* (n=10; 33.3%), *Streptococcus pyogenes* (n=5; 16.6%), *streptococci viridans* (n=1; 3.3%), *Haemophilus Influenzae* (n=6; 20%), *Mycobacterium tuberculosis*(n=2; 6.6%), and *Pseudomonas aeruginosa* (6n=2; 6%) were the isolated and identified microorganisms in the second age group (≥ 70 years). The results revealed that the isolated yeast from the first age group was *Candida albicans* (n=5; 25%) and *Candida glabrata* (n=3; 10%), while in the second age group, 1 (3.3%) *Candida albicans* was isolated (Table 1).

The results of this study proved that 30% and 10% of patients in the first and second age groups had invasive pulmonary *aspergillosis* co-infection by detecting Galactomannan (GM) in the blood serum (1.05 ±0.59,1.25 ±0.38), respectively (Table 2).

The results indicated that IgM and IgG levels in serum of (ICU) Covid-19 patients in the first age group (≤70 years) were 11.42±6.82 and 0.47±6.82, respectively. Moreover, the levels of IgM and IgG in the group aged ≥ 70 years were 14.84±9.21 and 0.12±0.11, respectively. Furthermore, IFɤ and IL6 levels were 98.37±65.70, 146.12±46.35 in the group aged ≤ 70 years, while IFɤ and IL6 were obtained at 110.69±47.60 and 133.28±116.94 in the group aged ≥70 years, respectively (Table 3).

Table 1. Distribution of bacterial and yeast species in (ICU) Covid patient's specimens

Age groups	Bacterial and yeast species	Percentage %
≤ 70	Streptococcus pneumonia	5 (25%)
	HaemophilusInfluenzae	7 (35%)
	Moraxella catarrhalis	3 (15%)
	Candida albicans	5 (25%)
≥ 70	Streptococcus pneumonia	10 (33.3%)
	Streptococcus pyogenes	5 (16.6%)
	Streptococcus viridians	1 (3.3%)
	HaemophilusInfluenzae	6 (20%)
	Mycobacterium tuberculosis	2 (6.6%)
	Pseudomonas aeruginosa	2 (6.6%)
	Candida albicans	3 (10%)
	Candida glabrata	1 (3.3%)

Table 2. Concentration of Galactomannan serum level in Covid-19 patients admitted to intensive care units

Age groups	No. of patients with aspergillosis/ total no. of patients (%)	GM serum level ±sd
≤ 70	6 (30%)	1.05±0.59
≥ 70	3 (10%)	1.25±0.38

Table 3. Concentrations of IgM, IgG, IL-6, and IFɣ serum levels in (ICU) Covid patients

Age groups	IgM ±sd	IgG±sd	IFɣ±sd	IL6±sd
≤ 70	11.42±6.82	0.47±6.82	98.37±65.70	146.12±46.35
≥ 70	14.84±9.21	0.12±0.11	110.69±47.60	133.28±116.94

4. Discussion

A study conducted by Dres, Hajage (8) pointed out that 28% of COVID-19 patients admitted to ICUs were in the age range of 72-78 years. In the same context, Gkoufa, Maneta (9) demonstrated that elderly patients constitute a considerable proportion of critically ill patients admitted to the ICU due to COVID-19. Fu, Yang (10) explained that secondary bacterial infection occurred in 13.9% (5 out of 36) of patients infected with Covid-19, and these patients were admitted to the ICU. Another researcher suggested that the patients who received mechanical gas ventilation were more susceptible to secondary infection (*P<0.0001*), where the most detected bacteria were Gram-negative bacteria (n=26; 50.00%), followed by Gram-positive bacteria (11), Gram-negative bacteria (n=14; 26.92%), virus (n=6; 11.54%), fungi (n=4; 7.69%), and others (n=2; 3.85%).

In a study performed by Bag Soytas, Cengiz (12), 1, 071 patients were assigned to two groups, Group 1 aged <60 years (n=902) and Group 2 aged ≥60 years (n=169). They reported that SARS-CoV-2 IgG antibody titers were higher in Group 2 (*P=0.001*). Luporini, Joice (13) demonstrated that people over the age of 65 had higher levels of IL-6 and IL-10, compared to younger patients. Along the same lines, in their study, Marta, Lorena (14) indicated that out of 300 patients who participated in the study, 35 cases were diagnosed with COVID-19 associated pulmonary aspergillosis (prevalence of 11.7%), and the death rate in these patients was reported to be 31.4%.

Studies have pointed out that elderly Covid-19 patients who suffer from chronic diseases or acute respiratory infection, diabetes, or obesity are more likely to be admitted to the ICU since advanced age is linked to a defect in the immune system. There may be a possible association between a higher risk of severe disease in the elderly and pre-existing disease, with high blood pressure, diabetes, chronic obstructive pulmonary disease, and obesity being the greatest usually recognized diseases (9). The immune system changes with age, leading to a marked decrease in immunity with age. Disease and mortality rates are associated with age, and data from China showed that age was a key factor associated with the development of the disease that leads to ARDS and peripheral organ failure (15).

Feldman and Anderson (16) reported that Covid-19 patients admitted to ICUs may develop a secondary infection. This justifies the need for antibiotic treatment and causes great concerns over complications resulting from excessive use of antibiotics due to hospital-acquired bacterial and fungal diseases. Bacteria can infect patients with COVID-19 accidentally, and a longer hospital stay leads to a greater risk of pneumonia. According to reports, 50% of patients who died had a bacterial or fungal infection (17). Several hypotheses, such as host immune changes, mechanical damage and spread, and mucus removal within the lungs, have been proposed about the cause of secondary infection, especially in patients suffering from a lung viral infection (18).

The link between fungal infection and Covid-19 is not well understood, and as demonstrated by a hypothesis, the virus causes harm to lung tissue with the presence of vesicular ulcers, which leads to an increase in fungal infection by inhalation and the lack of white cells caused by the virus. Immunoglobulin IgG and IgM play a major role in disease identification as early as 11-15 days after disease onset through an important decrease in the number of CD4 and CD8 lymphocytes and the raise of interleukins (IL-2R, IL-6, IL-10, and TNF-alpha) and other inflammatory markers (19). Epithelial

damage during viral infection leads to the removal of the mucous membrane and the accumulation of mucus where the bacterial cells spread in the mucus while the immune cells cannot (20).

Studies have revealed that the Covid-19 death rate is higher in patients aged 80 years and older. They have high levels of antibodies and high levels of nAB as a result of strong infection or fungal infection. Moreover, in patients admitted to ICUs, IL-1β, IL-6, IL-8, and sTNFR1 are elevated and formed at locations of tissue inflammation and released into the movement by macrophages, lymphocytes, and fibroblasts, as well as epithelial and endothelial cells. The generation of these cytokines leads to the activation of macrophages and phagocytes to get rid of the virus and infected cells (21).

Authors' Contribution

Study concept and design: S. R.
Acquisition of data: S. R.
Analysis and interpretation of data: S. R.
Drafting of the manuscript: S. R.
Critical revision of the manuscript for important intellectual content: S. R.
Statistical analysis: S. R.
Administrative, technical, and material support: S. R.

Ethics

The study design was approved by the ethics committee of Kufa University, Kufa, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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