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Detection and Assessment of Neutralizing Antibodies and Interleukin -6 among Healthcare Workers Post-infected Patients with Covid-19 and Candidiasis in Diyala Province

A Thesis

Submitted to The Council the College of Medicine , University of Diyala in Partial Fulfillment of The Requirements for The Degree Of Science In Medical Microbiology

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﴿ قُلْ آمِنُوا بِهِ أَوْ لَا تُؤْمِنُوا إِنَّ الَّذِينَ أُوتُوا الْعِلْمَ مِن قَبْلِهِ إِذَا يُتْلَىٰ عَلَيْهِمْ يَخِرُّونَ لِلْأَذْقَانِ سُجَّدًا ﴾

صدق الله العظيم

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Dedication

To .... Dear Father and Mother, My Lovely Children, Brothers and Sisters, Best friend who stood by me in my work and is still.

With great love and gratitude

Special thanks for Murtadha Ismaeel Ali who supported me throughout this work and I will always appreciate what he has done to me.

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Maysam

Supervisor Certification

I certify that this thesis entitled (**Detection and Assessment of Neutralizing Antibodies and Interlecin-6 among Healthcare Workers Post-infected Patients with Covid-19 and Candidiasis in Diyala Province** at the **College of Medicine-University of Diyala** was under my supervision as a partial fulfillment of the requirements for the degree of Master of Science in Medical Microbiology.

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Summary

Coronaviruses belonging to the family Coronaviridae . In 2019, Novel coronaviruses belonging to the Betacorona virus were the reason for many deaths in Wuhan, China. The term COVID -19 was given to symptomatic disease caused by SARS-CoV-2 . the aims of this study was to detect and assess the IgG, IgM antibodies titer in patients with COVID- 19 after convalescent ,to study the role of Interleucin-6 in patients with COVID- 19 after convalescent, to study the role of Vitamin D3 in patients with COVID-19 after convalescent and to study the relation between COVID-19 disease and candidiasis.

This study was carried out in two hospitals in Baquba. SARS-CoV-2 infection was detected in various clusters of HCWs. One hundred Convalescent HCWs with coronavirus PCR-confirmed HCWs were recruited who worked in Diyala Hospitals center (Al-Batool Teaching Hospital and Baquba General Hospital) between October 2020 and March 2021 were included. Each participant in this study had 3-5 milliliters of whole venous blood drawn via venipuncture with syringes after the region was disinfected with 70% ethanol. Blood samples were collected in plastic plane tubes (no anticoagulant) and arranged in a rack. These tubes were left at room temperature (15-25 C^0) to clot. Centrifugation at 3000g for 10 minutes at room temperature yielded serum. The samples were kept at 20°C until the beginning of ELISA analysis. Furthermore, the participants were asked to provide information about the nature, severity, and duration of their C-related symptoms (fever, nausea, diarrhea, loss of sense of smell or taste, fatigue, dyspnea, headache, cough, runny nose, sore throat, and myalgia). In a standardized questionnaire, they answered questions about their COVID-19 infection. All of these markers were

measured in the Public Health Laboratory in Baquba using the Enzyme-linked Immunosorbent Assay (ELISA).

Also , then after few days, One hundred swab samples were obtained from who are in the study sample from patients healing from Coronavirus at ages ranging from 23 to 59years old. The patient's name, age, and gender were written on each swab and identified instantly. Chloramphenicol was added to Sabouraud dextrose agar (SDA). After that, it was incubated for 48 hours. For the evaluation of Candida spp, produced slides from each sample were produced and stained with Lactophenol cotton blue stain.

As well as included 100 healthy individuals as control with ages ranging from (20 – 35 years) in order to detect Covid IgM and IgG titers. The healthy people were chosen at random from blood donors who came to the Central Blood Bank in Baquba and from healthy people who came to the Public Health Laboratory for a pre-marriage routine exam.

Serum antibodies titer were measured three months after the onset of the illness. The average titer of IgG after three months of infection was 204.18 IU/ml. Antibody remeasurements were performed on 100 patients, and serum IgG titers changed as COVID-19 progressed. It was observed that serum IgM declined significantly between weeks 8 to 12 after illness onset, but it was also observed that serum IgG began to rise 3 months after infection. In this study, the results showed no relation between IgM, IgG titer and gender, but there was a relation between IgG titer and age. Also, there was a relation between IgG titer after 3 months of infection and after 6 months of infection, The study's main finding is that IgG titer antibodies against SARS-CoV-2 can be kept in patients for at least 6 months. It was discovered that the antibody titer, particularly IgG levels, can persist at high levels, providing a positive signal for anti-secondary infection. Although IgG and neutralizing antibody levels continue to fall, their

residuals are still abundant, providing a reference point for the study of SARS-CoV-2 immunity. During the follow-up period, the overall seroconversion rate was 95 percent (95/100) among the 100 patients. After 3 months, 5 of the patients appeared to enter a plateau of IgG titer, 3 female and 2 male. while IgG after 6 months were 16(16%) negative . Our results showed that there was a highly significant difference in IgG titer 3 and 6 months after infection. Also this study showed there was no relation between IgG titer 3 months after infection and chronic disease because the age group (20-30years) had a higher percentage and had no chronic diseases, while the age group (50-60 years) had a lower percentage and therefore there was no relationship between chronic disease and Covid-19 IgG titer. Also in this study, there was a relation between IL6 and Vitamin D3 in convalescent patients, accounting to a range of previously described clinical predictors and, potentially directing future therapeutic strategies, including the relation to Vitamin D.

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List of Abbreviations

Abbreviation	Key
ACE2	Angiotensin-converting enzyme
ADCC	Antibody-dependent cell cytotoxicity
COVID-19	Coronavirus disease 2019
CDC	Centers for Disease Control
CoV	Coronavirus
DMVs	Double-membrane vesicles
EC	Extracellular domain
HCWs	Healthcare Workers
HLA	Human leukocyte antigen
ICU	Intensive care unit
TCR	T-cell redepter
IHA	Indirect Hemagglutination Antibody
IL	Interleukin
MasR	Mas receptor
NF-B	Nuclear factor B
Nsps	Non-structural proteins
ORFs	Open reading frames
PAMPs	Pathogen-associated molecular patterns
(pDCs)	Plasmacytoid dendritic cells
RBD	Receptor-binding domain
TMPRSS2	Transmembrane protease, serine 2
TAD	Transmembrane anchor domain
UVB	Ultraviolet B

Chapter One

Introduction

1.1Introduction

The global coronavirus pandemic (COVID-19), which started in the Chinese city of Wuhan, in December 2019 (Huang *et al*., 2020), has quickly spread to more than 58 countries (Yang and Jin, 2020). there have been 211,373,303 confirmed cases of COVID-19, including 4,424,341 deaths, reported to WHO. As of 20 August 2021, a total of 4,562,256,778 vaccine doses have been administered (Organization and (WHO, 2021).

Coronavirus was discovered to be a beta coronavirus related to the coronavirus which also causes severe acute respiratory syndrome (SARS-CoV) . SARS-CoV-2 is a virus that causes SARS (Varnaitė *et al*., 2020). These viruses are encased in positive-sense single-stranded RNA viruses with a diameter of 80–220 nm (Wu *et al*., 2020). Under electron microscopy, the envelop bears crown-like, 20-nm-long spikes that look similar to the corona of the sun, hence the name coronavirus (Hu *et al*., 2021).

The virus is capable of causing sickness in both animals and humans. Among the currently known RNA viruses, it has the largest genome (Su Eun Park, 2020). Furthermore, Coronaviruses is related to the Coronavirinae subfamily of the Coronaviridae family and the Nidovirales order. There are now six coronaviruses known to cause human illness. Humans are infected with four different coronaviruses. 229E, OC43, NL63, and HKU1 are human coronaviruses (HCV). SARS-CoV and MERS-CoV are two pandemic human coronaviruses (Siddell *et al*., 2019).

A nucleoprotein is present within the coronavirus particle (N), the viral envelop (E) surrounds this helical nucleocapsid (Van Doremalen *et al*., 2020). Coronavirus dissemination has been proposed from contaminated dry surfaces, including self-inoculation of the mucous membranes of the nose, eyes, and mouth, highlighting the significance of a complete understanding of coronavirus CoV persistence on inanimate surfaces (Vella *et al*., 2020).

The immune system is the best defense because it supports the body's natural ability to protect itself against pathogens such as viruses, bacteria, fungi, protozoans, and worms (Grigoryan and Pulendran, 2020). Individual differences in protective immunity can occur as a result of genetic differences. Human leukocyte antigen (HLA) molecules from a haplotype with higher binding specificities to SARS-CoV-2 peptides on antigen-presenting cells may confer a genetic advantage (Shi and Wang, 2020). The identification of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors trigger the antiviral immune response, which is normally coordinated by IFN cytokines that activate cells and boost the response against these invaders (PRRs). Nuclear factor B (NF-B) is activated by signaling downstream of these PRRs, resulting in the production of inflammatory cytokines and phosphate (Lee *et al.*, 2020).

Toll-like receptors (TLR) are important for pathogen identification and innate immune activation (Li *et al*., 2013). which is expressed on the surface of endosomes primarily in the lungs, placenta, and spleen, may play a key role in COVID-19 (Delneste, *et al.*,2007). This receptor has been shown to recognize single-stranded SARS-CoV-1 RNA quickly, causing plasmacytoid dendritic cells to produce pro-inflammatory cytokines such as TNF-, IL-6, and IL-12 (Libbey and Fujinami, 2014).

COVID-19 has been transmitted in Iraq mainly through people who had visited Iran (Mikhael and Al-Jumaili, 2020). On February 24, 2020, the first case of COVID-19 in Iraq was reported in Al-Najaf city, south of Baghdad. The Iraqi Ministry of Health (MOH) announced 101 confirmed cases of COVID-19 by the end of March 13, 2020 (Al-Jumaili and Hamed, 2020). Patients infected with SARS-CoV-2 were more likely to develop lymphocytopenia. These patients were also more likely to receive treatments that increased their risk of infection, such as intensive care unit (ICU) hospitalization, the use of broad-

spectrum antibiotics and corticosteroids, and any preexisting chronic diseases (diabetes, hypertension, etc.), make these patients' immune systems even more impaired (Salehi *et al*., 2020). These patients' exposure to any secondary infection is probable, such as viruses, bacteria, fungi, protozoan, and worms. *Candida albicans* is a common commensal fungus that colonizes the oropharyngeal cavity, gastrointestinal and vaginal tract, and healthy individuals' skin. *C. albicans* is found in the typical flora of the microbiome in 50% of the population.

Candida species can cause a variety of clinical symptoms, ranging from localized, superficial mucocutaneous illnesses to invasive, life-threatening infections that affect several organ systems (Talapko *et al*., 2021). Damage to the mucosal or skin barrier, as well as a weakened immune system, can allow these yeast to infiltrate the body and cause systemic infections. C. albicans, C. glabrata, C. tropicalis, C. parapsilosis, and C. krusei are among the species that cause the bulk of candidiasis cases globally (Singh *et al*.,2020). Although Candida pneumonia in immunocompromised patients is uncommon, the presence of Candida in the respiratory system of immunocompromised individuals should not be overlooked because it might increase morbidity and death (Pendleton *et al*., 2018).

1.2 Aims of the study

- To detect and assess the IgG, IgM antibodies titer in HCWs patients with COVID- 19 after convalescent.
- To study the role of IL6 in HCWs patients with COVID- 19 after convalescent.
- To study the relation between COVID-19 disease and candidiasis
- To study the role of Vitamin D3 in HCWs patients with COVID- 19 after convalescent.
- To study the role of CRP titer in HCWs patients with COVID- 19 after convalescent .