Relationship between Vitamin D AND IL6 in Convalescent Healthcare Workers with Covid-19 in Baquba Hospitals in Diyala Province

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Abstract

The outbreak of coronavirus disease 2019 (COVID-19) and pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has become a major concern globally. One hundred Convalescent HCWs patients with COVID-19 works in Diyala, Hospitals center, from October 2020 to March 2021 were included. We recruited 100 non-infected from healthy people and 100 PCR-confirmed infected HCWs. In this review, we have summarized and discussed recent immunological studies focusing on the response of the host immune system, cytokine storms such as IL6 have been discussed as part of immunopathology mechanisms in SARS-CoV-2 infection. This may help us understand patients' immune status with COVID-19, particularly those with severe clinical presentation, and form a basis for further immunotherapeutic investigations. This study calculate that there is the relation between IL6 and Vitamin D3 in convalescent patients, accounting for a range of previously described clinical predictors and, potentially directing future therapeutic strategies, including about Vitamin D.

Keywords: *IL6 and Vitamin D3 in convalescent healthcare workers.*

Introduction

The global pandemic of coronavirus disease 2019 (COVID-19), which began in Wuhan, China, in December 2019 (1). Has quickly spread to more than 58 countries(2). These viruses are enveloped positive-sense single-stranded RNA viruses sized 80–220 nm in diameter (3). The envelope bears crown-like, 20-nm in length spikes that look like the corona of the sun under electron microscopy, hence given its name coronavirus (4). The immune system is the best defence because it supports the body's natural capability to protect against pathogens (eg, viruses, bacteria, fungi, protozoan, and worms (5). An antiviral immune response is typically coordinated by IFN-type cytokines that activate cells and increase the response against these invading agents, triggered by the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), Signaling downstream of these PRRs induces the activation of nuclear factor κB (NF-κB) to produce inflammatory cytokines and phosphorylation of interferon regulatory factors (6). Such as toll-like receptors (TLR), fundamental for pathogen recognition and activation of innate immunity (7). Type 7 of TLR (TLR7) – expressed on the surface of endosomes predominantly in the lungs, placenta, and spleen – might play a central role in COVID-19 (8). This receptor has been reported to quickly recognize single-stranded SARS-CoV-1 RNA, inducing the production of pro-inflammatory cytokines such as TNF-α, IL-6, and IL-

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12 in plasmacytoid dendritic cells[9]. In Iraq, obtained COVID-19 primarily via people who have visited Iran [10]. Vasculitic processes essential organ damage in seriously ill patients, induced by the activation of inflammatory cascades, complement activation, and pro-inflammatory cytokines (i.e. interleukin (IL)-6) [11]. It has been proposed that ineffective early innate antiviral response followed by impaired adaptive immune responses and hyper inflammation may lead to micro thrombosis and tissue injury, resulting in ARDS, multiorgan failure, and death [12]. When produced Vitamin D3 in the skin through the action of UVB radiation reaching 7-dehydrocholesterol in the skin, followed by a thermal reaction, Vitamin D has been found to modulate macrophages' response, preventing them from releasing too many inflammatory cytokines and chemokines. Found that calcitriol (1,25 -dihydroxy vitamin D3) exerted a pronounced impact on the ACE2/Ang (1–7) MasR axis with enhanced expression of ACE2, Masr and Ang (1–7) generation [13], Another property of vitamin D relevant both to antibacterial and antiviral mechanisms are promoting autophagy. Autophagy is a fundamental biological process that maintains cellular homeostasis via intracellular membrane encapsulation of damaged organelles and misfolded proteins. Laboratory methods ready for the detection of IL6 include enzyme-linked immunosorbent assay (ELISA) [14].

Materials and Method

Collection of Sample

The present study was is taking place occurring in Baquba City, the center of Diyala province. The population of this study includes convalescent healthcare workers patients during the period from October 2020 to March 2021. It included 100 health workers patients previously diagnosed with coronavirus infection, their age range from (20-65 years) and 100 healthy humans as control, their age range from (20-65 years).

IL6 Detection

The IL-6-ELISA is a solid phase Enzyme Amplified Sensitivity Immunoassay performed on the microtiter plate. The amount of substrate turnover is determined colorimetrically by measuring the absorbance, which is proportional to the IL-6 concentration. A calibration curve is plotted and IL-6 concentration in samples is determined by interpolation from the calibration curve.

Table (1) show Laboratory diagnostic kits use in the study.

Diagnostic kits	Manufacture Company	Country of Origin
Interleukin-6 human ELISA(ELISA) kits	Demeditec Diagnostics GmbH	(Germany)
Elecsys Vitamin D total II	Roche COBAS E411	Japan

Vitamin D detection

The total duration of the assay: 27 minutes

· 1st incubation: By incubating the sample (12 µL) with pretreatment reagents 1 and 2, bound 25 hydroxy vitamin D is released from the VDBP.

· 2nd incubation: By incubating the pretreated sample with the ruthenium labeled vitamin D binding protein, a complex between the 25 hydroxy vitamin D and the ruthenylated VDBP is formed. A specific unlabeled antibody binds to 24, 25 dihydroxy vitamin D present in the sample and inhibits cross-reactivity to this vitamin D metabolite.

· 3rd incubation: After the addition of streptavidin-coated microparticles and 25 hydroxy vitamin D labeled with biotin, unbound ruthenium labeled VDBPs become occupied. A complex consisting of the ruthenylated VDBP and the biotinylated 25 hydroxy vitamin D is formed and becomes bound to the solid phase via the interaction of biotin and streptavidin.

· The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed with Pro Cell II M. Application of a voltage to the electrode then induces chemiluminescent emission which is measured by a photomultiplier.

· Results are determined via a calibration curve which is an instrument specifically generated by 2 point calibration and a master curve provided via the cobas link.

Statistical Analysis

Using SPSS (package or social science) statistical program. The significant value was chosen at the level (p=0.05).

Results

The immunity (IgM, IgG titer) against Covid -19 among control group

One hundred healthy individuals were included in this study to detect immunity. The healthy individuals were randomly chosen from blood donors who were attended to the Central Blood Bank in Baquba and from healthy individuals who were attended to the Public Health Laboratory for pre-marriage medical checkups, and all of them were negative for Covid IgM, IgG titer. As shown in table (2).

Table (2): The immunity against Covid-19 among control group.

		IgM titer	IgG titer	IgM of control group	IgG of control group
N	Valid	100	100	100	100
	Missing	0	0	0	0
Mean		.07813	263.15048	.0433	4.4800
Std. Error of Mean		.015049	31.121927	.00091	.22144
Median		.04800	141.91000	.0400	4.9500
Mode		.043	14.220	.04	6.00
Std. Deviation		.150486	311.219270	.00911	2.21442
Variance		.023	96857.434	.000	4.904
Skewness		8.339	1.695	.520	-.181-
Std. Error of Skewness		.241	.241	.241	.241
Kurtosis		76.205	2.713	-.100-	-1.064-
Std. Error of Kurtosis		.478	.478	.478	.478
Minimum		.032	4.780	.03	.50
Maximum		1.472	1536.210	.07	8.80
Sum		7.813	26315.048	4.33	448.00

The immunity in convalescent healthcare workers according to age

The results showed there is a significant difference at p-value ($p< 0.05$) in the immunity in convalescent healthcare workers according to age. As shown in table (3).

Table (3): The immunity in convalescent healthcare workers according to age

Age		COV19 IgG titer/after 6 ths
(20-30) Years	Mean	172.6334
	N	53
	Std. Deviation	236.28635
(31-40) Years	Mean	397.9152
	N	19
	Std. Deviation	303.28023
(41-50) Years	Mean	308.6800
	N	21
	Std. Deviation	334.00402
(51-60) Years	Mean	446.1157
	N	7
	Std. Deviation	538.77731
Total	Mean	263.1505
	N	100
	Std. Deviation	311.21927

Sig. P value after 6= 0.006

The immunity in convalescent healthcare workers according to gender

The study showed there is no significant difference at p-value ($p< 0.05$) in the immunity in convalescent healthcare workers according to gender. As shown in figure (1).

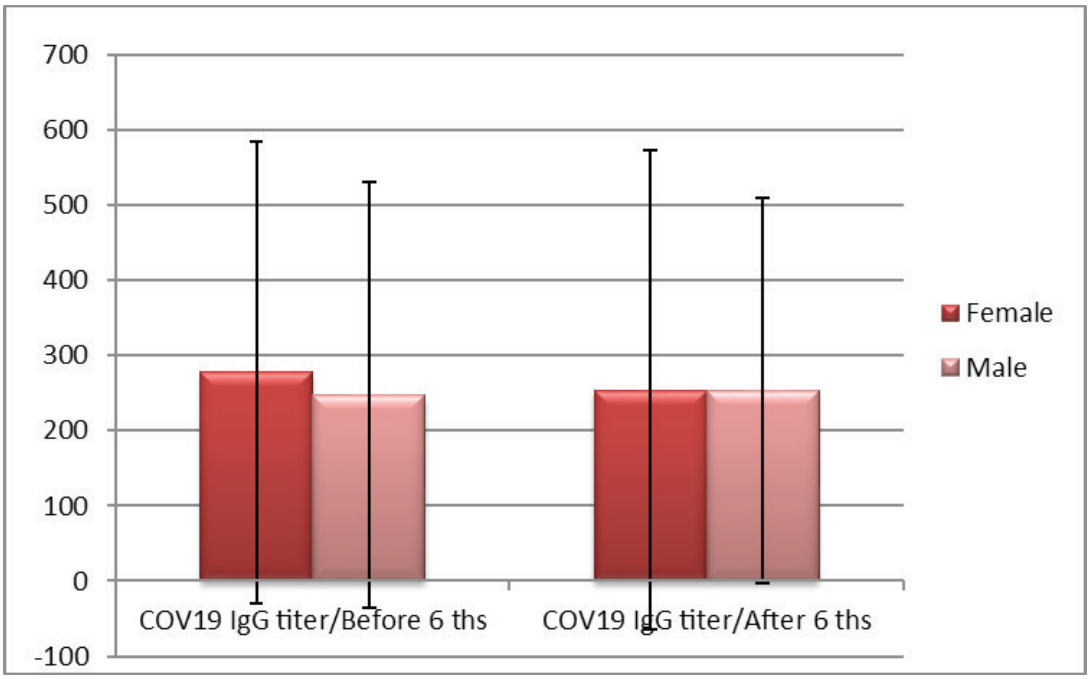

Figure (1): The immunity in convalescent healthcare workers according to gender

Correlations study in convalescent healthcare workers were investigated with IL6.VIT D3 titers

The present study shows the convalescent healthcare workers were investigated with IL6. VIT D3 titers there is the relation between IL6 and VITD3 at p-value ($p< 0.01$) as shown in table (4)

Table (4): Correlations study in convalescent healthcare workers were investigated with IL6.VIT D3 titers.

		IL6	D3 Ng/mL
IL6	Pearson Correlation	1	.282**
	Sig. (2-tailed)		.004
	N	100	100
D3 ng/mL	Pearson Correlation	.282**	1
	Sig. (2-tailed)	.004	
	N	100	100

Discussion

One hundred healthy people were included in this study to detect Covid IgM and IgG titer. None of these people was positive for Covid (IgM or IgG) titer. These results are obviously lower than those obtained in another study in the closer surrounding countries in the Turkish population the IgG antibodies against SARS-CoV-2 in serum samples of all participants were detected by chemiluminescent microparticles immunoassay. The rate of seroprevalence was 2.7% among non-infected [15]. This variation in occurrence between studies may be due to different epidemiological trends of Coronavirus infection in

different countries, which may be due to environmental factors geographical factors, the difference in host genetic susceptibility, Sampling size, immune status and detection technique and/or various viral strains circulating in different parts of the world. Environmental factors such as high temperature and high relative humidity are the first and most important causes [16]. According to age the results of this study revealed that there was a significant increase at ($p < 0.05$) in the immunity in convalescent healthcare workers according to age with age .similar findings were reported by others [17].

This observation can be explained by the fact that they showed neutralizing antibodies IgG have a positive correlation with age. According to gender, the obtained results from this study revealed that the number of females infected with Covid 19 was higher than the number of males. While statistical analysis showed no significant differences at ($p< 0.05$) between males and females. Because the patients in this study were in the convalescent stage and the small size of the sample, these results disagree with [18,19]. This observation can be explained by the fact that they showed neutralizing antibodies IgG have a positive correlation with gender, Male sex is also associated with a greater risk of more severe COVID-19 outcomes. Also, this study showed a positive significant correlation between Interleukin6 and Vitamin D3 [20]. that IL6 elevation lies at the nexus between low vitamin D status and higher risk of Covid-19 infection, severe morbidity and death in these vulnerable populations. It further proposes that these IL6-mediated risks can be ameliorated by vitamin D supplementation which appears to inhibit IL6 expression. This result was reported also by [21].

Conclusion

Positive relation between IL6 and Vitamin D3 has been calculated in convalescent patients, accounting for a range of previously described clinical predictors and, potentially directing future therapeutic strategies, including about Vitamin D.

Conflict of Interest: None

Funding: Self

Ethical Clearance: Not required

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