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Determination of Serum Vitamin D Levels in Patients with COVID-19

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ABSTRACT

Background: In 2019, the first cases of an acute respiratory infectious disease were announced in the city of Wuhan, China. In places where the speed of transmission and the resulting high prevalence and death from this virus is high, it is important to find things that help prevent and reduce the symptoms and complications of the disease, one of these things. Things are serum levels of vitamin D. As a result of this study, serum levels of vitamin D were measured in patients with Covid-19.

Methods: From December to March of 1400, 100 samples of people hospitalized in Khurshid laboratory were examined to identify the RNA of the Covid-19 virus by Real Time PCR method and at the same time to check the serum levels of vitamin D. The data was statistically analyzed using SPSS software.

Results: The average age of the patients was between 12.45 and 40.8 years. Comparison the two Covid-19 positive and negative groups in terms of symptoms, it was found that the patients with RH positive had more positive PCR percentage and a significant difference was reported between RH positive and Covid-19 ($p=0.006$). In comparing the relationship between disease symptoms and the rate of PCR positive reports of gastrointestinal symptoms, history of significant disease, cough, fever, a significant difference was reported ($p<0.001$). Also, 50% of the samples were PCR positive. Based on the t-test, a significant difference was reported between the serum levels of vitamin D and Covid-19 ($p<0.001$).

Conclusion: The results showed that the vitamin D is an acceptable protective factor against Covid-19 infection and its deficiency increases the probability of infection.

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Introduction

As a worldwide public health emergency, coronavirus disease 2019 (Covid-19) has had an effect on more than 180 million individuals in 222 countries (1) since the first outbreak in Wuhan, China (2). Since June 22, 2021, Oceania and Europe were reported with the lowest (n=70,000) and highest (n=47,500,000) numbers of confirmed cases (1).

The first confirmed Covid-19 case in Iran was identified in Qom on February 19, 2020. Since March 16, 2020, a total of 14,991 Covid-19 patients with 853 mortalities (CFR=5.69%) have been reported from all 29 provinces of Iran, among which Tehran has been announced with the highest incidence (n=3,774) (4).

By identifying people at high risk for severe forms of the disease, adequate prevention strategies can be applied to these people (3).

The evidences have shown that vitamin D deficiency is associated with the incidence of upper respiratory tract infections and mortality from the new coronavirus (5 , 14).

A large number of studies have previously examined the association between low levels of vitamin D and high rates of other types of respiratory infections (6). Vitamin D affects innate and adaptive immunity in a number of ways, with vitamin D receptors being continuously expressed at the surface of epithelial cells as well as immune system cells such as monocytes and macrophages (5). Therefore, vitamin D can have a protective role against Covid-19 (7). One of the roles of vitamin D is to suppress the over-release of inflammatory cytokines that may lead to cytokine storms. This complication is one of the main causes of mortality and complications of Covid-19 (8).

Currently, the most prevalent and reliable technique to detect Covid-19 is real-time polymerase chain reaction (PCR) (9). Different fragments of viral components are used by different manufacturers in this experiment, which is mostly related to one or more antigens of viral antigens such as coat, nucleocapsid, spike and ORF1 genes (10).

During the global epidemic of Covid-19 , various reports have been reported on the beneficial effects of vitamin D in the prevention of the disease and reduction of disease severity in infected people (11, 12). Today, a large part of the global population is at risk of vitamin D deficiency (13, 14).

Considering the high prevalence of vitamin D deficiency in Iran (15) and the high rate of death associated with Covid-19 in Iran, in order to identify the factors affecting the incidence and severity of the disease, this study aimed to assess the concentration of vitamin D in serum samples of Covid-19 cases. The probable association between the vitamin D concentration aand Covid-related mortality in Baqiyatallah Hospital patients was analyzed.

Materials and Methods

This study was performed on 100 people who were suspected of Covid-19 and admitted to khorshid laboratory in Tehran from January to March 1400.

Sampling

A specialist and skilled nurse, using a sterile swab of the nasopharyngeal secretions and 2 ml of blood clots from each patient is sampled. Respiratory specimens (including nasopharyngeal and oropharyngeal swabs) of symptomatic cases admitted to different medical centers were instantly placed into sterile tubes with 3 mL of viral transport media. The medium forms Hank's balanced salt solution at pH 7.4 that contained bovine serum albumin (1%), amphotericin (15 µg/mL), penicillin G (100 units/mL), and streptomycin (50 g/mL). The specimens (3 ml) were stored at 4-8 °C and -70 °C for short and long

periods, respectively. blood samples to clot tubes and referred to a hormonal laboratory.

RNA Extrcation and Real Time PCR

An RNA extraction kit (Gene All Biotechnology, Seoul, South Korea) was used to extract viral ribonucleic acid (RNA) from 300 µL of nasopharyngeal and oropharyngeal swabs of symptomatic Covid-19 cases, which was eluted in 50 µL of nuclease-free water. The RNA extracts were stored at − 70 °C for more examinations. RT-qPCR primer-probe sets designed on RdRp and N genes introduced by World Health Organization were employed to detect severe acute respiratory syndrome coronavirus 2 (49). Based on the manufacturer's instructions, RT-qPCR was carried out for N and RdRp genes by the one-step RT-qPCR kit (Sansure Biotech, Changsha/Hunan, China) for all samples. In addition, as an internal control, the RNase P gene was utilized to monitor the validity of sample collection and the RT-qPCR process to avoid false-negative results. Thermal cycle conditions included 30 minutes at 50 °C for reverse transcription, 1 minute at 95 °C for PCR initiation activation, and 45 cycles of 95 °C at 15 and 30 seconds at 60 °C (Table 2). The sequence of the primer-probe set is reported in Table 1.

Measurement of vitamin D levels

For the determination of the vitamin D level, blood samples taken from patients were transferred to Baqiyatallah Hospital laboratory, and after centrifugation, serum levels of vitamin D were examined by Pishtaz ELISA kit, and the results were recorded as below:

Vitamin D deficiency is defined as a serum 25(OH) D level of < 20 ng/mL according to several guidelines (50). Moreover, the measurements were carried out, and 30 ng/ml was determined as the cut-off point of 25(OH) D to define vitamin D deficiency (51). Serum 25 (OH)D concentration was measured using automated immunoassays by Architect i1000sr 2015 last calibration on June 2, 2020. Calculations were performed according to relevant guidelines and regulations.

Then, the significant effective factors were evaluated simultaneously using logistic regression model and data was analyzed using SPSS 16 software.

Result

The age of Covid-19 cases was between 12.45 and 40.8 years. The oldest patient was 69 years old. 22 female and 28 male has covid PCR positive. No significant difference was observed between age (P=0.811) and gender (P=0.841) with Covid-19 incidence (Table 3).

Based on clinical signs of patients with positive Covid-PCR, 60% of the samples had RH +, 89.36% of the samples reported weakness and lethargy, 77.27 % gastrointestinal symptoms, 93.33% a history of a specific disease, 88.64% a cough and 12.5 % a fever (Table 4).

Comparing the two groups of positive and negative Covid in terms of symptoms, patients with RH + had a higher percentage of positive PCR, and a significant difference was reported between RH + and Covid (p=0.006). In comparing the relationship between disease symptoms and positive PCR reporting rate of gastrointestinal symptoms, significant disease history, cough, fever, significant difference was reported (p<0.001) as showed in Table 5. In the PCR test on the samples, 50% of the samples were PCR positive (Table 5).

The average levels of vitamin D in Covid-19 positive and negative subjects were 26.24 and 33.93%, respectively. Based on t-test, there was a significant difference between serum vitamin D levels and Covid-19 (P<0.001) (Table 6).

Table 1. Primer–probe set sequences.

Gene	Primer (5′–3′)	Probe (5′–3′)
N	F:GAC CCCAAA ATCAGC GAA AT R: TCTGGT TAC TGC CAGTTGAATCTG	FAM-ACC CCG CAT TAC GTT TGGTGGACC -BBQ
RdRP	F: GTGARATGGTCATGTGTG GCGG R: CAR ATG TTAAASACACTATTAGCATA	FAM-CAG GTG GAACCTCATCAG GAGATG C-BBQ
RNAase P	F: AGATTTGGACCTGCGAGC G R: GAG CGG CTGTCTCCACAAGT	FAM-TTC TGACCTGAAGGC TCTGCGCG-BBQ

Table 2. Polymerase chain reactions programming.

Function		Temperature	time	Nu. of cycles
reverse transcription		50 °C	30 min	1
PCR initiation activation		95 °C	1 min	1
Real time PCR	Denaturation	95 °C	15 s	45
	Annealing	60 °C	30 s	
	Extension	72 °C	15 s	

Table 3. Comparison of two groups of Covid-19 positive and negative in terms of age and sex.

		Covid-19 PCR				
		Negative		Positive		P.value
		Count	Percent	Count	Percent	
Sex	Female	23	51.11	22	48.89	0.841*
	Male	27	49.09	28	50.91	
Age	Standard deviation	39.78±14.30		40.38±10.42		0.811**

Table 4. Comparison of two groups of Covid-19 positive and negative in terms of symptoms.

		Covid-19 PCR				P.value
		Negative		Positive		
		Count	Percent	Count	Percent	
RH	Negative	24	68.57	11	31.43	0.006
	Positive	26	40.00	39	60.00	
Sluggishness and lethargy	No	45	84.91	8	15.09	<0.001
	Yes	5	10.64	42	89.36	
Gastrointestinal symptoms	No	45	57.69	33	42.31	0.004
	Yes	5	22.73	17	77.27	
History of a specific disease	No	49	57.65	36	42.35	<0.001
	Yes	1	6.67	14	93.33	
Cough	No	45	80.36	11	19.64	<0.001
	Yes	5	11.36	39	88.64	
Fever	No	49	87.50	7	12.50	<0.001
	Yes	1	2.27	43	97.73	

Table 5. Frequency distribution of the samples according to the result of PCR test of Covid-19 disease.

		Count	Percent
Covid-19 PCR	Negative	50	50.00
	Positive	50	50.00
	Total	100	100.00

Table 6. Comparison of vitamin D levels in two groups of Covid-19 positive and negative.

		Covid-19 PCR		
		Negative	Positive	Total
Vitamin D Level	Mean	33.93	26.24	30.08
	Standard deviation	9.83	8.86	10.08
	Middle	34.50	26.50	30.40
	lowest	8.40	7.00	7.00
	highest	59.00	55.00	59.00
	Low limit	31.13	23.72	28.08
	Upper limit	36.72	28.75	32.08
	P-Value*	P<0.001		

Discussion

The Covid-19 outbreak has caused in a great public health challenge (16). Vitamin D, a central hormone, plays a role in regulating immune response and preventing inflammation and autoimmunity (17). Based on the evidence, vitamin D supplementation decreases disease severity in those who have a respiratory infection in clinical trials (18).

The 25(OH)D level and Covid-19 infection are possibly associated, according to recent studies (19). Although researchers try to find effective drugs for the treatment of Covid-19, supplemental vitamin D possibly has potential for the prevention of virus infection or reduction of disease severity (20).

The current study assessed the serum 25-hydroxyvitamin D level in Covid-19 cases. Based on the obtained findings, there was a significant decrease in the mean serum level of vitamin D in Covid-19 positive patients.

Hosack et al. also observed that deficient serum levels of Vitamin D3 are associated with elevated levels of predictive markers such as cytokine storm, indicating a higher risk of severe Covid-19 disease. Hence, the role of this substance in the prevalence and severity of the disease tend to be significant. Although no statistically significant difference was found between Vitamin D3 deficiency and the mortality rate attributed to coronavirus infection, so, further research is needed to fully elucidate the relationship (21). In this regard, Aygun in his study stated that vitamins D3 is prevented by cytokine storm by several mechanisms and exacerbation of Covid-19 disease and multiple organ failure syndrome (Multiple organ dysfunction syndrome) Prevention Does (22). In addition, Chandran et al. have highlighted the role that vitamin D3 playes in innate and adaptive immune function (Innate and adaptive), stated that the prescription of vitamins in preventing a cytokine storm is like water on fire (23).

Also Im et al., have analyzed the vitamins and minerals in patients with coronavirus, and showed that the serum vitamin D levels were remarkably lower in Covid-19 subjects than in controls (24). Additionally, the association of serum vitamin D levels and Covid-19 infection has been studied such that vitamin D supplementation may have the potential to prevent or treat Covid 19 disease (25).

Ghasemian et al. (2020) found that a remarkable number of Covid-19 cases suffer from vitamin D deficiency, which proves the relationship of vitamin D levels and Covid-19 (26). A study in 2021 assessed the serum vitamin D level in Covid-19 cases and highlighted the lower level of serum vitamin D level in Covid-19 cases comparing to those in healthy individuals and the frequency of vitamin D deficiency is higher in Covid-19 patients (27)

According to a study by Baktash et al., Which examined the status of serum vitamin D and the final outcome of the disease in patients with Covid-19. Patients with lower levels of 25-hydroxyvitamin D received vitamin D supplementation and serum levels than patients of the same age. Their vitamin D was normal and had a more adverse outcome (28).

A large body of evidence has shown the link of vitamin D deficiency with poor disease outcomes and lower Covid-19 incidence (29-30).

Furthermore, comparing the serum levels of vitamin D between patients infected with the Covid-19 virus and non-infected individuals has indicated that vitamin D serves as a significant protective factor against Covid-19 disease, and its deficiency escalates the risk of contracting the illness (31).

The most typical form of Covid-19 is respiratory involvement. Furthermore, fever, dry cough, shortness of breath, and sore throat are the most frequent symptoms of the disease (32, 33). In a study in Iran (34), fever and cough were reported as the most important symptoms of Covid-19, becoming more frequent with age (37). There are also non-respiratory forms of the disease, with

11% of patients having gastrointestinal symptoms (35).

A 2021 review study of the epidemiology, pathophysiology, and clinical signs of Covid 19 infection to guide policy-making and knowledge-based attitudes reported fever and cough as the most common symptoms (36).

In our study, fever, cough, lethargy and lethargy included a higher percentage of symptoms.

Kazeminia et al. (2020) aimed to assess the symptoms of fever and cough in patients with COVID-19. This study was performed on 2131 patients and the overall prevalence rates of cough and fever in COVID-19 cases were 54.9% and 2.78%, respectively. According to the findings of the aforementioned study, the prevalence of fever and cough are high as two important factors in identifying the cases with Covid 19 (34). In the study of Shi et al., Which was performed to evaluate the symptoms in Covid 19 patients, the prevalence of fever was 72.8% and the prevalence of cough was 59.2%, which were the most common symptoms (38). In the study of Tian et al., the prevalence of fever was 82% and the prevalence of cough was 45.8% (39). In the study of Song et al., The prevalence of fever was 96% and the prevalence of cough was 47% (40).

We now know that ABO and Rh blood types are among the factors that may cause susceptibility or resistance to viral attacks such as influenza, Ebola, intestinal viruses, and SARS-COV infections, as well as the prognosis of infectious diseases transition (41-44).

RH blood group (Rhesus) which is determined by the presence or absence of RH or D antigen (45). Some studies have not found a significant relationship between RH and the incidence of Covid 19 (46), while in some studies RH has been positively associated with disease progression, which seems to be a new finding and requires further study (43), which is in agreement with the findings of the current investigation.

In this study, a significant difference was reported in gastrointestinal symptoms in covid 19 patient. A 2020 study of 111 patients with clinical presentation, training, and mortality risk factors for the high prevalence of Covid 19 in the United States. The most common symptoms were fever, 80.2%, and cough 43.2%. 42.3% lethargy, 32.4% fatigue was also reported (47). A study was conducted in 2020 on 6119 patientsin which, the prevalence rates of gastrointestinal symptoms in the aforementioned subjects were 91.3%, 79.13%, 41.73%, and 18.89% for anorexia, nausea and vomiting, diarrhea, and abdominal pain, respectively. (48).

Conclusion

Real Time PCR is the golden standard for the detection of many pathogens (48). Real-time PCR leads to easy detection of products and helps with quantifying and qualification of a wide range of sequences of viral nucleic acids (49). ELISA test is considered as a preliminary and screening test for CMV infections, IgG detected mostly higher percentage than IgM for all CMV infections (50). It should be noted that the incidence of cytomegalovirus infection has increased with age. On the other hand, due to the significant relationship between molecular and serological methods for the diagnosis of cytomegalovirus, a stronger and faster molecular method for the diagnosis of this virus is recommended.

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Ethics approval and consent to participate

All the procedures were approved by the institutional and/or national research committee and in compliance with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Additionally, this study obtained the approval of the Ethics Committee of Azad University of Medical Sciences, Iran (Code no: IR.IAU.CHALUS.REC.1400.122).

Conflict of interest

The authors declare no conflict of interest.

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