European Review for Medical and Pharmacological Sciences 2022; 26: 3038-3045

Prevalence of vitamin D deficiency among patients attending Post COVID-19 follow-up clinic: a cross-sectional study

A.A.R. MOHAMED HUSSEIN[1], I. GALAL[2], M.T. AMIN[3], A.A. MOSHNIB[3], N.A. MAKHLOUF[4], H.A. MAKHLOUF[3], H.K. ABD-ELAAL[5], K.M.S. KHOLIEF[6], D.A. ABDEL TAWAB[4], K.A. KAMAL ELDIN[3], A.M. ATTIA[3], A.E.A. OTHMAN[3], J. SHAH[7], H. AIASH[8,9]

[1]Chest Department, Assiut University Hospitals, Assiut, Egypt
[2]Faculty of Medicine, Aswan University, Aswan, Egypt
[3]Faculty of Medicine, Assiut University, Assiut, Egypt
[4]Department of Tropical Medicine and Gastroenterology, Faculty of Medicine, Assiut University, Assiut, Egypt
[5]Faculty of Nursing, Assiut University, Assiut, Egypt
[6]Public Health and Community Medicine Department, Assiut Faculty of Medicine, Assiut, Egypt
[7]Medical Research Center, Kateb University, Kabul, Afghanistan
[8]Department of Family Medicine, Suez Canal University, Ismailia, Egypt
[9]Department of Cardiovascular Perfusion, State University of New York Upstate Medical University, Syracuse, New York (NY), USA

Abstract. – OBJECTIVE: Post-COVID-19 syndrome appears to be a multi-organ illness with a broad spectrum of manifestations, occurring after even mild acute illness. Limited data currently available has suggested that vitamin D deficiency may play a role in COVID-19 cases. However, to our knowledge, no study has examined the frequency of vitamin D deficiency in post-COVID-19 cases and its effect on the symptom severity. The aim of this study is to both screen the frequency of vitamin D deficiency in post-COVID-19 syndrome patients and to study its relation to persistent symptoms.

PATIENTS AND METHODS: A cross-sectional, single-center study was conducted involving all cases attending post-COVID-19 follow-up clinic from November 2020 to May 2021. Complete history, clinical examination, and laboratory analysis [kidney functions, serum calcium, C-reactive protein, serum ferritin, Serum 25-(OH) vitamin D] was done as well as HRCT chest.

RESULTS: The study included 219 post-COVID-19 cases, 84% had deficient vitamin D levels (< 20 ng/dL); 11.4% had insufficient level (20-30 ng/dL) and only 4.9 % reported normal level. There was no link between levels of vitamin D with either the acute or post-COVID-19 symptoms in the studied groups.

CONCLUSIONS: Despite the prevalence of vitamin D deficiency among the study population, no association was observed between the levels of vitamin D and post-COVID-19 symptoms. It appears that post-COVID-19 syndrome pathophysiology involves a more complex interaction with the immune system. Dedicated clinical trials are advised to better study vitamin D levels and the related disease severity in COVID-19 patients.

Key Words:
Vitamin D, Vitamin D deficiency, Post-COVID-19, Long-COVID-19, Symptoms.

Abbreviations
ELISA: Enzyme-Linked Immunosorbent Assay.

Introduction

Vitamin D has natural properties that are classified into two categories: first, the standard action in the metabolism of calcium and phosphorus; and second, the non-traditional pathway that principally affects the immunological system, inflammation, anti-oxidation, anti-fibrosis and other systems[1]. The anti-inflammatory role

Corresponding Author: Jaffer Shah, BS; e-mail: jshah6@pride.hofstra.edu

is considered to be through anticipation of endothelial injury provoked by augmented neutrophil extracellular traps development[2]. The anticipated positive effects of vitamin D on immunity are expanding the blood oxygen-carrying capacity, modulating the immune system, accelerating the acute lung injury recovery rate, and adjusting the renin-angiotensin system[3].

Studies have shown that the level of vitamin D is closely related to the incidence and progression of multiple chronic conditions, for instance, cancers, autoimmune disorders, metabolic disorders, and contagious diseases[4]. Its deficiency principally affects the progress of many chronic disorders and, as the age of the population increased, the numbers of cases with osteoporosis, cardiovascular disorders, malignancy, diabetes, and neuropsychiatric diseases noticeably increase, resulting in diminution of the life quality, considerable social and financial burdens, or even decease[5].

Recently, multiple reviews have illustrated a negative association between the serum vitamin D level in Europe with reported COVID-19 contagion rate[6] and non-significant negative association with unfavorable outcome[7]. Some evidence-based studies[8] recommended that improvement in vitamin D status by supplementation may diminish COVID-19 clinical hazards.

Post-COVID-19 appears to be a multi-systemic illness, sometimes occurring after a reasonably mild acute complaint[9]. In the lack of agreed definitions, for the clinical purposes, post-acute COVID-19 was cleared as extending beyond 21 days from the onset of the initial complaints and chronic COVID-19 as extending beyond 3 months[10]. It may be assumed that the majority of survivors with a mildly symptomatic presentation (80%) will not present long-term sequala and will eventually fully convalesce[11]. However, studies showed that even so-called mild COVID-19 may be related to persistent complaints, most frequently cough, mild fever, and lethargy, dyspnea, chest pain, headache, neuro-cognitive troubles, myalgia, weakness, gastrointestinal troubles, skin rash, metabolic abnormalities, thrombo-embolic disorders, hopelessness, and other mental diseases (with relapse or remission course)[12].

At the time of writing, we know of no study that has reported on the frequency of Vitamin D deficiency in post-COVID-19 cases, and the pathophysiology and natural history of post-acute and chronic COVID-19 is still unknown.

The aim of this work is to screen the frequency of vitamin D deficiency in post-COVID-19 and to study its relation to the persistent symptoms.

Patients and Methods

Setting

All adult participants ≥18 years of age who presented at post-COVID-19 clinic, Assiut University Hospital, for a follow-up program between November 2020 to May 2021 were recruited. The program measures the functional and clinical status, pulmonary functions, sleep disorders, and need for rehabilitation.

Written informed consent was obtained at the time of screening and the study was approved by Assiut Faculty of Medicine Ethical Committee. The research was in accordance with the Declaration of Helsinki and relevant national and international guidelines.

Study Design and Data Collection

A cross-sectional, single-center study was conducted. Data was collected and the medical records were generated using the case record format in the post-COVID-19 out-patients clinic. Complaints reported by participants were categorized into: constitutional (fever, myalgia, arthralgia, restriction of daily activity, tachycardia, headache, dizziness, and excessive sweating), respiratory (cough, sputum, dyspnea, chest pain, sore throat, and rhinorrhea), GIT (gastritis, anorexia, diarrhea, abdominal pain, and dysphagia) and neuropsychiatric (sleeping problems, tinnitus, anosmia and/or ageusia, memory loss, loss of concentration, anxiety and/or depression, and peripheral neuropathy). Patients were considered positive for the category if they reported the presence of one or more complaints among this category.

Symptoms scores, which were previously published for acute stage and post-COVID symptoms, were calculated to represent the severity of symptoms[13].

Inclusion criteria: All cases equal to or above 18 years of age with confirmed COVID-19 diagnoses in the prior 3 months attending the post-COVID-19 clinic from November 2020 to May 2021 were included.

Exclusion criteria: Recent COVID-19 < 90 days, recent acute infections, active malignancy, and pregnancy.

Laboratory Methods

1. 4 milliliters blood was withdrawn by venipuncture and disseminated into two plastic tubes that were allowed to clot for serum separation. Non-hemolyzed serum was divided by centrifugation, allowing one serum tube to be used for the determination of serum urea, creatinine, serum calcium, CRP, and serum ferritin on COBAS INTEGRA 400. Another serum tube was stored in aliquots at -20°C to measure vitamin D levels.
2. The level 25-hydroxy vitamin D in the serum was measured by the Enzyme-Linked Immunosorbent Assay (ELISA) method *via* a PerkinElmer Health Sciences kit (Catalogue No.: 10501). The vitamin D level was defined as sufficient if between 30-100 ng/mL, insufficient between 10-30 ng/mL and deficient if 0-10 ng/mL. The levels of 25(OH) D3 were < 20 ng/ml. Normal range levels of 25(OH) D3 were defined as ≥ 30 ng/ml and the serum levels between 20 and 30 ng/ml were classified as insufficiency category[14].

Statistical Analysis

Statistical analyses were performed using IBM SPSS Statistics version 20 (SPSS Corp., Armonk, NY, USA). Categorical data was presented as numbers and percentages and compared by Chi-square test, while continuous data was reported as means ± SD and/or median (min -max) and tested for normality using the Shapiro-Wilkes test. When quantitative data is normally distributed, Students' *t*-test is used while, for data that was not normally distributed, a Mann-Whitney test was used. In all statistical tests, *p*-value <0.05 was deliberated statistically considerable.

Results

The study included that in 219 post-COVID-19 cases, most of them had deficient vitamin D levels; 184 (84%) and 25 (11.4%), respectively (Figure 1). There was no statistically considerable variation between cases with normal, insufficient, and deficient vitamin D levels as regard demographic data, clinical data, and associated comorbidities (Table I).

Concerning the difference in other laboratory parameters, mean±SD levels in cases with normal vitamin D compared to low vitamin D were as follow: CRP was 5.0±3 *vs.* 6.2±0.4 ($p = 0.7$), serum calcium 8.6±1.8 *vs.* 8.4±2.1 ($p = 0.02$), serum ferritin 113.6±50 *vs.* 151±138.3 ($p < 0.001$), serum urea 67.2±54 *vs.* 69.4±75.0 ($p < 0.01$) and creatinine 1.6±1.0 *vs.* 5.2±1.5 ($p < 0.001$), respectively.

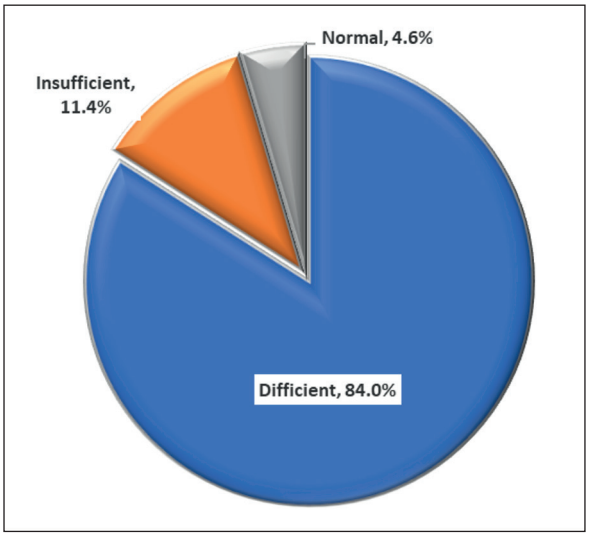

Figure 1. Distribution of Vitamin D levels of post-COVID cases included in the study (n=219). Normal > 30 ng/dL, insufficient 20-30 ng/dL, deficient < 20 ng/dL.

The associations between acute and post-COVID-19 symptoms according to the different levels of vitamin D (Table II and Table III) were presented.. As shown, there was no statistically considerable variance in either acute or post-COVID-19 symptoms between the three groups.

Discussion

This study examined 219 post-COVID-19 cases at Assiut University hospital out-patient clinic. The main results showed that vitamin D deficiency was established in 84% of cases and 11.4% were insufficient. 90% of cases with normal vitamin D levels received multi-vitamins during acute illness compared to 88% and 86.4% of cases with insufficient and deficient vitamin D, correspondingly. There was no statistically considerable variance in either acute or post-COVID-19 symptoms between the three groups.

The recorded frequency of abnormal vitamin D in the studied group was 95.4% (84% less than 20 ng/dL and 11.4% between 20-30 ng/dL). The living environments and circumstances, however, should be taken into account when considering the results, such as the typical weather conditions of the area which, for the majority of middle

Table I. Demographic and basic characteristics of post-COVID-19 participants (n=219).

	Total (n = 219)	Normal (n = 10)	Insufficient (n = 25)	Deficient (n = 184)	*p*-value*
Age (years)					
≤ 40	162 (74%)	9 (90%)	19 (76%)	134 (72.8%)	0.389
> 40	57 (26%)	1 (10%)	6 (24%)	50 (27.2%)	
Mean ± SD	35.1 ± 11.0	34.0 ± 4.9	36.0 ± 11.4	35.0 ± 11.3	0.890
Gender					
Male	65 (29.7%)	2 (20%)	10 (40%)	53 (28.8%)	0.408
Female	154 (70.3%)	8 (80%)	15 (60%)	131 (71.2%)	
BMI (213 cases#)					
Underweight (< 18.5kg/m^2)	85 (39.9%)	2 (22.2%)	10 (41.7%)	73 (40.6%)	0.467
Normal (18.5-24.9 kg/m^2)	76 (35.7%)	6 (66.7%)	10 (41.7%)	60 (33.3%)	
Overweight (25-29.9 kg/m^2)	26 (12.2%)	1 (11.1%)	2 (8.3%)	23 (12.8%)	
Obese (<30 kg/m^2)	26 (12.2%)	0	2 (8.3%)	24 (13.3%)	
Mean ± SD	21.1 ± 6.6	20.6 ± 3.4	20.8 ± 6.0	21.2 ± 6.8	0.984
Hospitalization					
Yes	34 (15.5%)	2 (20%)	3 (12%)	29 (15.8%)	0.820
No	185 (84.5%)	8 (80%)	22 (88%)	155 (84.2%)	
Need of oxygen therapy					
Yes	27 (12.3%)	1 (10%)	0	26 (14.1%)	0.128
No	192 (87.7%)	9 (90%)	25 (100%)	158 (85.9%)	
Comorbidities					
DM	11 (5%)	0	3 (12%)	8 (4.3%)	0.196
Hypertension	31 (14.2%)	1 (10%)	3 (12%)	27 (14.7%)	0.870
Cardiac disease	3 (1.4%)	1 (10%)	0	2 (1.1%)	0.051
Chronic pulmonary disease	17 (7.8%)	1 (10%)	3 (12%)	13 (7.1%)	0.663
Renal disease	6 (2.7%)	0	0	6 (3.3%)	0.556
Any chronic illness	51 (23.3%)	3 (30%)	7 (28%)	41 (22.3%)	0.716
No comorbidities	168 (76.7%)				

Data presented as numbers (%). #BMI was missing in 6 cases. *Student's *t*-test, Mann-Whitney & chi-square tests were used. BMI = body mass index; DM = diabetes mellitus.

eastern countries, are predominantly sunny. We should also consider the role of skin pigmentation in the production of vitamin D, and cultural and religious practices that require those to cover the whole, if not most, body[15]. A study[16] in Egypt reported high predominance of hypovitaminosis D among healthy women (77% deficient, 14% insufficient and only 9% normal). Furthermore, a study[17] of the nursing home residents in Egypt found major health troubles in the form of vitamin D deficiency and that this must be addressed with enduring strategies, such as public education, national health strategies for screening for adequate dietary vitamin D, calcium intake and public advice for the prevention of deficiencies, sufficient sun exposure for at least 10-15 minute

Table II. Differences in Acute stage symptoms in post-COVID-19 patients according to vitamin D levels (n = 219).

	Total (n = 219)	Normal (n = 10)	Insufficient (n = 25)	Deficient (n = 184)	*p*-value*
Reported symptoms (N & %)					
Constitutional	215 (98.2%)	10 (100%)	25 (100%)	180 (97.8%)	0.679
Respiratory	210 (95.9%)	9 (90%)	25 (100%)	176 (95.7%)	0.372
Gastrointestinal	204 (93.2%)	10 (100%)	24 (96%)	170 (92.4%)	0.543
Neuropsychiatric	215 (98.2%)	10 (100%)	25 (100%)	180 (97.8%)	0.679
Acute symptom score					
Mean ± SD	32.0 ± 14.8	35.1 ± 19.6	35.0 ± 11.9	31.5 ±14.9	0.484
Median (min – max)	30 (1-78)	32.5 (4-70)	30 (15-60)	30 (1-78)	

Data presented as numbers (%). *Mann-Whitney test and chi-square test were used.

Table III. Differences in post-COVID-19 symptoms in recruited patients according to vitamin D levels (n = 219).

	Total (n = 219)	Normal (n = 10)	Insufficient (n = 25)	Deficient (n = 184)	*p*-value*
Reported symptoms (N & %)					
Constitutional	197 (90%)	9 (90%)	24 (96%)	164 (89.1%)	0.563
Respiratory	148 (67.6%)	7 (70%)	21 (84%)	120 (81.1%)	0.168
Gastrointestinal	138 (63%)	8 (80%)	20 (80%)	110 (59.8%)	0.076
Neuropsychiatric	189 (86.3%)	9 (90%)	21 (84%)	159 (86.4%)	0.892
Post symptom score					
Mean ± SD	15.9 ± 13.9	19.2 ± 13.6	18.9 ± 12.3	15.3 ± 14.2	0.106
Median (min – max)	12 (1-77)	16 (1-45)	18 (1-49)	11 (1-77)	

Data presented as number (%). *Mann-Whitney test was used.

per day, and appropriate strength-training programs. Comparable vitamin D status reviews concluded that hypovitaminosis D is highly prevalent globally[18,19], in Northern India, China[20], and Africa[21]. This finding was in distinction with the extensively held view that the widespread sunlight in African and Asian countries was indicative of the most favorable vitamin D status[22].

In Western areas, hypovitaminosis D is common, affecting nearly half of the USA population, with higher rates among people with darker skin or reduced sunlight exposure, involving people living in high latitudes in the winter, health professionals[23], people existing in northern cities in the late winter[24], elderly adults, nursing home inhabitants[25], and populations who all have increased risk of vitamin D deficiency[26,27]. Incidence rates of severe hypovitaminosis D cleared as vitamin D level below 30 nmol/L of 5.9% in USA[28], 7.4% in Canada[29] and 13% in Europe[30] have been reported.

In the current study, cases with lower vitamin D had higher levels of inflammatory indices (CRP, $p = 0.7$ and ferritin, $p < 0.001$) and impaired renal function (higher levels of urea and creatinine, $p < 0.01$ and < 0.001 respectively). This is in accordance with studies showing that the level of vitamin D is closely related to the occurrence and development of many chronic conditions, such as malignancies, autoimmune diseases, metabolic disorders, and infectious diseases[4]. Vitamin D deficiency predominately affects disease development, causing impaired quality of life and considerable public and financial burdens and even loss of life[5]. The possible role of vitamin D may be explained by its regulatory role on acquired immunity and innate immunity where there is a complex interaction between vitamin D, infection, and the immune system[31]. Furthermore, comorbid conditions that are also related to hypovitaminosis D, such as diabetes, hypertension, overweight, and chronic kidney disorders tend to worsen COVID-19 contagion[32,33].

Vitamin D levels were not recorded during the acute stage in the recruited cases. It appears from both epidemiological data, biochemical, and immunological evidence that vitamin D could be an important illness-modifying agent in COVID-19. The effects of vitamin D on native immunity are likely to be valuable, but the overall suppression of adaptive immunity may interfere with the body's defense against the invading virus[34]. Hypovitaminosis D is associated with a poor prognosis for cases with COVID-19[35]. Plenty of clinical proof derived from some studies[36-38] advocate that vitamin D deficiency increases the hazard for severe illness or even decease among COVID-19 cases. Consequently, vitamin D supplementation has been shown to improve clinical outcomes in COVID-19 cases[39-42]. Vitamin D supplementation should have beneficial effects by reduction of the hyper-inflammatory response that is dependent on macrophage cells in the lungs of COVID-19 cases[43]. Furthermore, vitamin D supplementation might be allied with improved clinical outcomes, particularly when administered after the diagnosis of COVID-19[44].

As regards the association of lower serum vitamin D levels during post-COVID-19 with acute and post-COVID-19 symptoms, there was no statistically considerable variation in either acute or post-COVID-19 symptoms between the three studied groups (normal, insufficient and deficient Vitamin D).

Vitamin D deficiency seems allied with increased COVID-19 infection severity and fatality[45]. In a recent meta-analysis[46] of over 800 cases, patients with serum 25(OH)D levels <30 ng/ml

were one and half times more likely to test positive for COVID-19 compared to cases with favorable vitamin D levels. COVID-19 is more established among Afro-American individuals[47], and studies[48] have demonstrated that the COVID-19 contagion rate and fatality have been established to be elevated in areas with higher vitamin-D-deficient populations (Italy *vs*. Nordic countries). A study[49] showed a considerably lowered vitamin D level in COVID-19 positive cases in comparison to negative cases. Similarly, over the age of sixty-five years, there is a clear relationship of vitamin D deficiency with COVID-19 cases leading to worse clinical outcome[50]. One study[49] has suggested that Black individuals in England are more vulnerable to COVID-19, presumably due to their darker skin preventing the production of vitamin D with the help of sunshine.

However, the argument of this relationship has not been elucidated and some research has shown no relation between vitamin D deficit and COVID-19 contagion rate, hospitalization or even fatality, although some research suggests an association between lower levels of Vitamin D and fatality rates in certain countries[51].

Although the available evidence up till now, from principally several studies, may be viewed as presenting a trend for a relationship between lowered serum vitamin D levels and COVID-19 linked health consequences, this link was not established to be statistically considerable. Supplementation of Calcifediol may have a defensive effect on COVID-19 connected ICU admittance rate[45].

The current study has several limitations. First is a small sample size. Second, data about vitamin D level in the acute stage was not recorded. It would be helpful to know whether vitamin D deficiency was present before COVID-19 cases or occurred de novo after infection and worsened later on; third, the need to follow the effect of supplementation on the functional status of included patients.

Conclusions

Vitamin D deficiency is very common among post-COVID-19 cases; however, whether this relationship is causal or simply an association remains unclear from previous studies No association between vitamin D deficit and post-COVID-19 symptoms was established and it seems that post-COVID-19 syndrome pathophysiology is more complex than previously supposed. More studies are required to further examine its role and to elucidate the effect of vitamin D supplementation in the improvement of this clinical status.

Conflict of Interest

The Authors declare that they have no conflict of interests.

Ethics Approval and Consent to Participate

Research was in accordance with Declaration of Helsinki. The study was approved by Assiut Faculty of Medicine Ethical Committee. Informed written consent has been obtained from each patient following explanation of any study-related procedures.

Availability of Data and Materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Funding

Financial support was not obtained from any individual, institutions, agencies, drug industries or organizations.

Authors' Contribution

AAMH, IG, AAM, KMK, AMA and MTA conceived the study and designed the data collection tool and performed the study. The data was analyzed by AAMH, IG, NAMH, AEAO, HAM, DAATK and HA. The manuscript was written by AAMH, IG, KAME, AMA and JS. The manuscript was reviewed and edited by all authors.

References

1) Handono K, Sidarta YO, Pradana BA, Nugroho RA, Hartono IA, Kalim H, Endharti AT. Vitamin D prevents endothelial damage induced by increased neutrophil extracellular traps formation in patients with systemic lupus erythematosus. Acta Med Indones 2016; 16: 46-53.
2) Ebadi M, Montano-Loza AJ. Perspective: improving vitamin D status in the management of COVID-19. Eur J Clin Nutr 2020; 6: 856-859.
3) Xu J, Yang J, Chen J. Vitamin D alleviates lipopolysaccharide-induced acute lung injury via regulation of the reninangiotensin system. Mol Med Rep 2017; 1: 7432-7438.
4) Van Belle TL, Vanherwegen AS, Feyaerts D, De Clercq P, Verstuyf A, Korf H, Gysemans C, Mathieu C. 1, 25-Dihydroxyvitamin D3 and its analog TX527 promote a stable regulatory T cell

phenotype in T cells from type 1 diabetes patients. PLoS One 2014; 10: e109194.

5) Reddy KK. Reply to Glossmann: vitamin D compounds and oral supplementation methods. J Investig Dermatol 2013; 11: 2649-2651.
6) Ilie PC, Stefanescu S, Smith L. The role of vitamin D in the prevention of coronavirus disease 2019 infection and mortality. Aging Clin Exp Res 2020; 32: 1195-1198.
7) Ahmad A, Heumann C, Ali R, Oliver T. Mean Vitamin D levels in 19 European Countries & COVID-19 Mortality over 10 months. Cold Spring Harb Lab 2021; 11: 361.
8) Geddes L. Why strange and debilitating coronavirus symptoms can last for months. New Sci 2020; 24.
9) Assaf G, Davis H, McCorkell L. An analysis of the prolonged COVID-19 symptoms survey by Patient-Led Research Team. Patient Led Research, 2020.
10) Mohamed-Hussein A, Galal I, Saad M, Zayan HE, Abdelsayed M, Moustafa M, Ezzat AR, Helmy R, Abd Elaal H, Aly K, Abderheem S. Post-COVID-19 functional status: relation to age, smoking, hospitalization and comorbidities. MedRxiv 2020; 1.
11) COVID TS. Tedavi Rehberi. 2020. Available at: https://hsgm.saglik.gov.tr/depo/birimler/goc_sagligi/covid19/rehber/COVID-19_Rehberi20200414_eng_v4_002_14.05.2020.pdf
12) Dasgupta A, Kalhan A, Kalra S. Long term complications and rehabilitation of COVID-19 patients. J Pak Med Assoc 2020; 1: S131-135.
13) Galal I, Hussein AA, Amin MT, Saad MM, Zayan HE, Abdelsayed MZ, Moustafa MM, Ezzat AR, Helmy RE, Abd_Elaal HK, Al Massry NA. Determinants of persistent post-COVID-19 symptoms: value of a novel COVID-19 symptom score. Egypt J Bronchology 2021; 1: 1-8.
14) Kensarah OA, Azzeh FS. Vitamin D status of healthy school children from western Saudi Arabia. Pak J Nutr 2012; 3: 288-294.
15) Damanhouri LH. Vitamin D deficiency in Saudi patients with systemic lupus erythematosus. Saudi Med J 2009; 10: 1291-1295.
16) Botros RM, Sabry IM, Abdelbaky RS, Eid YM, Nasr MS, Hendawy LM. Vitamin D deficiency among healthy Egyptian females. Endocrinol Nutr. 2015; 7: 314-321.
17) Shelbaya S, Seddik S, Ahmed A, Roshdy N, Abbas M. Assessment of vitamin D status in different samples of an elderly Egyptian population. Egypt J Obes Diabetes Endocrimol 2017; 2: 53-60.
18) Hilger J, Friedel A, Herr R, Rausch T, Roos F, Wahl DA, Pierroz DD, Weber P, Hoffmann K. A systematic review of vitamin D status in populations worldwide. Br J Nutr 2014; 1: 23-45.
19) Durazo-Arvizu RA, Camacho P, Bovet P, Forrester T, Lambert EV, Plange-Rhule J, Hoofnagle AN, Aloia J, Tayo B, Dugas LR, Cooper RS. 25-Hydroxyvitamin D in African-origin populations at varying latitudes challenges the construct of a physiologic norm. Am J Clin Nutr 2014; 3: 908-914.
20) Zhang W, Stoecklin E, Eggersdorfer M. A glimpse of vitamin D status in Mainland China. Nutr 2013; 7-8: 953-957.
21) Mogire RM, Mutua A, Kimita W, Kamau A, Bejon P, Pettifor JM, Adeyemo A, Williams TN, Atkinson SH. Prevalence of vitamin D deficiency in Africa: a systematic review and meta-analysis. Lancet Glob Health 2020; 1: 134-142.
22) Luxwolda MF, Kuipers RS, Kema IP, Van der Veer E, Dijck-Brouwer DJ, Muskiet FA. Vitamin D status indicators in indigenous populations in East Africa. Eur J Nutr 2013; 3: 1115-1125.
23) Forrest KY, Stuhldreher WL. Prevalence and correlates of vitamin D deficiency in US adults. Nutr Res 2011; 31: 48-54.
24) Meltzer DO, Best TJ, Zhang H, Vokes T, Arora V, Solway J. Association of vitamin D status and other clinical characteristics with COVID-19 test results. JAMA Netw Open 2020; 9: e2019722-2019729.
25) COVID CD. Pandemic Planning Scenarios National Center for Immunization and Respiratory Diseases (NCIRD). Division of Viral Diseases 2020. Available at: https://stacks.cdc.gov/view/cdc/88617.
26) Huotari A, Herzig KH. Vitamin D and living in northern latitudes—an endemic risk area for vitamin D deficiency. Int J Circumpolar Health 2008; 2-3: 164-178.
27) Sowah D, Fan X, Dennett L, Hagtvedt R, Straube S. Vitamin D levels and deficiency with different occupations: a systematic review. BMC Pub Health 2017; 1: 1-25.
28) Schleicher RL, Sternberg MR, Looker AC, Yetley EA, Lacher DA, Sempos CT, Taylor CL, Durazo-Arvizu RA, Maw KL, Chaudhary-Webb M, Johnson CL. National estimates of serum total 25-hydroxyvitamin D and metabolite concentrations measured by liquid chromatography–tandem mass spectrometry in the US population during 2007-2010. J Nutr 2016; 5: 1051-1061.
29) Sarafin K, Durazo-Arvizu R, Tian L, Phinney KW, Tai S, Camara JE, Merkel J, Green E, Sempos CT, Brooks SP. Standardizing 25-hydroxyvitamin D values from the Canadian health measures survey. Am J Clin Nutr 2015; 5: 1044-1050.
30) Cashman KD. Vitamin D deficiency: defining, prevalence, causes, and strategies of addressing. Calcif Tissue Int 2020; 1: 1-6.
31) Gruber-Bzura BM. Vitamin D and influenza—prevention or therapy? Int J Mol Sci 2018; 8: 2419.
32) Nakashima A, Yokoyama K, Yokoo T, Urashima M. Role of vitamin D in diabetes mellitus and chronic kidney disease. World J Diabetes 2016; 5: 89.
33) Migliaccio S, Di Nisio A, Mele C, Scappaticcio L, Savastano S, Colao A. Obesity and hypovitaminosis D: causality or casualty? Int J Obes Suppl 2019; 1: 20-31.

34) Banerjee A, Ganguly U, Saha S, Chakrabarti S, Saini RV, Rawal RK, Saso L, Chakrabarti S. Vitamin D and immuno-pathology of COVID-19: many interactions but uncertain therapeutic benefits. Expert Rev Anti Infect Ther 2021; 2: 1-4.

35) Munshi R, Hussein MH, Toraih EA, Elshazli RM, Jardak C, Sultana N, Youssef MR, Omar M, Attia AS, Fawzy MS, Killackey M. Vitamin D insufficiency as a potential culprit in critical COVID-19 patients. J Med Virol 2021; 2: 733-740.

36) Mariani J, Giménez VM, Bergam I, Tajer C, Antonietti L, Inserra F, Ferder L, Manucha W. Association between vitamin D deficiency and COVID-19 incidence, complications, and mortality in 46 countries: an ecological study. Health Sec 2021; 3: 302-308.

37) Radujkovic A, Hippchen T, Tiwari-Heckler S, Dreher S, Boxberger M, Merle U. Vitamin D deficiency and outcome of COVID-19 patients. Nutr 2020; 12: 2757.

38) Annweiler G, Corvaisier M, Gautier J, Dubée V, Legrand E, Sacco G, Annweiler C. Vitamin D supplementation associated to better survival in hospitalized frail elderly COVID-19 patients: the GERIA-COVID quasi-experimental study. Nutr 2020; 11: 3377-3379.

39) Ling SF, Broad E, Murphy R, Pappachan JM, Pardesi-Newton S, Kong MF, Jude EB. High-dose cholecalciferol booster therapy is associated with a reduced risk of mortality in patients with COVID-19: a cross-sectional multi-centre observational study. Nutr 2020; 12: 3799-3804.

40) Giannini S, Passeri G, Tripepi G, Sella S, Fusaro M, Arcidiacono G, Torres MO, Michielin A, Prandini T, Baffa V, Aghi A. Effectiveness of in-hospital cholecalciferol use on clinical outcomes in comorbid COVID-19 patients: a hypothesis-generating study. Nutr 2021; 1: 219-223.

41) Castillo ME, Costa LM, Barrios JM, Díaz JF, Miranda JL, Bouillon R, Gomez JM. Effect of calcifediol treatment and best available therapy versus best available therapy on intensive care unit admission and mortality among patients hospitalized for COVID-19: A pilot randomized clinical study. J Steroid Biochemistry Mol Biol 2020; 1: 105751-105758.

42) Kloc M, Ghobrial RM, Lipińska-Opałka A, Wawrzyniak A, Zdanowski R, Kalicki B, Kubiak JZ. Effects of vitamin D on macrophages and myeloid-derived suppressor cells (MDSCs) hyperinflammatory response in the lungs of COVID-19 patients. Cell Immunol 2021; 360: 104259-104266.

43) Pal R, Banerjee M, Bhadada SK, Shetty AJ, Singh B, Vyas A. Vitamin D supplementation and clinical outcomes in COVID-19: a systematic review and meta-analysis. J Endocrinol Invest 2021; 24: 1-6.

44) Oscanoa TJ, Amado J, Vidal X, Laird E, Ghashut RA, Romero-Ortuno R. The relationship between the severity and mortality of SARS-CoV-2 infection and 25-hydroxyvitamin D concentration—a metaanalysis. Adv Respir Med 2021; 2: 145-157.

45) Bassatne A, Basbous M, Chakhtoura M, El Zein O, Rahme M, Fuleihan GE. The link between COVID-19 and VItamin D (VIVID): a systematic review and meta-analysis. Metab 2021; 24: 154753.

46) Garg S, Kim L, Whitaker M, O'Halloran A, Cummings C, Holstein R, Prill M, Chai SJ, Kirley PD, Alden NB, Kawasaki B. Hospitalization rates and characteristics of patients hospitalized with laboratory-confirmed coronavirus disease 2019—COVID-NET, 14 States, 2020. Morb Mortal Wkly Rep 2020; 15: 458-466.

47) Panarese A, Shahini E. COVID-19, and vitamin D. Aliment Pharmacol Ther 2020; 10: 993-996.

48) D'Avolio A, Avataneo V, Manca Al. 25-Hydroxyvitamin D concentrations are lower in patients with positive PCR for SARS-CoV-2. Nutrients 2020; 5: 1359-1364.

49) Mitchell F. Vitamin-D and COVID-19: do deficient risk a poorer outcome? Lancet Diabetes Endocrinol 2020; 7: 570-577.

50) Baktash V, Hosack T, Patel N. Vitamin D status and outcomes for hospitalised older patients with COVID-19. Postgrad Med J 2020; Epub ahead of print.

51) Daneshkhah A, Agrawal V, Eshein A, Subramanian H, Roy HK, Backman V. Evidence for possible association of vitamin D status with cytokine storm and unregulated inflammation in COVID-19 patients. Aging Clin Exp Res 2020; 10: 2141-2158.