Review

Vitamin D deficiency in association with endothelial dysfunction: Implications for patients with COVID-19

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There is emerging evidence to suggest that vitamin D deficiency is associated with adverse outcomes in COVID-19 patients. Conversely, vitamin D supplementation protects against an initial alveolar diffuse damage of COVID-19 becoming progressively worse. The mechanisms by which vitamin D deficiency exacerbates COVID-19 pneumonia remain poorly understood. In this review we describe the rationale of the putative role of endothelial dysfunction in this event. Herein, we will briefly review (1) anti-inflammatory and anti-thrombotic effects of vitamin D, (2) vitamin D receptor and vitamin D receptor ligand, (3) protective role of vitamin D against endothelial dysfunction, (4) risk of vitamin D deficiency, (5) vitamin D deficiency in association with endothelial dysfunction, (6) the characteristics of vitamin D relevant to COVID-19, (7) the role of vitamin D on innate and adaptive response, (8) biomarkers of endothelial cell activation contributing to cytokine storm, and (9) the bidirectional relationship between inflammation and homeostasis. Finally, we hypothesize that endothelial dysfunction relevant to vitamin D deficiency results from decreased binding of the vitamin D receptor with its ligand on the vascular endothelium and that it may be immune-mediated via increased interferon 1α. A possible sequence of events may be described as (1) angiotensin II converting enzyme-related initial endothelial injury followed by vitamin D receptor-related endothelial dysfunction, (2) endothelial lesions deteriorating to endothelialitis, coagulopathy and thrombosis, and (3) vascular damage exacerbating pulmonary pathology and making patients with vitamin D deficiency vulnerable to death.

Keywords
Coagulation; COVID-19; cytokines; endothelial activation; endothelial dysfunction; inflammation; SARS-CoV-2, vitamin D; von Willebrand factor.

1. Introduction
There is emerging evidence to suggest that vitamin D deficiency is associated with an increased risk of acquiring COVID-19 infection (Meltzer et al., 2020), as well as developing COVID-19-associated thrombosis (Weir et al., 2020). Moreover, vitamin D deficiency was shown to be a fatal co-morbidity in COVID-19 patients (Biesalski, 2020). On the other hand, increasing evidence suggests that vitamin D supplementation prevents COVID-19 infection-induced multi-organ damage (Aygun, 2020), coagulopathy (Ali, 2020), and mortality (Grant et al., 2020; Ilie et al., 2020). In addition, vitamin D supplementation is reported to reduce the risk and severity of COVID-19 (Hribar et al., 2020). Therefore it has been postulated that daily supplementation with moderate doses of vitamin D3 is safe treatment for COVID-19 patients (Zemb et al., 2020).

The mechanisms by which vitamin D deficiency leads to progression from its characteristic lesions (diffuse alveolar damage and airway inflammation) to the more complicated, clinically significant lesions (COVID-19-associated vascular inflammation and thrombosis) remain unclear. While multiple, interrelated mechanisms involving immune cells (T regulatory lymphocytes) and the renin-angiotensin system (RAS) have been proposed (Ali, 2020; Aygun, 2020), direct proof to elucidate the role of endothelial dysfunction in the pathogenesis of vitamin D deficiency are still lacking. Recently, a review suggests that endothelial dysfunction contributes to COVID-19-associated vascular inflammation and coagulopathy (Zhang et al., 2020), nevertheless, questions remain regarding the role of endothelial dysfunction in COVID-19 patients with vitamin D deficiency. The answers to these questions would provide not only essential insights into the mechanisms of COVID-19 in patients with vitamin D deficiency, but also potential therapeutic targets. Herein, we hypothesize endothelial dysfunction may play a role in COVID-19 patients with vitamin D deficiency.

2. Anti-inflammatory and anti-thrombotic effects of vitamin D
The classic role of vitamin D in calcium homeostasis and maintenance of bones is well recognized. Recent evidence suggests that vitamin D also has anti-inflammatory and anti-thrombotic effects. Mohammad et al. (2019) describe the role of vitamin D in inflammatory and coagulation pathways as well as its role in endothelial activation. They discuss the close connection between vascular inflammation and thrombosis in the context of vitamin D. The anti-inflammatory effects of vitamin D have been demonstrated by treating peripheral blood mononuclear cells from asthmatics with 1,25(OH)2D3 (Zhang et al., 2014). Doi so significantly inhibited IL-6 production, up-regulated the expression of
mitogen-activated kinase phosphatase-1 (MKP-1), and enhanced anti-inflammatory effects of corticosteroids in monocytes (Zhang et al., 2014). Further, treating humans at sufficient doses of vitamin D (≥ 30 ng/ml of 25(OH)D3) significantly inhibited IL-6 and tissue necrosis factor (TNF-α) production (Zhang et al., 2014). Another study reiterated that pretreatment with vitamin D inhibited IL-6 and TNF-α production by human monocytes and showed that the inhibition is mediated by MKP-1 (Zhang et al., 2012). Zhang and colleagues suggest that the upregulation of MKP-1 by vitamin D inhibits mitogen activated protein kinase p38 activation and pro-inflammatory cytokine production in monocytes (Zhang et al., 2012).

The role of endothelium as a target of vitamin D is demonstrated by the direct effects of vitamin D on endothelial function (Yancy, 2020). An in vitro study using human umbilical vein endothelial cell (HUVECs) by Uberti et al. (2014) showed the protective effect of vitamin D on the endothelium. They demonstrated that vitamin D prevents endothelial cell death by modulating apoptosis and autophagy through several actions including inhibiting superoxide anion generation and inducing nitric oxygen (NO) production. NO release induced by vitamin D during oxidative stress (imbalance between pro-oxidants and anti-oxidants) protects cells from death. They showed that pretreatment of HUVECs with vitamin D receptor (VDR) agonist, ZK191784, had a greater ability to reduce the apoptosis-related gene expression than vitamin D treatment only (Uberti et al., 2014). Another in vitro study using HUVECs by Teixeira et al. (2017) showed the protective effects of 1,25(OH)2D3 (the most active metabolite of vitamin D) on endothelial dysfunction induced by leptin. The effect is mediated by down-regulating vascular inflammatory mediators (MCP-1, VCAM-1, etc.) of antioxidant activity and inflammation, as well as decreasing nuclear factor-κB (NF-κB) subunit p65 to the nucleus. The protective effects of 1,25(OH)2D3 on the endothelium are dependent on the VDR.

3. Vitamin D receptor and VDR ligand

Vitamin D affects cellular proliferation, differentiation, apoptosis, and angiogenesis by binding with VDR and regulating gene expression (Uberti et al., 2014). VDR is expressed in the heart, lungs, kidneys, and other organs. It is distributed throughout the body in endothelial cells, macrophages, dendritic cells, and lymphocytes (Mammad et al., 2019; Teixeira et al., 2017). Using immunohistochemistry, Wong et al. (2008) detected VDR in endothelial and vascular smooth muscle cells of the aortic ring of rats. VDR activation reduces acute respiratory distress syndrome severity in patients with COVID-19 (Virizi et al., 2018).

The active form of vitamin D (1, 25(OH) D3) has a short half-life (approximately 15 hours) compared to 25(OH) D3, which has a much longer half-life (approximately 15 days). Both are moved to target organs and serve as natural ligands after binding to vitamin D-binding protein (Mammad et al., 2019). Antigen presenting cells (e.g., dendritic cells and macrophages) express enzymes that convert vitamin D (25(OH)D3) to its activated form, 1,25(OH)2D (Mammad et al., 2019). In this context, endothelial cells may also serve as antigen presenting cells by expressing MHC class I and II antigens on their surface (Virizi et al., 2018). Therefore, the endothelium, along with other antigen presenting cells, plays a role in innate and adaptive immunity of vitamin D.

4. The role of vitamin D in endothelial dysfunction

NF-κB activation induces pro-inflammatory genes and plays a role in endothelial activation and endothelial apoptosis. Additionally, endothelial apoptosis is associated with NO and peroxynitrite (Zhang et al., 2010). NO contributes to vascular homeostasis by opposing the actions of endothelium-derived contracting factors, such as angiotensin II and endothelin-1. The combination of reduced bioavailability of NO and its plethora of properties (vasodilative, antiplatelet, anti-proliferative, anti-adhesive, permeability-decreasing, and anti-inflammatory), plays a role in endothelial dysfunction (Zhang et al., 2017). Oxidative stress is another causative factor for vascular endothelial dysfunction (Zhang et al., 2017). In this context, Kanikarla-Marie and Jain showed that pre-treatment of HUVEC with 1,25(OH)2D3 inhibited reactive oxygen species, MCP-1, ICAM-1, and monocyte adherence. They suggest that vitamin D protects from endothelial dysfunction by reducing oxidative stress and NF-κB activation (Kanikarla-Marie and Jain, 2016). Further evidence in support of the role of NF-κB was obtained from Stio et al. (2007), who reported that TX 527, a vitamin D analogue, exerts an immunosuppressive effect on TNF-α production in patients with Crohn’s disease and that it may be mediated by NF-κB down-regulation. Kundu et al. (2017) confirmed that vitamin D inhibits NF-κB signaling in monocyte-derived dendritic cells following innate immune stimulation.

There is conflicting evidence about NF-κB activity between in vitro and in vivo studies. Although in vitro studies suggest that vitamin D reduces inflammation by NF-κB activity, clinical trials showed no effect of vitamin D supplementation on inflammatory markers or NF-κB activity in vivo in humans (Mousa et al., 2017). Recently, an updated systematic review with meta-analysis and meta-regression concluded that vitamin D supplementation does not improve endothelial dysfunction (Pincombe et al., 2019). At the present time, it is difficult to reconcile the results between in vitro and in vivo studies; however, the methodological differences in outcome assessment are worth noting. In in vivo studies, the effects of vitamin D supplementation are solely based on indexes, (e.g., flow-mediated dilation, pulse wave velocity, central augmentation index), all of which lack solid support from biomarkers of endothelial activation used in the clinical setting (Pincombe et al., 2019). Many biomarkers are released from activated and dysfunctional endothelial cells (Zhang et al., 2012), and are robust indexes for in vivo studies.

5. Risk of vitamin D deficiency

Vitamin D deficiency increases expression and secretion of pro-inflammatory cytokines and chemokines, and has been shown to increase the severity of respiratory viral infections. African-Americans experience inefficient absorption of ultraviolet light, resulting in suboptimal intake of vitamin D (Wong et al., 2008). In line with this, more than half of COVID-19 cases and approximately 70% of COVID-19 deaths were attributed to African-Americans in Chicago (Yancy, 2020).

Evidence suggests that vitamin D deficiency is related to increased risk for disease. Long term consequences of vitamin D deficiency are serious and include an increased risk of hypertension, diabetes, congestive heart failure, and peripheral arterial disease, as well as myocardial infarction, stroke, and death (Martinez-Miguel et al., 2014). Mildly insufficient vitamin D plasma lev-
els (approximately 25 ng/mL) increase the risk of hypertension, whereas severely deficient plasma levels (3-4.8 ng/mL) result in an increased risk for ischemic heart disease, myocardial infarction, and early death by 40%, 64%, and 57%, respectively, when compared to individuals with plasma vitamin D levels of 18.83-28.44 ng/mL (Mohammad et al., 2019).

6. Vitamin D deficiency in association with endothelial dysfunction

Vitamin D deficiency was shown to be associated with endothelial dysfunction in patients with stable systemic lupus erythematosus (SLE) and that endothelial function improved after 12 weeks of treatment with 1,25(OH)2D3 (Reynolds et al., 2016). Jablonski et al. (2011) provided the first evidence in humans that vitamin D deficiency is associated with increased pro-inflammatory NF-κB expression. Consistent with this, vitamin D deficiency also reduced VDR on vascular endothelial cells, indicating that reduced VDR may be a molecular mechanism linking vitamin D insufficiency to endothelial dysfunction. Moreover, vitamin D deficiency increases the downstream pro-inflammatory cytokine IL-6 on endothelial cells (Zhang et al., 2017). Likewise, Ngo et al. (2010) demonstrated that high-sensitivity CRP levels and asymmetric dimethylarginine (a marker of endothelial dysfunction) were associated with vitamin D deficiency. Further, Peterson and Heffernan (2008) reported that serum TNF-α concentrations were negatively correlated with vitamin D in healthy women.

Mandal et al. (2014) demonstrated that interferon-α (IFNa) is associated with SLE severity in patients with vitamin D deficiency. Activated dendritic cells are a primary source of IFNa in these patients, and vitamin D inhibits dendritic cell activation and production of IFNa. Endothelial dysfunction can result from locally and systemically elevated type-I IFNs in patients with SLE or other autoimmune disorders (Chen et al., 2020). Lee et al. (2007) confirmed that elevated IFN-I levels could lead to endothelial dysfunction by causing a reduction in the number of endothelial progenitor cells, by showing that SLE disease activity is associated with elevated serum levels of type-I IFN. A prevailing view is that type-I IFNs are immune modulators altering innate and adaptive immunity. Cytokines, such as TNF, can also induce type-I IFN expression through the TNF receptor-IRF1 signaling pathway (Lee et al., 2007). Taken together, it is likely that vitamin D-related endothelial dysfunction is immune-mediated via the IFNa pathway.

7. The characteristics of vitamin D relevant to COVID-19

The most important characteristics of vitamin D related to COVID-19 was discussed by Hribar et al. (2020). Activated vitamin D decreases pro-inflammatory cytokines and increases anti-inflammatory cytokines by binding with VDR, causing gene transcription to favor T helper (Th2) and regulatory T cell responses instead of Th1. Therefore, it is possible that the severity of COVID-19 could be mitigated through this process. In addition, vitamin D and VDR may directly down-regulate the angiotensin II converting enzyme (ACE2) receptor, thus decreasing the risk of infection with COVID-19 altogether (Hribar et al., 2020).

8. Role of vitamin D in innate and adaptive response

Vitamin D modulates innate and adaptive immune responses and binds to VDR to prevent inflammatory responses (Aranow, 2011; Trochoutsou et al., 2015). VDR is expressed by B and T lymphocytes and antigen-presenting cells (Aranow, 2011; Sassi et al., 2018). As these immunologic cells are able to locally convert 25(OH)D3 into its active form (1,25(OH)2D3), vitamin D may act in a paracrine or autocrine manner in an immune environment (Aranow, 2011; Sassi et al., 2018). Vitamin D metabolite 1,25(OH)2D3 has a genomic binding site, in which a transcriptional complex is formed modulating the expression of genes such as ACE and the VDR (Sassi et al., 2018).

Vitamin D down-regulates the production of pro-inflammatory cytokines through various mechanisms including inhibiting virus-induced NF-κB activation (Prietl et al., 2013). It suppresses T cell proliferation, changes the maturation of T cells (leading to a decrease in inflammatory Th17), increases T regulatory cells, and promotes Th2 cytokines in favor of Th1, which results in more anti-inflammatory and fewer inflammatory cytokines (Aranow, 2011; Greiller and Martineau, 2015). It also inhibits inflammatory cytokine production and preserves an immature phenotype of dendritic cells (Aranow, 2011).

Greiller & Martineau reviewed in vitro experiments and concluded that although vitamin D modulates expression and secretion of type 1 interferon, chemokines, and pro-inflammatory cytokines, vitamin D metabolites do not necessarily influence the clearance of respiratory viruses in human respiratory epithelial cell cultures (Prietl et al., 2013). In light of the potential role of vitamin D in the modulation of immune responses by decreasing pro-inflammatory cytokines and by increasing anti-inflammatory cytokines, vitamin D may reduce the risk of cytokine storm.

9. Biomarkers of endothelial cell activation contributing to cytokine storm

It has become evident that acute respiratory distress syndrome is associated with a cytokine storm, such as high concentrations of interleukin (IL)-1, IL-1B, IL-2, IL-6, IL-7, IL-8, IL-10, IL-12, IL-13, IL-17, granulocyte colony-stimulating factor (GCSF), interferon-γ inducible protein 10 (IP-10), macrophage inflammatory protein 1-α (MIP-1a), tumor necrosis factor-α (TNF-α), monocyte chemotactractant protein 1 (MCP-1), and increased C reactant protein (CRP) (Ali, 2020; Aygun, 2020). Vitamin D supplementation is capable of reducing this surplus of pro-inflammatory cytokines (Grant et al., 2020). It has generally been assumed that Th1 cells are the predominant cellular source for cytokine storms (Aygun, 2020). Previous studies suggest that these cytokines may be synthesized not only by T helper lymphocytes, but also by activated endothelial cells (Zhang et al., 2014). Therefore, endothelial activation may also be a cellular source of the cytokine storm in COVID-19. Seemingly, these cytokines serve as a modulation factor for T helper lymphocytes and promote vascular inflammation and coagulation. The cytokine storm is a double-edged sword in this scenario of immune and vascular response to COVID-19.

10. Bidirectional relationship between inflammation and homeostasis

Margetic proposed that there exists a bidirectional relationship between inflammation and homeostasis (particularly coagu-
Fig. 1. Schematic diagram showing a hypothesis of endothelial dysfunction in COVID-19 patients with vitamin D deficiency. A possible sequence of events may be described as (1) ACE2-related initial endothelial injury followed by VDR-related endothelial dysfunction, (2) endothelial lesions deteriorate to endothelialitis, coagulopathy, and thrombosis, and (3) vascular damage exacerbates pulmonary pathology and makes patients with vitamin D deficiency vulnerable to death. In the context of COVID-19 endothelialitis and coagulation, downstream pro-inflammatory mediators (E-selectin, ICAM-1, VCAM-1, JAM-1, and PECAM-1), cytokine and chemokines (IL-6 and IL-8) contribute to vascular inflammation, whereas downstream pro-coagulant mediators (P-selectin etc. and pro-coagulant family (vWF, vWFpp, TF, PAI-1) contribute to coagulation. In the process of vasculopathy and coagulopathy, their initial lesions can be amplified by the bidirectional relationship between vascular inflammation and coagulation. ICAM-1 = intercellular adhesion molecule-1, VCAM-1 = vascular cell adhesion molecule-1, JAM-1 = junctional adhesion molecule-1, PECAM-1 = platelet endothelial cell adhesion molecule-1, IL-6 and IL-8 = interleukin-6 and interleukin-8, vWF = von Willebrand factor, vWFpp = von Willebrand factor pro-peptide, TF = tissue factor, PAI-1 = Plasminogen activating inhibitor-1.

Inflammation leads to activation of the hemostatic system, which then influences inflammatory activity (Margetic, 2012). The concept may help explain the association of COVID-19 with vascular inflammation and coagulation. Margetic explains that the feedback loop between inflammation and coagulation involves vascular endothelial cells, platelets, plasma coagulation cascade, physiologic anticoagulants and fibrinolytic activity and that under inflammatory conditions, pro-inflammatory cytokines (e.g., TNF-α, IL-1, and IL-6) alert the hemostatic system to make a change. Such changes include increasing platelet activation, impairing function of physiologic anti-coagulants, and suppressing fibrinolytic activity. As a result, P-selectin, vWF, tissue factor, and plasminogen activating inhibitor-1 (PAI-1) become elevated (Margetic, 2012). Endothelial dysfunction acts as a signal to switch the anti-coagulants towards the pro-coagulants.

Conversely, an activated hemostatic system, particularly the coagulant system, also modulates inflammatory activity. For example, thrombin, a mediator released from activated endothelial cells has multilevel pro-coagulant activity (Zhang et al., 2012). In addition to converting fibrinogen to fibrin, thrombin activates inflammatory cells, causing increased production of inflammatory mediators and increased leukocyte adhesion and chemotaxis (Zhang et al., 2014). Tissue factor, a mediator released from activated endothelial cells, not only promotes coagulation (Zhang et al., 2012), but also modulates inflammatory activity (Mohammad et al., 2019; Yancy, 2020). Consequently, tissue factor is one of the links between inflammation and coagulation (and thus thrombosis) (Mohammad et al., 2019). The two interrelated, but conceptually distinct events may explain the co-existence of vascular inflammation and coagulation in COVID-19. Additionally, activated coagulation factors, Factor Xa (FXa) and Tissue Factor-Factor VIIa (TF-FVIIa) complex can directly stimulate cells involved in inflammatory response and increase production of pro-inflammatory mediators (Margetic, 2012).
11. Hypothesis: A cascade leads from angiotensin II converting enzyme receptors and vitamin D receptors to vascular inflammation and coagulation

Given that (1) endothelial cells could be infected by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) through ACE2 receptors on the endothelium, (2) vitamin D deficiency is due to the disorder of vitamin D-binding protein binding to reduced VDR on the endothelium, and (3) IFNa is involved in a vitamin D deficiency event, it is plausible that there exists a cascade from ACE2 and VDR to vascular inflammation and coagulation in COVID-19 patients with vitamin D deficiency (Fig. 1). The cascade is initiated by endothelial dysfunction, followed by the production of downstream pro-inflammatory and pro-coagulant mediators, and resulting in a bidirectional relationship between vascular inflammation and coagulation. The sequence of events may be postulated as (1) SARS-CoV-2 uptake into vascular endothelium by unregulated ACE2 receptors in which SARS-CoV-2-infected endothelial cells are susceptible to induce initial endothelial dysfunction; (2) ACE2-related initial endothelial injury is followed by VDR-related endothelial dysfunction. Induction of endothelial dysfunction may be also relevant to an inadequate degree of an active form of vitamin D (1,25(OH) 2D3), which cannot efficiently act as a ligand for VDR, resulting in the disorder of vitamin D-binding protein binding to the ligand for VDR on the endothelium. In addition, a cytokine storm (particularly TNF) increases IFNa. Activated IFNa pathways play a role in the induction of secondary endothelial dysfunction. Endothelial lesions deteriorate to endothelialitis, coagulopathy and thrombosis; and (3) vascular damage exacerbates pulmonary pathology and makes patients with vitamin D deficiency more vulnerable to death.

12. Summary

This review provides a summary of the literature on vitamin D deficiency as it pertains to COVID-19. The current status of research in vitamin D deficiency may support the role of vitamin D supplementation in prevention of COVID-19 infection-induced pulmonary pathology and vascular damage, although controversy remains.

Authors' contributions

Drs. Zhang and McCullough conceptualized this work. Dr. Zhang drafted the manuscript and Drs. McCullough and Tecson provided critical revisions. All authors approved the final version.

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Conflict of Interest

The authors declare no conflicts of interest statement.

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