

## **Adjuvant therapy with Serratiopeptidase and Vitamin D for COVID-19 patients: A new perspective**

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Type of Publication: Perspective Paper

Conflicts of Interest: Nil

### **ABSTRACT**

The inflammatory response plays a critical role in coronavirus disease 2019 and explosion of inflammatory cytokines increases the severity of COVID-19, leads to organ damage in late phase of disease. Hyperinflammatory response in COVID-19 patients leads to increase level of many proinflammatory protein markers which initiate the thrombus formation. Sudden death of COVID-19 patients suspects thrombo-embolic events. The postmortem report of COVID-19 patients shows evidence of thickening of blood vessels and microthrombosis. Serratiopeptidase is a protein enzyme (proteolytic) used to subside inflammatory response and degradation of inflammatory protein. It is always a treatment of choice in post-operative phase to reduce swelling and inflammation due to its proteolytic activity. In COVID-19 patients supplement with Serratiopeptidase prevents the formation of fibrin hence prevents microthrombi formation. Vitamin D, a fat soluble vitamin, steroid in nature also suppresses an explosive adaptive immune response. Vitamin D suppresses the endothelial cells to generate inflammatory markers. Low level of vitamin D linked with inflammation and vitamin D receptor agonist reduces proinflammatory cytokines production and release. Supplementation of vitamin D to sufficient level in subject of deficient and insufficient level of vitamin D dramatically leads to suppression of proinflammatory markers. So the combination of serratiopeptidase and vitamin D therapy in early stage of disease could be a new hope in the treatment of COVID-19.

**Keywords:** COVID-19, cytokine storm, immunosuppressant, microthrombosis, serratiopeptidase, vitamin D.

### **INTRODUCTION**

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV), which emerged as a novel human pathogen in 2019 [1], is responsible for coronavirus disease 2019 (COVID-19), which causes symptoms such as cough and fever, sore throat, fatigue, headache, myalgia, breathlessness later on severe pneumonia, and death.[2,3] In the current pandemic scenario, the number of COVID-19 cases are increasing continuously despite the effort of worldwide health agencies to prevent infection. It

affects the life of people in many ways and mortality has also increased with each passing day. WHO reported that globally as of 25 April 2021 there have been 146,054,107 confirmed cases of COVID-19 including 3,092,410 deaths (<https://covid19.who.int/>). In the second wave of COVID-19 pandemic the largest increase in cases has been reported by the South-East Asia region, largely from India followed by western pacific region(<https://covid19.who.int/>). As it become pandemic many ways to control

infection are not worthwhile. Though many patients of COVID-19 have developed mild to moderate symptoms and got health benefit with proper care and medication, but in critically ill patients there is hyperactive immune response which leads to coagulation cascade and intravascular clot formation.[4] Significantly elevated systemic levels of proinflammatory cytokine as IL-6, IL-18, IL-1  $\beta$ , C-reactive protein (CRP) and procalcitonin (PCT) have been reported in several COVID-19 patient cohorts and shown to correlate with disease severity.[5,6] Many data have been published stating the fact that interleukin storm and cytokine secretion leading to microthrombosis and coagulopathy increases the severity of COVID-19.[7] Increase level of lactate dehydrogenase (LDH) and D-dimer in COVID-19 patients also suggest thrombus formation in blood vessels.[8] Thrombus formation in COVID-19 patients may present with the complication of acute respiratory distress syndrome (ARDS) and other thrombo-embolic events, and eventually to death.[9,10] The post mortem findings of patients with deaths due to COVID-19 shows microthrombi and thickening of blood vessel which points towards suspected coagulopathy in the late stage of disease.[11] The current treatment strategies for COVID-19 should focus to avert the interleukin storm and to prevent the formation of microthrombus by degrading fibrin threads. Serratiopeptidase is a miracle discovery in medical science, used to reduce inflammation, swelling and pain from decades. Its use in COVID-19 patient helps to break down the inflammatory markers and reduce cytokine storm. Vitamin D acts as an immunomodulator and suppresses the formation of proinflammatory markers so prevents cytokine storm. By reducing cytokine storm its helpful to prevent formation of micro thrombus and patient's life can be saved.

### Serratiopeptidase

Serratiopeptidase or serrapeptase is a zinc containing metalloproteinase have proteolytic enzyme activity.[12] It was first isolated from the enterobacteria *Serratia* species *Serratia* E-15. This is nonpathogenic micro-organism and was originally isolated in the late 1960 from the silk worm *Bombyx mori*. [13] Serratiopeptidase is present in the silkworm (larval form of the silk moth) intestine and allows the emerging moth to dissolve its cocoon. Serratiopeptidase a serine protease belongs to the

group Serralyisin with a molecular weight 50-60 kDa has been significantly reported for its potent anti-inflammatory activity. Commercially it is produced by the fermentation technology by using nonpathogenic enterobacteria *Serratia* species *Serratia marcescens*. [14]

### Anti-inflammatory role of serratiopeptidase.

Since long time serratiopeptidase is used as anti-inflammatory agent to suppress pain and swelling. It is often used to reduce swelling in post-operative stage in various modalities of surgery.[15] Serratiopeptidase hydrolyses bradykinin, histamine, and serotonin shows its analgesic activity.[16] Its role alone or as an adjuvant with NSAID have been reported for many inflammatory disorders.[17] In animal model study *Rajinikanth B* 2014 found that on acetic acid-induced ulcerative colitis in mice when supplemented with serratiopeptidase, it reduces the process of inflammation and prevent shortening of colon. Further, they also found significant decrease in C-reactive protein level in mice treated with serratiopeptidase as compared to control group. [18] Being a proteolytic enzyme it has Fibrinolytic and caseinolytic activity and helps to dissolve blood clots, and atherosclerotic plaques.[19] It degrades the fibrin formed and also degrade the proinflammatory markers.[20,21] Fibrin degradation prevents formation of microthrombus in the vessels and reduces the risk of thrombo-embolic events. The clinical use of serratiopeptidase during allergic conditions was studied and it actually reduces the thickness and viscosity of the mucus and improves its elimination through bronchopulmonary secretions. In Japan it has been widely used as an anti-inflammatory and mucolytic agent to clear bronchopulmonary secretion [22] *Nakamura et al* found in chronic respiratory disease serratiopeptidase decrease the viscosity and elasticity of sputum.[23].

### Vitamin D

Vitamin D3 is a fat soluble vitamin, steroid in nature and is produced endogenously by exposure of skin to sunlight and can also be acquired from various non vegetarian sources.[24] 7-dehydrocholesterol, a serum circulating cholesterol precursor, is converted to cholecalciferol (the major natural source of the vitamin D) in the skin when exposed to ultraviolet B (UVB, 208 to 315 nm).[25] The active vitamin D metabolite calcitriol [26] mediates its biological

effects by binding to the vitamin D receptor (VDR),[27] which belongs to the nuclear receptor superfamily[28] and are expressed by cells in most organs,[29] including endothelial cells[30] and immune cells (B cells, T cells and antigen presenting cells ).[31] The recommended daily allowance is 400 IU (10 µg),[32] although higher requirement (800-1000 IU) may be needed in elderly.[33] The lower limit of adequate 25(OH)D levels should be 30 ng/mL [34] *Jabolanski KL et al* (2011)[35] categorized different level of serum conc. of 25(OH)D<sub>3</sub> as:

< 20 ng /ml (Deficient)

20- 29 ng /ml (Insufficient)

> 29 ng /ml (Sufficient)

### Anti-inflammatory role of vitamin D

Vitamin D is a steroid hormone in nature and suppresses the generation of inflammatory proteins. [36] Calcitriol in the blood, plays a major role in cell growth, neuromuscular, immune functions, and reduction of inflammation.[37,38,39] despite regulating the calcium and phosphate metabolism. Vitamin D has the ability to modulate both innate and adaptive immune response. [40] There are lots of study suggesting strong correlation between vitamin D deficiency and autoimmune disease, [41] inflammatory bowel disease,[42] diabetes mellitus[43] rheumatoid arthritis[44] and atherosclerosis.[45] Vitamin D has the ability to attenuate the disease progression in inflammatory bowel disease through anti-inflammatory immune responses.[46] It has been found that vitamin D influence the regulation of IL-6 synthesis and shows its significance as anti-inflammatory effects in cancer cells.[47] IL-6 was inversely related to 25(OH)D<sub>3</sub>. [48] Many recent studies establish beneficial role of vitamin D in cardiovascular health due to its immune suppression effects besides its role in skeletal health [49]. These include ability of vitamin D to affect function of endothelial cells through pro-inflammatory state [50,51]. Vitamin D inhibits vascular smooth muscle cell proliferation, vascular calcification, and atherogenesis via anti-inflammatory pathways [52]. Vitamin D deficiency is associated with endothelial dysfunction and plays an important role in the pathogenesis of arterial stiffness which leads to cardiovascular disease [53].

Epidemiological studies suggest an inverse association between circulating levels of 25(OH)D<sub>3</sub> and inflammatory markers, including CRP and interleukin (IL)-6. [54] Supplementation with vitamin D have been found to decrease the biomarkers of inflammation. [55,56].

### Conclusion

Coagulopathy and formation of microvascular thrombosis due to outburst release of proinflammatory proteins are the leading cause of morbidity and mortality in COVID-19 patients. Treatment protocol for COVID-19 patients should aim to degrade the already formed proinflammatory markers and to prevent the development of complication due to cytokine storm. Serratiopeptidase is good supplementary therapy to counteract cytokines storm and formation of fibrin hence it prevents the formation of microvascular thrombosis. In another way it is also helpful to clear mucous from air ways, and vitamin D as immunosuppressant suppresses the formation of cytokine storm. So combination therapy of serratiopeptidase and vitamin D from the initial stage of disease should be strongly considered to combat against the severe complication of COVID-19.

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