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SARS-Corona Virus-2 may initially infect brainstem through trigeminal ganglion-latency may be present-a new perspective

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Running title: Corona virus and trigeminal ganglion

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Highlights:

- SARS-Cov-2 virus enters the respiratory tract of the patient through nose, mouth and eyes
- It may also takes its route to trigeminal ganglion (TG) through its branches,V1,V2,V3
- TG is the center for brain respiratory control and releases important neurotransmitters including Substance P (SP)
- SP modulates the inflammation and initiates cytokine storming after being stimulated by nociceptive stimulus i.e virus
- SP along with its receptor Neurokinin-1 should be targeted to prevent cytokine storming

Abstract:

Novel severe acute respiratory syndrome coronavirus 2 infection (SARS-Cov-2) is an acute respiratory and infectious disease. This perspective aims to provide the basic understanding of the inflammation caused by SARS-Cov-2 and relation to trigeminal ganglion (TG). Virus enters through the mucous membranes of orofacial region and reach the TG where it resides and take control of its peptides including Substance P (SP).SP is the main neuropeptide, neuromodulator and neuro-hormone of TG, associated with nociception and inflammation under noxious stimulus. SP release is triggered and consequently, it affects the immune cells, blood vessels to release the mediators for inflammation. Cytokine storming is initiated and cause respiratory distress, bronchoconstriction and death in complicated cases. Neurokinin-1 Receptor (NK-1R) antagonist and glucocorticoids may be used to alleviate the symptoms and treat this infection. SP is the main culprit seem to be involved in the triggering of inflammatory pathways in SARS-Cov-2 infection. It has direct association with cardiorespiratory rhythm, sleep-wake cycle, nociception, ventilator responses and regulates many important physiological and pathological roles. Its over-secretion should be blocked by NK-1R antagonist. However, experimental work leading to clinical trials are mandatory for further confirmation.

Key words: Coronavirus, Substance P/ Neurokinin-1 Receptor, Respiratory illness, Infectious disease, Trigeminal ganglion

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Introduction:

Coronavirus infection (Covid-19) is caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which was first reported in Wuhan, China in December 2019 but later spreaded globally via international travelling and human-to-human transmission transmission[1]. It has become a pandemic as declared by WHO in March, 2020[2]. It has shattered the global psychy and economy in just 4 months. Despite of continued and collaborative efforts worldwide, there is no treatment, no vaccine, not a clear understanding of this infectious disease. The common symptoms include fever, cough, fatigue, acute respiratory distress syndrome, body aches etc [3, 4]. Complicated cases may undergo respiratory failure or even death [5]. Common and initial symptoms of Covid-19 infection include sore throat, loss of sense of smell and taste, pain in eyes, headache and flue[3] and similar functions are carried out by SP once it is released from trigeminal ganglion via TrN. It provides somatosensory innervation to the orofacial region and has three branches: ophthalmic (V1), maxillary (V2) and mandibular (V3). So any alteration in its seceretion in response to viral infection may result in symptoms in orofacial region[6].

So far, only prevention strategies are being adopted such as keeping a reasonable distance, staying at home and isolation. In my opinion, SP is responsible for the initiation of inflammatory pathways. It aggravates the condition by its over secretion by TG neurons which affects the immune cells as well as other cells in respiratory tract to release the mediators for cytokine storming which is responsible for further complications. The ventilatory role of SP is well established[7]. There may be less unlikely possibility of latency in SARS-Cov-2 and this virus may reach the TG via TrN in eyes, nose and mouth and controls the release of peptides including SP. It may remain in latent form in TG and may reactivate anytime causing infection or the person will remain asymptomatic in this condition. Virus remains dormant or latent within a cell and the replication of virus ceases after initial infection [8]. Viral genome may stay in the cell and may get reactivated anytime[9]. Reactivation may occur due to stress or UV etc [10]. The viruses may be retained in the cells of the host after initial infection despite of antibodies against it in the blood [11].

Substance P and Neurokinin--1 Receptor:

SP is a ‘brain-gut’ hormone and the first inflammatory neuropeptide discovered in 1931 by V Euler and Gaddum**.**[12] It belongs to Tachykinin (TK) family of proteins which is the largest of protein families having approximately 40 members. It has 11 amino-acids and is a neuromodulator, most potent vasodilator and neurotransmitter, involved in signal transmission, encoded by Tachykinin-1

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(TAC-1) gene.[13] SP is released in the trigeminal ganglion (TG) by the fifth cranial nerve (CN V) endings, known as trigeminal nerve (TrN). TrN provides the main afferent pathways for the transmission of nociception, pain and control the physiological mechanisms in orofacial region, including eyes, nose, mouth and associated with their physiological and pathological functions. SP is localized in the respiratory nuclei of the respiratory network which control the ventilatory functions, cardiac functions and sleep-wake cycle[6, 7, 14, 15]. They control, function of eyes, mouth, nose, tongue, lips, facial muscle, mastication, gustation, olfaction under normal circumstances. SP is present in perivascular neural plexuses of lung, skin and brain.So, its effects are not only limited to nervous system but are wide in distribution and expression [16]. Respiratory rhythm regulation is the main role of SP which is evidenced through many studies[14, 17]. SP along with serotonin is found to be innervating the medullary motoneurons involved in upper airways[7]. It was also found to be a sensory neurotransmitter in the laryngeal afferent system[18].

Neurokinin-1 Receptor (NK-1R) is the receptor for SP and all the functions of SP are elicited only after binding with its receptor. Both SP and NK-1R are abundant in CNS, PNS and enteric system.[19] It also regulates the immune system and cardiovasculatory system.[20, 21] SP is a neurotransmitter but can also affect the distant cells by acting as a modulator or hormone functioning in a autocrine, endocrine and paracrine manner. It is released from non-neuronal cells as well such as immune cells [22]. It is associated with respiratory inflammation e.g. asthma and chronic obstructive pulmonary disease (COPD) [23]. NK-1R is a 7-transmembrane, G-protein coupled receptor having 407 amino-acids. It is located on several cells in circulatory, digestive, respiratory and immune system[13]. It is mainly present in the brainstem region where it controls the key functions such as respiration and cardiac control after binding with SP[24].

Trigeminal Nociceptive pathways:

Any noxious stimulus from the orofacial structures such as eye, nose, mouth, is mainly transmitted by the TrN. TG neurons produce SP, CGRP which are largely involved in neuromodulation but in inflmammation and nociception as well[25]. The primary afferent neurons of the TrN are mainly located in the TG and partially in the mesencephalic trigeminal nucleus in the brainstem. The TrN consists of 3 branches: the ophthalmic (V1), maxillary (V2) and mandicular (V3) nerves, each providing innervation to their respective regions of head[25] (Figure 1).

Pain or anyother noxious stimulus such as SARS-Cov-2 activates the nociceptors which are the free nerve endings of trigeminal sensory afferents. These sensory nerve fibers are myelinated or non-myelinated C-fibers and their cell bodies reside in the TG [6, 26]. These signals are carried via afferent

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fibers to the trigeminal spinal caudalis (Vc) nucleus of the brainstem. Here, they synapse with the second order neurons that project to the somatosensory and limbic cortices via tha thalamus. Inflammation of orofacial tissues that are innervated by the TrN can modify the activity of trigeminal afferent neurons, consequently, causing ectopic firing, raised sensitivity of noxious stimuli. Sensitization is facilitated by many mediators such as neurotrophic factors, neuropeptides at nerve endings e.g SP, CGRP, serotonin[26]. SP and CGRP increase in TG and TrN in response to nerve injury or anyother noxoìous stimuli[25].

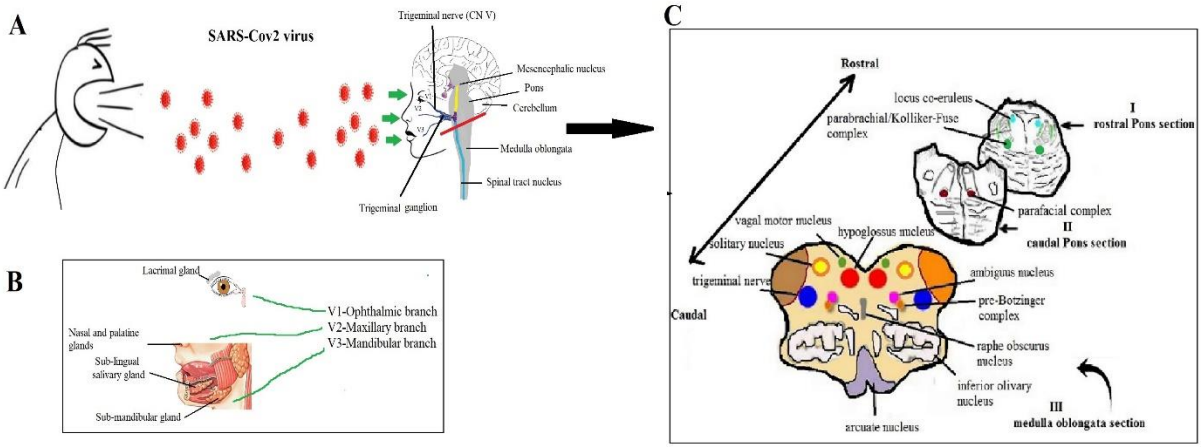

Figure 1-A: SARS-Cov-2 entry through orofacial structures, B) Trigeminal innervation in ophthalmic, maxillary and mandibular region, C) Schematic section of the main histological sections from the brainstem

Substance P immunomodulation in other viral infections and latency:

SP appears to contribute to disease pathology due to respiratory syncytial virus and encephalomyocarditis virus. It increases bronchoconstriction and cardiac inflammation following infection [27, 28]. SP is found to be directly related to inflammation. It can be used as a diagnostic and prognostic marker for Covid-19 infection. SP has been associated with various pathogenic diseases. Its role in HIV-AIDS has also been reported [29]. SP binds to NK-1R and cause an augmented HIV infection in macrophages via CD163 receptor [30]. NK-1R antagonists such as aprepitant show antiviral activities against HIV and may be used as a therapeutic strategy [31]. SP has also been reported in viral myocarditis which is a leading factor for heart failure. It is caused by encephalomyocarditis virus. Use of NK-1R antagonist may block the SP signaling pathway and serve as a drug target for treatment [28, 32]. It was also observed to have inflammatory role in infected rats with rat corona virus, parainfluenza virus 1[33].

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Herpes simplex virus type 1 (HSV-1) causes an infection of epithelial cells of nucosa tissues of the eye and orofacial region and then goes on to infect sensory neurons leading ultimately to a latent infection in TG . Virus passes through axons, reach the TG (Figure 2) and establishes life long latent infection where it encodes latency associated transcript (LAT) [34]. Reactivation of virus was found more in in TG as compared to brainstem in another study[35]. We may imagine a similar mechanism and pathology in Covid-19 infection as well. Previous studies have shown that latent HSV-1 infection of TG can alter the expression of many neuronal genes including those involved in the immune response, axonal remodeling, signal transduction and gene expression[36]. However, there is little evidence that HSV-1 LAT can affect the expression of neuropeptides in TG. The specificity of LAT action on TG may help in understanding the reason that the primary location for latent HSV1 infection is the TG [34].

Latent HSV-1 DNA has been detected in the CNS of humans postmortem, and infection with HSV has been correlated with the development of neurodegenerative diseases. However, whether HSV can directly reactivate in the CNS and/or infectious virus can be transported to the CNS following reactivation in peripheral ganglia has been unclear. Viral proteins were detected in neurons of the trigeminal ganglia. These results suggest that infectious virus is transported from the TG to the CNS following reactivation but do not exclude the potential for direct reactivation in the CNS [35]

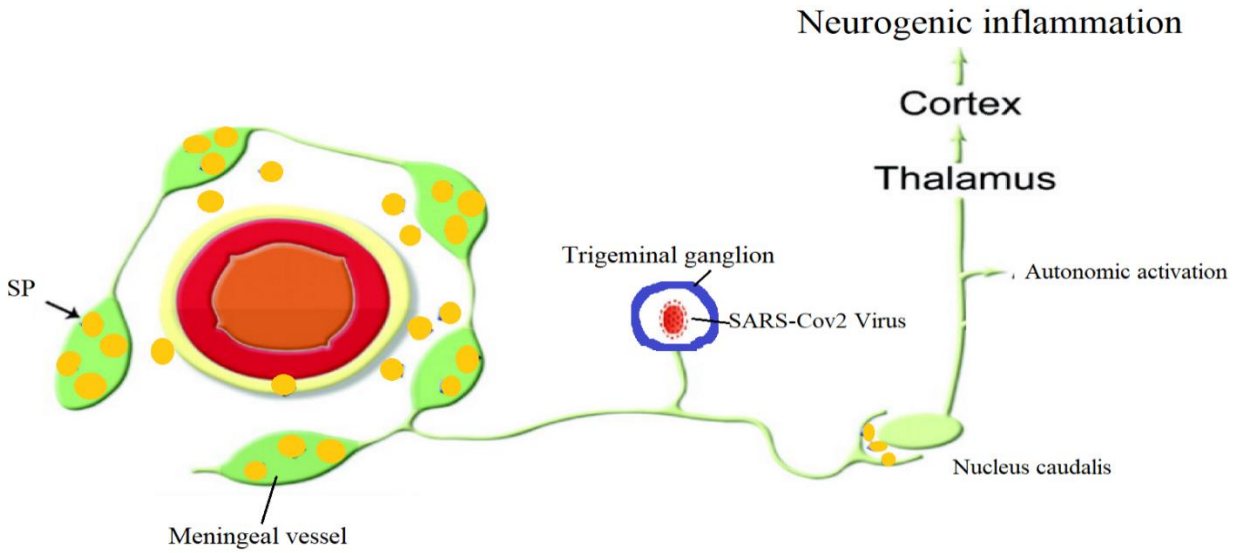

Figure 2: Corona Virus in Trigeminal ganglion

SP is the first to react in response to a noxious stimulus. It is rapid, immediate defense and survival system. In experimental studies, NK-1R deficient mice exhibited reduced pulmonary inflammation as

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compared to controls. [37] The immune response prevents the host cells by fighting against the pathogen but if continues uncontrolled, it may be fatal. It is known as “cytokine storming”. Inflammatory mediators continues to be secreted by immune cells and can cause acute respiratory distress syndrome (ARDS) in Covid-19 infection. So, it is not actually the pathogen that is fatal, but the cytokine storming. If prevented or reversed, it may save the infected patients [38], [39].

SP/NK-1R may be controlling the breathing activity in neonates, evident from the raised immunohistochemical SP expression in the brainstem tissues after postmortem studies in control infants as compared to Sudden Infant Death syndrome (SIDS) victims in my previous study at Centro Lino Rossi, University of Milan, Italy[6, 14]. SP expression was higher in sudden fetal deaths [14] and sudden death in adults[40]. SP and NK-1R regulates the breathing and cardiovascular control in medulla as a consequence of hypoxia.In a study conducted on SIDS victims, a significantly decreases in NK-1R binding within medullary nuclei in SIDS was observed as compared to control. Alteration in SP secretions and modulation may disturb the autonomic functionalities leading to lack of arousal and cause SIDS[41]. It explains a possible mechanism of causality in elderly patients due to Covid-19. As a neuromodulator, SP dilates the vessels, smooth muscle contraction in respiratory walls, increases the excitatory potential by neurons, increased vascular permeability[42] and saliva production. Under pathological conditions, it may cause bronchoconstriction.[43] My another study highlighted the fact that SP encoding gene TAC-1 has unconventional networking properties: being singleton, small protein interaction network and the members of tachykinin family have conserved aminoacid sequences, which make it vulnerable to be a causative agent for various diseases including fatal ones and death too[44].

Reactivation occurs in Herpes and HIV but not common in other types of viruses. It has not been speculated to exist in SARS-Cov-2, atleast in shorter duration of time. But if it proves to be the case then it may cause infection again at later stage in lifetime. Upon contracting the virus in orofacial parts, the virus reaches the TG through TrN and may reside there, taking control of the release of its peptides particularly SP. The vaccines may also not be of much help in eradication of the disease. Antibodies generated as an immune response to weakend viruses may only fight with the new active viruses. It will not kill the already present latent, inactive viruses inside the TG or other cells. These latent viruses may escape the immune system and become a continuous threat for disease.

Antibody therapies such as passive immunization and Intravenous immunoglobulin therapy may also not be useful in these circumstances. In that case, the promising strategy would be the use of antivial drugs, corticosteroids and SP/NK-1 blockers to cease the inflammatory responses and attack the latent viruses as well. Although this is one possibility which has less chances but cant be ruled out

totally due to its entry route through trigeminal nerves and getting toward TG like other latent viruses such as Hepes Simplex Viruses. We have to keep an eye on this phenomenon as well. Whether the virus becomes latent upon reaching TG or not is one aspect that needs to be explored but there are more possibilities that this virus reach TG and modulate SP release and inititates inflammatory mechanisms.

Another compelling argument is that these viruses are highly mutating and transforming, making it a difficult target for vaccines. Vaccines are successful for those viruses that mutate less and have strictly humans as host and have only one and stable antigenic type. Common cold viruses including SARS-Cov-2 have atleast 100 antigenic types and high mutation rate. So, vaccine development may be a challenging task in this scenario.

Serotonin and CGRP also co-exist with SP and should be explored as well. The abnormal release of SP from CN V may be associated with the acute respiratory symptoms in SARS-Cov-2 infection. SP may trigger the immune cells to release the inflammatory mediators in CNS as well as in respiratory system causing cytokine storming, inflammation, lung injury and bronchoconstriction. In complicated cases it may lead to cardiac failure. TG stimulation by virus may also increase the conductance of sodium ions and decrease the conductance of potassium ions in the cells leading to hypernatremia, hypokalemia and dehydration. This electrolyte and fluid balance will exert pressure on kidneys and pumping of heart causing them to failure and death. Maintaining electrolyte and fluid balance is necessary to manage these patients. Potassium and magnesium should be added in their dosage. High sunlight exposure, stress may also reactivate the virus. But all these phenomenons need to be evaluated and clinical trials are required.

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