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Selenium, a micronutrient can modulate viral diseases including COVID-19

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Selenium, a micronutrient is reported to play a very important role in fighting bacterial and viral infections. Selenium exerts its effects through incorporation into selenoproteins that are crucial for providing antioxidant defense and maintaining redox homeostasis. The deficiency of selenium in the diet leads to impaired immune response and increased pathogenesis of viral diseases. On the other hand, supplementation with selenium has been shown to be very effective in reducing infections and mortality against many pathogenic RNA viruses. This has encouraged researchers across the world to examine the role of selenium (if any) in the susceptibility and/or severity of the recent outbreak of coronavirus disease (COVID-19). Some of the emerging findings along with the future scope of research on using selenium for management of viral infections including COVID-19 are discussed.

Keywords: Ebselen, Pneumonitis, Selenium, Selenoneine, Selenoproteins

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Abbreviations: ACE2, Angiotensin-converting enzyme 2; AIDS, Acquired immune deficiency syndrome; AITD, Autoimmune thyroid disease; BCG, Bacillus Calmette–Guérin; CD, Crohn disease; $3CL^{pro}$, 3 Chymotrypsin-like protease; CoV, Coronaviruses; COVID-19, Coronavirus disease 2019; CVD, Cardiovascular disease; DHS, Dihydroxy-1-selenolane; DPD, Diphenyldiselenide; DSePA, Diselenodipropionic acid; E protein, Envelope protein; G-CSF, Granulocyte-colony stimulating factor; FDA, Food and drug administration; GPx, Glutathione peroxidase; GSeSeG, Selenoglutathione; HCV, Hepatitis C Virus; HIV, Human immune deficiency virus; H_2Se, Hydrogen selenide; IC_{50}, 50% inhibitory concentration; IAV, Influenza A virus; ICAM-1, Intracellular adhesion molecule-1; IFN-g, Interferon-gamma; IL, Interleukin; IL2R, Interleukin 2 receptor; LD_{50}, Median lethal dose; M^{pro}, Main protease; M protein, Membrane protein; MERS-CoV, Middle east respiratory syndrome coronavirus; MIP1A, Macrophage inflammatory protein 1 alpha; MSA, Methylseleninic acid; 2019-nCov, 2019 novel coronavirus; N protein, Nucleocapsid protein; NK, Natural killer; ORFs,Open reading frames; PMN, Polymorphonuclear neutrophils; PP, Polyprotein; pXSC, 1,4 Phenylenebis(methylene) selenocyanate; RNA, Ribonucleic acid; ROS, Reactive oxygen species; SARS-CoV, Severe acute respiratory syndrome coronavirus; SECIS, Selenocysteine insertion sequence; SeCys, Selenocysteine; S protein, Spike protein; TGF-β, Tumor growth factor-β; TMPRSS2, Transmembrane protease serine 2; TNFα, Tumor necrosis factor alpha; UTR, Untranslated region; SeM, selenomethionine; WNV, West nile virus; WHO, World health organization

Selenium is the 34th element belonging to Group 16 of the Periodic Table, which is also known as chalcogenide. Unlike other important elements of the group, oxygen, and sulfur, selenium is less abundant and a lesser-known element in biology and medicine. For quite some time, selenium was considered as the highly toxic element and several disorders, including cancers in humans, and animals have been attributed to the bioaccumulation of selenium[1]. If we observe the periodic table, it is interesting to note that this element is surrounded by more toxic elements like arsenic, antimony, tellurium, and bromine. Therefore, it is natural to presume that selenium is also toxic. This myth was however dispelled by the pioneering work of Swartz and Foltz in 1957 revealing that selenium is a micronutrient for mammals[2-5]. Subsequent research has confirmed the biochemical role of this trace element through its incorporation into 21st amino-acid known as selenocysteine (SeCys) and in turn constituting the active site of an important antioxidant enzyme, glutathione peroxidase (GPx)[5-8]. The physiological function of GPx is to catalyze the reduction of hydrogen peroxide by using glutathione as a co-factor. Over the years, researchers have identified at least eight different isoforms of GPx in mammals, out of which five (GPx1–4 and GPx6) are known to contain SeCys in their active site[7]. Along with GPx, humans have at least 24 other

proteins where SeCys is the constituent, and these are often termed as selenoproteins. Selenoprotein family, in human, constitute five GPx isoforms, thioredoxin reductases, thioredoxin-glutathione reductase, iodothyronine deiodinases, selenophosphate synthetase, selenoprotein F, selenoprotein H, selenoprotein I, selenoprotein K, selenoprotein M, selenoprotein N, selenoprotein O, selenoprotein P, methionine sulfoxidereductase B1, selenoprotein S, selenoprotein T, selenoprotein V and selenoprotein W[5].

The incorporation of selenium into selenoproteins occurs in a complex biosynthetic pathway catalyzed by several enzymes (Fig. 1)[5,9,10]. In brief, dietary selenium (organic or inorganic) undergoes reductive metabolism to produce hydrogen selenide (H_2Se). Subsequently, selenophosphatesynthetase converts H_2Se into selenophosphate which is incorporated into selenoproteins. The tRNA responsible for encoding SeCys is initially charged with serine by seryl-tRNAsynthetase to form seryl-tRNA. The seryl moiety is converted to O-phosphoseryl-tRNA by a phosphokinase. SeCys synthase replaces oxygen in the O-phosphoseryl-tRNA by a selenium atom generating selenocysteyl-tRNA. The selenocysteyl-tRNA is used for translating UGA codon of the mRNA into SeCys for selenoproteins.

Although UGA usually codes for the termination of protein synthesis, it also specifies SeCys in presence of a stem–loop structure called SeCys insertion sequence (SECIS) element downstream of UGA, in the mRNA of selenoproteins[10]. In mammals, the SECIS element is found in the 3′ untranslated region (UTR) of selenoprotein mRNAs. Apart from SeCys, selenomethionine (SeM) is another important selenium-containing amino-acid, which is incorporated into selenoproteins non-specifically in place of methionine by the initiation codon, AUG using methionine-tRNA. The nonspecific incorporation of SeM into proteins is more common in plants and yeast as they do not have the machinery for the biosynthesis of selenoproteins[11]. These selenoproteins play many important roles in cells, which include thyroid hormone metabolism, SeCys synthesis, transportation and storage of selenium, protein folding, immune-modulation, and protection from oxidative stress by neutralizing oxidizing peroxides, reducing oxidized proteins and membranes, controlling redox signaling, and maintaining redox homeostasis[5]. All the above physiological functions of selenoproteins are mediated through SeCys residue present in their active sites. SeCys has lower pKa as compared to analogous sulphur- containing amino acid, cysteine and therefore is catalytically more reactive under physiological conditions[12].

Selenium enters the environment through volcanic activity, weathering of rocks and soils, groundwater transportation, and metabolic uptake and release by plants and animals[11]. It enters the food chain through plant and meat products. The nutritional supply of

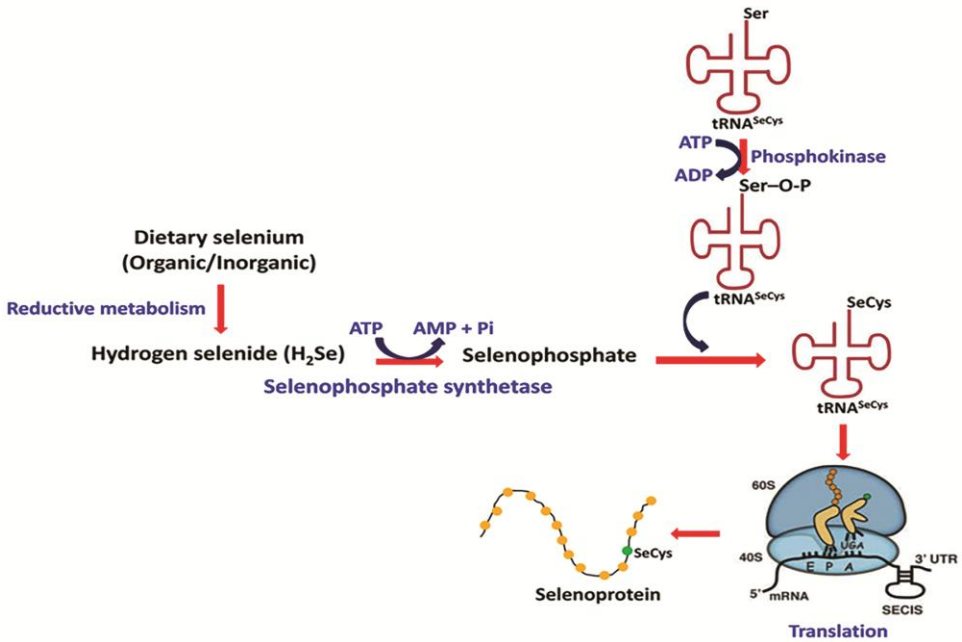

Fig. 1 — Schematic of the incorporation of dietary selenium into selenoproteins

selenium for humans depends on the selenium content of the soil, where it is mainly present in inorganic forms like selenite and selenate. It is subsequently accumulated in farmcrops and animals and finally to humans through the diet. Natural selenium sources are Brazil nuts, nuts, cereals, grains, legumes, selenised yeast, drum sticks, onion, garlic *etc*. Through plant products, selenium is taken up predominantly as SeM, methylselenocysteine or γ-glutamyl-selenium-methylselenocysteine, whereas the meat is the source of SeCys[9]. Selenoneine, a new organoselenium compound has been identified from sea fish[13].

The Recommended Daily Allowance of selenium is about 70 µg/day for men and 55-60 µg/day for women. Globally dietary selenium intake is usually below 55 µg/day. Notably, some European countries such as Spain, Greece, Poland have an average intake below 40 µg/day while in Argentina it is 32 to 24 µg/day. Most of the selenium- deficient countries have introduced selenium- enriched fertilizers to improve the selenium status in farm produce[14]. Selenium-enriched functional foods like vegetables, grains, eggs, milk, and meat have also been developed and introduced by these countries to overcome selenium deficiency. Overall, the intake of selenium up to 150 µg/day has been suggested to overcome its deficiency. According to National Research Council of USA, consumption of selenium in the range of 50 to 200 µg/day is considered safe, although the upper limit may vary from country to country. Similarly, some reports from China claimed that selenium intake even up to 750 µg/day did not produce any toxicity[15]. The level of selenium in the serum of healthy individuals ranges from 70 to 187 µg/dL depending on sex and age[15]. Notably, the expressions and activities of GPx and selenoprotein P in serum are highly responsive to the selenium status or availability and is used as an indicator to define the selenium deficiency or sufficiency in the body[8,15]. The deficiency of selenium has been associated with the onset of many chronic diseases like heart diseases, cancer *etc*.

As the selenium status or level in earth's crust is not the same at all the places, the dietary intake and bioavailability (serum level) of selenium vary significantly in different populations across the globe. China for example has two regions, with highly different selenium levels in soil[16]. About 72% of China (from northeast to southwest in the country) is designated by the world health organization (WHO) as selenium- deficient which affects over 70 million people, resulting in serious health consequences and endemic diseases like Keshan (endemic cardiomyopathy) and Kaschin-Beck (endemic osteoarthropathy). Dashan region located in the south of Anhui province, China is known as selenium-enriched area, where acute and chronic effects have been reported. Acute selenium toxicity causes gastrointestinal, respiratory, and cardiovascular problems, while chronic exposure results in mental problems, garlic-smelling breath, hair loss, fragile nails and excessive tooth decay[1,17]. Further, humans and animals consuming selenium-enriched foods have shown sporadic cases of selenium toxicity (selenosis). Additionally, few reports are indicating that selenium intake above the nutritional requirements may trigger the pathogenesis of type2 diabetes in human[17].

Selenium and immune functions

Mammals have the unique ability to protect themselves from pathogenic microorganisms by mounting an immune responses against them. The immune response constitutes of nonspecific immunity mediated by mononuclear phagocytes (monocytes/macrophages), polymorphonuclear cells (such as neutrophils), natural killer (NK) cells, and the complement system and specific immunity mediated by T (cellular immunity) and B (humoral or antibody response) cells. Classically these two types of the immune responses are referred to as innate and adoptive or acquired immunity, respectively. The successful elimination of pathogens from the body requires the coordinated functions of all the above effectors or cell types involved in innate and adoptive immunity. The loss in the functionality or the developmental defects in any of the above cell types increases the vulnerability of an individual for pathogens including bacterial and viral infections[18]. It is well established by the researchers that adequate nutritional status consisting of macro and micronutrients is needed for the optimal development and functionality of the immune cells. Selenium has been extensively studied for the correlation of its status (mainly deficiency) with the immune functions[5,6,8,19-22]. These studies have established an inverse correlation between selenium deficiency and the functionality of the cells of innate and adoptive immunity. For example, selenium deficiency in mice models is shown to cause cytotoxicity in lymphocytes, reduction in proliferation and differentiation (CD^{8+} and CD^{2+}) of T cells, decrease in the levels of serum IgG and IgM concentrations, and antibody responses[6,8]. The

effect of selenium deficiency on the T cell development has been attributed to the decrease in the expression of surface receptor- like interleukin 2 receptor (IL2R) and/or the loss of affinity of this receptor for its ligand, IL2 (a cytokine involved in the proliferation and maturation of T cells)[20]. Additionally, selenium deficiency also affects the innate immunity by reducing the ability of neutrophils to produce reactive oxygen species (ROS) needed for pathogen killing, decreasing the activity and count of NK cells, and inhibiting the phagocytosis of macrophages[19,21]. On the other hand, selenium supplementation in experimental animal models is shown to enhance immune functions by increasing the proliferation and differentiation of T cells and the activity of NK cells[6,8]. Indeed there are also reports of immune enhancement in human subjects through selenium supplementation. In brief, supplementation of adult human subjects with selenium (100 µg/day) enhanced a number of host immune responses like mitogen- activated proliferation of lymphocytes, antigen- induced proliferation and differential of T cells, increased production of interferon-gamma (IFN-g), up-regulation of IL-2 receptors, improved NK cell activity and increased antibody responses[8,22].

Viral infections and selenium

An effective immune response is necessary for the fight against viral infections. Considering the role of selenium in immune functions, its deficiency in humans or animals is expected to cause vulnerability to a wide variety of infections. Indeed, there are many reports in the literature, which confirmed that selenium deficiency is linked with the increased incidence, severity (virulence) and/or progression of viral infections[5,6,8]. Most of the human pathogenic viruses are ribonucleic acid (RNA) viruses including human immune deficiency virus (HIV), hepatitis C virus (HCV), Influenza A virus (IAV), poliovirus, West Nile virus (WNV), Ebola virus, and currently emerging corona viruses (CoV). Since the evolution, RNA viruses have shown the ability to mutate very fast under infective stages and this accounts for the high lethality rate associated with the pathologies caused by these viruses. The faster mutation is an adoptive strategy acquired by RNA viruses to survive under adverse conditions or to overcome the immune response of the host cells.

The earliest observations on the involvement of selenium in the progression of viral infections came from the study of human subjects with Keshan disease. Keshan disease is a pathological condition characterized by selenium deficiency, cardio-myopathy, and predisposition of infection to enterovirus. Bai *et al*. demonstrated that mice fed with selenium- deficient grains and infected with a strain of Coxsackie virus B4 (isolated from a Keshan disease victim), developed severe heart pathology[23]. Whereas similar viral infection in mice fed with selenium- enriched grains developed only mild heart pathology. This suggested that selenium deficiency is associated with increased infection of the Coxsackie virus and the onset of the pathogenesis of Keshan disease. This study also indicated that mice with adequate selenium levels showed high expression of GPx level in serum and immune cells, activated immune response, and prevented from viral infections. Similar observations have been reported with influenza virus infections, which are known to cause lung pathology. The mice deficient in selenium status were found to be more susceptible to influenza strains (influenza A/Bangkok/1/79/H1N1) and represented higher mortality as well as inflammatory score in the lung of these mice as compared to those with adequate selenium[24]. Notably, these studies also reported a decrease in GPx level of the lung of selenium-deficient mice and attributed this as one the factors responsible for the inflammatory responses in the lung following influenza virus infection[8,24].

Furthermore, it was also observed that benign strains of many viruses infected to selenium-deficient hosts were transformed into virulent ones by undergoing genetic mutations. Beck and colleagues made many important studies and reported that the amyocarditic strain of Coxsackie virus B3 (CVB3/0) mutated to virulence strain, when inoculated into selenium deficient mice[25-27]. The exact mechanism of such transformations is not understood. However, it was postulated that the reduced activity of antioxidant enzymes like GPx under selenium deficiency elevates the ROS level within host cells and this in turn causes the frequent damage-repair-mutation cycle of viral genome. Another explanation could be that impaired immune functions under selenium deficiency allows faster replication and in turn mutation of viral genome. All these hypotheses are still under validation.

Selenium has also been reported to play a crucial role in reducing the virulence of HIV infections[28-31]. Human HIV-1 and HIV-2 retroviruses are single-stranded RNA viruses and infect helper T (CD^{4+}) cells of individuals and in turn, suppress the adoptive immunity leading to the onset of a disease called acquired immune deficiency syndrome (AIDS). It has

been shown that the progression of HIV infection is accompanied by a concurrent decrease in plasma selenium levels. Such selenium deficient HIV patients are almost 20 times more susceptible to HIV-related mortality than those with adequate selenium levels[28,29]. Similarly, an inverse relation between plasma selenium levels and faster disease progression and mortality has also been seen in the case of HIV-infected children[30]. Daily supplementation with selenium, in HIV infected subjects, either as selenium salts or selenium- rich foods has been reported to suppress the viral burden, increase CD-4^{+} T cell counts, reduce oxidative stress, and to increase IL-2 production[31].

In the recent past, the outbreak of the Ebola virus in West Africa has also been linked with the deficiency of selenium in the susceptible population[32]. It is still not known whether the decrease in selenium status is the cause or effect of viral infections. However, researchers have proposed a new hypothesis of viral selenoproteins, according to which retroviruses can also incorporate selenium into viral proteins[33,34]. The group led by Taylor has performed extensive computational analysis of the viral proteins from HIV and Ebola and has confirmed the presence of UGA codon along with the SECIS in their genome[33,34]. His group has also suggested that some of the viral proteins show very high similarity with the mammalian GPx. As per his postulation, the growth of HIV viruses within host cells is restricted by their selenium status (high versus low). Under selenium deficiency, the intracellular environment of host cells becomes unfavorable for viral growth due to alteration in redox homeostasis. This in turn causes viral particles to escape from host cells and to spread the infection to neighboring cells. On contrary, selenium supplementation in HIV- infected subjects induces the expression of selenoproteins and reduces the level of oxidative stress in host cells prompting viruses to stay as dormant. The inhibition of viral multiplication also reduces the chance of their genome undergoing a mutation and acquiring virulence. Interestingly, Ebola viruses contain genes with several of SeCys insertion sites[33]. Therefore, it is expected that Ebola virus may compete with the host cells for the incorporation of selenium into selenoprotein leading to a physiological condition termed as "induced selenium deficiency". Indeed, a clinical study by Hou *et al*. indicated that selenium can effectively be used to treat an Ebola-like hemorrhagic fever[35]. In this study, administration of sodium selenite at a very high oral dose (2 mg for nineconsecutive days) reduced the death rate from 100% (untreated) to 37% (treated) in the very severe cases, and from 22% to zero in the less severe cases[35]. Overall above reports suggest that selenium controls the establishment and progression of viral infections by modulating the redox state (oxidizing versus reducing) and immune functions of host cells through antioxidant selenoproteins as presented in (Fig. 2).

CoV and selenium

Notably, CoV are the class of human pathogenic viruses that are known to cause fetal pneumonia- like pathology. These viruses have led to three major outbreaks of respiratory diseases since the beginning of the 21st century[36,37]. The first such outbreak was seen in the year 2003 with a fatality rate of 10% and the causative agent was named as Severe Acute Respiratory Syndrome Coronaviruse (SARS-CoV). Later in year 2012, another outbreak of CoV- induced pneumonia was reported from Arabian countries and this was named as Middle East Respiratory Syndrome Coronaviruse (MERS-CoV). The fatality rate associated with the disease was significantly higher of about 35%. Recently by the end of the year 2019, several unidentified cases of pneumonia were reported from Wuhan, China[37-43]. The viral genome analysis from these patients indicated the presence of a new variant of CoV named as 2019 novel coronavirus (2019-nCov) by WHO on January 12, 2020. Subsequently, it was found that the genome of these viruses shares a very high degree of sequence

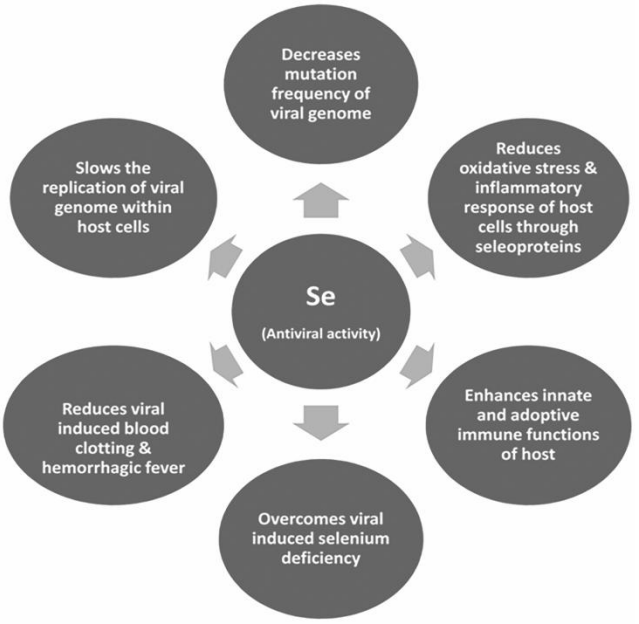

Fig. 2 — Mechanisms of the antiviral activity of selenium

homology (80%) with SARS-CoV and accordingly renamed by International Virus Classification Commission as SARS-CoV-2 on February 11, 2020. With the increasing number of infections and the spread of this virus to multiple countries, WHO named the respiratory disease caused by SARS-CoV-2 as COVID-19 and declared this outbreak a pandemic on March 11, 2020. At the time of writing this article, there are more than 7 million confirmed cases of COVID-19 worldwide and more than 400,000 deaths. The primary source for the human-to-human transmission of this virus is respiratory droplets from coughing or sneezing patients. The mortality rate associated with COVID-19 infections is in the range of 0.5% to 3% depending on age, sex, and co-morbid conditions[44].

The prediction about the structure and function of SARS-CoV-2 genome encoded proteins along with histopathological analysis of SARS-CoV-2 infected subjects have revealed some key points related to the pathophysiology of COVID-19 (Fig. 3)[37-45]. In brief, SARS-CoV-2 has positive- strand RNA as genetic material surrounded by a cage or envelop composed of lipoprotein. The genome size of SARS-CoV-2 is about ~30 Kb (30,000 bases) and carries the information of at least 10 open reading frames (ORFs) or genes[37]. Of these, ORF1ab translates two overlapping polyprotein 1a (PP1a) and polyprotein 1ab (PP1ab). ORF2-10 translates for viral structural proteins: spike (S) protein, envelope (E) protein, membrane (M) protein, nucleocapsid (N) protein, and other assisting proteins. The S, M, E proteins are involved in the formation of the viral coat and the N protein is involved in the packaging of the RNA genome[39,40]. The polyproteins (PP1a and PP1ab) undergo proteolytic cleavage to release function proteins involved in viral replication and transcription by viral proteases like 3 chymotrypsin-like proteases ($3CL^{pro}$) or main protease (M^{pro}) which by itself is released from polyproteins through autolytic cleavage[41]. The S protein present in viral coat interacts with surface receptors like angiotensin-converting enzyme 2 (ACE2) to facilitate its entry and to establish the infection of SARS-CoV-2 in host cells (like lung epithelium)[39,40]. Considering the wide distribution of ACE2 on cell types of different tissue origins like intestinal epithelium, endothelial cells of blood vessels, kidney epithelial cells, and nerve cells

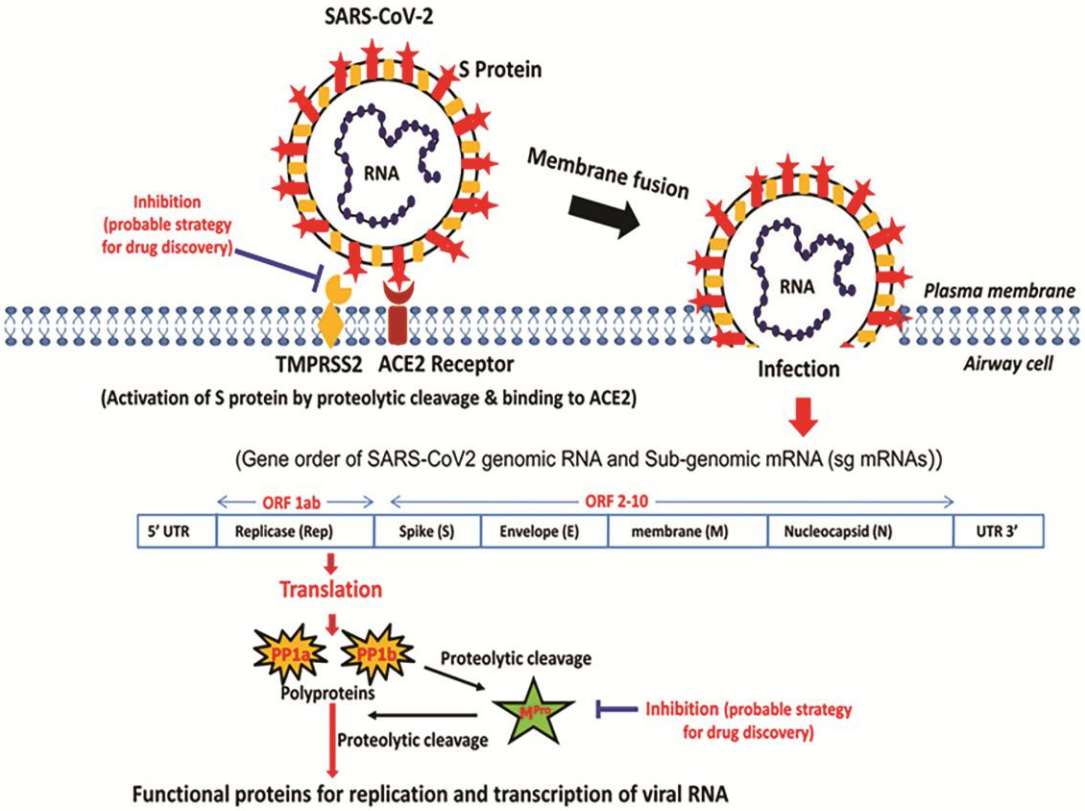

Fig. 3 — Probable SARS-CoV-2 proteins as therapeutic targets for organo selenium compounds

among others, it is believed that SARS-CoV-2 can infect to multiple organs apart from the lung. In addition to ACE2, another host protein called as transmembrane protease, serine 2 (TMPRSS2) is also involved in the entry of SARS-CoV into host cells[42]. This protein facilitates the proper docking of S protein on the ACE2 of host cells by trimming a part of it through proteolytic cleavage. Upon infection, the virus uses the host machinery to make new copies of its genome, reassembles itself, and comes out from the dead host cells to infect another cell. The dead lung cells mount an acute inflammatory response (classically referred to as pneumonia or cytokine storm) marked by the increase in the levels of pro-inflammatory cytokines like interleukin-6 (IL-6), granulocyte-colony stimulating factor (G-CSF), interleukin-10 (IL-10), interleukin-17 (IL-17), macrophage inflammatory protein 1 alpha (MIP1A), tumor necrosis factor (TNFα) among others[43,45]. Additionally, the activated immune cells start destroying the body's own cells triggering an autoimmune response. This is followed by respiratory and multi-organ failure and eventually mortality.

Since significant clinical benefits were observed with selenium supplementation in other similar RNA viral infections, researchers have started to examine the role of selenium in COVID-19 infections. Quickly in the last two to three months, a few papers already appeared on this subject. Prof, Rayman from UK along with her colleagues from China, in a letter to the Editor of the American Journal of Clinical Nutrition reported a correlation between selenium status and COVID-19 cure rates in few cities of Hubei province of China[46]. They used hair selenium concentration available in the database for correlating with the cure rate from COVID-19 and had two significant observations to report. First, the overall cure rate was significantly lower (13.2 % *vs* 40.6%) and the death rate was significantly higher (3% *vs* 0.6%) inside Hubei Province than that from outside-Hubei. Further, within Hubei province, Enshi city where high selenium status is reported notably had a high cure rate of 36.4%. The second interesting observation is that Heilongjiang Province in northeast China, with very low selenium status (16 µg/day), had a much higher death rate, than that of other provinces outside Hubei. The authors thus concluded a significant inverse association between cure rate and background selenium status in different cities of China. The authors however suggested validating these observations through proper clinical or epidemiological studies.

Apart from these reports, it is also interesting to look at the growing evidence about the effect of ageing and comorbid conditions on the selenium status of an individual[4,47-52]. In this context, Robberecht *et al*. have recently written an in-depth review summarizing various studies conducted on measuring selenium status in elderly people[47]. This group categorically concluded that serum selenium level is significantly reduced in an elderly population. Similarly, there are also clinical reports indicating that the selenium status of individuals suffering from chronic diseases is significantly lowered as compared to healthy individuals. For example, patients with Crohn disease (CD), cardiovascular disease (CVD), autoimmune thyroid disease (AITD), cancer and radiotherapy are shown to have impaired selenium status[4,48-52]. Despite many reports, it is still not clear, whether low selenium status is a cause or a consequence of the ageing process or disease. However, it can be speculated that inadequate selenium status in elderly individuals as well as in patients with co-morbid conditions like CVD, cancer, hypertension and auto-immune disorders may pose a higher risk for susceptibility as well as lesser cure rate from COVID-19. Therefore it is worth investigating such correlation study in the coming years.

Selenium- based therapeutics against COVID-19

To date no specific therapeutic medicine (herbal or allopathic), vaccine, and dietary supplement other than social distancing, and proper hygiene practices are available to protect from COVID-19. The evolving knowledge of the pathophysiology of COVID-19 has paved the way for evaluating several treatment strategies across the world[37-45,53]. Most of these treatment strategies are based on repurposing the already existing drugs for other clinical indications[37,38,41]. Considering the fact that drugs approved by Food and Drug Administration (FDA) are known for metabolic characteristics, dosages used, potential efficacy, and side effects, repurposing is the fastest way to develop medicines for the treatment of COVID-19. A few of the treatment approaches being used in the clinic to fight COVID-19 are as follows: (1) Broad-spectrum antiviral drugs like Remdesivir, Favipiravir, Lopinavir, Ritonavir, Viperin, Emodin, and Promazine among others being considered for the treatment of COVID-19. These drugs interfere with

the entry and molecular biology of the virus in the host cells[43,45]. (2) Anti-inflammatory drugs like glucocorticoids, IL-6 antagonists, and JAK inhibitors are being evaluated for efficacy against COVID-19[43,45]. These drugs suppress the inflammatory response or pneumonia associated with COVID-19 (3) As per the recent trials conducted in France, USA, China, and India (Jaipur hospital), hydroxychloroquine has shown potential for the treatment of COVID-19[43,45,53,54]. It has known anti-viral as well as anti-inflammatory activities. The combination of these activities is proposed to be responsible for its efficacy against COVID-19. However, there are also some concerns related to side effects (heart disorders) associated with this drug. (4) Bacillus Calmette–Guérin (BCG) vaccine against bacterium *Mycobacterium tuberculosis* is known to enhance the general immune response of a person against all kinds of infection including viruses. Recently few studies from the USA have reported a significantly lower infection and mortality rate and appearance of less severe symptoms among COVID-19 infected patients in countries, where BCG immunization is compulsory for all individuals as part of their health care programme[55]. Based on these observations, BCG vaccination is being suggested as the prophylactic measure against COVID-19. (5) The use of plasma from patients with COVID-19, who have made a full recovery (convalescent plasma) is another option being evaluated for management of COVID-19 globally[38,43,45]. Although, above treatment strategies have shown considerable success in the clinical setting, none of these have been approved by the FDA as a standard treatment protocol for COVID-19. This has kept all global pharmaceutical companies in the hunt for vaccine or specific drug against COVID-19.

Extensive research over the years has established that selenium in organic form is less toxic as compared to inorganic form and this has paved the way for a new research direction evaluating organoselenium- based compounds as potential therapeutic agents[4,56]. In this regard, a several organoselenium compounds such as ebselen, diphenyldiselenide (DPD), diselenodipropionic acid (DSePA), selenoglutathione (GSeSeG), dihydroxy-1-selenolane (DHS), methylseleninic acid (MSA) and 1,4 phenylenebis(methylene)selenocyanate among others are reported in the literature for various pharmacological activities ranging from antioxidant, anti-inflammatory, anticancer and radio-protective activities[4,56]. Ebselen is the most potent among the synthetic selenium compounds, which has been reported for excellent GPx-like activity in pre-clinical models[57]. It has also been demonstrated for anti-inflammatory, anti-cancer, and anti-oxidant properties using *in vivo* models. It is a modest immune-stimulant and induces several interleukins including interleukin-1 (IL-1), IL-6, IL-10 and interleukin-18 (IL-18). Ebselen has good blood-brain permeability and rapid absorption following oral administration. A number of studies demonstrated that ebselen attenuates neuronal cell death induced by ischemia/reperfusion. At present this compound has been approved by the FDA for the treatment of neurological bipolar disease/hearing loss and is also under clinical trials for the management of various other diseases. Similarly, our group has also been associated with the development of organoselenium compounds with the specific application as drugs for cancer radiotherapy[4]. One such compound, DSePA showed excellent results in preventing radiation-induced pneumonitis, one of the serious side effects of thoracic radiotherapy[4,58,59]. Mechanistically DSePA specifically increases selenium level in the lung tissue, elevates GPx level in the lung, and significantly reduces the radiation-mediated infiltration of polymorphonuclear neutrophils (PMN) and elevation in levels of cytokines such as G-CSF, interleukin-1-β (IL1-β), intracellular adhesion molecule-1 (ICAM-1), E-selectin, IL-17 and tumor growth factor-β (TGF-β) in the lung[58]. Additionally, the molecule also gains significance, as it is orally administrable. The lethal dose (LD_{50}) of DSePA is considerably higher than the known organoselenium compounds like SeM and methyl selenocysteine available in the market as health supplements[59].

Apart from the above pharmacological activities, the design and development of organoselenium-based therapeutics for anti-viral activities is also a fast-emerging area of research. The basic principle of designing such compounds is to use them as inhibitors to target viral proteins involved in entry, replication, and transcription within the host cells. The advantage of using viral proteins as targets is to improve the specificity of drugs as well as to reduce the side effect due to the absence of closely related homologues in humans. In the recent past, many synthetic organoselenium compounds such as diselenodi benzamides have been synthesized and evaluated for anti-HIV activity[60]. In brief, these compounds were reported to inhibit NCp7, a conserved retroviral protein of HIV that exerts essential and multiple functions in both early and late stages of viral replication. Similarly, a recent publication in "Nature journal" by a research group from China indicated that

synthetic organoselenium compounds could be potential candidate drug molecules for the management of COVID-19[41]. This group specifically proposed that the viral protein M^{pro} involved in the processing of the replicase proteins of SARS-CoV-2 is an attractive target to design drugs against COVID-19 (Fig. 3). Accordingly, the group performed structure-based docking of over 10000 FDA- approved drugs and other pharmacologically active compounds including Ebselen (organoselenium compound) as an inhibitor of M^{pro}. Subsequently, the most potent compounds were evaluated for anti-viral activities using recombinant M^{pro} and the cellular infection models of SARS-CoV-2. Additionally, the crystal structure of the inhibitor-protein (M^{pro}) complex was also established. Based on these-analysis, authors identified six compounds namely Ebselen, Disufiram, Tideglusib, Crmofur, Shikonin,and PX12 as potent inhibitors of the activity of M^{pro} with IC_{50} (50% inhibitory concentration) values ranging from 0.67 to 21.4 µM. Of these Ebselen was the best in terms of reducing the viral load in the cellular studies. This inhibitory action of Ebselen was mediated through its interaction with Cys^{145} and His^{41} residues present in the active site of M^{pro}. Finally, the authors concluded that Ebselen being FDA- approved drug is a candidate molecule for repurposing against COVID-19. This is landmark research and encourages synthetic researchers across the world to design newer and more potent organoselenium based derivatives for inhibition of viral proteins. Along with M^{pro}, another important SARS-CoV-2 protein which could be the possible targets of the organoselenium compound is S protein as it is involved in the viral entry into host cells (Fig 3). Additionally, it would also be worth evaluating organoselenium compounds for possible inhibition of host proteins such as ACE2 and/or TMPRSS2 (Fig. 3). However, these proteins are also involved in the normal functioning of various cell types and therefore side effects arising from the inhibition of these proteins may be a concern in drug development[37,39,40]. Finally, the pathogenesis of COVID-19 is marked by an acute inflammatory response and therefore several of synthetic organoselenium compounds including DSePA reported for anti-inflammatory activity in the lung may also be effective in suppressing or delaying pneumonia associated with COVID-19. However, this hypothesis needs to be rigorously tested using preclinical models.

Conclusion

The trace levels of several elements are required to maintain the redox and energy balance in the living systems. Selenium is one such trace element that has been proposed to be crucial not only for normal physiology but also for immunity to fight viral infections. Its function is mediated by the synthesis of selenoproteins.

Currently, when we are fighting the pandemic, we must contemplate and evaluate ourselves, where we as Indians stand as far as selenium nutrition is concerned. There is no systematic data on the statistics and mapping of selenium status in the Indian population, except for one study in some districts of the northern region[61]. This sole study documented that selenium levels were above normal where the soils are drier, alkaline, and less water is available for irrigation, while the soils of Punjab and Himachal Pradesh states showed low to normal levels, where plenty of irrigation water is available. An immediate task, therefore, is to evaluate selenium status among different populations and clusters and correct the deficiency if any through necessary supplementation. Since significant clinical benefits are achieved by supplementation through inorganic selenium compounds, the possibility of supplementation through organic forms should be explored. Organic selenium has the advantage of being less toxic than the inorganic forms.

Additionally, new research has to be initiated to explore the possibility of new selenium-based compounds for specific targeting of viral proteins. Initial promising results with Ebselen can be used as a possible themes for developing organoselenium- based drugs not only for COVID-19 infections but also for other viral diseases.

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Conflict of interest

All authors declare no conflict of interest.

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