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Review article

The dual impact of ACE2 in COVID-19 and ironical actions in geriatrics and pediatrics with possible therapeutic solutions

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ABSTRACT

The novel corona virus disease has shaken the entire world with its deadly effects and rapid transmission rates, posing a significant challenge to the healthcare authorities to develop suitable therapeutic solution to save lives on earth. The review aims to grab the attention of the researchers all over the globe, towards the role of ACE2 in COVID-19 disease. ACE2 serves as a molecular target for the SARS-CoV-2, to enter the target cell, by interacting with the viral glycoprotein spikes. However, the complexity began when numerous studies identified the protective response of ACE2 in abbreviating the harmful effects of vasoconstrictor, anti-inflammatory peptide, angiotensin 2, by mediating its conversion to angiotensin-(1–7), which exercised antagonistic actions to angiotensin 2. Furthermore, certain investigations revealed greater resistance among children as compared to the geriatrics, towards COVID-19 infection, despite the elevated expression of ACE2 in pediatric population. Based upon such evidences, the review demonstrated possible therapeutic interventions, targeting both the protective and deleterious effects of ACE2 in COVID-19 disease, primarily inhibiting ACE2-virus interactions or administering soluble ACE2. Thus, the authors aim to provide an opportunity for the researchers to consider RAAS system to be a significant element in development of suitable treatment regime for COVID-19 pandemic.

1. Introduction

The COVID-19 (CO-corona, VI-virus, D-disease, 19-of 2019) pandemic has created a chaos all over the globe, since its emergence from Hubei province in China in December 2019. Currently, the confirmed cases of COVID-19 have reached approximately 11,382,954 cases worldwide, with 533,477 deaths and 6,440,228 recovered, with USA, Brazil, Russia, India and Peru occupying the first five positions in terms of COVID-19 cases, which have been constantly rising each day [https://www.worldometers.info/coronavirus/]. Recent evidences have demonstrated greater risk of infection in hypertensive, diabetic, obese and elderly patients [1]. SARS-CoV-2 is responsible for COVID-19 infection in humans. This is a positive sense, single stranded ribonucleic acid (ssRNA) enveloped virus, comprising of glycoprotein spikes on the outer surface, which mediates its entry into the host cell [2]. The term SARS-CoV-2 has been given to the virus responsible for COVID-19 pandemic, on account of its similarity with the SARS-CoV of SARS (severe acute respiratory syndrome) 2003 pandemic, where both belong to the corona virus family, exhibit ACE2 mediated entry into the host cell and are known to originate from China. However, the mortality rate of SARS-CoV (9%) was greater than the SARS-CoV-2 (2.9%) of the COVID-19 pandemic [13]. All these phylogenetic similarities have led the scientists to investigate the entry mechanism of both the viruses, which was identical, as both mediated their host entry cell via attachment to ACE2 (angiotensin converting enzyme – 2) membrane receptors [3,4]. The organs like brain, heart, oral and nasal mucosa,

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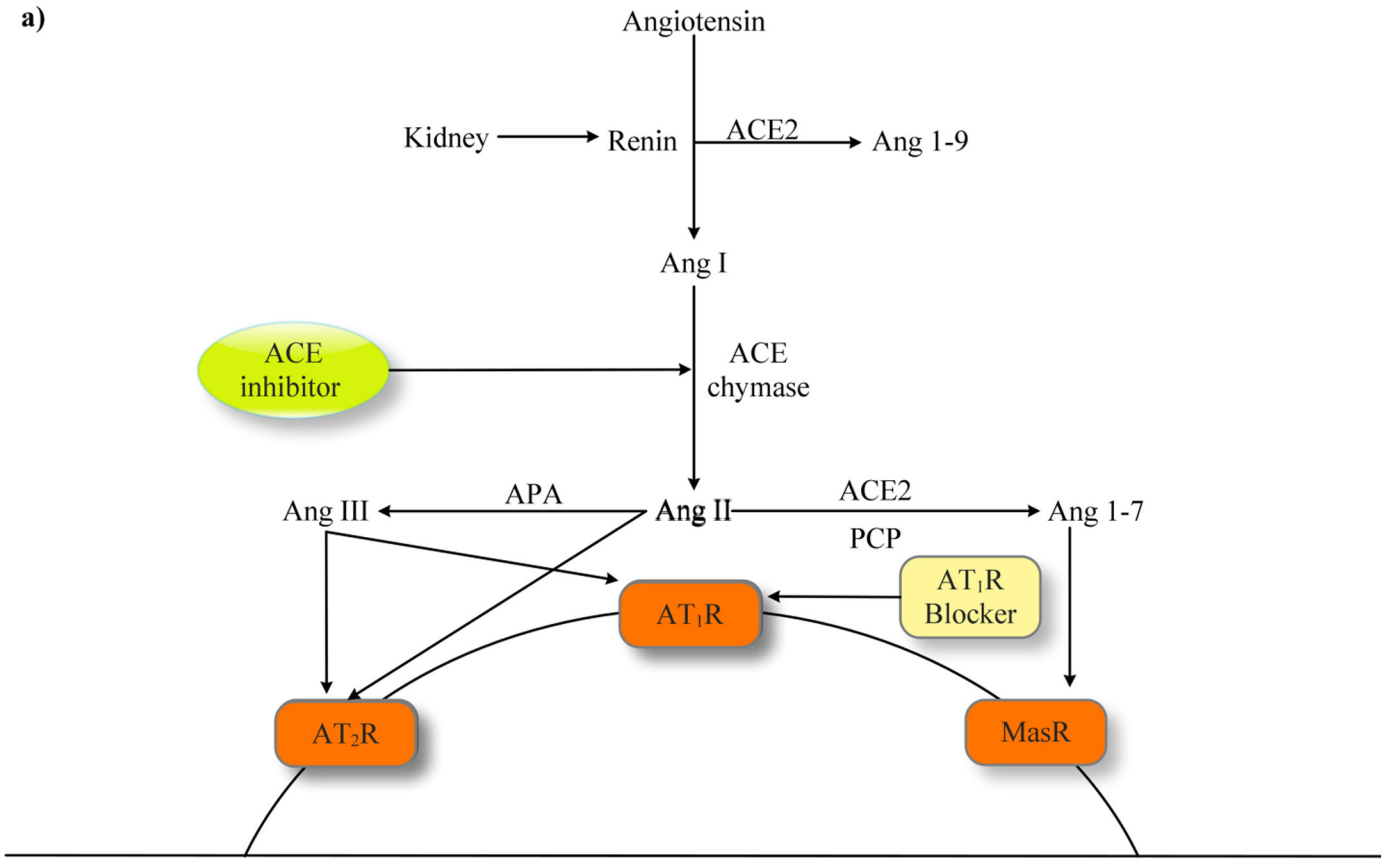
kidney, nasopharynx, colon, lymph nodes, small intestine, stomach, thymus, skin, spleen, bone marrow, liver and blood vessels, are all susceptible to be infected by COVID-19, on account of presence of abundant ACE2 in these areas of the body [5,6]. Besides, ACE2 expression is found in abundance in the lung alveolar epithelial cells, which accounts for most of the damage to the lungs, resulting in acute lung damage, acute respiratory distress syndrome (ARDS), pneumonia etc. [7]. Thus, it has been demonstrated that SARS-CoV-2 develops a relationship with renin angiotensin aldosterone system (hormonal cascade regulating primary processes involved in human physiology, like volume homeostasis and blood pressure), via ACE2 enzyme [8]. Angiotensinogen is a primary substrate for renin-angiotensin-aldosterone system (RAAS), which is produced in the liver, and is cleaved to produce angiotensin 1 (also called pro-angiotensin), by renin [1]. Angiotensin 1 is further activated to angiotensin 2, by ACE, which acts as peptidyldipeptidase, and transforms the decapeptide (angiotensin 1) to 8 amino acid peptide (angiotensin 2), which is one of the most commonly known vasoconstrictors in the body [9]. Furthermore, ACE2 is another 17 amino acid, enzymatic component of RAAS system with N terminal signal peptide along with a C terminal membrane anchor [1]. The C terminal amino acid of decapeptide (angiotensin 1) is cleaved by this transmembrane protein to a nonapeptide (angiotensin-(1–9)) [1]. Besides, ACE2 is also responsible for conversion of angiotensin 2 to a heptapaptide (angiotensin-(1–7)), which mediates its actions by G-protein coupled receptor (GPCR), i.e. Mas receptor [1]. The ACE2/angiotensin-(1–7)/Mas axis (significantly known as inhibitor system of RAAS), accounts for vasodilatory properties, as well as anti-inflammatory and antioxidant actions in the body [1]. The extracellular portion of the cell comprises of a catalytic domain of ACE2, which can be cleaved, followed by its release into the blood by ADAM17 (metallopeptidase and a disintegrin) [10,11]. Therefore, ACE2 counter acts the vasoconstriction, pro-inflammatory actions, sodium retention and pro-fibrotic actions mediated by angiotensin 2 by degrading this peptide to a heptapeptide (angiotensin-(1–7), thus, alleviating the effects mediated by angiotensin 2 [12]. Thus, the effects of ACE/angiotensin 2 (activator system of RAAS), are opposed by the inhibitor system [12]. Thus, it can be said that the ACE2 axis has the tendency to facilitate negative regulation of ACE axis [1].The interaction between ACE2 and SARS-CoV-2 has a significant contribution and relationship, with the intensity of infection developed in the body [14]. Understanding this interaction, can pave a way for the scientists and researchers to develop suitable therapeutic option, aiding in COVID-19 treatment.

Furthermore, the infection mediated by SARS-CoV-2 has been observed to vary according to difference in the age groups, where the older patients are more susceptible to COVID-19 than children [15]. Thus, numerous pathogenic differences of COVID-19 and severity of the infection have been observed between geriatrics and pediatrics, which has been discussed in the review. The protective mechanisms in children preventing against COVID-19 can serve as effective therapeutic target for susceptible elderly individuals, thus it is extremely important to promote high risk group identification in children, in order to facilitate development of appropriate therapeutics and vaccines can be developed [15]. Therefore, the review targets clinical manifestations, disease pathogenesis and possible factors, demonstrating the intensity of infection between pediatrics and adults. Also, on account of similarities between COVID-19 and SARS, certain results obtained from pathogenesis of SARS-CoV-1 are extrapolated to COVID-19 infection [15]. The review highlights the possible causes of better resistance to COVID-19 in children than in adults, on account of RAAS components and ACE2 expression, depicting dual role of ACE2 in providing protection against formation of inflammatory lesions and also exerting detrimental effects by serving as a molecular target facilitating entry of the virus. Also, the infants are found to possess greater threats to the infections by SARS-CoV-2, as compared to older children and adults, as discussed later in the review. The later sections focus on the development of therapeutic strategies, targeting RAAS system, in the management of COVID-19 and related anomalies, like delivery of recombinant human soluble ACE2 enzyme, that could compete for the binding to the SARS-CoV-2, with the membrane ACE2 receptors, co-infection with less pathogenic human corona virus, NL 63, compensatory mechanisms of angiotensin 2, synergistic effects of melatonin and vitamin D supplementation, ACE inhibitors, angiotensin receptor blockers (ARBs) and morphine administration. The variability of actions mediated by membrane bound and soluble ACE2 generates a need to clearly understand the direct and compensatory mechanisms associated with this receptor. The authors aim to provide a reliable information on ACE2 actions against or in favor of COVID-19 progression to portray the complicated actions of ACE2 enzyme in the infection, in order to provide an opportunity to the researchers to figure out a dominant action of ACE2 in the COVID-19 disease, which could further aid in the development of suitable vaccine and therapeutic solutions. This, in turn, would also promote clarity in investigating the ironical roles of ACE2 in three age groups, i.e. infants, children and adults/geriatrics, thus making it extremely necessary to study the receptor actions in the current pandemic.

2. ACE2 and COVID-19

Proteases or peptides are non-specific enzymes located in extracellular domains, which are responsible for degradation of certain peptides in human body [16]. These membrane proteins are further divided into two classes, endopeptidases and exopeptidases, where the former is responsible for releasing N- or C-terminal amino acids, while the latter deals with cleavage of peptide chain [16]. Angiotensin converting enzymes (ACE) belong to the category of exopeptidases (carboxypeptidases), which may be common to several peptides [16]. ACE2 is mainly located in the membrane cell or are found as circulating forms, and is homologous to ACE (now designated as ACE1). ACE1 was introduced in 2000 [17,18]. The ACE discovery brought angiotensin 2 into the picture, which is an active peptide and is also known as angiotensin-(1–8) [16]. This peptide is responsible for mediating events like vasoconstriction (increased blood pressure), pro-inflammatory action, pro-fibrosis and stimulation of aldosterone secretion, by binding to angiotensin 1 (AT1) receptor. ACE2 acts by deactivating angiotensin 2, followed by its conversion to angiotensin-(1–7), which is also an active peptide that antagonizes the actions of angiotensin 2 (see Fig. 1) [19]. Unlike angiotensin-(1–8) (angiotensin 2), which mediated its actions by interacting with AT1 receptor, angiotensin-(1–7) induced anti-fibrosis, anti-inflammatory and vasodilatory actions by Mas receptor interaction (see Fig. 1) [20]. Furthermore, angiotensin 2 is also known to be deactivated by aminopeptidases enzyme, which mediates its transformation into angiotensin 3, resulting in actions like, enhanced natriuresis and bradykinin, and vasodilatory actions, by interacting with angiotensin 2 (AT2) receptors with affinity 30 times greater than that for AT1 receptors [21,22]. Also, it has been found that ACE2 is responsible for conversion of angiotensin 1 (angiotensin-(1 − 10)), to angiotensin-(1–9), whose actions are still unknown. Angiotensin-(1–9) is further transformed to angiotensin-(1–7), via ACE1 [16]. All these components form a significant part of renin-angiotensin aldosterone system, which comprises of an ‘activator’ and an ‘inhibitor’ pathway, where the former comprises of angiotensin 2/ACE1/AT1 receptor/aldosterone pathway, while the latter comprises of angiotensin-(1–7)/ACE2/Mas receptor pathway, where the inhibitor pathway was able to block the actions of angiotensin 2 in the body and counter act its effects (see Fig. 1) [16]. A complete understanding of both these inhibitor and activator system, as well as effect of angiotensin-(1–7)/ACE2/Mas receptor, over angiotensin 2/ACE1/AT1 receptor/aldosterone pathway, has not been explored to a significant extent [16]. Moreover, ACE2 has also been found to interact with AT1 receptors, which are also targeted by angiotensin 2 receptor blockers, which oppose the actions induced by this receptor, as mediated by angiotensin 2, therefore, occupying the site of interaction of angiotensin 2 [16]. This, in turn promotes liberation of angiotensin 2,



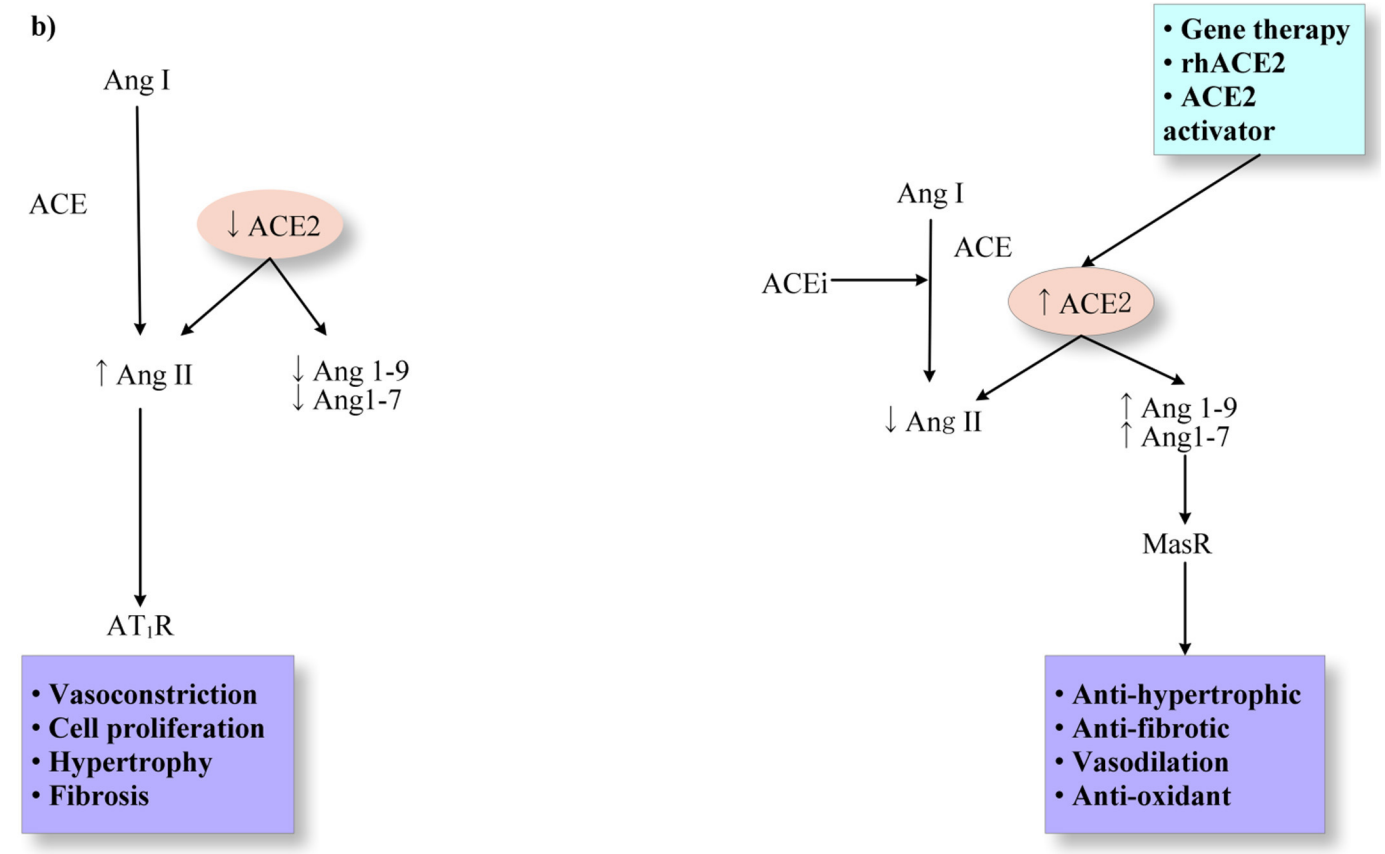


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Fig. 1. Renin angiotensin system enzymatic cascade depicting primary receptor systems, biological actions of Angiotensin 2 and Ang-(1–7) and balance between ACE and ACE2. a) RAS system demonstrating metabolic pathway of angiotensin peptide, as an initiator compound, cleaved to form Ang 1, by renin (kidney). This is followed by cleavage of Ang 1 to Ang 2, by ACE, followed by ACE2 mediated cleavage of Ang 2 to Ang-(1–7). Ang 2 acts on AT1 and AT2 receptors, while Ang-(1–7) acts on Mas receptors, and antagonizes the actions of Ang 2/AT1R axis. b) Curbed levels of ACE2 results in shifting of balance, in the RAS, towards Ang 2/AT1R axis (activator), facilitating infection progression. Enhanced ACE2 levels, by rhACE2, ACE2 activators or gene therapy, shifts the balance towards Ang-(1–7)/MasR axis (inhibitor), resulting in protection from the viral infection.

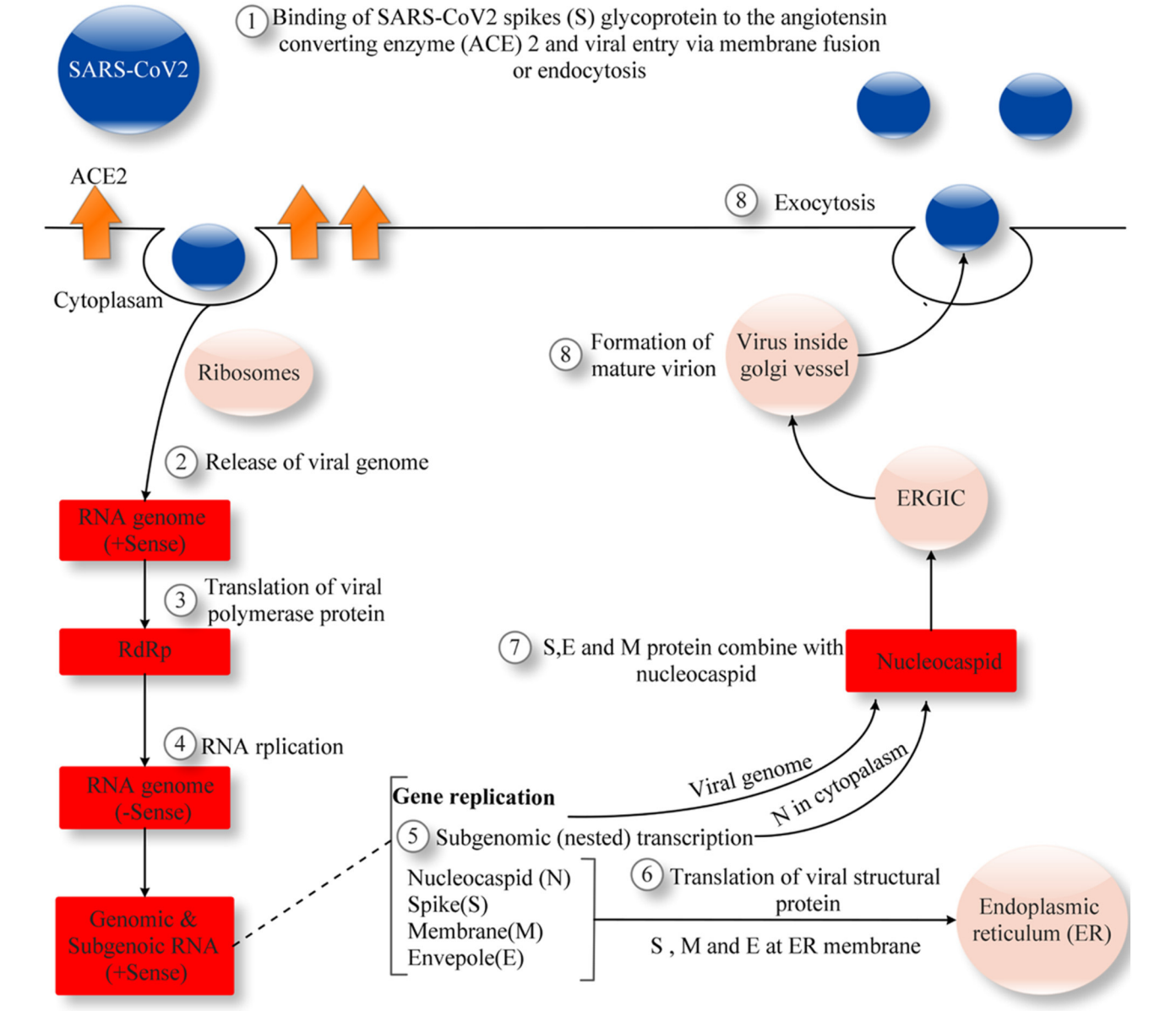

Fig. 2. Entry mechanism and life cycle of SARS-CoV-2 in host cells. The glycoprotein spikes of the virus bind to its receptor human angiotensin converting enzyme (ACE2), through receptor-binding domain (RBD), proteolytically activated by human proteases, and enters the cell through endosomal pathway. This is followed by uncovering of viral RNA in the cytoplasm and translation of ORF1a and ORF1ab to produce pp1a and pp1ab polyproteins. These proteins are cleaved by RTC proteases, which facilitate the production of full length (−) RNA copies and serve as templates for full length (+) RNA genomes. A set of sub-genomic RNAs (sgRNAs) is produced via fragmented transcription, having numerous open reading frames (ORFs), where only the closest (5′ end) is translated. The nucleocaspids are mustered in the cytoplasm, after the production of viral structural proteins. This is followed by budding in the lumen of intermediate compartment of endoplasmic reticulum (ER) and golgi apparatus (ERGIC). This is followed by exocytosis mediated release of virion from the infected cell.

followed by enhanced expression of ACE2 enzyme in the body [23]. Also, ACE2 can hetero-oligomerize with AT1 receptor, via Mas receptor, resulting in inhibition of the actions mediated by angiotensin 2, therefore, playing a significant role in regulation of this peptide in the body [24]. Various organs of the body like kidney, blood vessels, heart, brain, testicles, gastro-intestinal tract (GIT), adipose tissue and lungs, express the ACE2 enzyme, where lungs is the primary organ of consideration in the COVID-19 pandemic [16]. The glycoprotein spikes on the surface of SARS-CoV-2 utilize membrane ACE2 receptors and cellular protease, transmembrane protein serine 2 (TMPRSS2), in order to enter the target cells (see Fig. 2) [26,27]. Thus, ACE2 acts as a transmembrane enzyme with extracellular domain and provide a target site for the virus to mediate its actions in the human body(see Fig. 2) [16]. There is a certain degree of similarity between SARS-CoV-2 and

SARS-CoV-1 (of SARS pandemic of 2003), which accounts for similar entry mechanism of the two [28]. However, the similarity is 80%, with numerous distinctive features between the two viruses of the corona virus family [16], where SARS-CoV was confirmed to be a member of Beta-corona virus subgroup [22]. The SARS-CoV-2 possesses 10–20 times greater binding affinity towards ACE2 receptors, unlike SARS-CoV-1 [29,30]. Thus, the extent of binding affinity of SARS-CoV-2 towards ACE2 is directly proportional to ease with which the virus transmits from one human to another in the pandemic. The protein of SARS-CoV-2 is activated by TMPRSS2 proteases which mediate its binding to the extracellular region of transmembrane ACE2 receptors, promoting interaction with the virus, followed by penetration into the cell [16]. On the other hand, the circulating soluble ACE2 enzyme has the tendency to, without inducing any infectious actions, thus possibly introducing a therapeutic approach in alleviating COVID-19 progression in the human body [16]. Certain antibodies aim to block the interactions between SARS-CoV-2 and ACE2 receptors, promoting suitable therapeutic approach [31]. Therefore, ACE2, being the molecular receptor for facilitating entry of SARS-CoV-2, establishes an association between RAAS system and COVID-19 disease. Certain in vitro investigations have demonstrated positive relationship between tissue activity and membrane expression of ACE2 receptors, and susceptibility towards COVID-19 disease [32]. Furthermore, downregulation of tissue activity of ACE2 is induced by the virus where it binds to membrane ACE2 receptors in the infected cells, further aggravating the inflammatory responses, mediated by COVID-19, in organs, specifically lungs, and also reducing the risk of SARS-CoV-2 infection in other organs of the body [33]. An in vitro study depicted abbreviated ACE2 expression in lungs of mouse after administration of SARS-CoV which mediated respiratory impairment [14]. ARBs like losartan, if administered resulting in improved function of the respiratory system, maybe by restoring the ACE2 tissue activity and membrane expression, and permitting the interaction between AT1 receptor in the lungs and angiotensin 2, therefore, mitigating the risks of pulmonary infection [16]. Thus, it is evident that ACE2 tissue activity and membrane expression enhances the COVID-19 risks, along with inflammatory injuries in the body tissues [16]. However, in a recent retrospective Chinese study analysis in 175 patients infected with COVID-19, 62% patients showed hypokalemia [16]. The authors explained this to be a result of decreased tissue activity of ACE2 under COVID-19, which led to the imbalance between ACE1 and ACE2 levels in RAAS system with enhanced concentration of ACE1, resulting in altered deactivation of angiotensin 2, leading to enhanced angiotensin 2 mediated actions, like aldosterone synthesis, which resulted in hypokalaemia in patients [34]. On account of this hypothesis, the effects of mineralocorticoid receptor (MR) antagonists must be investigated due to their protective effects in hypokalaemia [16]. Therefore, angiotensin 2 levels were also reported to exert both protective and harmful effects in lung injury [16]. The COVID-19 infection primarily revolves around the tissue activity and membrane expression of ACE2 enzyme, making it complex to understanding the exact role of ACE2, which is harmful as well as protective in human body [35,36]. The receptor action of ACE2 promoting entry of COVID-19 marks its disastrous actions in the current pandemic, whereas its potential in inflammatory lesion phase promotes its useful impact against the virus [19,37]. This, in turn poses many questions, which are yet to be answered. Currently, there are no recognized specific pharmacological activators or inhibitors of ACE2 in humans [16]. However, there are some in vitro used ACE2 activators, or for veterinary purpose, like diminazineaceturate and xanthenone [38]. ACE2 activators have been found to exert anti-fibrotic and anti-inflammatory actions, serving as a suitable therapeutic approach in the current pandemic against SARS-CoV-2 infection [39]. Also, the circulating soluble ACE2 enzyme has opened a therapeutic window, which binds to the virus, decreasing its availability to the membrane ACE2 receptors, and thus, minimizing cell infection [16]. Engineered recombinant soluble ACE2 has been found as useful therapeutic option according to certain in vitro studies [27,40]. This soluble ACE2 enzyme activates RAAS inhibitor pathway and deactivates the RAAS activator pathway, thus preventing the formation of inflammatory tissue lesions [14]. Fig. 2 depicts the mechanism of entry SARS-CoV-2 in the host cell, and role of ACE2, as molecular target for virus propagation.

3. The irony in geriatric and pediatric population

Previously it has been discussed, how ACE2 has been recognized as a molecular target, but it can also serve as an ally to fight against this deadly disease. It was observed that the effects of COVID-19 disease were reduced in ACE2 mutant mice, thus paving a way for a therapeutic strategy in COVID-19 by promoting systemic treatment with recombinant ACE2 [14]. However, ACE2 is found in abundance in children, whereas it is defiant in the tissues of geriatric population, but ironically the latter is found to be at greater risks towards COVID-19 infection as compared to the children, so the question arises how? This ironical effect of ACE2 is based upon the post-translational actions, which regulate the protein levels in the body as well as the balance between the soluble and membrane forms of ACE2 [41]. ACE2 exhinitsADAM17 (metalloproteinase 17 and a disintegrin) mediated shedding from the endothelial cells, that results in ectodomain release with bioactive and catalytic power into the circulation [43]. The soluble circulating ACE2 enzyme has been found to protect against acute lung injury induced by influenza A (H7N9) virus, as per a study conducted in 2014 [44]. This has been evidently supported by severe lung damage in H5N7 infected mice, in which the gene for ACE2 has been turned off, whereas the mice which were treated with human ACE2 exhibited lesser lung damage comparatively [44]. Potential benefits in patients with pulmonary arterial hypertension are observed by administering a single dose of recombinant human ACE2 enzyme in clinical and pre-clinical results [45]. Furthermore, certain studies depict variations in the genetic sequences in the gene for ACE2 have the ability to affect the levels of ACE2 enzyme in the human body [41]. The activities of neutral endopeptidase (NEP) and both ACE enzymes (ACE1 and ACE2) were measured in the plasma, in a Leeds family study, carried out on 534 subjects. It demonstrated that phenotypic variation, of up to 67%, could be accounted for by genetic factors, in circulating soluble ACE2 [46]. Moreover, ACE2 rs2106809 was found to exhibit primary effects on levels of ACE2 in the body among different polymorphisms [41]. The CC and CT genotype tend to possess higher circulating ACE2 levels, unlike the TT genotype [41]. Regulating functions of endothelial cells via translational repression and post-transcriptional degradation is another possible mechanism that can be mediated by microRNA [41]. Numerous differences were observed in the distribution and frequency of ACE2 enzyme depending upon difference in races and ethnicity in the individuals. Asian males were reported to exhibit higher expression of tissue ACE2 in single cell RNA sequencing analysis [47]. A negative relationship was reported between the activity of serum ACE2 and body mass index (BMI), estrogen levels, pulse pressure in female hypertensive patients, according to an investigational study carried out on North Eastern Chinese Han population [48]. These results indicated the protective role of circulating soluble ACE2 in the cardiovascular system and also depicted the involvement of estrogen in the enhancement of tissue activity and membrane expression of ACE2 [49]. This in turn indicates greater protection in female individuals against COVID-19 disease as compared to male individuals [41]. Such a trend was also observed in pediatric population which has higher levels of ACE2 than geriatrics, but still offered more protection against the virus [50]. The levels of ACE in children are 13–100 U/I, unlike the levels of ACE in adults, i.e. 9–67 U/I, as analyzed using N-[3-(2-Furyl) acryloyl]-*L*-phenylalanyl-glycyl-glycine (FAPGG) based enzymatic activity assay [41]. The COVID-19 symptoms in children were observed to be mild as compared to the adults, and also the overall cases have been less severe in children, unlike the adults, as per studies conducted in China [41]. The category of < 9 years of age comprised of about 0.9%

of the confirmed cases, as per the study analysis in China out of 1099 patients, whereas, only 1.2% of patients were between 10 and 19 years old [51]. Similarly, Baric et al. demonstrated that even though, SARS-CoV can undergo normal replication, but the younger animals possess greater resistance to the infection, as observed from the results of a study conducted in micein North Carolina [41]. The severity of COVID-19 disease was found to be greater in older individuals [52]. Therefore, the immune system or immunosenescence might not be the only cause of greater risks of COVID-19 in geriatrics [41]. Besides this, the plasma profile of ACE plays a significant role in defining the severity of COVID-19 disease in an individual [41]. Furthermore, enhanced levels of ACE2 were found in the urine and plasma of pregnant women in mid to late terms of pregnancy [41]. This enhanced production and actions of ACE2 depicted its role in systemic hemodynamic system, resulting in the upregulation of placental fetal blood flow and contribution in rapid fetal growth [53]. Therefore, the mother is capable of transferring her immunity to the baby as ACE can pass the placental barrier, along with other protective soluble factors [41]. In younger patients and children, there was no notable gender difference in susceptibility towards COVID-19 disease as per the epidemiological features and transmission patterns of pediatric patients with COVID-19 [54]. The might be due to the effect of degree of sexual maturation in adolescents and children [41]. Although estradiol is considered to regulate the actions of ACE1 and ACE2 as well as expression of AT1 and AT2 receptor in the body, but ACE has also been found to be connected to the male reproductive system [41]. The catalytic actions of testis ACE comprise of the carboxy terminal only, which have exhibited unrecognized actions on the substrate, besides angiotensin 1 [55]. Therefore, besides factors like cross immunity, which is offered by previous viral infections in children or having a strong immune system, the protective influence in younger patients against COVID-19 disease, unlike adults has been also due to the plasma profile of ACE2 [41]. The circulating soluble ACE2 levels aids children and asymptomatic individuals, to counter act the spreading of the virus in the body, by buffering effect, more like neutralizing antibodies [41]. This, in turn, could help them to contain the infection spread. Also, there are many other questions, which arise as a result of this pandemic, revolving around why certain individuals develop resistance, while others are more susceptible to the infection? Therefore, understanding the physiology of such resistant patients, could further lead the scientists to develop effective vaccines for the current pandemic [41]. ACE2 has been able to indicate alterations in BP by serving as a potential marker aiding in the prediction and prevention of cardiac functions in the body, since the previous years [41]. Now, circulating soluble ACE2 might have a prognostic effect in COVID-19 regulation and genetic analysis of ACE2 polymorphism might play a vital role in prevention, diagnosis and treatment of an individual [41].

Enhanced levels of angiotensin 2 were related to inflammation and elevated pulmonary vascular permeability, resulting in lung injury events [56,57]. Elevated levels of angiotensin 2 were found in patients with COVID-19, as compared to healthy patients [36]. There is a positive relationship between concentration of angiotensin 2 in the body and severity of SARS-CoV-2 mediated lung injury, mainly due to downregulation of ACE2 expression [36]. Enhanced levels of angiotensin 2 have been observed in avian influenza pneumonia and respiratory syncytial virus [59,60]. The levels of ACE2 retard with age and co-morbidities, like diabetes and hypertension, which significantly explains the susceptibility of older people, diabetic and hypertensive individuals towards COVID-19 infection [61]. A phase 2 clinical trial was conducted in adults with ARDS [15]. Alleviated concentration of angiotensin 2 was observed. Enhanced surfactant protein-4 and angiotensin-(1–7) levels were observed in the study [62]. Increased ACE2 levels might exert protective actions in infected children, facilitating lesser severity in this age group unlike the older population [15].The infected adults exhibited retarded SARS-CoV-2 clearance [63].The levels of total lymphocytes, CD4+ (cluster of differentiation 4) and CD8+ (cluster of differentiation 8) T cells, helper T (hT cells) and memory T cells, are significantly reduced in infected patients, leading to retarded adaptive immunity [64]. The levels of blood neutrophils and cytokines in infected patients, resulting in dysfunctional innate response, mediating lung injury [65]. Over activity of innate immunity was also observed in infected type 2 alveolar cell cultures of humans, depicting mRNA over expression of interferon γ, interferon β and various other pro-inflammatory cytokines [66]. These responses resulted in elevated lung injury mediated by virus and cytokines storm. On account of normal lymphocyte count in children, these individuals exhibit healthy immune system response against COVID-19 infection [67]. Events like sequential viral loads, lymphocyte and alveolar lining fluid cytokine profile are absent in younger patients depicting lesser susceptibility of this set of population towards COVID-19 infection [15]. About 416 patients were < 10 years of age and 549 were between 10 and 19 years of age out of the total 44,762 laboratory confirmed cases in China. Similarly, in public health laboratories (USA), about 0.5% patients were categorized between 0 and 4 years of age, and 1.3% individuals between 5 and 17 years of age, out of the total 32,437 positive cases [68]. Moreover, the median duration of fever in children was much less (3 days) as compared to adults (10 days), depicting shorter course of illness in younger patients [51,67,70]. Also, lesser number of children were hospitalized as compared to the adults, between 18 and 64 years of age as per the reports from Centre for Disease Control and Prevention (CDCP). However, the infants had greater susceptibility towards COVID-19 infections and hospitalization rates as compared to the older children and adults [71]. Out of the total mortality of 2.27%, 0.1% was contributed by the children, among which one of the case reports from Illinois was an infant [71,72]. Furthermore, out of 171 children with confirmed COVID-19 infection, death of a 10 months old child with multiple organ failure depicted mortality risks in infants, as compared to older children and adults [67]. Out of 728 laboratory confirmed cases of children infected with COVID-19, a Chinese study demonstrated severe and critical cases with severity index of 8.2%in less than 1 year, 2.5% in 1–5 years, 0.6% in 6–10 years, 1.1% in 10–11 years and 5.1% in greater than 15 years of age, depicting highest severity rate in infants [73]. On one hand, the ACE2 defiant mice depicted acute lung injury with Avian influenza virus and respiratory syncytial virus, whereas on the other hand the ACE2 defiant rats depicted lesser inflammatory lesions, viral entities and injury score with SARS-CoV unlike the wild type rats [59,60]. There was no significant difference in ACE, ACE2 and the ratio between the actions of two, among children, neonates, adults and older patients with ARDS [74]. With the current reports from various studies and complicated dual effects, it is unclear that which effect of ACE2 is more dominant in the current pandemic, the protective effect (protection against lung injury induced inflammation) or destructive effect (molecular target for viral entry) [15].

4. Therapeutic portfolio associated with RAAS system

Various treatment strategies have been developed based upon the hypothesis discussed in this review. Administration of soluble ACE2 (inhaler or nebulizer) is a potential therapeutic intervention in COVID-19 pandemic, as it competes for the binding to the SARS-CoV-2, with the membrane ACE2 receptor, and can potentiate the inhibitor pathway of RAAS, preventing formation of inflammatory lesions [14,75]. Human recombinant soluble ACE2 (human rACE2) is an Food and Drug Administration (FDA)-approved therapy since 2013, with a 2017 phase 2 trial in ARDS [16], which would allow rapid transfer to COVID-19 application [16]. Furthermore, co-infection with human corona virus NL 63, can prevent the occurrence of COVID-19 infection, as NL 63 binds to the same receptor as SARS-CoV-2, and is less pathogenic to human body [75]. The researchers from Sweden claim the significance of morphine as an alternative treatment in COVID-19, for management of pain, coughing and short breath in patients, as it suppresses the release of inflammatory cytokines and hyper-inflammatory status,

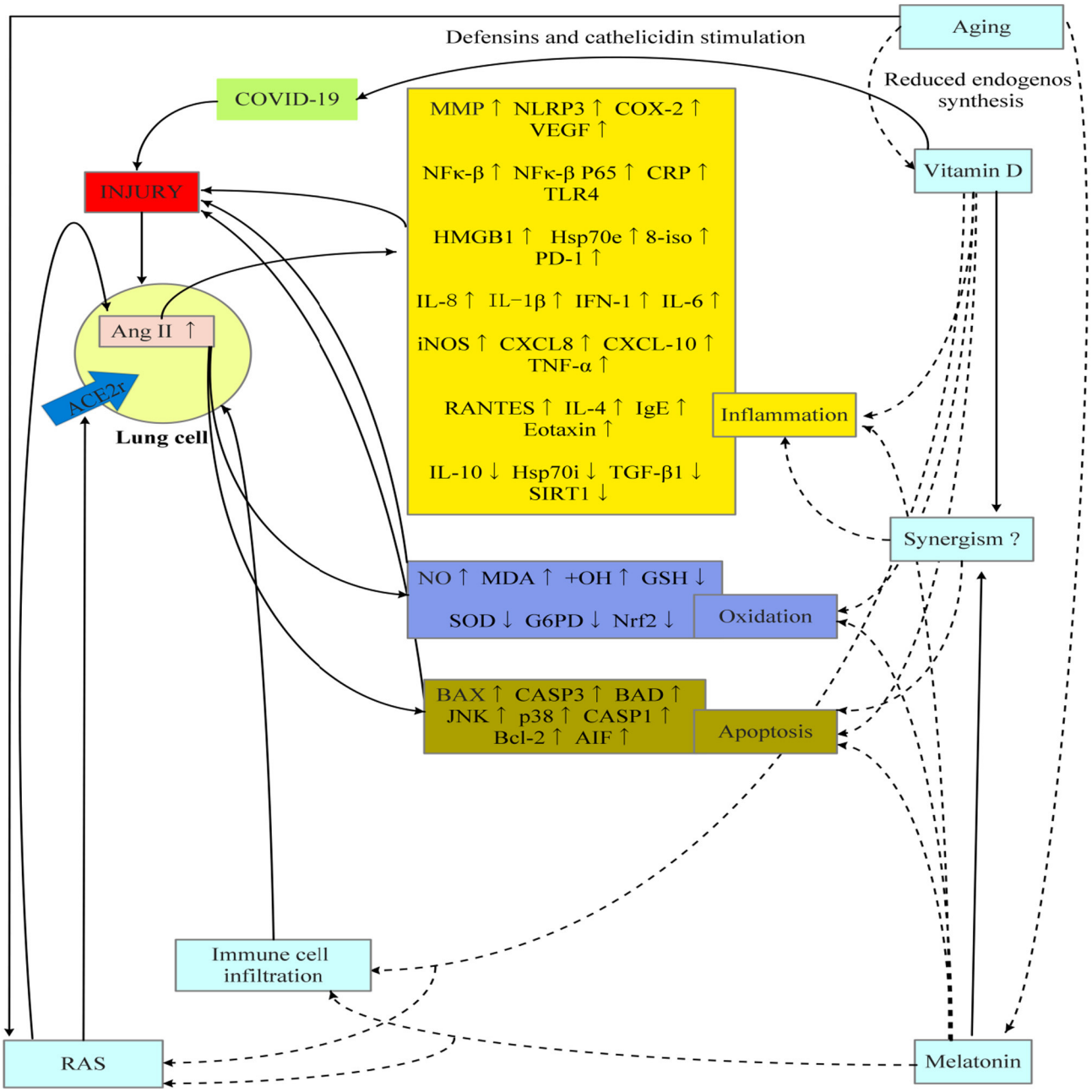

Fig. 3. Synergistic role of vitamin D and melatonin in mediating anti-inflammatory, antioxidant and anti-apoptotic actions, as well as preventing immune cell infiltration in mitigating COVID-19 infection. The solid lines indicate stimulatory, while the dashed lines indicate inhibitory actions; [MMP = matrix metalloproteinases, NLPR3 = nod like receptor protein-3, COX-2 = cyclooxygenase-2, VEGF = vascular endothelial growth factor, NF-κB = nuclear factor kappa-light-chain-enhancer of activated B cells, CRP = C-reactive protein, TLR4 = toll-like receptor 4, HMGB1 = high mobility group box 1, HSP70e/i = heat shock protein 70e/i, PD-1 = programmed cell death protein 1, IL-8/6/4/10/1β = interleukin-8/6/4/10/1β, INF-1 = interferon-1, iNOS = inducible nitric oxide synthase, CXCL = chemokine (C-X-C motif) ligand, TNF-α = tumor necrosis factor-alpha, RANTES = regulated on activation, normal T cell expressed and secreted, IgE = immunoglobulin E, TGF-β1 = transforming growth factor-β1, SIRT1 = sirtuin 1, NO = nitric oxide, MDA = malondialdehyde, +OH = hydroxide, GSH = glutathione, SOD = superoxide dismutase, G6PD = glucose-6-phosphate dehydrogenase, Nrf2 = nuclear factor erythroid 2-related factor 2, CASP3/1 = caspase 3/1, AIF = apoptosis-inducing factor, p38 = p38 mitogen activated protein kinases, JNK = c-Jun N-terminal kinases, Bax/Bcl-2/Bad = Bcl-2 family of apoptotic proteins].

according to certain experimental investigations [76–78]. Moreover, angiotensin 2 is also hypothesized to provide protection in the current pandemic, as it represents a primary ACE2 substrate, and may compete with the COVID-19 virus, for binding on to the ACE2 receptor [16].

4.1. RAAS inhibitors and COVID-19

ACE1 is significantly inhibited by ACE inhibitors, resulting in blockage of release of angiotensin 2. Unlike ACE1, ACE2 is insensitive to the actions mediated by ACE inhibitors [79,80]. Certain ACE inhibitors are prescribed as maintenance therapy, like ARBs, to facilitate treatment of cardiovascular diseases like, heart failure, increased blood pressure and diabetic nephropathy [16]. The membrane expression of ACE2 was found to be enhanced by treatment with ARBs and ACE inhibitors, especially in organs like heart [81,82]. However, results of some studies, performed in healthy human subjects, did not confirm

these results [83–85]. Some in vitro studies depict reduced concentration of circulating soluble ACE2, despite its great membrane expression and tissue levels [86]. However, currently there is no significant evidence regarding the impact of ACE inhibitors and ARBs in pulmonary ACE2 expression, regarding COVID-19 infection.

Certain investigations portray the compensatory role of ACE2 by increasing ACE2 expression [87], thereby, by acting at different stages of the system, RAAS inhibitors generated heterogeneous effects on the contributing peptides and enzymes [16]. Numerous clinical and in vitro models have demonstrated enhanced expression of ACE2 in humans by administration of ACE inhibitors and mineralocorticoid receptor antagonists [88,89], however, the effects of ACE inhibitors are more complex [90,91], on account of inconsistent finding in animals [92]. In humans, certain studies have indicated that only ARBs have the tendency to elevate ACE2 levels in the body, as ACE2 inhibit angiotensin 2 release [23], and currently, human studies have not been able to draw definitive conclusions [92]. Furthermore, ARBs may facilitate conversion of angiotensin 2 to angiotensin 3, by blocking AT1 receptor, elevating the potential benefits mediated by AT2 receptor activation [16]. The cardiovascular conditions in individuals led to increased ACE2 expression and actions in non-COVID-19 animal models, independent of ACE inhibitors and ARBs [93–95]. Also, the clinical situation, history of cardiovascular problems and COVID-19 disease plays a pivotal role. Certain studies demonstrated elevated levels of ACE inhibitors and ARBs in individuals with severe COVID-19 infections, requiring ICU admission [16]. Although it is noteworthy that in case of arterial hypertension (common in older population), ACE inhibitors and ARBs are prescribed in 25–30% cases [19]. Therefore, the impact of RAAS inhibitors in COVID-19 patients is highly complicated and remains unknown [96]. A retrospective cohort study performed in China has portrayed protective effects of ACE inhibitors and ARBs on overall mortality [97]. Some in vitro reports depict the potential benefits of ARBs in COVID-19, rather than ACE inhibitors, where the former formation of severe tissue lesions [35,98]. Losartan based clinical trials in COVID-19 (with or without hospitalization), are yet to be launched (NCT04312009 and NCT04311177) [16].

However, medical societies, like European Society of Cardiology [100] and French Society of Arterial Hypertension [99], suggest to discontinue RAAS inhibitors based maintenance therapy in order to prevent BP imbalance and heart failure in COVID-19 patients, thereby, posing complications in understanding the exact role of these agents in COVID-19 infection [16]. Furthermore, cardiovascular and anti-hypertensive drugs, like dihydropyridines, mineralocorticoid receptor antagonists and thiazide diuretics, were found to affect in vitro tissue expression of ACE2 [16]. No significant impact of beta-blockers, like atenolol, has been recorded on aortic tissue expression of ACE2, as per single in vitro study [101]. Moreover, no significant effect of loop diuretics has been reported on of ACE2 expression, till now [16].

4.2. Synergistic effect of vitamin D and melatonin

The combined supplementation of vitamin D with melatonin, might offer an attractive therapeutic approach in the current pandemic, as both the molecules regulate similar signaling pathways, leading to immunomodulatory, anti-inflammatory, anti-apoptotic, antioxidant and anti-fibrotic actions, thus, antagonizing the effects mediated by angiotensin 2 (see Fig. 3) [102]. These natural agents occupy a superior position in the therapeutic interventions on account of their safety and limited side effects. RAAS is strongly associated with melatonin [103,104], which can provide an effective treatment against COVID-19, by abbreviating the expression of AT1 in the body and bringing the angiotensin 2 levels to normal concentration in model of renal disease [1–5]. Also, a local pineal RAS contributes to melatonin synthesis in the body, via angiotensin 2, which acts on the AT1 receptors located in cells of the pineal gland, and modulate the actions and expression of enzyme tryptophan hydroxylase, which in turn mediates melatonin synthesis [102]. Furthermore, melatonin mediates antioxidant, anti-inflammatory and anti-apoptotic effects, which oppose the actions of angiotensin 2 [106]. It has been demonstrated that patients with kidney disorders exhibited lower nocturnal levels of melatonin than healthy patients [16]. Enhanced nocturnal intrarenal activation of RAS and kidney damage in patients occurred as a result of altered secretion in patients with renal disorders. Therefore, a close antagonistic relationship exists between melatonin and RAS [107]. Also, the antioxidant actions mediated by melatonin has been reported to prevent over-activation of intra-renal RAS and renal injury in 5/6 nephrectomized rat model with renal impairment, as reactive oxygen species are significant activators of intra renal RAS [108]. Melatonin is considered to prolong the time of survival of infected patients, since it indirectly targets several vital points of human corona virus, like ACE2, BCL2L1, JUN and inhibition of nuclear factor kappa B (NF-κB) kinase subunit beta [109]. However, there is not sufficient evidence available to specifically confirm the relationship between melatonin and RAS but further studies should be conducted in order to extract more information related to this relationship. The interaction between vitamin D and RAS has been demonstrated by the involvement of ACE2/Ang-(1–7), Mas receptor in neuroprotection mediated by vitamin D in the brain of hypertensive rats [110]. Vitamin D has been recognized as a cofactor in the mitigation of atrial fibrillation by RAAS inhibition [111]. Also, normalizing vitamin D deficiency in patients, facilitates inhibition of peripheral RAS [112]. Furthermore, risks of liver dysfunction and diabetes mellitus are high in case of over activation of RAS at hepatic levels. Thus, calcitriol (active form of vitamin D) regulates the over activation of liver RAS during of insulin resistance [113]. Furthermore, vitamin D significantly suppresses the production of renin. Thus, D hypovitaminosis is associated with renin production, resulting in over activation of RAAS, and angiotensin 2 formation, and vice versa [114,115]. D hypovitaminosis has been reported to cause overexpression of ACE in the body [116]. The mice models defiant of vitamin Dreceptor, resulted in the production of more severe lung injury than wild type mice, with enhanced levels of angiotensin 2 in the lungs and renin. However, pre-treatment of the mice, defiant in vitamin D receptor, with losartan, abbreviated the intensity of the lung damage [117]. These evidences significantly support the potential actions of melatonin and vitamin D, synergizing to fight against COVID-19 pandemic and related pathologies. Thus, this information promotes analyzing these two natural compounds and their synergism, to overcome the effects of SARS-CoV-2, and facilitating development of a definitive treatment and effective vaccines (see Fig. 3) [102]. It is, therefore, important to access the relationship between vitamin D, melatonin and RAAS, and their link with the entry mechanism of COVID-19, in order to promote the development of effective therapeutic approaches to mitigate viral infection in humans.

5. Future perspectives

The review highlights the role of ACE2 and other RAAS components in COVID-19 disease, specifying the dual actions mediated by ACE2 from two different levels, one protective and the other destructive, posing a significant challenge to the researchers to investigate the dominant effect of ACE2 and act in accordance with it, to develop an appropriate therapeutic solution, which is essentially required to minimize the effects of the current pandemic. Quantification of soluble circulating ACE2, based upon ELISA, should be proposed as a rapid test screening process, in all body fluids in humans, in order to facilitate genetic analysis of ACE2 polymorphism, that might play a pivotal role in prevention, treatment and care of an individual in the current pandemic [41]. Also, there is a need to follow a standardized protocol for sampling, transport and storage of samples, to facilitate reliability and accuracy of intra- and inter-individual quantitation, during the disease condition [41]. With a significant progress, being made in diagnostics, repurposing drugs like remdesivir, immunotherapeutic strategies and production of vaccine, the review considers it important to understand

the relationship between ACE2 expression and COVID-19 infection, to facilitate development of a suitable therapeutic intervention. Moreover, significant investigational studies are needed, especially to study the disease progression in children, and understand the cause of increased resistance in pediatrics [118].

6. Conclusion

The COVID-19 pandemic has posed a great challenge to the healthcare systems worldwide, and elevated pressure among the researchers to develop a suitable vaccine and a therapeutic solution to fight against the pandemic. The review, in this regard, aims to highlight the role of ACE2 in the COVID-19 disease, in converting angiotensin 2 to angiotensin-(1–7), which opposed the vasoconstrictor and inflammatory actions of the former.ACE2 also served as a molecular target, facilitating entry of SARS-CoV-2, where the glycoprotein spikes present on the outer envelope of the virus bind to the membrane ACE2 receptor, along with TMPRSS2 proteases, which mediate its binding to the extracellular domain of ACE2, resulting in penetration of virus into the target cell (see Fig. 2). As depicted by various investigations, that have portrayed a positive relationship between ACE2 expression and susceptibility towards COVID-19 disease. However, several studies have evidently indicated that downregulation of ACE2 has led to aggravation of inflammatory events due to over-expression of angiotensin 2 in the RAAS system. ACE2 activators have been found to exert anti-inflammatory and anti-fibrotic actions, which could be of therapeutic importance in the current pandemic [39]. Also, the circulating soluble ACE2 enzyme has the tendency to bind to SARS-CoV-2, without mediating its effect, therefore, it reduced the availability of the virus for membrane bound ACE2 receptor, mitigating the actions mediated by the virus in humans. The review further demonstrates the difference in the intensity of infection in geriatrics and pediatrics, which created an irony because the infection risks in children were lesser, despite the increased ACE2 levels, unlike the adults. Therefore, it was observed that immunosenescence might not play a significant role in providing resistance to children, but the plasma profile of ACE2, might play a pivotal role in providing greater resistance in pediatric population. Also, the adults with limited ACE2 levels in the body, acquired greater levels of angiotensin 2, resulting in inflammation and elevated pulmonary vascular permeability, that accelerated lung injury events. Treatment strategies paved a way for RAAS inhibitors, like ACE inhibitors and ARBs to serve as therapeutic agents in mitigating the events mediated by COVID-19 disease. The review further depicted the therapeutic paradigm, associated with COVID-19 in treating the disease progression in humans, mainly focusing on inhibition of ACE2-virus interaction or administration of soluble ACE2. In the current scenario during the pandemic, there is a dire need to develop a suitable vaccine and a treatment, in order to abbreviate the mortality rate across the globe. Therefore, the authors aim to grab the attention of the researchers worldwide, towards contribution of RAAS components, in order to pave a way for the development of treatment strategy, revolving around ACE2 and RAAS, facilitating improved conditions in COVID-19 patients.

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