

Original article**Angiotensin converting enzyme 2 in the gastrointestinal tract: binding with coronavirus and its consequences**Jesmine Khan¹, Wan Nor I'zzah Wan Mohamad Zain², Mohammed Nasimul Islam³**Abstract**

Introduction: Angiotensin converting enzyme 2 (ACE2) is expressed in several cell types in the body including the gastrointestinal (GI) epithelium. **Objective:** To provide an overview of the normal distribution of ACE2 in the GI tract, altered ACE2 expression notably in coronavirus infection and its consequences. **Materials and Methods:** Pubmed and google scholar were searched using the key words ACE2 paired with GI tract, intestinal permeability, gut microbiota, inflammatory bowel disease. **Results and Discussion:** ACE2 is highly expressed in the ileum and colon in human being as well as in rodents. In this current situation of COVID-19 pandemic, downregulation of ACE2 has been reported due to internalization of the ACE2-virus complex within the cells. Although researches are still in infancy in this topic, altered luminal microbiota, increased intestinal permeability, higher level of inflammatory markers and deficient nutrient transport has been reported due to altered ACE2 expression. **Conclusion:** Altered expression of ACE2 has the possibility to hamper normal physiological function of the GI tract and might affect GI disease progression and prognosis.

Keywords: ACE2; corona virus; gut microbiota; intestinal permeability; inflammatory bowel disease

Bangladesh Journal of Medical Science Vol. 21 No. 04 October'22 Page : 808-812
DOI: <https://doi.org/10.3329/bjms.v21i4.60276>

Introduction

Angiotensin converting enzyme 2 (ACE2) is a transmembrane glycoprotein derived from the duplication and fusion of two genes; ACE and collectrin (TMEM27). ACE2 is homologous with ACE at the catalytic domain and with collectrin in the membrane proximal domain¹.

Other than the lung alveolar cells, ACE2 and transmembrane serine protease 2 (TMPRSS2) are expressed in several cells like the pancreas, pituitary gland, adrenal glands, thyroid, ovary testes and the gastrointestinal (GI) epithelium². In the GI tract, ACE2 mRNA and protein are highly expressed in the small intestinal enterocytes but not in the goblet cells or intestinal immune cells^{3,4}. ACE2 has been identified as the cellular binding site for the novel severe acute respiratory syndrome coronavirus 2

(SARS-CoV-2), the causative agent of COVID-19⁵. The cellular entry of SARS-CoV-2 requires binding of the SARS-CoV-2 spike protein to the membrane-bound form of ACE2 which acts as a receptor on the cell surface. This is followed by the priming of the spike protein by the host TMPRSS2 to facilitate cell entry⁶. Attachment of the virus to ACE2 triggers the internalization of the ACE2-virus complex into the target cell, leading to the downregulation of ACE2⁷. Alteration of the expression of ACE2 in different systems might contribute to increase in COVID-19 related morbidity and mortality^{2,8,9}.

In this review, we will discuss the distribution of ACE2 in the GI tract and the recent studies associated with alteration or involvement of ACE2 in the GI tract in different physiological and pathological conditions and their consequences.

1. Jesmine Khan
2. Wan Nor I'zzah Wan Mohamad Zain
3. Mohammed Nasimul Islam

Faculty of Medicine, Sungai Buloh Campus, Universiti Teknologi MARA, Jalan Hospital, Selangor 47000, Malaysia

Correspondence: Jesmine Khan, Faculty of Medicine, Sungai Buloh Campus, Universiti Teknologi MARA, Jalan Hospital, Selangor 47000, Malaysia. Email: jesminek@uitm.edu.my

Materials and Methods

Electronic literature search was conducted to identify the articles related to ACE2 expression in the GI tract. Pubmed and google scholar were searched. Search terms were ACE2 paired with gastrointestinal tract, corona virus, intestinal permeability, gut microbiota, inflammatory bowel disease and their derivatives and Medical Subject Heading (MeSH) terms. Only English language articles were selected. Abstracts of selected articles were scrutinized and those meeting the inclusion criteria were retrieved for further evaluation by the authors. Articles reporting the findings from human being, animal experiments and in vitro studies were included.

Ethical clearance: Not applicable

Results and Discussion

Beside its well-known protective effects on the cardiovascular and respiratory system as anti-inflammatory, antifibrotic, vasodilator and blood pressure regulation, ACE2 has several other beneficial functions in almost all the organs of the body⁹. Presence of ACE2 in the GI epithelium suggests its important function however, few studies have investigated its role in this system. Clinical studies from COVID-19-positive patients revealed that as high as 46% presented with gastrointestinal symptoms such as loss of appetite, nausea, diarrhea and abdominal pain but little is known about the underlying mechanisms⁶. Understanding the mechanism of GI disturbances is of utmost importance for exploring better clinical care for COVID-19 patients.

Distribution of ACE2 in the GI tract

Due to recent COVID-19 pandemic researchers become interested to search and analyze the data available in public databases about the distribution of ACE2 in the GIT. Hao Zhang et al analyzed 4 datasets with single-cell transcriptomes of different organs such as lung, esophagus, stomach, ileum and colon and have described that ACE2 was not only highly expressed in the type 2 alveolar cells (AT2) of lung, but also in the upper part of esophagus and stratified epithelial cells, absorptive enterocytes of ileum and colon cells¹⁰.

GTEX RNA-Seq gene expression profiling datasets (RSEM normalized) of 31 human healthy tissues from the University of California Santa Cruz genomic institute Xena project found that highest ACE2 was present in the small intestine, testis, kidneys,

heart, thyroid, and adipose tissue. Additionally, ACE2 expression levels were positively associated with the immune signatures in the skin, digestive system, brain, and blood vessels in both males and females^{11,12}.

RNA-sequence profiles from two public databases namely, The Cancer Genome Atlas (TCGA) and Functional Annotation of The Mammalian Genome Cap Analysis of Gene Expression (FANTOM5 CAGE) were identified and confirmed with single-cell transcriptomics from an independent data by a group of researchers reported that ACE2 is expressed in the mucosa of oral cavity. Out of the 32 different oral sites evaluated, the tongue epithelium, the fungiform and the surrounding papillae were found to have the highest level of ACE2 expression. Among these three sites, this receptor was particularly highly enriched in the epithelial cells of tongue¹³.

ACE2 mRNA and protein were expressed in the brush border membrane of small intestinal enterocytes in human neonates as early as 0–4 days of age. Its expression increased with age of the individuals. Small intestinal ACE2 mRNA expression was almost 1.5 times higher in human adults as compared to the neonates. Duodenal biopsies collected during routine endoscopy from 43 healthy human adults also found that ACE2 gene expression was directly correlated with age (Spearman's $r=0.317$, $p=0.039$). With each year increase of age, duodenal ACE2 expression increased by 0.083 RU¹⁴.

ACE2 expression in rodents was reported way back in 2005. Both mRNA and ACE2 expression by immunohistochemistry was highest in the ileum and mouse kidney but weak in the rat kidney¹⁵. *In vitro* RNA-sequence analyses of gut organoids in three-dimensional cell culture model, which possessed all the key features of intestinal tissue reported highest expression of ACE2 at the brush border of mature intestinal enterocytes, predominantly colocalized with the protein villin, a tissue-specific marker for intestinal epithelium¹⁶.

Altered ACE2 level in the tissues due to several reasons notably, binding and internalization by corona virusin this current situation might lead to several gastrointestinal changes such as altered intestinal luminal microbiota, intestinal permeability and susceptibility to higher inflammation¹⁷.

Altered intestinal microbiota

Maintaining the normal microbial flora in the GI lumen is of utmost importance in GI as well as overall

health and well being of an individual. Shotgun metagenomic sequencing analyses of fecal samples from 15 patients with COVID-19 in Hong Kong reported enrichment of opportunistic pathogens and depletion of beneficial commensals, at the time of hospitalization and during hospitalization, which persisted even after clearance of SARS-CoV-2 from throat swabs and resolution of respiratory symptoms. There was an inverse correlation between abundance of *Faecalibacterium prausnitzii*, an anti-inflammatory bacterium with disease severity. Over the course of hospitalization several bacteria such as *Bacteroides dorei*, *Bacteroides thetaiotaomicron*, *Bacteroides massiliensis*, and *Bacteroides ovatus*, which were reported to downregulate the expression of ACE2 in murine gut, correlated inversely with SARS-CoV-2 viral load in fecal samples from the COVID-19 patients¹⁸.

Gut microbiota analysis by 16S ribosomal RNA gene V3-V4 region sequencing of fecal samples of 30 COVID-19 patients from China reported significantly reduced bacterial diversity, significantly higher relative abundance of opportunistic pathogens and a lower relative abundance of beneficial symbionts¹⁹. Another study with 62 COVID-19 patients found reduced alpha diversity in GI luminal microbes. However, this study did not analyze species or strain-level bacterial identification²⁰. Although all these studies reported alteration of bacterial flora during the infections but none of them reported whether the bacterial flora changed back to normal after recovery from COVID-19.

In contrast to the effect of altered ACE2 induced gut microbial alteration, gut microbiome itself was also reported to be one of the important factors regulating the level of ACE2 in the GI tract. Germ free mice had significantly higher levels of ACE2 throughout the small intestine and colon as compared to the specific pathogen free (SPF) mice. Gut microbiota depletion of SPF mice by administration of antibiotic for 10 days had higher intestinal ACE2 expression compared to the mice which were not treated with antibiotics, which suggests the role of microbiome on the expression of ACE2. Variability in intestinal ACE2 expression was observed in gnotobiotic mice colonized with different types of microbiota, suggesting that differences in ACE2 expression may in part be attributable to the composition of the gut microbiome²¹.

Adult male germ-free Sprague Dawley (SD) rats when

co-housed with conventional SD rats for 10 days had colonization of 9 bacterial phyla of the conventional rats. Reconstitution of the gut microbiota markedly decreased the colonic ACE2 expression compared to the germ-free rats. The alteration of the bacterial population was also associated with significantly higher neutrophil count and systemic inflammatory marker lipocalin 2. The underlying mechanism of these changes are still unknown²¹.

Inflammatory Bowel Disease (IBD)

Both up and downregulation of ACE 2 expression in IBD model has been reported. ACE2 expression was elevated in the colon during experimental dextran sodium sulphate (DSS) induced colitis²² and a chemical inhibitor of ACE2 (GL1001) was able to reduce the colitis severity in animal model. In contrast to the higher expression of ACE2 in DSS colitis, enhanced susceptibility to DSS colitis in ACE2 deficient animal model was also reported²³. ACE2 deficiency related inflammation in colitis might be due to altered immune cell trafficking, changes in innate immunity and cytokine production as well as alterations of the gut microbiota. with IBD are treated with immunomodulating medications. During this pandemic of COVID-19, it is of great concern whether these patients are more susceptible to infection or disease. A recent study found that inflammation in IBD led to downregulation of epithelial ACE2 in animal model. But expression of ACE2 and TMPRSS2 were not increased in samples from patients with IBD as compared to the control subjects. Treatment of the IBD patients with anti-tumor necrosis factor drugs such as vedolizumab, ustekinumab and steroids were linked to significantly lower expression of ACE2 in CD11b-enriched cells²⁴. These findings might alleviate the tension and anxiety among patients and the physicians treating IBD patients. Physicians can reassure their patients that they do not have higher expression during inflammation and their treatment was not associated with higher levels of ACE2.

Intestinal barrier permeability

Although COVID-19 enters the cells via the ACE-2 receptor, translocation of the virus from the gut to the systemic circulation should be considered if the intestinal barrier is compromised prior to or during COVID-19 infection. Permeability of the colon of ACE2 knockout mice and *ace2^{-/-}* organoids was markedly increased as compared with the *ace2^{+/+}* mice and organoids. In addition to that ACE2 knockout mice were more susceptible to IBD

than *ace2^{+/-}* mice, including the signs and symptoms of IBD such as early episodes of blood in the stool, compromised intestinal architecture and marked weight loss²⁵.

Nutrient transport and consequences

ACE2 is situated in close proximity to B⁰AT1, the neutral amino acid transporter and regulates its activity²⁶. In addition to B⁰AT1, ACE2 also interacts with the sodium-dependent imino-acid Transporter 1 (SIT1), which is a transporter for proline, sarcosine, and betaine²⁷. In ACE2 deficient mice, B⁰AT1 was not expressed in the small intestine. Reduced ACE2 caused low plasma level of neutral amino acid tryptophan, increased likelihood of developing intestinal inflammation due to the disruption of dietary amino acid homeostasis, innate immunity and gut microbial ecology²⁸. Altered ACE2 expression might have impact in the metabolism of amino acids. A metabolomic analysis showed that germ free conventionalized rats had higher levels of tryptophan metabolites, kynurenic acid, and hydroxy kynurenine as compared to the germ-free rats²⁹. Altered level of ACE2 might also have impact on the transport of all these amino acids and their derived biomolecules which might have considerable impact on health and well-being of an individual.

Conclusion

In normal physiological conditions, ACE2 has several beneficial effects in different organs of our

body including the GI tract. Due to internalization of the virus-receptor complex, ACE2 expression/level is down regulated in COVID-19 patients. Altered ACE2 level in the GI tract have been reported to alter gut microbiota, intestinal permeability, gut inflammation, nutrient transport, all of which might hamper normal physiological function of the GI tract and ultimately affect GI disease progression and prognosis.

Acknowledgement

This work was supported by MyRA 2020 grant (600 RMC/MyRA5/3/Lestari 9045/2020).

Source of fund: MyRA 2020 grant (600 RMC/MyRA5/3/Lestari 9045/2020)

Conflict of interest: None

Authors' contribution:

Data gathering and idea owner of this study: Jesmine Khan

Study design: Jesmine Khan, Wan Nor I'zzah Wan Mohamad Zain

Data gathering: Jesmine Khan, Mohammed Nasimul Islam

Writing and submitting manuscript: Jesmine Khan

Editing and approval of final draft: Jesmine Khan, Wan Nor I'zzah Wan Mohamad Zain, Mohammed Nasimul Islam.

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