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Relevance of enriched expression of SARS-CoV-2 binding receptor ACE2 in gastrointestinal tissue with pathogenesis of digestive symptoms, diabetes-associated mortality, and disease recurrence in COVID-19 patients

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Keywords: SARS-CoV2, digestive symptoms, COVID19, diabetes, recurrence, mortality, amino acid transporter, glucose transporter

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Abstract

Introduction

COVID-19, is caused by a new strain of coronavirus called SARS-coronavirus-2 (SARS-CoV-2), which is a positive sense single strand RNA virus. In humans, it binds to angiotensin converting enzyme 2 (ACE2) with the help a structure on its surface called the S-spike. COVID-19 poses intriguing issues with imperative relevance to clinicians. The pathogenesis of GI symptoms, diabetes-associated mortality, and disease recurrence in COVID-19 are of particular relevance because they cannot be sufficiently explained from the existing knowledge of the viral diseases. Tissue specific variation of ACE2 expression in healthy individuals can help in understanding the pathophysiological basis the aforementioned collection of symptoms.

Materials and Methods

The data were downloaded from the Human Protein Atlas available at (https://www.proteinatlas.org/search/ACE2) and the tissue specific expression (both mRNA and protein) of ACE2 as yielded from the studies with RNA sequencing and immunohistochemistry (IHC) was analyzed as a function of the various components of the digestive tract. A digestive system specific functional enrichment map of ACE2 gene was created using g:profiler (https://biit.cs.ut.ee/gprofiler/gost) utility and the data were visualized using Cytoscape software, version 3.7.2 (https://cytoscape.org/).

Results

The correlated expression (genomic and proteomic) of ACE2 (to which SARS-CoV-2 binds through the S-spike) was found to be enriched in the lower gastrointestinal tract (GIT) (highest in small intestine, followed by colon and rectum), and was undetectable in the upper GIT components: mouth cavity (tongue, oral mucosa, and salivary glands), esophagus, and stomach. High expression of ACE2 was noted in the glandular cells as well as in the enterocytes in the lining epithelium (including brush border epithelium). Among other digestive system organs, gallbladder (GB) showed high expression of ACE2 in glandular cells, while any protein expression was undetectable in liver and pancreas.

Conclusions

Based on the findings of this study and supportive evidence from the literature we propose that a SARS-CoV-2 binding with ACE2 mediates dysregulation of the sodium dependent nutrient transporters and hence may be a plausible basis for the digestive symptoms in COVID-19 patients. ACE2 mediated dysregulation of sodium dependent glucose transporter (SGLT1 or SLC5A1) in the intestinal epithelium also links it to the pathogenesis of diabetes mellitus which can be a possible reason for the associated mortality in COVID-19 patients with diabetes. High expression of ACE2 in mucosal cells of the intestine and GB make these organs potential sites for the virus entry and replication. Continued replication of the virus at these ACE2 enriched sites may be a basis for the disease recurrence reported in some, thought to be cured, patients.

Highlights

- **SARS-CoV-2 binding receptor ACE2 expression is enriched in human intestine**

- **ACE2 regulates neutral amino acid (B0AT1) and glucose transporter (SGLT1) in intestinal epithelium**

- **Mucus secreted from GI cells may protect SARS-CoV-2 from harsh pH levels of the digestive juices**

- **Continued replication of SARS-CoV-2 in GI cells may cause disease recurrence**

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Graphical Abstract
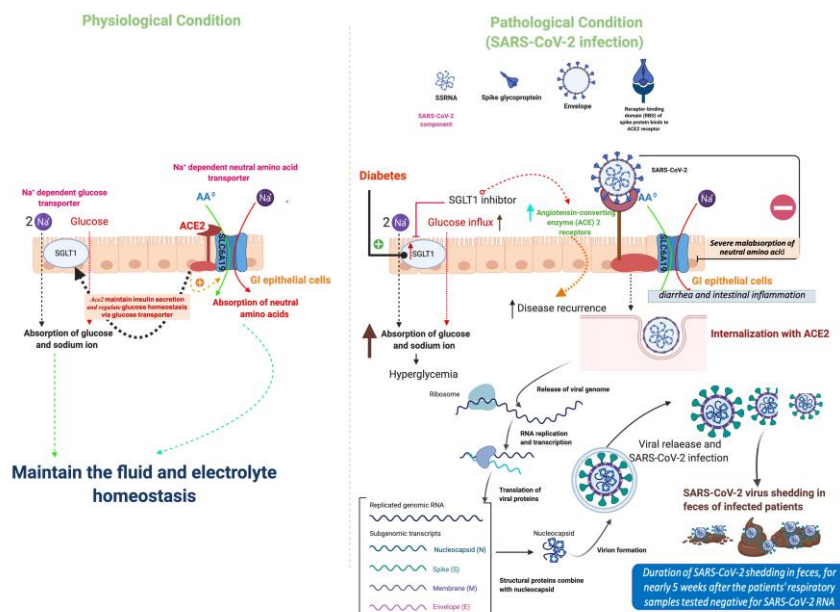
Introduction

The world is currently reeling in an alarming outbreak of novel coronavirus disease 2019 referred to as COVID-19. COVID-19 is caused by a new coronavirus strain severe acute respiratory syndrome coronavirus 2 (SARS CoV-2)—a positive sense single strand RNA virus. Recent studies which decoded structure of the virus showed binding of its S-spike protein to a human protein- angiotensin converting enzyme 2 (ACE2).[1-3] Clinical presentation in COVID-19 patients is highly diverse and majority of them primarily presents with pulmonary symptoms (cough, fever, shortness of breath).[4] In addition, some of the patients present with digestive symptoms like diarrhea, nausea, vomiting and abdominal pain (3.8%).[4] Digestive symptoms have been the only presentations in some of the patients.[5,6] Digestive symptoms are not unique to the COVID-19 and usually present in the gastroenteritis caused by many other respiratory syndrome viruses like SARS-CoV-1 and influenza A and B.[7,8] However, how SARS-CoV-2 makes entry into the gastrointestinal (GI) tissue leading to gastroenteritis-like features, does not imbibe sufficient and coherent explanation in the light of the existing literature. Some investigators have speculated a fecal-oral route of transmission but any conclusive evidence is lacking in this regard.[6]

Knowing the expression pattern of ACE2 in gastrointestinal tract (GIT) may explicate the pathogenesis of digestive symptoms in COVID-19. Digestive juices and enzymes secreted from the liver, gallbladder (GB) and pancreas play an important role in maintenance of the secretions and absorption of nutrients across intestinal epithelium. Hence their possible dysfunction in COVID-19 patients needs to be examined in order to understand pathogenesis of the digestive symptoms which, in turn, prevent some COVID-19 associated mortality.

Existing literature on the role of ACE2 in regulation of the ion transporters which maintain secretion/absorption across intestinal epithelium provide a clue that digestive symptoms in COVID-19 may have an ACE2 based etiogenesis.[9-12] Investigating the ACE2 expression pattern of digestive system components may also help to explain exacerbated diabetic complications and mortality in COVID-19 patients. Diabetes has been noted as a co-morbidity (16.2%) in COVID-19 and has contributed to increased mortality (22%).[13] Existing literature implicates ACE2 mediated dysregulation of sodium dependent glucose transporter (SGLT1 or SLC5A1) at intestinal epithelium in the pathogenesis of the diabetes mellitus.[14,15]

In this study, we aim at examining the plausibility (based on the tissue specific expression of ACE2) whether any of the digestive system components can be involved in the continued replication of the SARS-CoV-2 after pulmonary symptoms are relieved.

Many incidences of disease recurrence have been reported in COVID-19 patients even after being discharged from the hospital. Studies have reported continued shedding of SARS-CoV-2 in the feces of COVID-19 patients up to five weeks after disappearance of the pulmonary symptoms bolstering the indication that a residual persisting of virus inside the digestive system components may be a reason for the disease recurrence.[16]

We aimed to validate genomic and proteomic expression of ACE2 in the components of human digestive system (including liver, GB, and pancreas) in tissues derived from the healthy individuals to understand pathophysiological basis of the digestive symptoms in COVID-19 patients.

Materials and Methods

We analyzed the tissue specific distribution of ACE2 receptor expression (mRNA and protein) in digestive system components (GIT, liver & GB, and pancreas) using RNA sequencing and immunohistochemistry (IHC) data available in Human Protein Atlas (https://www.proteinatlas.org/search/ACE2). A digestive system specific functional enrichment map of ACE2 gene was constructed using g:profiler (https://biit.cs.ut.ee/gprofiler/gost) utility and viewed with Cytoscape software, version 3.7.2 (https://cytoscape.org/).

Human Protein Atlas methods

Estimation of mRNA expression and localization of human proteins were performed by the source laboratory using deep sequencing of RNA (RNA-seq) and IHC in normal tissue.

IHC

As described by the source labs, specimens containing normal tissue were collected and sampled from anonymized paraffin embedded material of surgical specimens, in accordance with approval from the local ethics committee. The specimens were derived from surgical material, normal was defined by morphological parameters and absence of

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neoplasia. IHC staining was performed using a standard protocol on normal tissue microarray (https://www.proteinatlas.org/download/IHC_protocol.pdf). Antibodies against human ACE2 (HPA000288, CAB026174) were labeled with DAB (3, 3'-diaminobenzidine) stain. Protein expression score was done based on the staining intensity (negative, weak, moderate or strong) and fraction of stained cells (<25%, 25-75% or >75%). For each protein, the IHC staining profile was matched with mRNA expression data and gene/protein characterization data to yield an 'annotated protein expression' profile.

Transcriptomics

The Human Protein Atlas collects transcriptomic data from the three databases (HPA, GTEx and FANTOM5). HPA RNAseq was performed on human tissue samples from healthy individuals (Accession no: PRJEB4337, Ensembl: ENSG00000130234 (version 92.38). Total RNA was extracted from the tissue samples using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The extracted RNA samples were analyzed using either an Experion automated electrophoresis system (Bio-Rad Laboratories, Hercules, CA, USA) with the standard-sensitivity RNA chip or an Agilent 2100 Bioanalyzer system (Agilent Biotechnologies, Palo Alto, USA) with the RNA 6000 Nano Labchip Kit. Only samples of high-quality RNA (RNA Integrity Number 7.5) were used for the mRNA sample preparation for sequencing. mRNA sequencing was performed on Illumina HiSeq2000 and 2500 machines (Illumina, San Diego, CA, USA) using the standard Illumina RNA-seq protocol with a read length of 2x100 bases. Transcript abundance estimation was performed using Kallisto v0.43.1 (https://pachterlab.github.io/kallisto/about). The normalized Tags Per Million (TPM) for each gene from the three databases were calculated and included in the Human Protein Atlas. Each tissue was categorized for the intensity of gene expression using a cutoff value of 1 NX as a limit for detection across all tissues. A tissue was categorized (i) enriched if it had NX level at least four times higher than other tissues, (ii) low specificity if NX $\geq$ 1 in at least one tissue, (iii) Not detected if NX < 1 in all tissues. Further details of the assays and annotation used by the Human Protein Atlas can be accessed at: https://www.proteinatlas.org/about/assays+annotation#ihk.

Gene enrichment analysis and visualization

Functional enrichment analysis of the ACE2 gene was performed with g: profiler web server (https://biit.cs.ut.ee/gprofiler/gost) and p-value computed using a Fisher's exact test with multiple-test correction. Enrichment map visualization was done with the help of Cytoscape software, version 3.7.2 (https://cytoscape.org/).

Results

The genomic and proteomic expression of ACE2 displayed high enrichment in the lower GIT (small intestine, colon, and rectum) (Fig. 1, 2e-h, Table 1). It was highest in the parts of small intestine followed by the colon and the rectum, and nearly absent (negligible/low mRNA expression and undetectable protein expression) in the upper GIT components: mouth cavity (including tongue, oral mucosa, and salivary glands), esophagus, and stomach (Fig. 1, 2a-d). GB showed high glandular expression of ACE2, while any protein expression was undetectable in appendix, liver (hepatocytes and bile duct), and pancreas (exocrine and endocrine glandular tissue) (though minimal mRNA expression was noted) (Fig. 3). Intense ACE2 expression was noted in the glandular cells as well as in the enterocytes in the lining epithelium of the lower GIT (Fig. 2e-h). The cellular expression of ACE2 was visible in the enterocyte cytoplasm and in the apical brush border (Fig. 2e-h, marked with arrow heads). The digestive system specific functional enrichment map for ACE2 gene were related to digestive functions like enzyme activity, amino acids transport, and peptide metabolism at the brush border membrane of enterocytes in the intestinal epithelium (Fig. S1, Table S1).
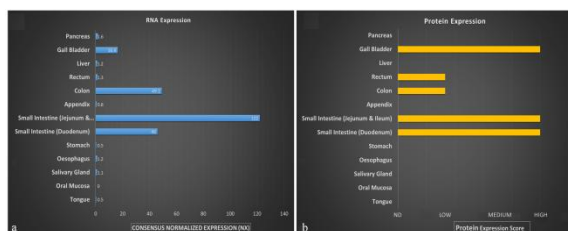

Figure 1 Physiological expression of SARS-CoV2 binding receptor ACE2 in human digestive system a. mRNA b. Protein. Data Source: The Human Protein Atlas.

Table 1 Physiological expression (mRNA and protein) of SARS-CoV-2 binding receptor ACE2 in human digestive system.

| Tissue	Cellular components	RNA Expression (NX)	Protein Expression
Tongue	Squamous epithelial cells	0.5	Not detected
Oral mucosa	Squamous epithelial cells	0	Not detected
Salivary gland	Glandular cells	1.1	Not detected
Esophagus	Squamous epithelial cells	1.2	Not detected
Stomach	Glandular cells	0.5	Not detected
Small Intestine (Duodenum)	Glandular cells	46.0	High
Small Intestine (Jejunum&Ileum)	Glandular cells	122.0	High
Appendix	Glandular cells	0.8	Not detected
	Lymphoid tissue		Not detected
Colon	Endothelial cells	49.1	Not detected
	Glandular cells		Low
	Peripheral nerve/ganglion		Not detected
Rectum	Glandular cells	1.3	Low
Liver	Bile duct cells	1.2	Not detected
	Hepatocytes		Not detected
Gallbladder	Glandular cells	16.4	High
Pancreas	Exocrine glandular cells	1.6	Not detected
	Islets of Langerhans		Not detected
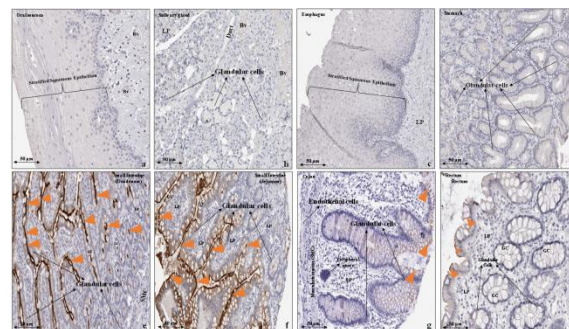
Figure 2 Immunohistochemical expression of ACE2 protein in human gastrointestinal tract a. Oral mucosa b. Salivary gland c. Esophagus d. Stomach e. Duodenum f. Small intestine g. Colon h. Rectum. Orange arrow heads show antibody stained cells. Data Source: The Human Protein Atlas.

Abbreviations: GC- goblet cells, Bv - Blood vessels, LP - Lamina propria, SMC - Smooth muscle cells.
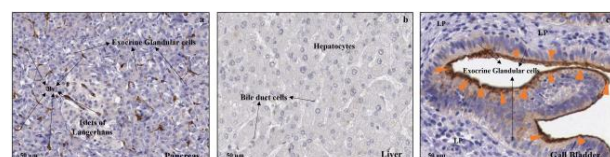
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Figure 3 Immunohistochemical expression of ACE2 protein in Human tissue a. Pancreas b. Liver c. Gallbladder. Orange arrow heads show antibody stained cells. (In pancreatic tissue blood vessels (Bv) but not in the exocrine or endocrine glandular cells can be seen expressing ACE2.) Data Source: The Human Protein Atlas.

Abbreviations: Bv - Blood vessels, LP - Lamina propria, SMC - Smooth muscle cells.

Discussion

We found enriched genomic and proteomic expression of SARS-CoV-2 binding receptor ACE2 in lower GIT (small intestine, colon, and rectum) and GB (Fig. 1-3, Table 1). The digestive system specific functional enrichment map of the ACE2 gene suggests its role in regulating secretory/absorptive functions at the brush border membrane of the enterocytes in the intestinal lining epithelium (Fig.S1, Table S1). ACE2 is a homologue of angiotensin-I converting enzyme (ACE), the key enzyme of the renin-angiotensin system (RAS). It is an integral membrane protein and localizes predominantly at the apical surface of polarized epithelial cells where it is proteolytically cleaved within its ectodomain to release a soluble form.[17,18]

Currently, SARS-CoV-2 mediated binding of ACE2 and the following downstream events leading to tissue damage are little known. Presumptive understanding of SARS-CoV-2 driven pathology is being borrowed from SARS-CoV-1 which was the etiological basis of SARS pandemic in 2003. Uniquely, it acted on the same receptor as SARS-CoV-2 and led to many clinical manifestations similar to COVID-19.[19] Studies utilizing cell lines to decipher SARS pathology in lung tissue showed that the spike protein of SARS-CoV-1 (SARS-S) induced TNFα production which facilitated virus entry.[20] TNFα also led to inflammation of the cell membrane and consequently tissue damage.[18,20] SARS-CoV-1 was also showed to cause downregulation of ACE2 expression at the cell membrane level.[18,21] Existing literature regarding expression of ACE2 in human tissues are rare. Hamming et al, studied ACE2 protein expression in human tissues in reference to SARS-CoV-1.[22] Our findings for ACE2 protein expression in digestive system components are in line with the findings of their study.[22] Enriched expression of SARS-CoV-2 binding receptor ACE2 in the mucosal glands and enterocytes (including brush border cells) in the lining epithelium (Fig. 2e-h, Table 1) of the lower GIT indicates that GI cells are potential sites for virus replication. Evidence of the viral shedding in the feces shown in some studies indicates possible replication of the virus inside the GI cells which, in turn may explain GI manifestations of COVID-19 in addition to disease recurrence.[23,24]

How the virus reaches the GI is arguable. Some authors speculated a fecal-oral route of entry.[6] We examined possibility of this route of entry based on the expression pattern of ACE2 along the length of the GIT (Fig. 1, 2, Table 1). Negligible or very low mRNA expression and undetectable proteomic expression of ACE2 in the mouth cavity (including tongue, oral mucosa, and salivary glands), esophagus, and stomach (Fig. 1, 2a-d, Table 1) indicate these parts of GIT can be resistant for the virus entry. But this observation does not negate a possible site of virus entry through the ACE2 receptors present in the lower GIT in case of fecal-oral transmission. It is then intriguing that how SARS-CoV-2 survives extremes of pH within the digestive system milieu (gastric-1.5 to 3.5, pancreatic-7.5, bile acid-7-8) while passing along the length of GIT. Recently, Chin *et al.,* 2020 showed *in vitro* that SARS-CoV-2 can survive at wide range of pH values at room temperature (pH3-10).[25] This can be further explained by an earlier study by Hirose et al, who, in an experimental, model demonstrated that RNA viruses like influenza A and B (when swallowed) can survive extremes of pH and maintain infectivity with help of the mucus cover lining GIT allowing their safe passage and even excretion in feces.[26] Mucus cells are abundant all along the length of the GIT which can contribute to the carriage and survival of SARS-CoV-2 thereby contributing to the so hypothesized fecal-oral transmission. This also hints that shedding of the virus in feces always may not be indicative of its replication in GI cells; all those patients who shed virus in stools don't necessarily present with digestive symptoms.[23]

Healthy intestinal mucosa may not be well conducive for the entry of the virus due to the presence of unique multi-layer barrier system, though a prior inflammatory condition which disrupts mucosal barrier may render the lower GI entry of the SARS-CoV-2 using ACE2 receptor and its replication inside tissue plausible.[27] Inflammatory conditions in GIT enhance the expression of ACE2 in the luminal epithelium which can provide additional support for the entry of the virus.[28] Once inside the GI cells, the virus can replicate there and may orchestrate viral toxin mediated cell injury ensuing further inflammation, thereby, giving rise to gastroenteritis like symptoms (diarrhea, nausea, and vomiting, abdominal pain).[18,20,29] Other than the fecal-oral route, an alternative route of viral entry to the GI cells may be through the tissue microvasculature. Though this may not be highly probable but this premise does warrant consideration. In that case, fecal viral shedding can happen after sloughing of the inflamed/necrosed intestinal mucosa. Currently, data is limited which support presence of SARS-CoV-2 in the blood, however such evidence is available for other coronaviruses infections like SARS and MERS.[23,30-32]

ACE2 is known to regulate sodium-dependent amino acid and glucose transporters in the enterocytes brush border which physiologically engage in the absorption of nutrients from the digested food, and maintain osmotic and electrolyte balance across the GI lining epithelium.[9,10] In a recent study Yan et al., 2020 showed that SARS-CoV-2 can bind to the complex of ACE2 with B0AT1(Slc6a19)—a major sodium dependent neutral amino acid transporter present in the epithelial lining of human intestine (and also in kidneys).[1,33] The dysregulation of the intestinal ion transporters has been implicated in the pathophysiology of infectious diarrhea and malabsorption disorders.[11,12] Literature

also suggests that a dysregulation of these transporters can ensue interleukin/cytokine mediated intestinal inflammation and can give rise to digestive symptoms.[10] An enhanced GI expression of ACE2 is known in inflammatory bowel diseases (IBDs) which present with similar symptoms as in COVID-19 patients.[10,34]

Based on the findings of this study and supportive evidence from the literature, we propose that a virus binding-ACE2 mediated dysregulation of the sodium dependent nutrient transporters may be a plausible basis for the digestive symptoms in COVID-19. Prior intestinal inflammatory conditions like IBD may raise the susceptibility of SARS-CoV-2 infection through fecal-oral transmission. ACE2 mediated dysregulation of SGLT1 and/or SLC5A1 at intestinal epithelium also links it to the pathogenesis of diabetes mellitus.[14,15] The SGLT1 transporters are physiologically involved in active absorption of glucose across the intestinal epithelium and its virus binding receptor ACE2 mediated dysregulation may exacerbate the existing impaired glycemic control in COVID-19 patients with diabetes mellitus.[15] [Sufficient data on glycemic control in COVID-19 patients is lacking for now, impaired glycemic control was stated as an independent risk factor predicting morbidity and mortality in SARS patients with diabetes mellitus.[35]] ACE2 mediated downregulation of SGLT1 in intestinal epithelium prevents hyperglycemia in rat models of the diabetes mellitus.[36,37] Though direct evidence is lacking in terms of the effect of SARS-CoV-2 binding on ACE2 on its signaling cascades, however, substantiation from SARS-CoV-1 studies (for SARS) suggests that it can downregulate ACE2 expression.[21] Such an eventuality can lead to upregulation of SGLT1 thereby precipitating hyperglycemia.[36,37] [SGLT1 inhibitors are being used in treatment of diabetes mellitus, their use in COVID-19 patients may need a rethinking for the dose adjustments.[38]]

Our data showed undetectable expression of ACE2 protein in insulin producing Islets of Langerhans of the pancreas raising an insulin independent possibility of dysregulated intestinal SGLT1 transporters. This bolsters the rationale behind diabetes related increased morbidity/mortality in COVID-19 patients. Apart from intestine SGLT1 is known to be widely expressed in other human tissues like proximal tubule of kidney, heart, and liver (proteinatlas.org/ENSG00000100170-SLC5A1/tissue) where it regulates the glucose absorption. An ACE2-mediated dysregulation of SGLT1 in COVID-19 patients warrants further investigation.

High expression of ACE2 in glandular cells of the GB indicates that this also can be a potential site for the virus replication. GB has a luminal connection to the duodenum through cystic and common bile duct (CBD). Though this connection is guarded by a sphincter (of Oddi) present in duodenal mucosa, it doesn't create an anatomical barrier and, therefore, a viral invasion along the mucosal epithelium remains a possibility.

GB is the physiological storage site for the bile secreted from the hepatocytes, and pathology of this organ can also contribute to the digestive symptoms present in COVID-19 patients. GB has been a known reservoir for *Salmonella typhi*, a bacterium causing enteric fever, and one of the cited reasons for disease recurrence.[39] The thick mucin secreted from its glandular cells can provide a protective environment for survival of SARS-CoV-2 (as we discussed above for GI lining epithelium).[26] Hence, GB homing may act as a mechanism for the replication of the virus even without ensuing a local tissue injury.

Continued replication of the SARS-CoV-2 in the intestinal tissue, and possibly in GB, may be a potential reason for the recurrence of SARS-CoV-2 in the light of the diagnostic tests as has been noted in some COVID-19 patients after being discharged from the hospital.[31,40] A post-mortem study of these organs in COVID-19 patients may provide some confirmation in this regard.

Based on the observed pattern of tissue specific expression of ACE2 (which binds to SARS-CoV-2) in the components of the digestive system in normal individuals, we propose that an ACE2 based mechanism may be involved in the pathogenesis of digestive symptoms, increased diabetes-associated mortality risk, and disease recurrence in COVID-19.

Limitations

All the aspects of the plausible SARS-CoV-2 binding receptor ACE2 mediated pathology in the digestive system which we have discussed above are based on the distribution of the virus binding receptor in the normal tissue. Hence, this study presents indirect evidence which needs to be validated in actual patients before reaching any conclusion.

Future directions

Further studies are advisable to understand the molecular mechanisms involved in the SARS-CoV-2 binding receptor ACE2 mediated dysregulation of the intestinal nutrient transporters and finding out COVID-19 specific drug targets. Inter-individual variations in frequency of the digestive symptoms, diabetes associated mortality, and recurrences may depend upon the genotype specific variations in ACE2 expression and other patient specific characteristics (like age, sex, and comorbidity). A study of these variables in the disease pathogenesis may help in deciding personalized therapeutic management for the COVID-19 cases.

Conflict of Interest

All the authors declare "No Conflict of Interest".

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Author Contributions

Funding

Data Availability

Data used for this study can be accessed at the following link:
https://www.proteinatlas.org/about/download

Acknowledgments

References:

1. Yan, R., Zhang, Y., Li, Y., Xia, L., Guo, Y., & Zhou, Q. (2020). Structural basis for the recognition of SARS-CoV-2 by full-length human ACE2. Science, 367(6485). doi: 10.1126/science.abb2762.

2. Shang, J., Ye, G., Shi, K. et al. (2020). Structural basis of receptor recognition by SARS-CoV-2. Nature.https://doi.org/10.1038/ s41586-020-2179-y.

3. Walls, Alexandra C., Young-Jun Park, M. Alejandra Tortorici, Abigail Wall, Andrew T. McGuire, and David Veesler. (2020). "Structure, function, and antigenicity of the SARS-CoV-2 spike glycoprotein." Cell. pii: S0092-8674(20)30262-2. doi: 10.1016/j.cell.2020.02.058.

4. Guan, W. J., Ni, Z. Y., Hu, Y., et al. (2020). Clinical characteristics of coronavirus disease 2019 in China. New England Journal of Medicine. DOI: 10.1056/NEJMoa2002032

5. Pan, L., Mu, M., Ren, H. G., Yang, P., Sun, Y., & Wang, R. (2020). Clinical characteristics of COVID-19 patients with digestive symptoms in Hubei, China: a descriptive, cross-sectional, multicenter study. Am J Gastroenterol, 20. doi:10.14309/ajg.0000000000000620

6. Hindson, J. (2020). COVID-19: faecal–oral transmission?. Nature Reviews Gastroenterology & Hepatology, 1-1. doi: 10.1038/s41575-020-0295-7.

7. Minodier, L., Charrel, R. N., Ceccaldi, P. E., et al. (2015). Prevalence of gastrointestinal symptoms in patients with influenza, clinical significance, and pathophysiology of human influenza viruses in faecal samples: what do we know?. Virology journal, 12(1), 215. doi: 10.1186/s12985-015-0448-4.

8. Leung, W. K., To, K. F., Chan, P. K., et al. (2003). Enteric involvement of severe acute respiratory syndrome-associated coronavirus infection. Gastroenterology, 125(4), 1011-1017. DOI: 10.1016/s0016-5085(03)01215-0.

9. Bröer, S., & Fairweather, S. J. (2011). Amino acid transport across the mammalian intestine. Comprehensive Physiology, 9(1), 343-373. doi: 10.1002/cphy.c170041.

10. Hashimoto, T., Perlot, T., Rehman, A., et al. (2012). ACE2 links amino acid malnutrition to microbial ecology and intestinal inflammation. Nature, 487(7408), 477-481. doi: 10.1038/nature11228.

11. Das, S., Jayaratne, R., & Barrett, K. E. (2018). The role of ion transporters in the pathophysiology of infectious diarrhea. Cellular and molecular gastroenterology and hepatology, 6(1), 33-45. doi: 10.1016/j.jcmgh.2018.02.009.

12. Milne, M. D. (1971). Disorders of intestinal amino-acid transport. Journal of Clinical Pathology. Supplement (Royal College of Pathologists), 5, 41. PMC1176258.

13. Fang, L., Karakiulakis, G., & Roth, M. (2020). Are patients with hypertension and diabetes mellitus at increased risk for COVID-19 infection?. The Lancet Respiratory Medicine, 8(4):e21. doi: 10.1016/S2213-2600(20)30116-8.

14. Bindom, S. M., & Lazartigues, E. (2009). The sweeter side of ACE2: physiological evidence for a role in diabetes. Molecular and cellular endocrinology, 302(2), 193-202. doi: 10.1016/j.mce.2008.09.020.

15. Navale, A. M., & Paranjape, A. N. (2016). Glucose transporters: physiological and pathological roles. Biophysical reviews, 8(1), 5-9. doi: 10.1007/s12551-015-0186-2.

16. Wu, Y., Guo, C., Tang, L., et al. (2020). Prolonged presence of SARS-CoV-2 viral RNA in faecal samples. The Lancet Gastroenterology & Hepatology, pii: S2468-1253(20)30083-2. doi: 10.1016/S2468-1253(20)30083-2.

17. Ren, X., Glende, J., Al-Falah, M., et al. (2006). Analysis of ACE2 in polarized epithelial cells: surface expression and function as receptor for severe acute respiratory syndrome-associated coronavirus. Journal of general virology, 87(6), 1691-1695. DOI: 10.1099/vir.0.81749-0.

18. Jia, H. P., Look, D. C., Tan, P., et al. (2009). Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. American Journal of Physiology-Lung Cellular and Molecular Physiology, 297(1), L84-L96. doi: 10.1152/ajplung.00071.2009.

19. Petrosillo, N., Viceconte, G., Ergonul, O., Ippolito, G., & Petersen, E. (2020). COVID-19, SARS and MERS: are they closely related?. Clinical Microbiology and Infection, pii: S1198-743X(20)30171-3. doi: 10.1016/j.cmi.2020.03.026.

20. Haga, S., Yamamoto, N., Nakai-Murakami, C., et al. (2008). Modulation of TNF-α-converting enzyme by the spike protein of SARS-CoV and ACE2 induces TNF-α production and facilitates viral entry. Proceedings of the National Academy of Sciences, 105(22), 7809-7814. doi: 10.1073/pnas.0711241105.

21. Kuba, K., Imai, Y., Rao, S., et al. (2005). A crucial role of angiotensin converting enzyme 2 (ACE2) in SARS coronavirus–induced lung injury. Nature medicine, 11(8), 875-879. DOI: 10.1038/nm1267.

22. Hamming, I., Timens, W., Bulthuis, M. L. C., Lely, A. T., Navis, G. J., & van Goor, H. (2004). Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. The Journal of Pathology: A Journal of the Pathological Society of Great Britain and Ireland, 203(2), 631-637. DOI: 10.1002/path.1570.

23. Young BE, Ong SWX, Kalimuddin S, et al. (2020). Epidemiologic Features and Clinical Course of Patients Infected With SARS-CoV-2 in Singapore. JAMA. Published online. doi:10.1001/jama.2020.3204.

24. Gu, J., Han, B., & Wang, J. (2020). COVID-19: Gastrointestinal manifestations and potential fecal-oral transmission. Gastroenterology, pii: S0016-5085(20)30281-X. doi: 10.1053/j.gastro.2020.02.054.

25. Chin, A., Chu, J., Perera, M., et al. (2020). Stability of SARS-CoV-2 in different environmental conditions. The Lancet Microbe, 0(0). DOI:https://doi.org/10.1016/S2666-5247(20)30003-3

26. Hirose, R., Nakaya, T., Naito, Y., et al. (2017). Mechanism of human influenza virus RNA persistence and virion survival in feces: mucus protects virions from acid and digestive juices. The Journal of infectious diseases, 216(1), 105-109. doi: 10.1093/infdis/jix224.

27. Okumura, R., & Takeda, K. (2018). Maintenance of intestinal homeostasis by mucosal barriers. Inflammation and regeneration, 38(1), 5.

28. Khajah, M. A., Fateel, M. M., Ananthalakshmi, K. V., & Luqmani, Y. A. (2016). Anti-inflammatory action of angiotensin 1-7 in experimental colitis. PLoS One, 11(3). doi: 10.1016/j.dci.2017.05.005.

29. Gu, J., & Korteweg, C. (2007). Pathology and pathogenesis of severe acute respiratory syndrome. The American journal of pathology, 170(4), 1136-1147. DOI: 10.2353/ajpath.2007.061088.

30. Chang, L., Yan, Y., & Wang, L. (2020). Coronavirus disease 2019: coronaviruses and blood safety. Transfusion Medicine Reviews. pii: S0887-7963(20)30014-6. doi: 10.1016/j.tmrv.2020.02.003.

31. Chen, D., Xu, W., Lei, Z., et al. (2020). Recurrence of positive SARS-CoV-2 RNA in COVID-19: A case report. International Journal of Infectious Diseases. 93:297-299. doi: 10.1016/j.ijid.2020.03.003.

32. Kim, S. Y., Park, S. J., Cho, S. Y., et al. (2016). Viral RNA in blood as indicator of severe outcome in Middle East respiratory syndrome coronavirus infection. Emerging infectious diseases, 22(10), 1813. doi: 10.3201/eid2210.160218.

Observational study

33. Singer, D., & Camargo, S. M. (2011). Collectrin and ACE2 in renal and intestinal amino acid transport. Channels, 5(5), 410-423. doi: 10.4161/chan.5.5.16470.

34. Sueyoshi, R., Ignatoski, K. M. W., Daignault, S., Okawada, M., & Teitelbaum, D. H. (2013). Angiotensin converting enzyme-inhibitor reduces colitis severity in an IL-10 knockout model. Digestive diseases and sciences, 58(11), 3165-3177. doi: 10.1007/s10620-013-2825-4.

35. Yang, J. K., Feng, Y., Yuan, M. Y., et al. (2006). Plasma glucose levels and diabetes are independent predictors for mortality and morbidity in patients with SARS. Diabetic medicine, 23(6), 623-628. DOI: 10.1111/j.1464-5491.2006.01861.x

36. Wong, T. P., Ho, K. Y., Ng, E. K., Debnam, E. S., & Leung, P. S. (2012). Upregulation of ACE2-ANG-(1–7)-Mas axis in jejunal enterocytes of type 1 diabetic rats: implications for glucose transport. American Journal of Physiology-Endocrinology and Metabolism, 303(5), E669-E681. doi: 10.1152/ajpendo.00562.2011.

37. Chan, L. K. Y., & Leung, P. S. (2015). Multifaceted interplay among mediators and regulators of intestinal glucose absorption: potential impacts on diabetes research and treatment. American Journal of Physiology-Endocrinology and Metabolism, 309(11), E887-E899. doi: 10.1152/ajpendo.00373.2015.

38. Tahrani, A. A., Barnett, A. H., & Bailey, C. J. (2013). SGLT inhibitors in management of diabetes. The lancet Diabetes & endocrinology, 1(2), 140-151. doi: 10.1016/S2213-8587(13)70050-0.

39. Gonzalez-Escobedo, G., Marshall, J. M., & Gunn, J. S. (2011). Chronic and acute infection of the gallbladder by Salmonella Typhi: understanding the carrier state. Nature Reviews Microbiology, 9(1), 9-14. doi: 10.1038/nrmicro2490.

40. Zhou, L., Liu, K., & Liu, H. G. (2020). Cause analysis and treatment strategies of" recurrence" with novel coronavirus pneumonia (COVID-19) patients after discharge from hospital. Chinese journal of tuberculosis and respiratory diseases, 43, E028. doi: 10.3760/cma.j.cn112147-20200229-00219.