Serotonin is elevated in COVID-19-associated diarrhoea

We read with great interest the recent publication by Lin *et al*,[1] which reported diarrhoea as the most common GI symptom in patients with COVID-19. Diarrhoea as a symptom of COVID-19 has been commonly observed,[2 3] but the cause remains unknown.

Diarrhoea occurs from excessive production of serotonin (5-hydroxytryptamine, 5-HT) in the GI tract.[4] Ninety-five per cent of 5-HT is produced and released from the enterochromaffin cells within the epithelium of the GI tract. 5-HT localised to the GI tract is a key modulator of GI peristalsis.[5] We hypothesised that plasma 5-HT levels are increased in patients with COVID-19 with diarrhoea.

Table 1 summarises the COVID-19 severity, interleukin 6 (IL-6), IgM/IgG antibodies, fever, GI symptoms, plasma 5-HT, 5-hydroxyindoleacetic acid (5-HIAA), underlying diabetes, body mass index, gender and age measured in 80 patients with COVID-19 (10 patients with diarrhoea) and 18 healthy donors (see online supplemental material). Correlation analysis identified 5-HT, 5-HIAA and IL-6 as the most noticeable features in the COVID-19 cohort with diarrhoea. Patients with COVID-19 had significantly increased plasma 5-HT and 5-HIAA levels compared with healthy donors (figure 1A,D). When patients were categorised by disease severity, 5-HT and 5-HIAA levels increased with higher severity of symptoms (figure 1B,E). Patients with COVID-19 with diarrhoea had substantially higher 5-HT and 5-HIAA levels compared with patients without diarrhoea and the healthy group (table 1 and figure 1C,F). Diarrhoea was present in 10 of 80 cases with COVID-19, and all 10 cases with diarrhoea had elevated 5-HT and 5-HIAA levels (figure 1C,F). The ratio of 5-HIAA to 5-HT was significantly lower in those with critical symptoms (figure 1G) and was substantially lower in those with diarrhoea compared with healthy donors or patients without diarrhoea (figure 1H and table 1). A Spearman's rank correlation matrix showed that 5-HT and 5-HIAA levels were correlated with diarrhoea (0.81 and 0.80; p=0.0017 and p=0.0021), but the 5-HIAA to 5-HT ratio was negatively correlated (−0.43; p=0.0094) (figure 1I). Additionally, 5-HT, 5-HIAA, 5-HIAA to

Table 1 Clinical characteristics of patients with COVID-19 with GI symptoms

Variables	Patients with COVID-19 with diarrhoea	Patients with COVID-19 without diarrhoea	Healthy control†
Total number of cases, n (% of total cases)	10 (12.5)	70 (87.5)	18
SARS-CoV-2 test	Positive	Positive	–
IgM‡	0.40±0.12	0.29±0.12	0.07±0.04*
IgG‡	0.30±0.10	0.34±0.14	0.10±0.09*
Fever	5 (50.0)	28 (54.2)	–
COVID-19 severity, n (%)§			
Asymptomatic	–	7 (10.0)	–
Mild	3 (30.0)	38 (54.3)	–
Severe	5 (50.0)	25 (35.7)	–
Critical	2 (20.0)	–	–
IL-6 (pg/mL/1 µg of protein)	102.7±7.2*	67.4±9.3	4.9±5.1**
GI symptoms			
Nausea	3 (30.0)	3 (4.3)	–
Abdominal pain	2 (20.0)	5 (7.1)	–
Vomiting	–	2 (2.9)	–
GI bleeding	–	1 (1.4)	–
Diarrhoea	10 (100)	–	–
5-HT (pg/mL/1 µg of protein)	127.0±18.2**	17.9±11.4	13.7±16.2
5-HIAA (pg/mL/1 µg of protein)	93.1±8.8**	20.3±9.6	8.1±5.1
5-HIAA:5-HT ratio	0.73±0.1*	1.2±0.2	1.3±0.3
Underlying disease			
Diabetes	3 (30.0)	26 (38.2)	–
BMI (kg/m^2)	36.3±8.7	34.1±10.9	22.4±3.1
Gender			
Male	4 (40.0)	40 (60.0)	10 (55.5)
Female	6 (60.0)	30 (40.0)	8 (45.5)
Age (male)			
<40 years	2 (50.0)	6 (15.0)	2 (20.0)
40–60 years	–	15 (37.5)	3 (30.0)
>60 years	2 (50.0)	19 (47.5)	5 (50.0)
Age (female)			
<40 years	–	5 (16.7)	1 (12.5)
40–60 years	1 (16.7)	10 (33.3)	1 (12.5)
>60 years	5 (83.3)	15 (50.0)	6 (75.0)

*P<0.05 and **P<0.01, vs the group of patients with COVID-19 without diarrhoea.

†Healthy control plasma samples were collected before the COVID-19 pandemic.

‡The negative control is less than 0.25.

§The classification of COVID-19 severity was based on WHO guidelines.

BMI, body mass index; 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine.
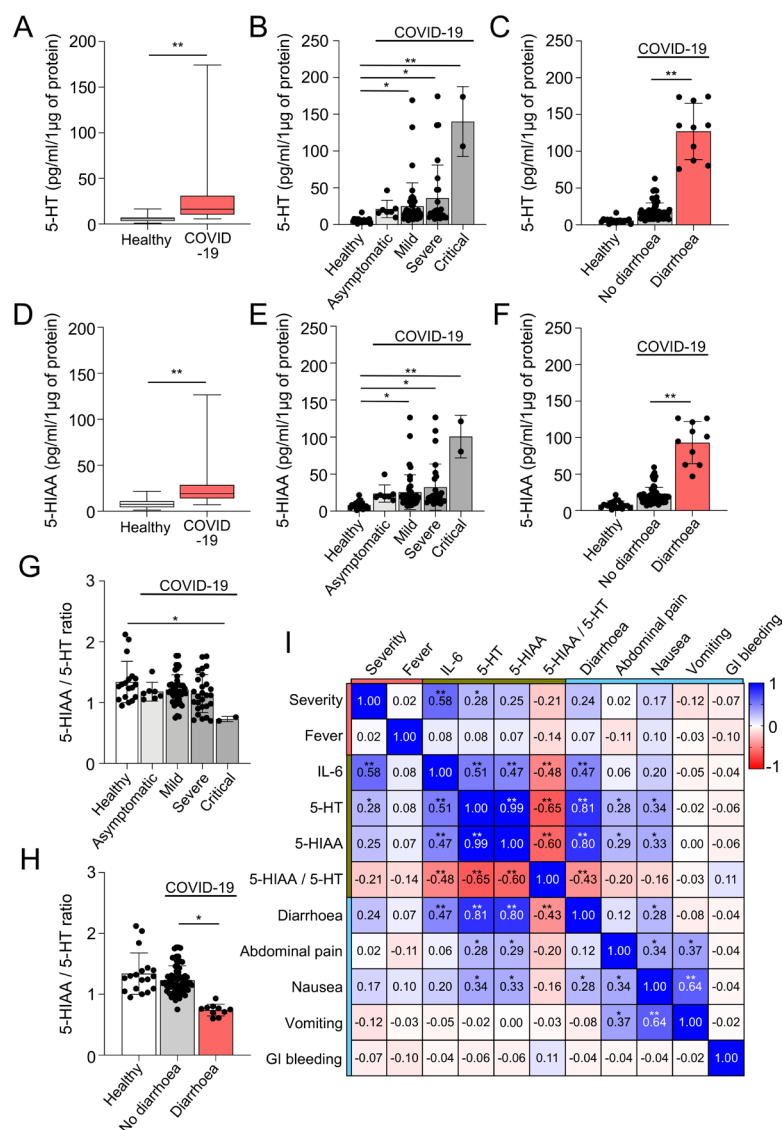
5-HT ratio and diarrhoea were also highly correlated with IL-6 (0.51, 0.47, –0.48, 0.47; p=0.0078, p=0.0092, p=0.0087, p=0.0089, respectively) and symptom severity (0.58; p=0.0065) (figure 1I).

5-HT synthesised and released from the enterochromaffin cells is an important GI signalling molecule that regulates GI motility and inflammation.[5] Released 5-HT is taken up by enterocytes in the gut and platelets in the blood via the serotonin reuptake transporter and metabolised by the liver and kidney to its metabolite 5-HIAA, which is eventually excreted to urine.[6] 5-HT and 5-HIAA levels are stable in plasma, serving as useful markers to study patients with IBS.[7] Increased 5-HT in the gut leads to diarrhoea in patients with IBS-D.[8] GI symptom studies in patients with COVID-19 have shown diarrhoea is common.[9] In the present study, we found elevated 5-HT and 5-HIAA levels in patients with COVID-19 are correlated with diarrhoea. Interestingly, the diarrhoea group (0.73; p=0.0409) had a significantly lower ratio of 5-HIAA to 5-HT than the non-diarrhoea (1.2) and healthy donor (1.3; p=0.1105) groups (table 1). This finding suggests 5-HT in the diarrhoea group remains elevated due to delayed or insufficient breakdown to 5-HIAA. In addition, our data indicate 5-HT and 5-HIAA levels are positively correlated with a proinflammatory cytokine IL-6, which is an essential pathogenic cytokine for COVID-19 severity.[10] IL-6 levels were substantially higher in patients with diarrhoea (102.7; p=0.0384) compared with patients without diarrhoea (67.4) or healthy donors (4.9; p=0.0003) (table 1).

In summary, we found 5-HT levels are markedly elevated in COVID-19-associated diarrhoea. Elevated 5-HT levels in diarrhoea are also correlated with increased IL-6. These data suggest increased 5-HT may contribute to diarrhoea and the severity of COVID-19.

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Figure 1 Profiles of plasma 5-HT and 5-HIAA in patients with COVID-19 with different severity and diarrhoea. (A–C) Plasma levels of 5-HT in a group of healthy individuals (n=18) and patients with COVID-19 (n=80) (A), in a group of healthy individuals and patients with COVID-19 with asymptomatic, mild, severe and critical conditions (B), and in a group of healthy individuals and patients with COVID-19 with and without diarrhoea (with diarrhoea n=10, without diarrhoea n=70) (C). (D–F) Plasma levels of 5-HIAA in a group of healthy individuals (n=18) and patients with COVID-19 (n=80) (D), in a group of healthy individuals and patients with COVID-19 with asymptomatic, mild, severe and critical conditions (E), and in a group of healthy individuals and patients with COVID-19 with and without diarrhoea (F). (G and H) Comparison of plasma 5-HIAA to 5-HT ratio in patients with COVID-19 with different severity and in a group of healthy individuals (G) and patients with COVID-19 with and without diarrhoea (H). (I) Correlation matrix of severity, fever, IL-6, 5-HT, 5-HIAA and GI symptoms in patients with COVID-19. Correlation matrix shows the Spearman's rank correlation among severity (asymptomatic, 1; mild, 2; severe, 3; critical, 4), fever, IL-6, 5-HT and GI symptoms (diarrhoea, abdominal pain, nausea, vomiting and GI bleeding) in patients with COVID-19 (n=80). A correlation number of 1.0 or −1.0 indicates 100% of patients with a positive (blue) or negative (red) correlation. Statistical analysis was performed with two-way analysis of variance. P<0.05 was considered significant (*p<0.05, **p<0.01). 5-HIAA, 5-hydroxyindoleacetic acid; 5-HT, 5-hydroxytryptamine; IL-6, interleukin 6.

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Ha *et al*. Serotonin is elevated in COVID-19 associated diarrhea

This supplement contains the following item:

Methods

Human Samples

Plasma samples were collected from COVID-19 patients in the Renown Regional Medical Center, Reno Nevada. Plasma samples from healthy volunteers were collected prior to the pandemic at Stanford University School of Medicine. All patients provided informed consent, and all study procedures were approved by the Stanford University Institutional Review Board, the Institutional Review Board at University of Nevada Reno and Renown's Clinical Research Department. SARS-CoV-2 infection was confirmed by RT-PCR from nasopharyngeal swab specimens (Cepheid or thermo fisher) and SARS-CoV-2 IgG Antibody ELISA (antibodies-online Inc, Pennsylvania, USA) or SARS-CoV-2 IgM Antibody ELISA Kit (antibodies-online Inc, Pennsylvania, USA) test. Diarrhea symptom was defined as loose stools >2 or 3 times a day.

Enzyme-linked immunosorbent assay (ELISA)

Plasma protein (for detected ELISA kit) was isolated using Trizol LS (Thermo Fisher Scientific, Massachusetts, USA), according to the manufacturer's specifications. 5-HT, 5-HIAA and IL-6 levels were measured by the serotonin ELISA (LDN Labor Diagnostika Nord GmbH & Co, Nordhorn, Germany), 5-HIAA ELISA (Novus Biologicals, Colorado, USA), and human IL-6 ELISA (Invitrogen, Carlsbad, USA), according to the manufacturers' specification.

Statistics

All data obtained in the present study were compared using the one-way (versus critical group) and/or two-way analysis of variance (ANOVA) in order to determine whether differences were statistically significant. Measured variables were expressed as the mean ± standard errors of the mean (SEM).