Gut-related metabolites are associated with respiratory symptoms in COVID-19: a proof-of-concept study

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Abstract

Gut-related metabolites have been linked with respiratory disease. The crosstalk between the gut and lung suggests that gut health may be compromised in COVID-19. The aims of the present study were to analyse a panel of gut-related metabolites (acetyl-L-carnitine, betaine, choline, L-carnitine, trimethylamine and TMAO) in COVID-19 patients, matched with healthy subjects, and non-COVID-19 respiratory patients. As results, metabolites from this panel are impaired in COVID-19, associated with symptoms (breathlessness and temperature) and able to differentiate between COVID-19 and asthma. Preliminary results show lower levels of betaine appear to be associated with poor outcomes in COVID-19 patients suggesting betaine as a marker of gut microbiome health.

Introduction

Increasing evidence suggests the gut may be compromised by COVID-19 [1, 2]. The gut microbiome is essential to health and wellbeing, whilst gut microbiota perturbations are linked with many diseases, included respiratory [3]. Despite the anatomic distinctions between the gut and lungs, recent evidence into the lung microbiota has identified crosstalk with the gut microbiota [4]. One mechanism of gut-lung crosstalk is by the distribution of gut metabolites. A gut-derived metabolite, trimethylamine N-oxide (TMAO) has been identified as a novel marker of gut health and disease in particular in cardiometabolic diseases, showing associations with disease severity and outcome, diet, ethnicity, gender and lifestyle; and would therefore be suitable to investigate the associations of the gut in COVID-19 [5]. The aims of the present study were to investigate the association of circulating levels of gut-related metabolites (acetyl-L-carnitine, betaine, choline, L-carnitine, trimethylamine and TMAO) in COVID-19 patients. We hypothesise that COVID-19 patients will have different levels of metabolites compared to healthy individuals due to compromised gut health. Furthermore, we compared COVID-19 with patients affected by other acute respiratory diseases (i.e., acute asthma, pneumonia).

Methods

Patients with acute COVID-19 (n=41) were recruited on admission at Glenfield General Hospital, Leicester (UK) and matched by age and gender with plasma samples from healthy (n=28), acute asthma (n=30), and pneumonia (n=24) patients. Each patient consented to have blood samples taken and outcomes surveyed. Gut-related metabolites were measured using liquid chromatography-tandem mass spectrometry [6].

Statistical analyses were performed using IBM SPSS Statistics (V26, IBM Corp., Armonk, New York, USA). Association and distribution of metabolites between the groups

and with clinical features were analysed using Spearman's rank correlation and Kruskal-Wallis test. Comparisons between COVID and non-COVID groups were performed using Mann-Whitney test for continuous variables and chi-square test for categorical. A one-way ANOVA was used to compare metabolite levels across each of the study groups. A p-value <0.05 was considered statistically significant.

Results

Study demographics showed that the COVID-19 cohort were majority male (68%) with a median age of 54 years. The majority of patients reported breathlessness (90%) and a new or continuous cough (76%) (Table 1). Distribution of metabolites showed no significant difference between COVID-19 and healthy patients for acetyl-L-carnitine, choline, L-carnitine, trimethylamine and TMAO ($p \geq 0.056$); however, there was a difference in betaine levels between these groups ($p=0.010$), with higher levels in the healthy cohort, but not after Bonferroni correction (adj. $p=0.058$). No differences between COVID-19 and pneumonia were observed ($p \geq 0.059$, adj. $p \geq 0.352$). Furthermore, patients with asthma demonstrated lower levels of acetyl-L-carnitine, betaine, and choline, and higher levels of trimethylamine when compared with COVID-19 patients (adj. $p<0.001$) (Table 2A). Similar patterns were observed when asthma and healthy patients were compared (adj. $p \leq 0.030$). There was an overall significant difference in metabolite distribution between the study groups ($p \leq 0.028$), with a notable difference in distribution of acetyl-L-carnitine, betaine, choline, L-carnitine and TMA, between COVID-19 and asthma ($p \leq 0.020$) (Figure 1). Distribution of metabolites with COVID-19 symptoms (breathlessness, fatigue, loss of smell and/or taste, new, continuous cough and runny nose) were analysed and showed elevated levels of acetyl-L-carnitine and L-carnitine in patients who reported with breathlessness. There were no other significant observations (Table 2B). Additionally, betaine and TMAO showed negative correlations with temperature ($r_s = -0.395$ and -0.344 respectively, $p \leq 0.028$), whilst betaine

also correlated with O_2 saturation levels ($r_s = 0.312$, p=0.050) (Table 2C). Comorbidities were grouped into three groups; diabetes, cardiac disease and chronic pulmonary disease. We found no significant differences in metabolite levels for patients who reported to have chronic pulmonary disease (n=10, p≥0.191) and also for diabetes (n=9, p≥0.295). However, in patients who had cardiac disease (n=6), we found elevated levels of betaine, choline, L-carnitine, and TMAO (p≤0.049). Furthermore, there was no significant difference in metabolite levels when at least one comorbidity (i.e., diabetes + cardiac disease + chronic pulmonary disease) was present (p≥0.101). Kaplan-Meier survival analysis showed that when betaine levels were split by the median, a trend was observed between lower betaine levels and reduced survival (greater than 2-fold lower than those with higher betaine levels) (p=0.407).

Discussion

To the best of our knowledge, this is the first study investigating associations of gut-related metabolites with COVID-19. The main finding from the present study is that metabolites from the choline/carnitine-TMAO pathway are associated with COVID-19 symptoms (breathlessness and temperature) and severity (O_2 saturation levels), and were able to differentiate between COVID-19 and acute asthma.

Growing evidence indicates a key crosstalk that allows maintaining homeostasis and disease evolution. The *gut-lung axis* concept however, is not completely understood despite recent evidence identifying host-microbe as well as microbe-microbe interactions [7]. Patients with respiratory infections typically have gut dysfunction or secondary gut dysfunction complications, suggesting a gut-lung interaction [3]. Gut microbiota influence on lung microbial composition and disease has been proposed by two crosstalk mechanisms i) direct seeding of the respiratory tract with bacteria and ii) the distribution of bacterial

metabolites [8]. Increasing evidence supports a “common mucosal response” where the effects of the gut microbiota on the mucosal immunity may influence an immune response on distal mucosal sites including the lung, whilst gut bacterial cells and metabolites may also elicit immune response at distal sites [9]. An alternative mechanism of the gut-lung interaction is by systemic dissemination of metabolites. Current research has investigated the role of short chain fatty acids (SCFA) which can suppress lung inflammation through the activation of G protein-coupled receptors and exert anti-inflammatory properties [10].

In all, we investigated whether the distribution of metabolites was associated with COVID-19. We focused on the choline and carnitine pathways, which are derived from dietary sources of red meat, fish and eggs, and converge into a common metabolite, TMAO. TMAO is formed from the bacterial cleavage of trimethylamine [6]. Differences in metabolite levels were only observed between COVID-19 and asthma patients. Previous studies have shown that choline, betaine, and L-carnitine are lower in asthmatic patients and suggest the possibility of supplementation to improve symptoms of asthma. Mechanistically, these metabolites are thought to modulate immune inflammation and suppress oxidative stress and improve airway inflammation [11]. Findings in asthma subjects have shown significantly elevated and decreased metabolites from tracheal wash and exhaled breath samples, including trimethylamine [12]. Among microbial-derived metabolites, evidence supports SCFA, bile acids, polyunsaturated fatty acids, and now those from this investigation, gut-derived metabolites, as contributors to asthma pathophysiology [10]. Markers of inflammation have also been associated with COVID-19 symptoms, use of respiratory support and mortality whilst, TMAO has been reported to be a mediator in systemic inflammation via the NLRP3 inflammasome by activating NF-kB and reactive oxygen species signaling. By this mechanism, it is suggested that TMAO levels would correlate with inflammation exacerbated by the COVID-19 infection [13].

Limitations of the study include the proof-of-concept design, with a small sample size coupled with low numbers of mortality that could influence the results. As consideration, these metabolites are related to the gut microbiota, it is therefore necessary to take into account dietary data, treatment (i.e., antibiotics), and whether the patients take supplements or not, since the detected levels could come not only from the metabolism of the intestinal microbes but also directly from these sources.

In conclusion, we showed for the first time that gut-related metabolites, in particular betaine, are associated with COVID-19 symptoms. Lower levels of metabolites in asthma and COVID-19, in particular betaine, suggests a role for betaine as a surrogate marker for gut health in COVID-19, and hypothesises that dietary intervention against the gut microbiome could improve outcomes, and enhance immunity. Validation studies are warranted.

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DISCLOSURES

The authors report no disclosures.

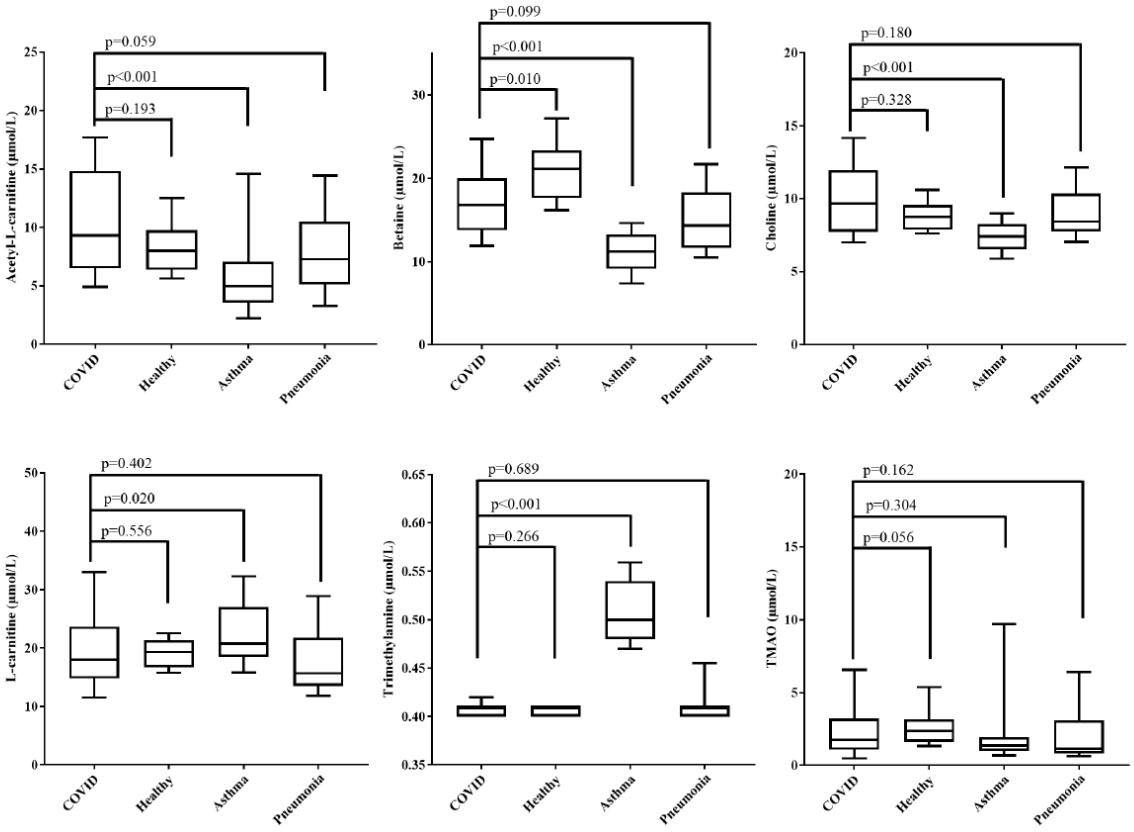
REFERENCES

1. Tay MZ, Poh CM, Rénia L, MacAry PA, Ng LF: The trinity of COVID-19: immunity, inflammation and intervention. *Nature Reviews Immunology* 2020:1-12.
2. Gao QY, Chen YX, Fang JY: 2019 novel coronavirus infection and gastrointestinal tract. *Journal of Digestive Diseases* 2020, 21:125-126.
3. Chunxi L, Haiyue L, Yanxia L, Jianbing P, Jin S: The gut microbiota and respiratory diseases: new evidence. *Journal of Immunology Research* 2020, 2020.
4. Budden KF, Gellatly SL, Wood DL, Cooper MA, Morrison M, Hugenholtz P, Hansbro PM: Emerging pathogenic links between microbiota and the gut–lung axis. *Nature Reviews Microbiology* 2017, 15:55-63.
5. Salzano A, Cassambai S, Yazaki Y, Israr MZ, Bernieh D, Wong M, Suzuki T: The Gut Axis Involvement in Heart Failure: Focus on Trimethylamine N-oxide. *Heart Failure Clinics* 2020, 16:23-31.
6. Israr MZ, Bernieh D, Salzano A, Cassambai S, Yazaki Y, Heaney LM, Jones DJL, Ng LL, Suzuki T: Association of gut-related metabolites with outcome in acute heart failure. *American Heart Journal* 2021, 234:71-80.
7. Enaud R, Prevel R, Ciarlo E, Beaufils F, Wieërs G, Guery B, Delhaes L: The gut-lung axis in health and respiratory diseases: a place for inter-organ and inter-kingdom crosstalks. *Frontiers in Cellular and Infection Microbiology* 2020, 10:9.
8. Marsland BJ, Trompette A, Gollwitzer ES: The gut–lung axis in respiratory disease. *Annals of the American Thoracic Society* 2015, 12:S150-S156.
9. Ipci K, Altıntoprak N, Muluk NB, Senturk M, Cingi C: The possible mechanisms of the human microbiome in allergic diseases. *European Archives of Oto-Rhino-Laryngology* 2017, 274:617-626.

10. Lee-Sarwar KA, Lasky-Su J, Kelly RS, Litonjua AA, Weiss ST: Gut microbial-derived metabolomics of asthma. *Metabolites* 2020, 10:97.
11. Mehta AK, Singh BP, Arora N, Gaur SN: Choline attenuates immune inflammation and suppresses oxidative stress in patients with asthma. *Immunobiology* 2010, 215:527-534.
12. Bazzano M, Laghi L, Zhu C, Magi GE, Serri E, Spaterna A, Tesei B, Laus F: Metabolomics of tracheal wash samples and exhaled breath condensates in healthy horses and horses affected by equine asthma. *Journal of Breath Research* 2018, 12:046015.
13. Terruzzi I, Senesi P: Does intestinal dysbiosis contribute to an aberrant inflammatory response to SARS-CoV-2 in frail patients? *Nutrition* 2020:110996.

FIGURE LEGENDS

Figure 1. Box and whisker plot showing the distribution of gut-related metabolites between COVID-19, asthma, pneumonia and healthy subjects



Whiskers show 10-90% percentile. Statistical significance between the COVID-19 cohort and healthy, asthma and pneumonia groups are also displayed.

TABLES

Table 1. Study demographics and characteristics

	COVID-19 (n=41)	Non-COVID (n=82)	p Value
Age	54 (43-65)	57 (47-66)	0.520
Male	68%	65%	0.840
Heart rate	91 (75-104)	81 (68-97)	0.113
Respiratory rate	19.5 (16-20)	18 (14-20)	<0.001
O_2 saturation	96 (94-98)	97 (95-98)	0.061
Temperature (°C)	36.7 (36.3-37.3)	36.6 (36.2-37.0)	<0.001
Lymphocyte	1.3 (0.9-1.8)	1.5 (1.1-2.2)	<0.001
Eosinophil	0.10 (0.03-0.23)	0.16 (0.08-0.29)	<0.001
C-reactive protein	54 (24-171)	47 (16-124)	0.049
Breathlessness	90%		
Fatigue	51%		
New, continuous cough	76%		
Runny nose	15%		
Loss of smell and/or taste	15%		
Mortality	17%		
Metabolites (µmol/L)			
Acetyl-L-carnitine	7.4 (5.4-11.1)	6.6 (5.0-9.8)	0.001
Betaine	15.9 (12.3-19.9)	14.6 (11.4-19.9)	0.067
Choline	8.5 (7.5-9.9)	8.1 (7.4-9.2)	0.001
L-carnitine	19.2 (15.8-22.7)	19.3 (16.0-22.1)	0.318
Trimethylamine	0.41 (0.40-0.46)	0.41 (0.41-0.49)	0.003
Trimethylamine N-oxide	1.6 (1.1-3.0)	1.6 (1.1-3.0)	0.853

Non-COVID group include asthma, healthy and pneumonia patients

Data are reported as median (interquartile range) for continuous variables and as a % for categorical.

Table 2. A. Distribution of metabolites in COVID-19. **B.** Distribution of metabolites with COVID-19 signs and symptoms. **C.** Correlations

	Acetyl-L-carnitine		Betaine		Choline		L-carnitine		TMA		TMAO	
Table 2A												
Covid-asthma	<0.001		<0.001		<0.001		0.020		<0.001		0.304	
Covid-pneumonia	0.059		0.099		0.180		0.402		0.689		0.162	
Covid-healthy	0.193		0.010		0.328		0.556		0.266		0.056	
Healthy-asthma	0.005		<0.001		<0.001		0.114		<0.001		0.006	
Healthy-pneumonia	0.548		<0.001		0.706		0.196		0.177		0.003	
Asthma-pneumonia	0.037		0.004		0.002		0.005		<0.001		0.681	
Table 2B												
Breathlessness	0.007		0.130		1.000		0.009		0.352		0.160	
Fatigue	0.048		0.454		0.752		0.083		0.396		0.654	
Loss of smell/taste	0.578		0.449		0.558		0.383		0.521		0.081	
New, continuous cough	0.790		0.262		0.396		0.476		0.625		0.134	
Runny nose	0.292		0.782		0.507		0.218		0.564		0.507	
Table 2C												
	r_s	p Value	r_s	p Value	r_s	p Value	r_s	p Value	r_s	p Value	r_s	p Value
Temperature	-0.020	0.897	-0.395	0.011	-0.139	0.387	0.212	0.183	-0.171	0.285	-0.344	0.028
Heart rate	-0.135	0.445	-0.147	0.408	-0.120	0.499	-0.088	0.620	0.091	0.610	-0.284	0.103
Respiratory rate	-0.264	0.151	-0.227	0.220	-0.078	0.678	-0.080	0.667	-0.045	0.812	-0.099	0.596
O_2 saturation	-0.096	0.555	0.312	0.050	-0.159	0.327	-0.077	0.635	0.069	0.671	0.054	0.741
Lymphocytes	0.035	0.827	0.026	0.870	-0.020	0.899	-0.099	0.539	0.080	0.621	0.052	0.745
Eosinophils	-0.114	0.478	0.263	0.097	0.289	0.067	-0.077	0.633	-0.025	0.874	0.241	0.129
C-reactive protein	-0.203	0.223	0.066	0.695	0.301	0.066	-0.127	0.447	-0.004	0.980	-0.136	0.414