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Original Paper

GUT MICROBIOME DIFFERENCES REGARDING LIFESTYLE AND THE HISTORY OF COVID-19 DISEASE IN ULCERATIVE COLITIS PATIENTS

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The microbiome's role in ulcerative colitis pathogenesis is established. The influence of lifestyle on gut microbiome composition remains unclear, and interplay with COVID-19 disease warrants investigation. In a cross-sectional study conducted from June to December 2021, 49 outpatients from Rīga East Clinical University Hospital were included. Patients were categorised based on COVID-19 disease status (positive vs. negative) within the preceding six months. Lifestyle factors (smoking, alcohol consumption, physical activity, stress levels, and dietary patterns) were assessed and evaluated. Taxonomic classification of gut microbiome metagenome data was performed using MetaPhlAn v.2.6.0, with subsequent analysis conducted using SPSS 20.0. Thirty-one (63%) were male, and 18 (37%) were female patients. Fourteen patients (28.6%) tested positive for COVID-19. Gut microbiome composition differences were not observed between COVID-19 disease groups. Twenty-four (49%) patients engaged in sports activities and 30 (61.2%) patients reported a history of smoking. No significant microbiome differences were observed in groups regarding physical activity or smoking. Thirty-five (71.4%) were alcohol users, for whom Firmicutes abundance was significantly higher compared to non-users, p *= 0.041. Patients reporting lower stress levels (18, 36.7%) exhibited higher Actinobacteria abundance compared to those with higher stress levels (31, 63.3%),* p *= 0.03. COVID-19 disease status did not affect gut microbiome composition, alcohol consumption and stress levels demonstrated significant associations.*

Keywords: *lifestyle factors, alcohol, smoking, stress, diet.*

INTRODUCTION

Ulcerative colitis (UC) is an inflammatory bowel disease (IBD) that is multifactorial and polygenic. Genetics, lifestyle, and environmental factors play an important role in the risks of development of the disease and progression (Yamamoto-Furusho, 2007; Abegunde *et al*., 2016). Some of the risk factors that have been investigated in IBD, include smoking, alcohol, stress, diet, food additives, air and water pollution, infections. These factors are referred to as exposomes (Abegunde *et al*., 2016).

In the European Union (EU), lifestyle factors such as tobacco and alcohol use, unhealthy diets, physical inactivity,

and environmental factors have been identified as responsible for two-thirds of premature deaths. Such data are similar in other EU countries such as Lithuania, Romania, and Bulgaria. According to the European Commission's published data in 2021 on the state of health in Latvia, the high mortality rates and poor health status of the Latvian population also are largely due to high prevalence of behavioral and lifestyle risk factors that can be modified (European Observatory on Health Systems and Policies, 2021; European Commission, n.d.).

The microbiome contributes an important role in the pathogenesis of inflammatory diseases including inflammatory bowel diseases like ulcerative colitis (UC). The contribution of different lifestyle and environmental factors to the compositional variability of the gut microbiome is unclear. Intestinal microbiota of a healthy individual is dominated by Firmicutes, Bacteroidetes, Actinobacteria, Proteobacteria and Verrucommicrobia phylum, with Firmicutes and Bacteroidetes representing 90% (Rinninella *et al*., 2019). It can be argued that intestinal dysbiosis may be a component of IBD pathogenesis, including UC. When imbalance of the intestinal microbiome occurs, the intestinal defense function and immunoregulatory function are decreased (Piovezani Ramos and Kane, 2021) Research conducted in China demonstrated that the intestinal flora was significantly less rich and diverse in UC patients than in healthy control subjects (Zhu *et al*., 2022).

Microbiome of the gut plays an important role in infectious diseases like coronavirus disease 2019 (COVID-19). COVID-19 is an infectious disease caused by severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). Most people infected with SARS-CoV-2 were observed to have respiratory symptoms, but up to 20% of patients reported having gastrointestinal symptoms such as diarrhea, nausea, vomiting, and abdominal pain as well (Devaux *et al*., 2021; Xia *et al*., 2023). Severe form of COVID-19 disease was associated with decreased bacterial diversity, for which also hypertension, type-2 diabetes, autoimmune diseases, and metabolic syndrome, obesity were significant risk factors (Magalhães *et al*., 2021; He *et al*., 2022; Ishizaka *et al*., 2024). It is important to investigate whether ulcerative colitis patients may also have risk of developing severe COVID-19 disease by evaluating microbiome changes regarding different lifestyle and environmental factors, as reported in other studies.

Several environmental factors have been studied, cigarette smoking being the most widely described. Smoking currently is associated with a lower incidence of UC, but these contradictory effects are not fully understood and the protective effect of cigarette smoking in UC is temporary (Berkowitz *et al*., 2018). Excess risk of COVID-19 progression is found to be 30–50% in current and former smokers compared with never-smokers. Smoking cessation in COVID-19 prevention and disease progression is advised (Gallus *et al*., 2023).

Alcohol has been reported to be the most-avoided diet item by the IBD patient population. Patients report worse gastrointestinal symptoms following alcohol consumption, but heavy alcohol consumption has been associated with worse IBD outcomes (Piovezani Ramos and Kane, 2021). In addition, an increased risk of symptomatic COVID-19 disease and hospitalisation has been reported in current drinkers and excessive drinkers, respectively. Alcohol consumption intensifies COVID-19 severity and deteriorates its clinical outcome (Wei *et al*., 2023).

Stress is also an important lifestyle factor that plays an important role in the pathogenesis of IBD (Sun *et al*., 2019; De Sousa *et al*., 2022). Ulcerative colitis patients have higher levels of daily stress and a lower quality of life compared with the general population. The beneficial role of exercise in such cases has been proven (Bilski *et al*., 2014; Cataldi *et al*., 2022). Exercise is associated with increased biodiversity and a beneficial metabolic function. Microbiomes of active individuals have increased bacterial diversity (Koutouratsas *et al*., 2021; Cataldi *et al*., 2022).

Bacterial diversity is an important factor, and it has been recognised that diet may play a role in the development of UC by changing microbiome composition and diversity. Diet is an important part of UC management because it can either aggravate or alleviate symptoms along with medical therapy (Knight-Sepulveda *et al*., 2015). IBD diet limits certain carbohydrates, such as refined sugar, gluten-based grains, and particular starches, which are thought to stimulate the growth of pro-inflammatory bacteria in the digestive tract. A low-fermentable oligosaccharide, disaccharide, monosaccharide, and polyol (FODMAP) diet initially consists of eliminating foods high in FODMAPs for six to eight weeks (Abbas *et al*., 2023). The Mediterranean diet, which emphasises plant-based foods and healthy fats, was shown to reduce clinical disease activity and improve quality of life for IBD patients, including those with UC (Abbas *et al*., 2023). A ketogenic diet consists of a high proportion of fat and low proportion of carbohydrates. Research on mice in China demonstrated that a ketogenic diet worsened colitis and disease specific activity scores, altered the bacterial abundance, and increased abundance of pathogenic taxa such as Proteobacteria, Enterobacteriaceae, and *Escherichia-Shigella* (Li *et al*., 2021). It has been reported that an Atkins diet, which is an extreme low-carbohydrate diet, can lead to development of new-onset ulcerative colitis (Chiba *et al*., 2016).

MATERIALS AND METHODS

Over a period of six months (from June 2021 to December 2021), a total of 49 UC outpatients from Rīga East Clinical University Hospital were enrolled in a cross-sectional study. The patients were categorised based on their COVID-19 status, distinguishing between those who tested positive and those who tested negative within the preceding six months. The study aimed to assess the impact of various lifestyle factors including smoking, alcohol consumption, physical

activity levels, dietary patterns, and the presence of high psychological stress within the last month on the gut microbiome. Furthermore, the study investigated the influence of COVID-19 disease on the gut microbiome composition in patients with ulcerative colitis. Specifically, it aimed to analyse microbiome changes in relation to COVID-19 status and the presence of different lifestyle factors.

Stool samples were collected in two aliquots by participants at home, using sterile collection tubes without buffer, and within 24 hours delivered to the closest clinical or research laboratory, where samples were frozen at –80 °C. Microbial DNA from the stool samples was extracted using the MagPure Stool DNA LQ Kit (MGITech) reagent kit and the automated platform MGISP-960 (MGI Tech). Further shotgun metagenomic library preparation was done with MGIEasy Universal DNA Library Prep Set (MGI Tech Co. Ltd). The end-products were sequenced using DNBSEQ-G400RS sequencing platform (~30M reads/sample).

Statistics. Data were analysed using R 4.2.1 (R Core Team, 2022; R Foundation for Statistical Computing, Vienna, Austria). Categorical variables were expressed as numbers (n) and percentages (%). Independent groups were analysed using the Pearson's chi-square test (if expected frequencies > 5) and Fisher's exact test (if expected frequencies < 5). Odds ratio (OR) calculation was used to evaluate 2×2 tables. A binomial test was used to compare two categorical proportions. A 95% confidence interval (95% CI) was calculated to evaluate the accuracy of statistical parameters. The normality of the distribution of continuous variables was checked using the Shapiro–Wilk test. Normally distributed continuous variables were presented as the mean (M) and standard deviation (SD). Data without a normal distribution were presented as the median (Md) and interquartile range (Q1–Q3). Considering the peculiarities of calculating the median used for characterising individual quantitative variables (where the median is equal to 0), the mean value was also calculated in cases where the quantitative variable did not conform to a normal distribution. Spearman's correlation coefficient (denoted Rs) analysis was used to evaluate the correlation. In all statistical analyses, a p-value < 0.05 was considered statistically significant. For taxonomical classifying of the gut microbiome metagenome data, the MetaPhlAn v.2.6.0 tool was used. In further analysis, gut microbiome (mostly bacteria phyla) and related data were analysed with SPSS 20.0.

RESULTS

Of 49 patients, 31 (63%) were male and 18 (37%) were female; the median age was Md = 38 [IQR: 34; 51]. Fourteen patients (28.6%) were tested COVID-19 positive and 35 (71.4%) COVID-19 negative. Spearman's correlation coefficient analysis between age and *Proteobacteria* showed a positive and statistically significant correlation (Rs = 0.47; p < 0.001) (Fig. 1).

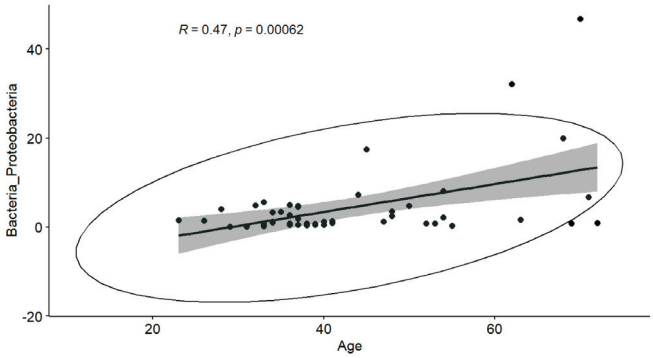

Fig. 1. Spearman's correlation coefficient analysis between age and Proteobacteria.

COVID-19 disease and microbiome. Of the 49 enrolled patients, 14 (28.6%) tested positive for COVID-19 within the last six months, comprising 7 (22.6%) men and 7 (38.9%) women. The median duration of symptoms was 7 days [IQR: 3.50; 18.8]. Among them, twelve (85.7%) patients reported experiencing COVID-19 symptoms. Abdominal pain, lack of appetite, and vomiting were reported exclusively by female patients, with each symptom group represented by one patient (7.14%). Diarrhoea was reported by three patients (21.4%), including one male (3.23%) and two female (11.1%). Constipation and postprandial fullness were each reported by one male patient in their respective symptom categories. Statistical analysis revealed no significant differences between COVID-19 positive and negative patients regarding their gut microbiome (see Table 1), nor were there significant differences observed between COVID-19 positive patients experiencing symptoms (12; 24.5%) and those who were asymptomatic (37; 75.5%) in terms of their gut microbiome.

Dietary patterns. Twenty-five patients (51%) of all interviewed participants reported following a specific diet. Among them, sixteen (32.7%) adhered to an IBD diet, while two (4.08%) followed a low-sugar, low-fat diet, and a lactose-free diet concurrently. Additionally, one patient (2.04%) in each diet category group followed a fermentable oligosaccharide, disaccharide, monosaccharide, and polyol (FODMAP) diet, a vegan diet, a vegetarian diet, and a

Table 1. COVID-19 disease status and microbiome differences

Bacteria	No Md [Q1; Q3]	Yes Md [Q1; Q3]	p
	n = 35	n = 14	
Acidobacteria	0 [0; 0]	0 [0; 0]	0.366
Actinobacteria	10.1 [4.58;15.2]	9.19 [3.66;13.7]	0.707
Bacteroidetes	21.8 [9.41;35.8]	20.5 [9.38;29.5]	0.825
Candidatus Saccharibacteria	0 [0; 0]	0 [0; 0]	0.811
Chlamydiae	0 [0; 0]	0 [0; 0]	0.527
Chlorobi	0 [0; 0]	0 [0; 0]	0.366
Deinococcus Thermus	0 [0; 0]	0 [0; 0]	0.816
Firmicutes	62.0 [43.6; 68.9]	64.8 [55.9; 68.8]	0.580
Proteobacteria	1.32 [0.79; 4.66]	1.21 [0.61; 3.88]	0.757
Verrucomicrobia	0 [0; 0.11]	0 [0; 0]	0.276

gluten-free diet, respectively. None of the respondents reported to follow a Mediterranean diet pattern (MDP), a Western type of diet, a ketogenic diet, a Stockholm diet, an Atkins diet, or a flexitarian diet. There were no statistically significant differences between COVID-19-infected or uninfected patients and their preferred diet type. However, two patients who preferred a low-sugar, low-fat diet were COVID-19 positive, compared to none in the COVID-19 negative group ($p = 0.077$). COVID-19 positive patients reported minimal sweet intake in 13 cases (92.9%), excessive sweet intake in one case (7.14%), and none reported abstaining from sweets. In contrast, in the COVID-19 negative group, five patients (14.3%) reported abstaining from sweets, excessive sweet intake was reported by 10 patients (28.6%), and minimal intake by 20 patients (57.1%) ($p = 0.066$).

No statistically significant differences were observed in the microbiome of patients who followed any form of specific diet, including the IBD diet. However, patients who followed a low-sugar, low-fat diet exhibited higher amounts of *Verrucomicrobia* phylum bacteria ($p = 0.03$) (see Table 2).

Physical activity. Out of the total 49 respondents, 24 (49%) were engaged in sports activities. Among those patients who were practicing any form of physical activity, 18 (75%) tested negative for COVID-19, compared to 17 (68%) who did not practice sports ($p = 0.82$). There were no statistically significant differences observed in the duration of COVID-19 infection or in the preferred type of physical activity. Notably, COVID-19 negative patients more frequently reported engaging in some form of physical activity (see Table 3).

When evaluating differences in the gut microbiome between patients who practiced any form of physical activity, no statistically significant differences were found in their microbiome.

Smoking history. Of the 49 patients, 30 (61.2%) had smoked before, while 19 (38.8%) had never smoked. At the time of the interview, 11 (22.4%) were active smokers. The median smoking duration was 8 years [IQR: 4.50–20.0]. Two (14.3%) COVID-19 positive patients were smokers, compared to nine (25.7%) in the COVID-19 negative group ($p = 0.475$). The median pack years in COVID-19 positive patients were 2.50 [IQR: 0.00; 8.50], and in the COVID-19 negative group, it was 3.60 [IQR: 0.00; 8.00] ($p = 0.982$).

Smoking history was reported by 21 (60%) of COVID-19 negative patients and nine (64.3%) of COVID-19 positive patients ($p > 0.05$). There were no statistically significant differences observed in the gut microbiome between smokers and non-smokers, or in the length of smoking history.

Alcohol consumption. Out of the total 49 respondents, thirty-five (71.4%) reported using alcohol, while 14 (28.6%) did not. Among the 49 patients, 15 (30.6%) consumed wine, 14 (28.6%) consumed vodka, nine (18.4%) consumed beer, and three (6.12%) consumed liquor.

Table 2. Low sugar, low fat diet and microbiome differences

Bacteria	No Md [Q1; Q3] n = 47	Yes Md [Q1; Q3] n = 2	*p*
Acidobacteria	0 [0; 0]	0 [0; 0]	0.768
Actinobacteria	9.77 [3.91; 14.8]	14.5 [11.5; 17.4]	0.448
Bacteroidetes	21.3 [9.29; 35.8]	18.7 [16.2; 21.2]	0.920
Firmicutes	62.0 [48.1; 69.2]	65.8 [65.4; 66.2]	0.613
Proteobacteria	1.43 [0.79; 4.65]	0.69 [0.59; 0.80]	0.245
Verrucomicrobia	0 [0; 0.03]	0.21 [0.15; 0.27]	0.03

Table 3. Physical activity summary in COVID-19 positive or negative patients

Physical activity	COVID-19 negative; n (%)	COVID-19 positive; n (%)		*p*
Sports:				0.821
No	17 (48.6%)	8 (57.1%)	Ref.	
Yes	18 (51.4%)	6 (42.9%)	0.72 [0.19;2.54]	
Walking:				0.312
No	31 (88.6%)	14 (100%)	Ref.	
Yes	4 (11.4%)	0 (0%)	. [.;.]	
Running:				0.085
No	27 (77.1%)	14 (100%)	Ref.	
Yes	8 (22.9%)	0 (0%)	. [.;.]	
Yoga:				1.000
No	32 (91.4%)	13 (92.9%)	Ref.	
Yes	3 (8.57%)	1 (7.14%)	0.89 [0.03;8.47]	
Tennis: No	35 (100%)	14 (100%)	Ref.	.
Basketball: No	35 (100%)	14 (100%)	Ref.	.
Volleyball: No	35 (100%)	14 (100%)	Ref.	.
Swimming:				1.000
No	29 (82.9%)	12 (85.7%)	Ref.	
Yes	6 (17.1%)	2 (14.3%)	0.84 [0.10;4.46]	
Gym:				0.494
No	34 (97.1%)	13 (92.9%)	Ref.	
Yes	1 (2.86%)	1 (7.14%)	2.56 [0.06;105]	
Football:				1.000
No	34 (97.1%)	14 (100%)	Ref.	
Yes	1 (2.86%)	0 (0%)	. [.;.]	
Cycling:				1.000
No	29 (82.9%)	12 (85.7%)	Ref.	
Yes	6 (17.1%)	2 (14.3%)	0.84 [0.10;4.46]	
Fitness:				0.334
No	32 (91.4%)	11 (78.6%)	Ref.	
Yes	3 (8.57%)	3 (21.4%)	2.83 [0.43;18.8]	
Kart racing:				1.000
No	34 (97.1%)	14 (100%)	Ref.	
Yes	1 (2.86%)	0 (0.00%)	. [.;.]	
Skiing:				1.000
No	34 (97.1%)	14 (100%)	Ref.	
Yes	1 (2.86%)	0 (0%)	. [.;.]	
Snowboarding:				0.286
No	35 (100%)	13 (92.9%)	Ref.	
Yes	0 (0%)	1 (7.14%)	. [.;.]	

Twenty-two patients (44.9%) reported alcohol consumption once a month, 13 (26.5%) reported consuming alcohol once to twice a week, two (4.08%) reported consuming alcohol three times a week, and none reported daily alcohol consumption. Alcohol consumption was reported by 26 (74.3%) COVID-19 negative patients and nine (64.3%) COVID-19 positive patients ($p = 0.503$). Among COVID-19 negative patients, eight (22.9%) consumed beer, 11 (31.4%) consumed wine, and 10 (28.6%) consumed vodka. No statistically significant differences were observed between COVID-19 positive and negative patient groups regarding the regularity of alcohol intake and the type of preferred alcoholic beverage.

There was no statistically significant relationship between alcohol use and COVID-19 symptoms ($p = 0.280$); however, there was a trend indicating that COVID-19-associated symptoms are 54% more common in non-alcohol users than in alcohol users.

Those who consumed alcohol had statistically significantly more *Firmicutes* in their gut microbiome (Md = 64.8 [IQR: 53.7–70.4]) than non-users (Md =4 8 [IQR: 37.5–66.3], $p = 0.041$). Regarding the preferred beverage type (beer or wine), no statistically significant differences in microbiomes were observed.

Stress level assessment. Regarding reported high stress levels during the last month, individuals with lower stress levels (18, 36.7%) exhibited higher levels of Actinobacteria in their gut microbiome, with a median of 11.7 [IQR: 6.92–14.8], compared to those experiencing higher stress levels (31, 63.3%), with a median of 6.03 [IQR: 2.23–13.8] ($p = 0.03$). There were no statistically significant differences observed between genders ($p > 0.05$) or COVID-19 status ($p = 0.815$).

DISCUSSION

The gut microbiome primarily consists of two bacterial phyla: the gram-negative Bacteroidetes and the gram-positive Firmicutes. Actinobacteria, Fusobacteria, Proteobacteria, and Verrucomicrobia phyla vary among individuals (Belizário and Napolitano, 2015; de Vos *et al*., 2022). Gut microbial abundance in inflammatory bowel disease patients compared to the healthy controls is characterised by an increased abundance of Firmicutes and Actinobacteria and decrease of Proteobacteria, and no differences in levels of Bacteroidetes in ulcerative colitis patients in some of the studies. Lower abundance of anaerobic bacteria — *Clostridium* cluster and a higher abundance of Proteobacteria — have been reported. Overall, the biodiversity of the gut microbiome is diminished, with variable imbalances observed across IBD studies (Sheehan *et al*., 2015; Nishida *et al*., 2018; Alam *et al*., 2020; Cortes *et al*., 2022; Dahal *et al*., 2023; Fu *et al*., 2024). Firmicutes and Proteobacteria are considered as pathogenic since they negatively affect glucose and fat metabolism within the gut. On the other hand, Verrucomicrobia, Actinobacteria, and Bacteroidetes influence gut health positively by promoting the host immune response to infectious diseases (Ozsoy *et al*., 2022). Some studies have shown that decreased bacterial diversity increases the risk of severe COVID-19 disease, which is exacerbated by comorbidities such as hypertension, type-2 diabetes, autoimmune diseases, metabolic syndrome, and obesity (Magalhães *et al*., 2021; He *et al*., 2022; Ishizaka *et al*., 2024). Based on a study done in China in 2020, the *Enterococcus*/Enterobacteriaceae ratio is altered in approximately 74% of patients with severe COVID-19 disease, and increasing in non-survivors compared with survivors, and it was concluded that dysbiosis occurred in COVID-19 patients and was associated with disease severity (Tang *et al*., 2020). Our study did not report a severe course of COVID-19 disease among our patients, and no statistically significant differences were found in the gut microbiome between symptomatic and asymptomatic SARS-CoV-2 infected patients.

Human enterocytes express the highest levels of the SARS-CoV-2 receptor angiotensin-converting enzyme, which is present on the luminal surface of enterocytes in the ileum and colon, as well as in the epithelial and gland cells of the oesophagus, serving as the entry point for the SARS-CoV-2 virus (Lamers *et al*., 2020). Gastrointestinal symptoms are frequently observed in patients with COVID-19 disease — abdominal pain, diarrhoea, nausea, and vomiting (Devaux *et al*., 2021). In our study, abdominal pain and vomiting were reported exclusively by female patients, with one patient in each symptom group (7.14%), and diarrhoea was reported by three patients (21.4%).

The dietary pattern is an essential regulatory factor of gut microbiome composition and plays an important role in its diversity, which might be associated with many inflammatory disorders including IBD. Evidence does not currently support any dietary pattern that is associated with a specific microbiome profile in IBD patients (He *et al*., 2022). Our study did not include detailed dietary evaluation; instead, we based our analysis on subjective assessments of patient dietary patterns. Of the total 49 patients, 21 (51%) followed a specific diet, with the inflammatory bowel disease (IBD) diet being the most common, reported by 16 (32.7%) respondents. The IBD diet is characterised by low carbohydrate intake and includes solid proteins, fats, nuts, fruits, and vegetables, while excluding grains, processed meats, starches, and most dairy products. The food is typically soft, well-cooked, seed-free, and may be further developed and advanced in subsequent dietary plans (He *et al*., 2022).

The Westernised type of diet is a high-fat, high-sugar and processed food containing diet, and some dietary components are found to be linked with an increased incidence of IBD and dysbiosis (Yan *et al*., 2022). Our study group patients did not report having a Western type of dietary pattern.

A high-sugar diet was found to increase gut permeability, decrease microbial diversity, and elevate Verrucomicrobiaceae and Porphyromonadaceae at the family level, reduce

Firmicutes and Tenericutes at the phyla level and Anaeroplasmataceae, Prevotellaceae and Lachnospiraceae at the family level (Laffin *et al*., 2019). Studies have shown that an increased abundance of the Verrucomicrobiaceae family has been linked to increased mucin degradation in patients with neurodegenerative disorders (Parkinsons disease and Alzheimer's disease). Such degrading bacteria can lead to compromised gut barrier integrity (Heravi *et al*., 2023). Two (4.08%) of our UC patients had a low sugar, low fat diet. Only this type of diet showed statistically significant data suggesting those having such a dietary pattern had higher amounts of Verrucomicrobia in their microbiome. In a 2024 study done in China, the presence of the Verrucomicrobiaceae family was linked to significant risk of diabetic nephropathy. Analysis found significant associations between the gut microbiome and type two diabetes, including Verrucomimicrobiae (Yan *et al*., 2024). We believe that these findings should be re-evaluated after including a control group in further data collection and analysis to draw appropriate conclusions.

None of the study group patients had a Mediterranean Diet Pattern (MDP). The diet is described to promote gut microbiome alterations associated with the maintenance of clinical remission in patients with inactive UC. This diet could be recommended as a maintenance diet and adjunctive therapy for UC patients in clinical remission (Haskey *et al*., 2023).

Our study did not reveal Bacteroidetes abundance in the ulcerative colitis current smoker patient group nor decreased abundance of Firmicutes and Proteobacteria phyla, as reported in a population-based cross-sectional study done in 2018 in Korea between current and never smokers. No microbiome differences between former and never smokers were seen (Lee *et al*., 2018). Such results were also reported in the United Kingdom — the smoker's microbiome consisted of significantly higher Bacteroides abundance than in the control group and non-IBD control group (Benjamin *et al*., 2012). Our study analysed current smokers and others including those who had smoked before. No statistically significant microbiome differences in smokers and non-smokers nor differences between their length of smoking were observed.

One of inflammatory bowel disease pathogenesis mechanisms is increased gut permeability. Alcohol impairs intestinal absorption and results in increased gut permeability (Bode and Christian Bode, 2003; Mutlu *et al*., 2009). Ulcerative colitis relapses are associated with high alcohol intake but not with medium intake (Jowett, 2004). In our study most patients (44.9%) reported alcohol consumption once a month; 13 (26.5%) once to two times a week; two (4.08%) reported three times a week but none reported daily alcohol consumption. The most consumed alcoholic beverages were wine (30.6%), vodka (28.6%), and beer (18.4%). The precise amount of alcohol was not included in our questionnaire. There are some studies showing positive effect of alcohol consumption, i.e. the component resveratrol polyphenol found in red wine (Shah *et al*., 2010). In the literature a trend is seen toward more negative effects of alcohol use in patients with IBD than positive. Although some alcoholic beverages (red wine) may have a beneficial impact on inflammation when used in moderation, it is still ambiguous and should not be promoted (Piovezani Ramos and Kane, 2021).

In our study we did not reveal a statistically significant relationship between alcohol use and COVID-19 symptoms. However, there was a trend that COVID-19 symptoms were more common in non-alcohol users than in alcohol users — most likely the effect of confounding factors or a simple coincidence. In a 2023 study done in China it was reported that current drinkers had an increased risk of symptomatic COVID-19 disease and excessive drinkers were at high risk of hospitalisation (Wei *et al*., 2023). Our study group patients did not report any hospitalisation nor excessive alcohol consumption. An explanation for this is that the most avoided dietary item in IBD patients is alcohol, which has been reported in the literature (Piovezani Ramos and Kane, 2021). Regarding the microbiome, those who were using alcohol had statistically significantly more Firmicutes in their gut than those who did not consume alcohol. Marques *et al*. in 2022 reported that the most abundant phyla in the group using non-alcoholic beer and alcoholic beer were Firmicutes followed by Bacteroidetes and Actinobacteria (Marques *et al*., 2022). Studies have shown that low or moderate beer consumption, with or without alcohol, shows positive health effects by development of a healthy microbiome, increasing microbial alpha-diversity (Hernández-Quiroz *et al*., 2020).

Stress in an important lifestyle factor that is associated with developing inflammatory diseases. Studies done during the outbreak time reported higher stress in the general population and healthcare workers (Hossain *et al*., 2020; Wang *et al*., 2020; 2021; Gao *et al*., 2022). Our study revealed that patients who did not report high stress levels in the last month, had more Actinobacteria in their gut microbiome compared to those who reported higher stress. Increased faecal Actinobacteria abundance was observed to be linked to a higher quality of life in individuals aged 50–80 years in a study conducted in Spain (de Cuevillas *et al*., 2022). Our finding regarding stress levels could suggest that stress (emotional or physical) might have an important impact on gut microbiome changes regarding IBD patients dysbiosis, which has been reported many times in other studies (Nishida *et al*., 2018; Alam *et al*., 2020; Dahal *et al*., 2023). In a study done in 2024, stress reactivity in UC patients suggested that the microbiome may contribute to stress-induced UC flares by differences in faecal and plasma metabolite levels in high or low stress states, without changes in microbial abundances, suggesting that stress is an important lifestyle factor that may have a wide range of impact on one's health (Jacobs *et al*., 2024).

Physical activity may lead to alteration in the gut microbiome, but the mechanisms are poorly understood. Studies have evaluated aerobic physical activity in relation to the changes induced in the gut microbiome. High-intensity and

moderate-intensity physical activities can promote reduction in intestinal inflammation with changes in the microbiome — decreased ratio of Firmicutes/Bacteroidetes, *Clostridium* genus, and increased Bacteroidetes (Cataldi *et al*., 2022). In study done in swimmers the most abundant bacterial phyla reported were Bacteroidetes and Firmicutes, with an average ratio of Firmicutes: Bacteroidetes of 2:1 at the peak of the training programme (Puce *et al*., 2022). Our study did not find specific bacterial abundance patterns in the gut microbiome of patients who engaged in physical activity.

LIMITATIONS

Our results are representative of a patient's subjective evaluation of their stress level during the last month. Objective evaluation scales for stress level evaluation were not included during our interview. The smokers group of patients could be reevaluated, and new data should be calculated to analyse patients who have never smoked, who have smoked any previous time, and current smokers, and their microbiome differences. The study will include healthy control group participants in further analysis to evaluate differences in the UC patient's microbiome.

CONCLUSIONS

There were no changes in the gut microbiome in COVID-19 positive and COVID-19 negative patients, nor in smokers and no smokers ($p > 0.05$). Alcohol users had more Firmicutes in their gut microbiome Md = 64.8 [IQR: 53.7–70.4] than non-users Md = 48 [IQR: 37.5–66.3]. There were no differences in the gut microbiome in UC patients participating in or lacking sports. Patients with less stress had more Actinobacteria in their gut microbiome 11.7 [IQR: 6.92–14.8], compared to those with higher stress levels, with a median of 6.03 [IQR: 2.23–13.8]. Lifestyle factors may play a role in ulcerative colitis patients gut microbiome composition and it is important to re-evaluate each patient's risks and harmful lifestyle habits, educate about possible outcomes and advice best practices.

ETHICS

Ethical approval for this study was obtained from the Rīga East Clinical University Hospital Medical and Biomedical Research Ethical Committee (No. 14/2021).

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ZARNU MIKROBIOMU ATŠĶIRĪBAS ATTIECĪBĀ UZ DZĪVESVEIDU UN COVID-19 SLIMĪBAS VĒSTURI ČŪLAINĀ KOLĪTA PACIENTIEM

Mikrobioma loma čūlainā kolīta (ČK) patoģenēzē ir nozīmīga. Dzīvesveida ietekme uz zarnu mikrobiomu joprojām ir neskaidra, un tā mijiedarbību ar ČK un Covid-19 slimību ir nepieciešams pētīt. Šķērsgriezuma pētījumā, kas tika veikts no 2021. gada jūnija līdz decembrim, tika izmeklēti 49 ČK ambulatorie pacienti no Rīgas Austrumu klīniskās universitātes slimnīcas. Pacienti tika iedalīti kategorijās, pamatojoties uz Covid-19 slimības statusu (pozitīvs vai negatīvs) iepriekšējo sešu mēnešu laikā. Tika apskatīti dzīvesveida faktori (smēķēšana, alkohola patēriņš, fiziskā aktivitāte, stresa līmenis un uztura paradumi) un to ietekme uz zarnu mikrobiomu. Zarnu mikrobiomu metagenomu datu taksonomiskā klasifikācija tika veikta, izmantojot MetaPhlAn v.2.6.0, un turpmākā analīze tika veikta, izmantojot SPSS 20.0. Trīsdesmit viens (63%) respondents bija vīrieši un 18 (37%) sievietes. Četrpadsmit pacienti (28,6%) bija Covid-19 pozitīvi, taču statistiski nozīmīgas atšķirības zarnu mikrobioma sastāvā netika novērotas. Divdesmit četri (49%) pacienti nodarbojās ar sportu un 30 (61,2%) pacienti atzīmēja smēķēšanu, bez nozīmīgām mikrobioma atšķirībām abās grupās. Trīsdesmit pieci (71,4%) lietoja alkoholu regulāri un *Firmicutes* pārpilnība bija ievērojami augstāka, salīdzinot ar alkohola nelietotājiem. Pacientiem, kuri ziņoja par zemāku stresa līmeni, bija vairāk *Actinobacteria* mikrobiomā. Covid-19 slimība neietekmēja zarnu mikrobioma sastāvu. Alkohola lietotājiem bija augstāks *Firmicutes*, savukārt zemāks stresa līmenis korelēja ar palielinātu *Actinobacteria* daudzumu.