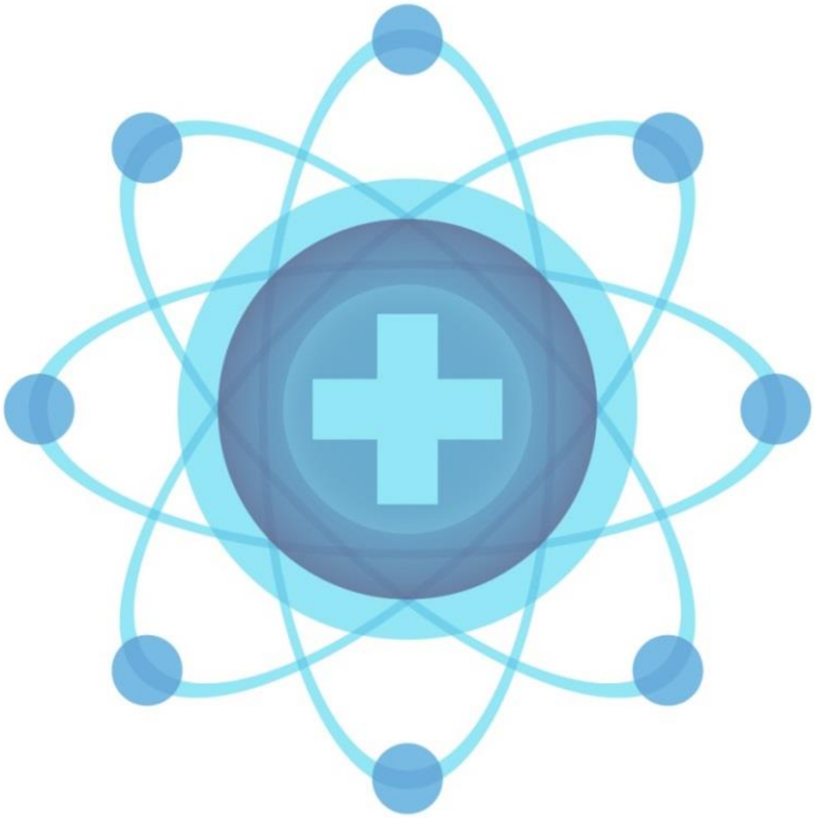

INTERNATIONAL MEDICAL SCIENTIFIC JOURNAL

ART OF MEDICINE

Founder and Publisher **North American Academic Publishing Platforms**

Internet address: http://artofmedicineimsj.us

E-mail: info@artofmedicineimsj.us

11931 Barlow Pl Philadelphia, PA 19116, USA +1 (929) 266-0862

Available at https://www.bookwire.com/

ISBN: 978-0-578-26510-0

Art of Medicine *Volume-2*
International Medical Scientific Journal Issue-*3*
10.5281/zenodo.7219791

METHODS OF DETERMINING THE INTESTINAL MICROBIOTA IN PATIENTS AFTER COVID-19

[1]Karimov Mirvosit Mirvasikovich

[2]Sobirova Guzal Naimovna, Zargarova Nargiza Rustamovna

[1]Republican Specialized Scientific and Practical Medical Center for Therapy and Medical Rehabilitation, Tashkent, Uzbekistan

[2]Tashkent Medical Academy, Tashkent, Uzbekistan

Abstract. The global and regional impact of the SARS-CoV-2 pandemic is of great concern. Although the availability of vaccines against SARS-CoV-2 has partially alleviated this concern in some parts of the world, it remains a global public health crisis. Diagnosing gut microbiota in patients after infection with COVID-19. The research was conducted on 120 (54 men and 66 women) patients aged 29 to 70 years and 20 healthy people treated for dysbacteriosis in the gastroenterology department of the Multidisciplinary Clinic of the Tashkent Medical Academy. The composition of intestinal microorganisms in patients with dysbacteriosis is almost the same, but its quantitative ratio differs according to whether or not they have had COVID-19.

Keywords: SARS-CoV-2, intestinal microbiota, methods, determine.

Introduction. The typical course of COVID-19 is characterized by fever, dry cough, shortness of breath, fatigue and chest tightness. Myalgia, numbness, headache, and palpitations may occur during the acute phase of the disease [1].

Some researchers have described the gastrointestinal symptoms of SARS-CoV-2 along with the detection of the virus in feces. Thus, two large clinical studies in China focused on gastrointestinal symptoms and virus detection in feces [7]. In one of them, Jin X et al studied 74 SARS-CoV-2-infected patients with clinical manifestations of diarrhea, nausea, and vomiting. Up to 28% of individuals with gastrointestinal symptoms had no respiratory symptoms. The researchers found that compared to patients without gastrointestinal symptoms (n=577) in the surveyed group, overall, patients had a more severe or severe condition, body temperature greater than 38.5°C, and elevated aminotransferase levels. Symptoms of liver or intestinal damage were observed in 11.4% of the total number of patients tested with SARS-CoV-2 (n=651) [7,8]. In another study, Lin L. et al studied data from 95 patients infected with SARS-CoV-2, where 61.1% showed signs of various gastroenterological symptoms, the most important of which were diarrhea (24.2%), nausea (17.9 %), vomiting (4.2%) and liver dysfunction (32.6%). It should be noted that SARS-CoV-2 was detected in the biopsy material obtained during esophagogastroduodenoscopy in three out of six people. In two patients, the virus was found in the esophagus, stomach, duodenum, and rectum, which proved that the virus was present throughout the gastrointestinal tract. In 52.4% (n=22) cases, the virus was detected in the examined stool samples [9]. Thus, two publications highlighted the prevalence of symptoms in patients with COVID-19.

In another study, Xiao F. et al. studied the medical records of 73 hospitalized patients with SARS-CoV-2 in China, and found that 53.4% of individuals had a positive stool test between days 1 and 12 of infection [10]. It should be noted that more than 20% of infected patients had positive virus in their feces even after clearing the virus in the respiratory tract. Similar results from Singapore showed that 50% of patients with COVID-19 had faecal virus, but only half of these patients complained of diarrhea [11].

In some cases, diarrhea may precede the clinical presentation of COVID-19. Y. Song et al. It is described that a 22-year-old patient was admitted to the hospital with a complaint of subfebrile temperature in the absence of 3-4 separate liquid stools per day and no other clinical symptoms. Radiologically, bilateral pneumonia was detected, and SARS-CoV-2 RNA was detected in a nasopharyngeal swab. After specific antiviral treatment (lopinavir and ritonavir), diarrhea stopped in parallel with the disappearance of changes in the lungs [12]. Thus, evidence is accumulating that OIT symptoms are common and that SARS-CoV-2 can be detected in the feces of approximately 50% of people infected with SARS-CoV-2, but there is no clear correlation between gastroenterological symptoms and the presence of the virus in feces.

It is not clear why SARS-CoV-2 causes gastrointestinal symptoms. However, the question remains whether SARS-CoV-2 can be transmitted through the digestive system in addition to the respiratory tract. A prerequisite for infection is its entry into host cells. Like its predecessor SARS-CoV, SARS-CoV-2 uses angiotensin-converting enzyme-2 (ACE2) as a viral receptor to enter host cells, and ACE2 is an important regulator of intestinal inflammation [13, 14].

The purpose of the study. Diagnosing gut microbiota in patients after infection with COVID-19.

Research material and methods. Researches were conducted in 120 (54 men and 66 women) patients aged 29 to 70 years and 20 healthy people treated for dysbacteriosis in the gastroenterology department of the Multidisciplinary Clinic of the Tashkent Medical Academy. Patients were divided into two groups: the first group included patients with COVID-19 and dysbacteriosis (n=58), and the second group included patients with dysbacteriosis without COVID-19 (n=62). The indicators of patients with dysbacteriosis (according to whether they had or did not have COVID-19) were compared with each other.

Thus, during the implementation of this study, identification of clinically important microorganisms was carried out using MALDI-TOF mass spectrometry and fecal culture. Traditional bacteriological tests based on phenotypic characteristics of microorganisms and efficiency of mass spectrometric system were compared.

Research results. 120 patients suffering from dysbacteriosis were selected for the study. Their average age and gender ratio were studied (Table 1).

Table 1

Age and gender of patients with dysbacteriosis who had COVID-19 / who did not have COVID-19

Gender	Age				Total
	29-39	40-49	50-59	60-70	
males	12 (10±2,8)	16 (13,3±3,1)	12 (10±2,8)	10 (8,3±2,5)	12 (10±2,8)
females	20 (16,7±3,4)*	14 (11,7±2,9)	17 (14,2±3,2)	19 (15,8±3,3)*	20 (16,7±3,4)*

Note: * – $p<0.05$ is statistically significant compared to the male group

Table 2 presents a comparative analysis of fecal indicators based on the results of coprological research in patients with dysbacteriosis, taking into account the fact that they passed COVID-19 or did not pass COVID-19.

Table 2

Results of coprological examination of patients in research groups

Indicators	**Patients with dysbacteriosis who did not transmit COVID-19 (n=58)**	**Patients who have passed COVID-19 and have dysbacteriosis (n=62)**
Altered muscle fibers (points)	0,497±0,008	2,998±0,056***
Intact muscle fibers (points)	0,301±0,006	0,7±0,014***
Digested Plant Fiber (points)	0,104±0,002	2±0,034***
Undigested Plant Fiber (points)	0,081±0,002	2,003±0,039***
Intracellular starch, points	0,099±0,002	2,003±0,037***
Extracellular starch (points)	0,067±0,001	2,8±0,054***
Fatty acids (points)	0,051±0,001	0,01±0***
Soap (points)	0,01±0	0,301±0,007***
Neutral fats (points)	0,099±0,002	3,004±0,058***
Mucus (points)	0,021±0,001	0,1±0,002***

Note: * – $p<0.05$, ** – $p<0.01$, *** – $p<0.001$ statistically significant compared to the group of patients without COVID-19

The obtained results showed that the studied groups differed in terms of coprogram indicators ($p<0.001$). Indicators such as altered and unchanged muscle fibers were higher in patients with dysbacteriosis who underwent COVID-19.

Plant fiber (digestible and non-digestible) was found to be higher in the group of patients with dysbacteriosis who underwent COVID-19 than in the control group. The above-mentioned differences were found at an almost absolute level ($p<0.001$). Neutral fat was significantly higher ($p<0.001$) in the group of patients with COVID-19. Also, mucosal coprogram scores were found to be naturally higher in the group of patients with COVID-19 ($p<0.001$) (Table 3). This indicates that patients with COVID-19 have defecation disorders and inflammatory-edematous changes in the colon mucosa, mainly in the form of constipation.

The features identified on the basis of coprological examination of feces in both groups of patients are more relevant to the group of patients with dysbacteriosis who have undergone COVID-19, which indicates the presence of manifestations of maldegistia syndrome. It is assumed that there is a deficiency of exocrine function of the pancreas in patients with COVID-19 who have observed bowel movements and defecation disorders.

Table 3

Results of bacteriological examination of feces of patients with dysbacteriosis who underwent COVID-19

Criteria		**Dysbacteriosis of the 1st degree**	**Dysbacteriosis of the 2nd degree**	**Dysbacteriosis of the 3rd degree**
There is a dysbacteriosis caused by COVID-19	Bifidobacterium	18%	55%	27%
	Lactobacillus	21%	51%	28%
	Escherichia coli	18%	46%	36%
	Conditionally pathogenic microflora	19%	43%	38%

When the groups of patients with dysbacteriosis who had and who did not have COVID-19 were subjected to traditional bacteriological examination, a decrease in the quantitative indicators of bifido, lacto-, propion- and eubacteria was observed in most of the patients with dysbacteriosis in both groups. The number of propionic bacteria in the studied stages of patients with dysbacteriosis did not exceed 50% of the indicator in the control group (r<0.05) (Table 4).

Table 4

Results of bacteriological examination of feces in patients with dysbacteriosis who did not pass COVID-19

Criterion		**Dysbacteriosis of the 1st degree**	**Dysbacteriosis of the 2nd degree**	**Dysbacteriosis of the 3rd degree**
There is a dysbiosis that does not transmit COVID-19	Bifidobacterium	18%	25%	57%
	Lactobacillus	21%	28%	51%
	Escherichia coli	18%	33%	49%
	Conditionally pathogenic microflora	19%	34%	47%

However, when using the traditional bacteriological examination method, it will be possible to determine the level of dysbacteriosis and cultivate the actually existing microbes in only 20% of cases. The remaining 80% of microorganisms are not grown in pure form, or the environmental conditions are not selected correctly. Therefore, at the next stage of the research, the fecal mass spectrometry method, which is not yet widely used in clinical practice, was used.

Analysis of fecal microflora in examined patients revealed Enterobacter spp. (>108 KOE); Citrobacter spp. (>105KOE), E. coli showed a predominance of enteropathogenic (>108KOE) proteolytic flora, as well as a lack of Bifidobacterium (<108KOE) bacteria. The number of bifidobacteria decreased with increasing severity of dysbacteriosis, with significant differences compared to group 2 indicators (r<0.05). The latter have been proven to belong to conditionally pathogenic flora. A lower than normal number of bifidobacteria and lactobacteria indicates the presence of pathological changes in the body, such as inflammation and a decrease in immune protection.

In the group of patients with dysbacteriosis who did not transmit COVID-19, there was a tendency to decrease bifidobacteria (r=0.05). The amount of lactobacilli in patients who did not have COVID-19 and in the second group was almost the same and statistically significantly higher (r=0.05) than in the group of patients who had COVID-19. The absolute highly significant difference of lactobacilli in patients with dysbacteriosis who did not undergo COVID-19 compared to patients with dysbacteriosis who underwent COVID-19 is noteworthy (r=0.05). The maximum level of eubacteria was recorded in patients with the most severe manifestations of dysbacteriosis (r=0.062), in patients with the initial stages of the disease, the values of this indicator were lower than in the control group (r=0.045).

When analyzing the composition of intestinal clostridial and coccal flora depending on the severity of dysbacteriosis, ruminococci, staphylococci, enterococci and Str. an increase in the number of cocci due to an increase in the amount of mutans was found. A significant increase in the total number of microscopic fungi was also observed in the group of patients with dysbacteriosis who underwent COVID-19. It should be noted that this law does not apply equally to all microscopic fungi: the number of aspergills was higher in patients with dysbacteriosis compared to controls (r<0.05). Prevotella spp. and Kingella spp. an increase in the number of gram-negative flora, which are considered to be the main representatives, was noted. In rare cases, other gram-negative bacteria such as Flavobacterium spp., Campylobacter mucosalis, Moraxella sp.

Thus, during the implementation of the study, a comparative increase in many indicators of microbial markers was found in the group of patients with dysbacteriosis who had COVID-19, with several differences from the group of patients with dysbacteriosis who did not have COVID-19.

In patients with dysbacteriosis, the composition of intestinal microflora was analyzed by MALDI-TOF mass spectrometry, and the genus, type, and subspecies of microorganisms were determined. In addition, the analysis of mass spectrometric indicators showed that the maximum level of eubacteria was recorded in patients with dysbacteriosis who did not pass COVID-19, while the values of eubacteria were lower in patients with dysbacteriosis who passed COVID-19 (r<0.05). Analyzing the composition of clostridial and coccal intestinal flora in patients with dysbacteriosis according to whether they had/did not have COVID-19, ruminococci, staphylococci, enterococci and anaerobic Str. An increase in the number of cocci was found as a result of an increase in the amount of mutans. It was found that both the total number of actinomycetes and their individual representatives increased in patients with dysbacteriosis who underwent COVID-19 (r<0.05).

Dysbacteriosis patients who did not have COVID-19 also had a significant increase in the total number of microscopic fungi. However, their number was lower compared to the comparison group.

Prevotella spp. and an increase in the number of Gram (–) flora, which are considered the main Kingella spp. In rare cases, Campylobacter mucosalis, Flavobacterium spp., Moraxella spr. Other Gram (–) bacteria were also detected.

In addition, in both groups of patients with dysbiosis, the amount of Enterobacteriaceae (especially Enterobacter, Klebsiella, Escherichia), Enterococci, Clostridium Perfringens increased, and the number of bacteria such as Bifidobacterium, Lactobacillaceae, Bacteroidaceae and Prevotellaceae decreased.

Analysis of fecal microflora revealed a lack of Bifidobacterium (<108KOE) in the examined patients. Usually, the normal composition of the intestinal microflora includes lactic acid bacteria - lactobacilli, bifidobacteria and propionic acid bacteria, with a predominance of bifidobacteria. They play an important role in maintaining the optimal composition of the biocenosis and its functions. A lower than normal number of bifidobacteria and lactobacteria indicates the presence of pathological changes in the body, such as inflammation and decreased immune protection.

In addition, Echerichia (>108 KOE) in the examined patients. it has been proved that the number has increased and that they belong to conditionally pathogenic flora. In healthy people, it does not cause direct changes. However, if they exceed the norm and/or reduce the effectiveness of the immune defense, they can cause serious changes.

Conditionally pathogenic flora competing with beneficial bacteria can become part of the intestinal microbial film and cause functional disorders, allergic and inflammatory diseases. Through the intestinal wall, conditionally pathogenic microflora can pass into the bloodstream and spread throughout the body.

Conclusion. The composition of intestinal microorganisms in patients with dysbacteriosis is almost the same, but its quantitative ratio differs according to whether or not they have had COVID-19. Firmicutes/Bacteroidetes was reliably higher in the group of dysbacteriosis patients with COVID-19 and significantly different from the group of dysbacteriosis patients without COVID-19 ($p<0.05$).

A significant reduction of butyric acid-producing bacteria such as Roseburia, Faecalibacterium prausnitzii in the group of patients with dysbacteriosis who underwent COVID-19 compared to the control group may be important in the exacerbation of inflammation and dysbacteriosis.

The number of Enterobacteriaceae (especially Enterobacter, Klebsiella, Escherichia), Enterococci, Clostridium Perfringens increased, Bifidobacterium, Lactobacillaceae, Bacteroidaceae and Prevotellaceae decreased in the group of patients with dysbacteriosis who underwent COVID-19.

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