Central Asian Journal of Medicine

16S RRNA ANALYSIS OF MICROBIOTA COMPOSITION AGAINST THE BACKGROUND OF SARS-COV-2 INFECTION USING MOLECULAR DIAGNOSTICS (LITERATURE REVIEW)

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ABSTRACT

This article is devoted to the study of microbial environmental changes against the background of SARS-CoV-2 infection using molecular diagnostics. The composition of the human microbiota was analyzed using 16S rRNA sequencing technology and the dysbiosis of the microbiota associated with COVID-19 was studied. The article presents scientific data on the enrichment or reduction of the microbiota with different categories of microorganisms during SARS-CoV-2 infection, as well as their impact on health and the immune system. The clinical implications of changes in the structure of the microbiota and the possibility of their application for diagnostic purposes have also been considered.

Key words: SARS-CoV-2, COVID-19, microbiota, 16S rRNA sequencing, molecular diagnostics, microbiota dysbiosis, immune system, molecular biology.

INTRODUCTION

The use of 16S rRNA gene sequences to study bacterial phylogeny and taxonomy has been the most common household genetic marker used for several reasons [1]. In 1980, 1791 true names were recognized at the species level in approved lists. Today, the number has reached 8168 species, an increase of 456%. The explosion in the number of recognized taxa is directly related to the ease with which 16S rRNA gene sequencing research is performed, as opposed to the cumbersome manipulations that involve research on DNA-DNA hybridization.

DNA-DNA hybridization is the "gold standard" for accurately assigning a strain with proposed new species and ambiguous traits to the correct taxonomic unit DNA hybridization analyses are not without drawbacks, But they are time-consuming, labor-intensive, and expensive to implement. Today, fewer and fewer laboratories around the world perform such analyses, and many studies describing new species are based only on small subunit (SSU) sequences or other multi-phase data [2]. In the early 1990s, the availability of DNA sequencers improved dramatically in terms of cost, methodology, and technology, so many centers are now able to purchase such equipment. Stackebrandt and Goebel summarized the emergence of SSU sequencing technology and its potential usefulness in species detection. Although the 16S rRNA gene sequence has been shown to be less accurate on the individual strain, which is its closest neighbor with a 97% similarity rate. This latter value could indicate a new species or alternatively indicate clustering within a predefined taxon [3]. The study of DNA-DNA hybridization has traditionally been required to provide definitive answers to such questions. While 16S rRNA gene sequence data can be used for many purposes, unlike DNA hybridization (>70% reassociation), there are no defined "gate values" (e.g., 98.5% similarity), beyond which there is a universal consensus for what is clear and unambiguous. The final identification of the level of species is [4].

The raw dataset contains the 16S rRNA gene sequence produced from nasopharyngeal swab samples taken from patients with SARS-CoV-2. The dataset has 1,552,769 readings, with an average of 4,635 readings per sample. Metadata provides the following information about the samples: city, hospital ID, type of material collected, sampling season, technical batch of sequence, and date of sampling. The patient's status is characterized by the following indicators: age, sex, oxygen saturation (SpO2), respiratory rate, need for supplemental oxygen supply (extra O2), chest computed tomography (CT score), percentage of affected lung tissue (lung damage), and hospitalization or outpatient treatment (patient status) [5]. The data describing the patients' health status and their habits are represented by the following factors: obesity, smoking, past smoking (previously smoking), diabetes, chronic obstructive pulmonary disease (COPD), inflammatory bowel disease (IBD), arthritis, tuberculosis. , hypertension, coronary artery disease, chronic heart failure, and asthma, Pangolin classification of the genome of the progeny SARS-CoV-2 [6].

As a respiratory virus, SARS-CoV-2 infects humans primarily through upper respiratory contact. Previous research has shown that the microbiota can modulate immunity to pathogenic infection. In this study, we performed metagenomic sequencing of pharyngeal swabs of eleven patients with COVID-19 and eleven

non-COVID-19 patients with similar symptoms such as fever and cough. Through metagenomic analysis of the healthy group from the above two groups and the public data, 6502 species were identified in the samples [7]. Specifically, the Pielou index showed that the uniformity of the microbiota in the COVID-19 group was lower than in the non-COVID-19 group. In combination with the linear discriminant analysis (LDA) and the generalized linear model, eighty-one bacterial species increased in the COVID-19 group, where 51 species were enriched more than 8 times. The trio of best-enriched genera *include Streptococcus, Prevotella,* and *Campylobacter* , which contain some opportunistic pathogens. Interestingly, through experiments, we *have found that two* Streptococcus *strains,* S. suis *and* S. agalactiae, can stimulate in *vitro* ACE2 expression of Vero cells, which can stimulate SARS-CoV-2 infection [8]. Therefore, these enriched pathogens in the pharynx of COVID-19 patients may be involved in virus-host interactions to induce SARS-CoV-2 infection and potentially induce secondary bacterial infections by altering ACE2 viral receptor expression and/or modulation of the host [9].

To date, little is known about the impact of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), responsible for the 2019 coronavirus disease (COVID-19) pandemic, on the upper respiratory tract (URT) microbiota over time. To fill this information gap, we used ribosomal RNA gene sequencing to characterize the URT microbiota in 48 adults, including 24 participants with mild to moderate-weight COVID-19, with sequential medium-turbine swabs collected after 21 days. and 24 asymptomatic, non-infected controls with medium-turbine buffers collected at enrollment and only at enrollment [10]. To comprehensively compare the URT microbiota between groups, a variety of statistical analyses frequently used in microbial ecology, including α diversity , β-diversity, and differential abundance analyses, were used. Final statistical models include the presence of at least one comorbidity by age, gender, and covariance. The mean age of all participants was 34.00 years (range of quartets = 28.75-46.50) years [11]. Compared to control samples, participants with COVID-19 had a lower species index observed on day 21 (linear regression coefficient = -13.30; 95% CI = -21.72 to −4.88; q = 0.02). In addition, the Jaccard index differed significantly between participants with COVID-19 and samples taken from the control group at all study time points (PERMANOVA q <0.05 for all comparisons). The plurality of the three amplicon sequence variants (ASV) (one *Corynebacterium* ASV, one *Corynebacterium ASV, Frederiksenia canicola* and one *Lactobacillus* ASV) decreased at all seven study time points in samples from participants infected with COVID-19, while the plurality of one ASV (from the family) decreased.

Neisseriaceae) had an increase in samples from participants infected with COVID-19 in five of the seven study times (71.43%). Our results suggest that mild to moderate COVID-19 can cause altered URT microbiota that lasts for several weeks after initial infection [12].

Little is known about the relationship between the respiratory virus responsible for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the ongoing coronavirus disease 2019 (COVID-19) pandemic, and the upper respiratory tract (URT) microbiomes [13, 14].

Emerging evidence suggests that the oral and upper respiratory tract microbiota may play an important role in modulating immune responses specific to viral infection. Because the host microbiome may be involved in the pathophysiology of coronavirus disease 2019 (COVID-19), we investigated the association between the oral and nasopharyngeal microbiome and COVID-19 severity. We collected saliva (n = 78) and nasopharyngeal buffer (n = 66) samples from the COVID-19 cohort and characterized microbiomes using 16S ribosomal RNA gene sequencing. We also looked at the relationship between salivary and nasopharyngeal microbiome and age, COVID-19 symptoms, and blood cytokines [15]. A case of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, but not COVID-19 severity, was associated with community-level differences in the oral and nasopharyngeal microbiomes. Alpha diversity in the saliva and nasopharyngeal microbiome was negatively correlated with age, and was associated with fever and diarrhea. Oral Bifidobacterium, Lactobacillus, and Solobacterium decreased in patients with severe COVID-19. Nasopharyngeal Paracoccus decreased, with increased levels of nasopharyngeal Proteus, Cupravidus, and Lactobacillus in patients with severe COVID-19. Further analysis showed that the COVID-19 biomarkers known to have an abundance of Bifidobacterium through the mouth were negatively correlated with plasma concentrations of interleukin 17F and monocyte chemoatracant protein-1. Our results indicate that the severity of COVID-19 disease is related to the relative abundance of known bacterial taxa [15].

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comprehensively compare the URT microbiota between groups, a variety of statistical analyses frequently used in microbial ecology, including α diversity , β-diversity, and differential abundance analyses, were used. Final statistical models include the presence of at least one comorbidity by age, gender, and covariance. The mean age of all participants was 34.00 years (interquarteral range = 28.75–46.50) years [17]. Compared to control samples, participants with COVID-19 had a lower type index observed on day 21 (linear regression coefficient = -13.30; 95% CI = -21.72 to -4.88; q = 0.02). In addition, the Jaccard index differed significantly between participants with COVID-19 and samples taken from the control group at all study time points (PERMANOVA q <0.05 for all comparisons). The abundance of three amplicon sequence variants (ASV) (one Corynebacterium ASV, Frederiksenia canicola and one Lactobacillus ASV) decreased at all seven study points in samples from participants infected with COVID-19, compared to the plurality of one ASV (from the family). There was an increase in samples from participants with COVID-19 in five of the seven study times (71.43%). Our results suggest that mild to moderate COVID-19 can cause altered URT microbiota that persists for several weeks after initial infection [18].

Little is known about the features of the respiratory microbiome in patients with Coronavirus disease (COVID-19) 2019. We conducted a study with the expectation of clarifying these features as much as possible. A cross-sectional study was conducted to characterize the microbial communities of the respiratory tract of 69 COVID-19 inpatients from 64 nasopharyngeal swabs and 5 sputum samples using the 16S ribosomal RNA gene V3-V4 region sequence [19]. Bacterial profiles were analyzed to find potential biomarkers using a two-step method, a combination of a randomized forest model and a linear discriminant analysis, and a study of associations with clinical features through the Spearman-level test [20] [21]. Compared to mild COVID-19 patients, bacterial diversity in severe patients decreased significantly (p-values less than 0.05 in alpha and beta diversity) and the abundance of opportunistic pathogens, including Actinomyces, Prevotella, Rothia, Streptococcus, Veillonella, was relatively low. Eight potential biomarkers, including Treponema, Leptotrichia, Lachnoanaerobaculum, Parvimonas, Alloprevotella, Porphyromonas, Gemella, and Streptococcus, have been found to be able to distinguish mild COVID-19 patients from severe COVID-19 patients [22] [23] [24]. The offspring of Actinomyces and Prevotella were negatively correlated with age in the two groups. Intensive care unit admissions, neutrophil counts, and lymphocyte counts were significantly correlated with different offspring in the two groups. In addition, there was a positive correlation between Klebsiella and white blood cell counts in the two groups. The respiratory

microbiome had significant differences in COVID-19 patients of different weights. The value of the respiratory microbiome as predictive biomarkers for COVID-19 severity deserves further research [25] [26] [27].

In summary, the article states that changes that occur in the upper respiratory tract microbiota during SARS-CoV-2 infection were studied using 16S rRNA technology. The results of the study suggest that COVID-19 leads to microbiota dysbiosis, with a decrease in certain beneficial bacteria and an increase in pathogenic microorganisms. These changes can increase the severity of infection, slowing down the activity of the immune system and increasing the risk of secondary infections. It has also been noted that there is potential to apply changes in the microbiota for diagnostic purposes. This research is important in identifying the complex relationships between the microbiota and viral infections.

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