Pengaruh Rasio Kadar Sitokin Pro-Inflamasi dengan Anti-Inflamasi dan Karakteristik Mikrobiota di Saluran Napas Atas terhadap Beratnya Manifestasi Klinis COVID-19 = Effect of Pro-Inflammatory to Anti-Inflammatory Cytokine Levels Ratio and Microbiota Characteristics in Upper Airway on COVID-19 Severity

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Abstrak

COVID-19 merupakan penyakit penyebab pandemi pada akhir 2019. Perbedaan manifestasi klinis pada infeksi SARS-CoV-2 ini memicu banyak pertanyaan di kalangan peneliti dan medis. Perbedaan klinis COVID-19 tersebut dapat dipicu oleh faktor hospes, patogen maupun lingkungan. Infeksi SARS-CoV-2 terutama melalui saluran napas atas, tempat kolonisasi mikroba komensal dan patogen. Bagaimana interaksi antara mikroba yang berkolonisasi dengan SARS-CoV-2 dalam menimbulkan respons inflamasi di saluran napas atas masih belum diketahui dengan jelas. Penelitian ini bertujuan menganalisis hubungan antara karakteristik mikrobiota, serta rasio kadar sitokin pro- dan anti-inflamasi dari saluran napas atas dengan beratnya COVID-19.

Penelitian ini merupakan studi potong lintang menggunakan 74 swab nasofaring dan orofaring di dalam viral transport medium (VTM) dari pasien COVID-19 berusia 18–64 tahun. Profil mikrobiota di saluran napas atas dan kadar IL-6, IL-1, IFN-, TNF- dan IL-10 diperiksa dengan metode sekuensing 16S ribosomal RNA dan Luminex assay, secara berurutan. Selanjutnya dilakukan analisis hubungan antara beratnya COVID-19 dengan OTU, keragaman alfa dan beta dari mikrobiota saluran napas atas.

Lima filum terbanyak di saluran napas pasien COVID-19 di Indonesia berusia 18-64 tahun adalah Firmicutes (32,3%), Bacteroidota (27,1%), Fusobacteriota (15,2%), Proteobacteria (15,1%) dan Actinobacteria (7,1%). Analisis indeks Shannon dan ACE menunjukkan bahwa tidak ada penurunan keragaman microbiota saluran napas atas dengan bertambah beratnya penyakit. Namun, ada perbedaan bermakna keragaman beta pada mikrobiota saluran napas atas antara COVID-19 ringan dan berat. Keberlimpahan filum Firmicutes ($p = 0,012$), dan genus Streptococcus ($p = 0,033$) dan Enterococcus ($p = 0,031$) lebih tinggi pada COVID-19 berat dibandingkan yang ringan, sedangkan keberlimpahan filum Fusobacteriota ($p = 0,021$), Proteobacteria ($p = 0,030$), Campilobacterota ($p = 0,027$), genus Neisseria ($p = 0,008$), dan Fusobacterium ($p = 0,064$), spesies Porphyromonas gingivalis ($p = 0,018$), Fusobacterium periodonticum ($p = 0,001$) dan Fusobacterium nucleatum ($p = 0,022$) lebih tinggi pada COVID-19 ringan dibandingkan berat. Keberadaan bakteri Prevotella buccae ($p = 0,005$) dan Prevotella disiens ($p = 0,043$) lebih rendah pada COVID-19 berat. Rasio TNF-/IL-10 lebih tinggi pada COVID-19 berat ($p < 0.05$). Selanjutnya, rasio IL-6/IL-10, IFN-/IL-10, dan IL-1/IL-10 juga lebih tinggi pada COVID-19 berat, namun tidak berbeda bermakna jika dikaitkan dengan beratnya penyakit.

Penelitian ini mendukung adanya hubungan antara karakteristik mikrobiota di saluran napas atas dengan beratnya COVID-19 pada pasien dewasa. Studi lebih lanjut diperlukan untuk memeriksa mekanisme bagaimana mikrobiota mencegah beratnya COVID-19. Rasio TNF-/IL-10 dari saluran napas dapat menjadi prediktor beratnya penyakit dan sebagai alternatif pemeriksaan kadar sitokin pada COVID-19 yang kurang invasif dibandingkan serum.

.....COVID-19 is a disease that caused a pandemic at the end of 2019. Clinical manifestations difference in SARS-CoV-2 infection has raised many questions in research and medical provider. The clinical differences in COVID-19 can be triggered by host, pathogen and environmental factors. SARS-CoV-2 mainly enters through the upper airway, with colonization of commensal and pathogenic microbes. How the interaction between colonized microbes and SARS-CoV-2 in causing an inflammatory response in the upper airway is still not clearly known. Therefore, we examined the association between the diversity of microbiota, pro- and anti-inflammatory cytokines ratio of upper respiratory and COVID-19 severity.
This research is an observational cross-sectional study using 74 nasopharyngeal and oropharyngeal swabs in viral transport medium from COVID-19 patients aged 18-64 years. We examined microbiota profile in the upper airway using 16S ribosomal RNA sequencing method and levels of IL-6, IL-1, IFN-, TNF- and IL-10 were examined by Luminex assay. We also examined the association between COVID-19 severities with OTU analysis, alpha and beta diversity of upper respiratory microbiota.
The top five phyla in upper respiratory tract of Indonesian COVID-19 patients with aged of 18–64 years old were Firmicutes (32,3%), Bacteroidota (27,1%), Fusobacteriota (15,2%), Proteobacteria (15,1%) and Actinobacteria (7,1%). Shannon and ACE index analysis showed no decline of microbiota diversity in upper airway with the increase of disease severity. However, there were significant differences of beta diversity in the upper airway microbiota between mild and severe COVID-19. The abundance of the Firmicutes phylum ($p = 0,012$), Streptococcus ($p = 0,033$) and Enterococcus ($p = 0,031$) genera were significantly higher in severe COVID-19 than mild, while the abundance of the Fusobacteriota ($p = 0,021$), Proteobacteria ($p=0,030$), and Campilobacterota ($p = 0,027$) phyla, Neisseria ($p = 0,008$), and Fusobacterium ($p = 0,064$) genera, Porphyromonas gingivalis ($p = 0,018$), Fusobacterium periodonticum ($p = 0,001$) and Fusobacterium nucleatum ($p = 0,022$) species were significantly higher in mild. The presence of Prevotella buccae ($p=0.005$) and Prevotella disiens ($p=0.043$) bacteria was lower in severe COVID-19. The TNF-/IL-10 ratio was significantly higher in severe COVID-19 ($p < 0.05$). Furthermore, IL-6/IL-10, IFN-/IL-10, and IL-1/IL-10 ratio was also higher in severe, but those were not significantly related to the disease severity.
This research supports the relationship between the severity of COVID-19 and microbiota diversity in the upper airway in adults. Further studies are needed to examine the mechanism by which microbiota prevents the COVID-19 severities. The ratio of TNF-/IL-10 from upper airway swab may be as a predictor of disease severity and alternative for examining cytokine levels in COVID-19 which is less invasive than serum.