MINI REVIEW

RECORDS OF PHARMACEUTICAL AND BIOMEDICAL SCIENCES

Unveiling the Smoking and Obesity Impact on the Orointestinal Axis Microbiome: Pilot study

Esraa K. Hassan[1], Salah Salah Abdalla[2], Ali Abdellah[2], Marwa Azab[2*], Kareem A. Ibrahim[1], Mohammed Ramadan[3]

[1]Department of Microbiology and Immunology, Faculty of Pharmacy, Egyptian Russian University, Badr City, Egypt; [2]Department of Microbiology and Immunology, Faculty of Pharmacy, Suez Canal University, Ismailia, Egypt; [3]Department of Microbiology and Immunology, Faculty of Pharmacy, Al-Azhar University, Assiut, Egypt.

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* Correspondence Author:

Tel: +201024299630

E-mail address:

Marwaazab2515@yahoo.com

Abstract

According to WHO, obesity comes in the fourth place as a death risk factor, after hypertension, dietary risks and smoking. The influence of obesity and smoking on oral and gut microbiome -the two largest microbial ecosystems in the human body- needs better understanding.

Using V3-V4 16S rRNA next-generation sequencing, we examined the orointestinal axis microbiome of obese-smokers (ObSm), smokers, and healthy patients in addition to the relationship between oral and gut bacteria. oral and gut microbiomes of ObSm and smokers had higher diversity than healthy subjects and oral microbiomes were significantly more diverse than gut microbiomes. The phyla *Firmicutes* and *Proteobacteria* were significantly more abundant in the oral microbiome of healthy subjects than in ObSm and smokers, while the oral microbiome of ObSm the phylum *Bacteroidetes* was significantly showed higher relative abundance. Furthermore, *Firmicutes* was enriched in stool microbiome of smokers compared to ObSm and healthy subjects, but the phylum *Bacteroidetes* was reduced in stool microbiome of ObSm compared to smokers and healthy subjects. The phylum *Proteobacteria* was enriched in the stool microbiome of ObSm than in smokers and healthy subjects at the genus level; *Streptococcus*, *Veillonella*, and *Prevotella* were presented with higher relative abundance in the oral microbiome of smokers than in ObSm and healthy subjects. interestingly, stool microbiome of ObSm and smokers showed retracted representation of *Bacteroides* and *Faecalibacterium* in comparison to healthy subjects.

Keywords: Obesity, Smoking, Orointestinal axis, Oral microbiome, Gut microbiome, 16S rRNA gene.

1. Introduction

The human body is composed of nearly ten trillion cells, with at least 10 microbial cells sharing our body space for each cell. Although the overall number of base pairs in the human body is close to a billion, protein-coding genes constitute approximately 20,000 to 25,000 genes in human cell genetic material (Gaudet et al. 2017). Microbial populations exist in the GIT, ranging in diversity from the mouth to the anus. The proportional proportions of microbes vary according to anatomical site (Xu et al. 2007).

The "human microbiome" is the term used for the genomes of commensal bacteria that live symbiotically on and within numerous places of the human body. Our mouth cavity, genitalia, respiratory tract, skin, and digestive system are all examples of occupied habitats (Kho and Lal 2018). The human microbiome, made up of bacteria, archaea, viruses, and eukaryotes, plays a vital role in maintaining the host's immune system homeostasis and resisting infections at various body sites while also defining the host's metabolic characteristics (Ogunrinola et al. 2020), (Zhang et al. 2021), (Shabayek et al. 2022). The human microbiome can be divided into two types: 'core' microbiome and 'variable' microbiome. Every individual has a core microbiome, which is composed of the predominant species that inhabit different parts of the body in a healthy environment and normally, The microbial taxa that are present in two or more samples from the same host or environment are referred to as core microbiomes (Zarco, Vess, and Ginsburg 2012), (Neu, Allen, and Roy 2021). Conversely, the individual's variable microbiome is distinct and has developed in response to certain lifestyle, phenotypic, and genotypic factors. Although individuals share microbiota at similar regions throughout the body, there are variances at the species and strain levels of the microbiome that can be as unique to the individual as a fingerprint (Zarco, Vess, and Ginsburg 2012).

These terms, "microbiome" and "microbiota," can be used interchangeably; the former refers to the collective genomes of microorganisms, while the latter refers to the organisms themselves (Frank and Pace 2008). Therefore, by investigating microbiota variances, it may be possible to better understand how lifestyle and environmental factors affect commensal microbial communities in healthy and ill individuals (Ogunrinola et al. 2020), (Shabayek et al. 2022), (Huang and Shi 2019). These insights are being expanded in order to better understand the intricate interactions that exist between the host and the related microbiome (Ogunrinola et al. 2020), (Shabayek et al. 2022). The microbiome, in particular, is regarded as the "new" biomarker of human health due to its critical function in maintaining normal body physiology while developing and teaching the immune system (Antinozzi et al. 2022).

The two largest microbial ecosystems in the human body are the oral and gut microbiomes (Park et al. 2021). According to the human microbiome project (HMP), more than half of the bacteria in the human body live in the gastrointestinal (GI) tract (29%) and the mouth cavity (26%), where the 15 distinct habitats that are biologically rich and taxonomically varied in the body include the oral and fecal microbiomes (Park et al. 2021). Dysbiosis of such communities precede several oral and systemic disorders, including cancer, autoimmune disorders, and inflammatory conditions (Khor et al. 2021).

Although the gut microbiome is complex and challenging to research, it is essential to human health because it affects the emergence of chronic illnesses, such as metabolic disorders and GI problems as colorectal cancer and pathogen colonization resistance (Fan and Pedersen 2021; Hills et al. 2019; Seekatz, Safdar, and Khanna 2022). Changes in the composition and functionality of the gut microbiome impair colonization resistance and have been linked to a number of GI and non-GI disorders (Fan and Pedersen 2021; Seekatz, Safdar, and Khanna 2022).

The whole collection of genomes from microorganisms living in the mouth cavity is known as the oral microbiome (Lim et al. 2017). The human mouth cavity serves as a home for oral microbial populations (Li et al. 2022). The oral cavity has the second-largest and most varied microbiota after the digestive tract. It is home to a diverse array of microorganisms, including as viruses, bacteria, fungi, and protozoa (Deo and Deshmukh 2019). The mouth's warm, humid environment fosters the growth of a variety of bacteria and supplies nutrients that are obtained from the host, including glycoproteins, saliva proteins, and gingival crevicular fluid (Kilian et al. 2016). Approximately one thousand different species of bacteria may be found in the oral cavity, most of which are members of the following phyla: *Firmicutes*, *Proteobacteria*, *Euryarchaeota*, *Fusobacteria*, *Bacteroidetes*, and *Tenericutes* (Li et al. 2022) .

Obesity is a systemic disease that affects the entire body and is caused by an imbalance between energy intake and expenditure (Muluke et al. 2016). Obesity was associated with significant changes in oral and gut microbiome. Furthermore, the inclusion of salivary samples, gut microbiome, and consideration of microbiome community structure may increase our understanding of the mechanisms linking microbiota to obesity and the influence of gut microbiome on nutritional status (Bombin et al. 2022; Zsálig et al. 2023).

Smoking is a major public health issue that currently exists throughout the world, where such a preventable cause of premature death impacts almost every organ system in the body (Al-Zyoud et al. 2020; Al Bataineh et al. 2020). The oral cavity is one of the first areas of the body to be exposed to cigarettes smoke, making it particularly vulnerable to increased carcinogenesis, reduced mucosal immunity, and changes to the oral microbiome (Al Bataineh et al. 2020). Moreover, The

toxins of cigarettes smoke can disrupt the mouth's microbial ecology by antibiotic actions, oxygen deprivation, or other potential pathways (Wu et al. 2016).

The hand acts as a conduit for the transfer of bacteria from the feces to the mouth, as evidenced by the high correlation between the human hand microbiota profile and the patterns of the oral and gut microbiomes (Park et al. 2021), (Shaffer and Lozupone 2018). The microbial ecosystem of both environments can be shaped or reshaped by this bidirectional relationship, which will ultimately affect physiological and pathological processes in the GI system. Despite the oral-gut barrier and the diversity of microbiome species in terms of kinds and quantities in both habitats, their natural relationship supports bacterial translocation mechanisms, leading to dysbiosis in either habitat; and although the links between the human microbiome and systemic disease are becoming more perceptible, the complex interaction will become clearer through continuous research and multiple-discipline cooperation (Khor et al. 2021).

Previously, investigating the microbiome was limited to culture-dependent approaches, but the extensive bacteria present in the oral cavity could not be cultured using traditional cultivation method and also the most significant features of the gut microbiome are not well understood due to a lack of scientific tools for identifying non-cultivable microbes; however, it has been exposed by the development of new genomic technologies such as next-generation sequencing and bioinformatics (Tawfik et al. 2023; Deo and Deshmukh 2019; Sreevatshan et al. 2022).

Traditional culture-dependent methods, such as isolating and sequencing individual bacteria from lab-based cultures, can be used to characterize microbes, identify a limited range of microorganisms and leave many bacteria uncharacterized (Deo and Deshmukh 2019), (Sreevatshan et al. 2022), (Yang et al. 2021). Although culture-based approaches provide a low-cost gold standard for the isolation and phenotypic characterization of many microorganisms, the number of fastidious or uncultivable 10 times outnumbers that of cultivable microbes. This fact prompted greater efforts to improve culture methods that enable surveying and detection of other uncultivable microorganisms (Browne et al. 2016). Interestingly, these microorganisms can be detected using metagenomic sequencing, which enables the extraction of genomic sequences from a mixture of microbial DNA using next-generation sequencing (NGS) in a culture-independent manner (Willis and Gabaldón 2020), (Yang et al. 2021).

NGS is a novel technique for variant/mutation identification and DNA and RNA sequencing that combines the benefits of distinct sequencing chemistries, various sequencing matrices, and bioinformatics technology to enable massive parallel sequencing of various lengths of DNA or RNA sequences or even whole genomes in a relatively short period of time (Qin 2019). Previously, investigating the microbiome was limited to culture-dependent approaches (Sreevatshan et al. 2022). The introduction of next generation sequencing (NGS) technology has opened up new possibilities for large-scale metagenomic studies in varied populations, allowing for definition of microbiome structure and, in some cases, functional roles and implications for health (Willis and Gabaldón 2020). 16S rRNA sequencing to taxonomically characterize microbial communities; entire Genome Shotgun (WGS) metagenomic sequencing of body-site specific entire community DNA (Kho and Lal 2018). Six major phyla were identified by 16S rRNA profiling of the oral cavity in healthy individuals, namely *Firmicutes*, *Actinobacteria*, *Proteobacteria*, *Fusobacteria*, *Bacteroidetes*, and *Spirochaetes*, which accounted for 96% of the total oral bacteria (Deo and Deshmukh 2019)

Metagenomic analysis and sequencing tools for characterization of community compositions:

In Greek, meta means "transcendent" (combination of separate analysis) and the study of the genome is known as genomics. The field of metagenomics– which has the potential to reveal the secrets of the microbial world- employs a range of genomic technologies and bioinformatics techniques to obtain direct access to the genetic structure of whole organism populations (Thomas, Gilbert, and Meyer 2012). Throughout the last five to ten years, the field of metagenomics has significantly advanced our understanding of microbial ecology, evolution, and diversity. Currently, several research labs are actively working in this subject (Thomas, Gilbert, and Meyer 2012). The conventional procedure involved gathering the sample, plating it, and then sequencing the growth. The primary constraint was that the sequencing process could only be used to growing samples. With the use of current technology, we may potentially extract nucleic acids straight from a sample, avoiding the need for culture and giving us access to 100% of the sample's genetic information (Escobar-Zepeda, Vera-Ponce de León, and Sanchez-Flores 2015). Metagenomic analysis can be performed on amplified 16S rRNA PCR fragments or on all DNA isolated from an environmental sample (shotgun MGA) (Sharma, Tripathi, and Chandra 2021). Metagenomic can be

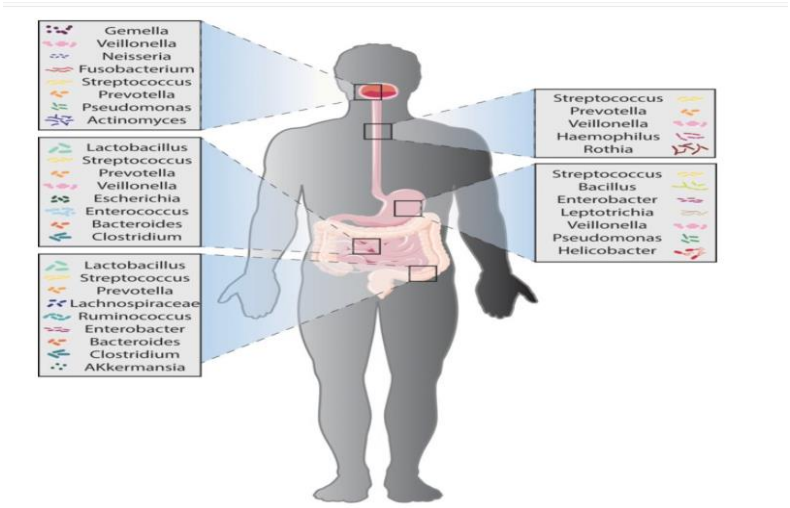

Figure 1. The composition of the human microbiome differs depending on location in the GI tract. The main bacterial genera found in the stomach, esophagus, colon, small intestine, and mouth are identified in this figure. Copyright © 2020 the Digestive Diseases and Sciences (Ruan et al. 2020).

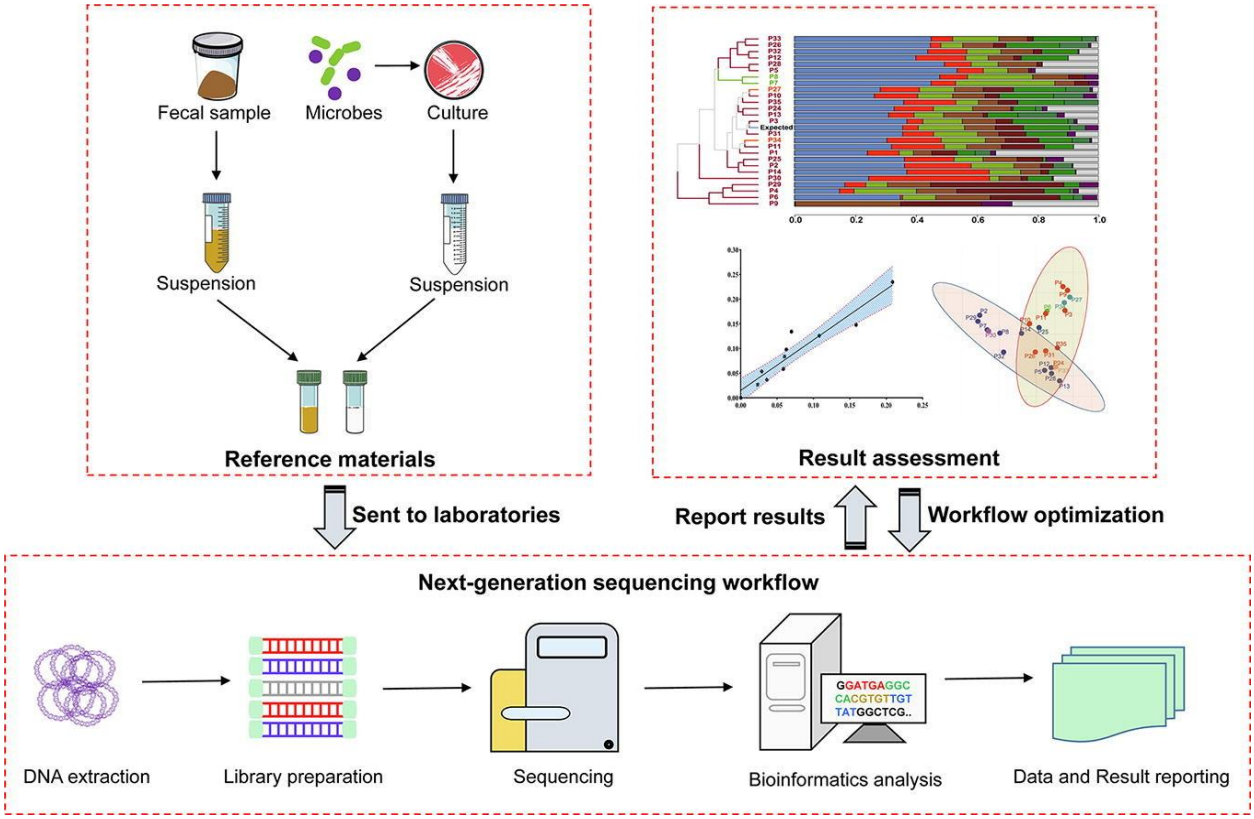

Figure 2. Schematic diagram explains experimental design for metagenomic analysis. Copyright © 2020 the Journal of Advanced Research (Han et al. 2020).

used for discovery of new genes and these genes may be used to create bioengineered probiotics and perhaps find new biotherapeutics for application in human medicine. Functional metagenomics is the second major aspect of metagenomic analysis (Culligan et al. 2014). Metagenomic sequencing, as opposed to amplicon sequencing, yields potentially functional information in addition to extending taxonomic resolution to the species- or strain-level (Liu et al. 2021).

Sanger Sequencing

Sanger sequencing is a technique used to determine the nucleotide sequence of DNA. It is often referred to as the "chain termination method." 2′,3′-dideoxynucleotides are used in the Sanger sequencing method to synthesize DNA. DNA cannot be further synthesized when the 2′,3′-dideoxynucleotides lack a 3′-hydroxyl group because no phosphodiester link can be formed with the subsequent dNTP, causing the chain to end. The four DDNTPs (dideoxynucleotide phosphates such as ddATP, ddTTP, ddCTP, and ddGTP) are tagged with different fluorochrome dyes to facilitate laser beam detection. Each fluorescent-labeled terminated segment of DNA is recorded, and the DNA sequence is determined based on this information (Sanger, Nicklen, and Coulson 1977).

Pyrosequencing

The pyrosequencing procedure involves the sequential addition of all four deoxynucleotide triphosphates, which are integrated by a DNA polymerase if they are complementary to the template strand. This polymerization event produces pyrophosphate, which is then turned into light via two enzymatic reactions. Light generation may be observed in parallel using a charge-coupled device camera and is then applied to the template's real sequence (Fu, Sun, and Li 2010).

ILLUMINA/SOLEXA Technology

Illumina (www.illumina.com) released the Illumina genome analyzer in 2006. Eight DNA libraries are hybridized to an eight-lane flow cell. The flow cell surface, which was intended to display the DNA in a way that facilitates enzyme access, ensures high stability of the surface-bound template, and has low non-specific binding of fluorescently labelled nucleotides, is where single-stranded library molecules hybridize to complementary oligos covalently bound in each lane. One of the main distinctions between Illumina sequencing and earlier techniques was the use of bridge amplification, which allowed for the creation of millions of clonal sequencing clusters connected to a sequencing flow-cell (Goodwin, McPherson, and McCombie 2016). The HiSeq platform has become the standard technique for shotgun metagenomic sequencing because of its enhanced read depth. But because the MiSeq generates longer sequence reads and its speed and affordability fit the objectives of academics, it is most effective in 16S rRNA gene sequencing investigations (Caporaso et al. 2012).

Ion Torrent Next-Generation Sequencing Technology

Ion Torrent is a novel technique that detects protons emitted during DNA polymerization when nucleotides are inserted during synthesis. DNA fragments with specified adaptor sequences are joined to and then clonally amplified on the surface of 3-micron diameter beads known as Ion Sphere Particles using emulsion PCR. The templated beads are placed into proton-sensing wells created on a silicon wafer, and the adapter sequence is primed from a specified point (Quail et al. 2008).

Pacific Biosciences: Realtime Single Molecule Sequencing

The PacBio RS sequencing platform, which is considered a "third-generation" technology, generates data with read lengths that are significantly longer than those of "second-generation" technologies (Chin et al. 2010). PacBio RS long-read sequencing device promises enhanced read length and unbiased genome coverage, with the ability to generate completed grade genome sequence data with fewer gaps and longer contigs. These benefits, however, are accompanied by a noticeably higher mistake rate and a relatively higher cost per nucleotide (Ferrarini et al. 2013). Pacific Biosciences introduced the PacBio RS sequencing technology, which allows for real-time sequencing of single polymerase molecules (SMRT) which doesn't require previous amplification of the DNA template since the polymerases synthesize DNA from a template utilizing four fluorescently-labelled nucleotides (Eid et al. 2009).

Bioinformatics tools for analysis of metagenomic data

The bioinformatics tools used for analysis have evolved and the output of a Sanger sequencing run allowed to BLAST, all reads against a database such as NCBI. Whereas the same procedure is impractical with hundreds of millions of short reads delivered by one run on an Illumina next generation sequencing machine. There are bigger databases containing known sequences for organisms that have been cultivated and isolated from the environment, such as

Green Genes (DeSantis et al. 2006), SILVA (Pruesse et al. 2007), and the Ribosomal Database Project (Cole et al. 2014).
Software packages such as the widely used Quantitative Insights into Microbial Ecology (QIIME) (Caporaso et al. 2010), muther (Schloss and Handelsman 2006), and MG-RAST (Aziz et al. 2008) can analyze millions of 16S rRNA gene sequences from microbial communities. Using microbial community analysis software called QIIME, nucleic acid sequence data from bacterial, viral, fungal, and archaeal communities have been analyzed and interpreted. Raw sequences are sent into QIIME algorithms, which perform quality filtering based on the properties of each sequence, deleting any low quality or confusing readings, selecting Operational Taxonomic Units (OTUs) based on sequence similarity within the reads, and selecting a representative sequence from each OTU (Kuczynski et al. 2012).

Conclusion:

We study the orointestinal axis microbiomes of obese-smokers, smokers, non-smokers and non-obese participants in addition to the correlation between their oral and gut microbiomes. Both obesity and smoking greatly affect and be affected by their oral and gut microbiomes, which are supported by significant differences in the relative abundance of many taxa. Obesity and smoking are associated with structural and compositional shifts along the orointestinal axis microbiomes and potentially contributed to definite perturbations in host associated immunopathological factors or and normally balanced microbial ecology.

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