

Role of Human gut microbiota in Inflammatory bowel disorders, Diabetes mellitus and Obesity- A Review

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Abstract:

With recent advances in gene sequencing and gnotobiotic model, interest in the field of gut microbiota has taken a leap. It is found that a dysbiotic state of gut microbiome is playing a pivotal role in pathological conditions and metabolic disorders such as Inflammatory bowel disorder, Diabetes mellitus and Obesity. Faecal microbiota transplant is found useful for treatment of recurrent Clostridium difficile infections. Human gut microbiome is a vital organ by itself and not many studies have able to show complex functional properties. This review aims to provide an insight on how gut microbiome dysbiosis has been linked to conditions like inflammatory disease, diabetes mellitus and obesity and various recent methods for characterization of gut microbiota.

Key words: Human gut microbiome, dysbiotic, inflammatory bowel disorder, Clostridium difficile

Introduction

Human gut contains many organisms like bacteria, fungi and viruses which accounts for 10^{14} microbes¹. Recent advances in genetic sequencing and culturing techniques has led to important findings between associations of composition of human gut microbiota with disorders of gastrointestinal, respiratory, psychiatry, metabolic, hepatic, autoimmune and oncologic spectra². New-born's intestinal tract is sterile, later gets colonized by maternal and environmental microbes³. Mode of delivery like assisted and vaginal delivery, feed like breast milk and formula feed, antibiotic intake and level of sanitation have known to influence the gut microbiota^{4,5}. Gut microbiome is required to maintain integrity of epithelium of the gut by regulating permeability of tight junction⁶. In case of loss of integrity of gut epithelium, there is shift of bacterial toxins, gut microbiome, proteins and partially digested fats to enter blood stream, leading to inflammatory response and symptoms like excessive gas, abdominal bloating cramps and food sensitivities; leaky gut syndrome exhibits these characteristics. Microbiome in gut helps in the release of short chain fatty acids (SCFA) from indigestible dietary fibers⁷, SCFA is important for modulating immune responses and important source of energy for intestinal mucosa. Butyrate, which is a bioactive SCFA in gut, is found to play a complex role in cancer of colon. In studies it was found that butyrate played a role in promoting tumorigenesis in transgenic mice along with mismatch repair gene and tumor suppressor gene. It was founded that tumor formation reduced by low carbohydrate diet or antibiotic treatment, both these decreased butyrate levels and increased by giving butyrate to antibiotic-treated mice⁷.

In mice deficient in Grp109a, a receptor for butyrate had increased tumor-genesis induced by APC mutation or induced by inflammatory stimuli and signaling through Grp109a, and inhibited tumorigenesis⁸. Environmental factors like dietary intake, treatment with drugs, intestinal motility, consistency and frequency of stools can influence the gut microbiota composition⁹. Gut microbiota and diseases. There is increasing evidence suggesting that gut microbiome dysbiosis has linked to number of diseases, like obesity, inflammatory bowel disease, allergy, cardiovascular diseases, and diabetes mellitus. Alteration in gut microbiota has lead to mood and behavior^{9, 10, 11}. Inflammatory bowel disorders like Crohn's disease (CD) and ulcerative colitis (UC) have found to be associated with gut microbiota dysbiosis. Studies have shown that bacteria like *Raecalibacterium prausnitzii* and *Roseburia hominis* are lowered in UC patients compared to controls¹². In one study, it was found out that there was five dysbiotic bacterial species in CD patients than their relatives¹³. Faecal microbiota transplantation was done in recent clinical trials in patients with UC and was found succesful¹⁴.

Clostridium difficile infection

Clostridium difficile is a Gram-positive bacteria belonging to *Firmicutes* which are normal constituents of gut microbiota. *Clostridium difficile* infection (CDI) develops due to alteration in gut microbiota, recurrent infection is treated by faecal microbiota transplant¹⁵. After faecal microbiota transplant, the recipient microbiota showed increase in bacteria like *Bacteroides* and *Firmicutes*, decrease in *Proteobacteria*¹⁶. Recent studies have postulated that overgrowth of *clostridium difficile* is prevented by dominant gut microbiome by bio-conversion of primary bile to secondary bile acids. Secondary bile acid inhibits vegetative growth *C. difficile* while primary bile acid serves as a germinant for spore of the bacilli¹⁷.

Gut microbiota and Type 2 Diabetes milletus

Recent studies have showed that type 2 diabetes in humans had increase in abundance of Lactobacillus species and lowered abundance of butyrate producing bacteria and significantly fewer *Clostridia* and *Firmicutes* as compared to non diabetic persons^{18,19}. A strong correlation exits between blood glucose levels and ratios of *Frimicutes* and *Bacteroides* and of *Prevotella* - *Bacteroides* group to *Clostridium coccoides*-*Eubacterium* rectal groups. *Betaproteobacteria* was found more abundant in the patients of type 2 diabetes compared to the control group.

Gut microbiota and obesity

Studies have shown that *Frimicutes* and *Bacteroides* ratio changed with the change in body mass index (BMI) and between genders. When BMI less than 33; males had higher F/B ratio, in contrast when BMI was greater than 33 men had lower F/B ratio than women. At genera level groups with BMI>33: higher *Bacteroides* genus in females, but decrease in male²⁰. Differences in gut microbiome of infants in the age 6 and 12 months have found to be associated with the higher risk of being obese or overweight at 7 years of age²¹. Normal weight children had lower *Staphylococcus aureus* and higher *Bifidobacterial* concentrations at ages of 6 and 12 months than the children who became obese/overweight. These results showed that there was difference in the microbiota exists preceding the obese/overweight.

Sample collection, processing and storage of samples

Specimen collection, processing and storage of faeces for study of gut microbiota are a challenge as composition of gut microbiota undergoes marked change over the time. Hence there is no consensus regarding sample collection, storage and processing or protocol for sequencing. Gorzelak et al had proposed faeces freezing within fifteen minutes of defecation and storage in frost-free for 3 days. He also suggested homogenisation of faeces to reduce the variability of microbiota across the sample microenvironment²². Thomas et al. suggested immediate freezing of the specimen at -80 °C, storage at 4 °C followed by freezing at -80 °C or addition of stabilizing agents²³.

16s RNA and gut microbiota

Earlier, culture methods were used for identification of bacteria which often failed to identify bacteria that grow in common media²⁴. The molecular method like 16s r RNA sequencing is simple and effective alternative compared to microbial culture. 16S r RNA is a gene which codes ribosomal subunit that contains hyper-variable regions interspersed in conserved regions of the sequence²⁵. These regions which are hyper-variable are unique to each species of bacteria which allows for easy taxonomical classification. The conserved region allows developing primers that is universal and binds to known sequence among the bacteria. There are nine hyper-variable regions, out of which some characterize the bacteria better hence selection of primers is an important step in designing the study²⁶.

Metagenomics and gut microbiota

Metagenomics is a genome sequencing which detects all DNA from the specimen followed by either assembling of the sequencing reads or mapping to a reference database then followed by annotations of genes. There two types targeted and shot gut metagenomics, targeted metagenomics amplifies a selected sequence before sequencing and allows taxonomic description of all bacterial components of the analyzed specimen.

Shot gun metagenomics relies on sequencing of all DNA present in the specimen without prior knowledge of its contents and allows a complete description of the sample including bacteria, parasites and virus²⁷. Functional metagenomics can detect novel functional genes, antibiotic resistant genes, microbial pathways, functional dysbiosis of gut microbiota and establish interaction as well as co-evolution between host and gut microbiomes²⁸. Though metagenomics allows extensive and rapid exploration of human gut microbiome, it has its limitations like DNA extraction bias. Study by Angelakis et al, showed that bacterial taxonomic distribution differed due to different extraction protocols²⁹ and cannot differentiate between active or quiescent bacteria.

Metaproteomics and gut microbiota

In recent years, metaproteomics has been used to analyze human gut microbiome. It is a large -scale profiling of a whole protein complement which is expressed by microbial ecosystems³⁰. It reveals functional traits, which is relevant for the underlying physiological states, and hence providing in detail insights of the connections between microbial functions, diversity and the impact on host biology. Recently Young et al. prepared a detailed faecal metaproteome profile of preterm infant, hence shedding more light on the host-microbiota interactions and functional clues during early developmental stage³¹. Metaproteomics will be able to go into the depth of microbial functionality, which will help to understand in detail about the underlying patho-physiology and give guidelines for targeted approach in improvement of health and disease.

Culturomics and gut microbiota

Culturomics was developed due to limitations in metagenomics; it can detect maximum number of bacteria per specimen. In this method serial dilutions of specimen are placed on the agar with different culture conditions and then each colony is isolated. First the colony is identified by Matrix Assisted Laser Desorption Ionisation - Time of Flight (MALDI-TOF) mass spectrometry and later if identification is not possible by this method, 16s RNA amplification and sequencing is used for taxonomic characterisation²⁷. Bacteria which couldn't be detected by metagenomics were detected by culturomic and vice versa³².

Conclusion

Human gut microbiota evolves throughout the life and appears to play a pivotal role in health and disease. There is need for exploration in identification and characterisation of gut microbiota, its patho-physiological role in health and disease by utilizing various recent genomic analyses. More upcoming research can focus on personalised treatment in accordance to patient's microbiome and ascertain safety and usefulness of probiotic interventions³³.

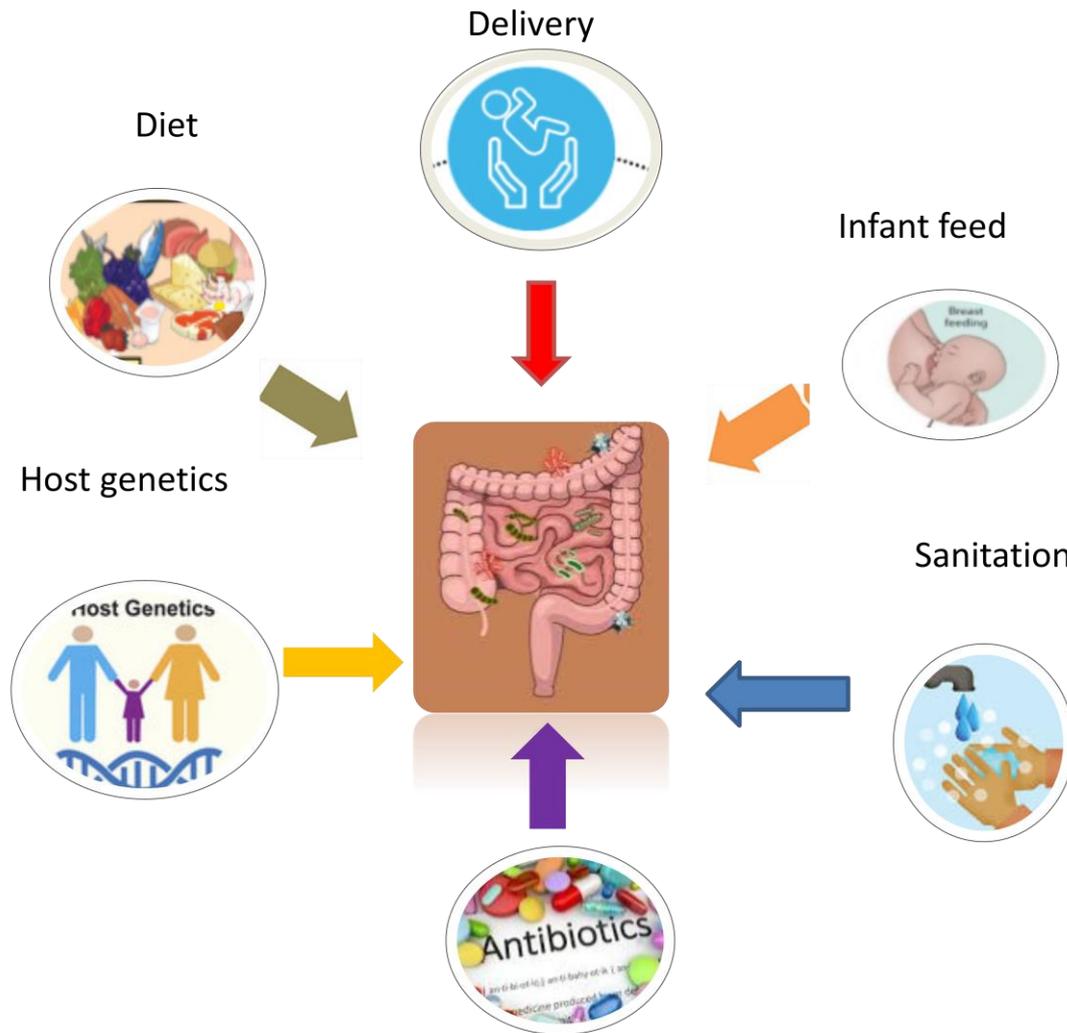


Figure 1: Figure showing multiple factors affecting Human gut microbiota

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