CHAPTER

33

Clinical Benefits of Manipulating the Gut Flora

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Introduction

The gastrointestinal (GI) tract represents a complex ecosystem in which a balance exists between the intestinal microflora and the host. The microflora comprises predominantly of facultative anaerobes e.g. lactobacillus and obligate anaerobes e.g. bifidobacterium. Aerobic organisms are not present in the GI tract of healthy individuals except Pseudomonas in very small numbers. Most of the gut micro-organisms reside in the colon in a concentration of 10^{11}- 10^{12} colony forming units per ml. The gut microflora is important for maintaining human health. It helps in the development of normal intestinal morphology, maturation of immune system, re-enforcing the barrier function of intestinal mucosa, (thus preventing the attachment of pathogenic micro-organisms and entry of allergens) and for maintaining a chronic and immunologically balanced inflammatory response. In addition, the microbes also contribute to body's requirement for certain vitamins such as biotin, pantothenic acid and vitamin B_{12}.

The dietary use of live micro-organisms has a long history. Mention of cultured dairy products is found in sacred books of Hinduism and the Bible. Soured milk and cultured dairy products such as kefir, kourniss and dahi were often used therapeutically even before the existence of micro-organism was recognized. The use of micro-organisms in food fermentation is one of the oldest methods for producing and preserving food. Much of the world depends on various fermented food that are staples in diet.

Elie Metchnikoff, the father of modern immunology spoke highly about the possible health benefits of lactic acid bacteria (LAB). In his book 'Prolongation of life' he wrote that the consumption of live bacteria such as Lactobacillus Bulgaricus and Streptococcus thermophillus in the form of yogurt was beneficial for GI health as well as health in general and longevity. Recent work has shown that certain live micro-organisms have immuno-modulatory and anti-carcinogenic effects. Modern medicine has only recently started recognizing the tremendous potential of gut microbes not only for health promotion but also for treatment of diseases.

Probiotics & Prebiotics

Microbial cultures have extensively undergone scientific scrutiny for their ability to prevent and cure a variety of diseases. This has led to coining of the term pro-biotics or "pro-life". **Probiotics** are live non-pathogenic micro-organisms which when administered in adequate amounts confer a health benefit to the host. In clinical practice, LABS are the most commonly used microbes. Their role has

been gradually evolving in management of diarrheal diseases including those due to antibiotic use and Cl. difficile, irritable bowel syndrome, inflammatory bowel disease, prevention of bacterial translocation (stimulation of mucosal barrier function), modulation of immune function, and prevention of GI cancers. **Prebiotics** are substances predominantly carbohydrates that encourage the growth of such 'health beneficial' or useful bacteria in GI tract (like Bifidobacterium and Lactobacillus). This effect is brought out by physiochemical changes in luminal environment like pH changes due to promotion of lactic acid fermenting bacteria, production of short chain fatty acids especially butyrate, and by preventing attachments of enteropathogens by acting as receptor homologous. Inulin, fructo-oligosaccharide, oligofructose, difructose anhydride, galacto-oligosaccharides, soyabean oligosaccharide, raffinose, beta-glucan hydrolysate, amylase resistant starch, pectin, fibregum TAN, sugar alcohols, polydextrose, isomalto-oligosaccharides and psyllium are some of the agents. When probiotics and prebiotics are used judiciously together they are called **symbiotic**.

Almost all probiotics are lactic acid fermenters and most belong to genera Bifidobacterium and Lactobaccilus. Some of the commonly useful probiotics are Bifidobacterium bifidum, Bifidobacterium infantis, Bifidobacterium breve, Bifidobacterium longum, Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus salivarius, Lactobacillus rhamnosus GG, Lactobacillus bulgaricus, E. coli bacteria (nissle 1917 strain), Lactococcus lactis, Enterococcus faecium and Saccharomyces Boulardi. Preparations of probiotics may be monostrain (one species), multistrain (more than one strain of same species or at least genera) and multispecies (different strains from one or more genera). Multistrain and multispecies probiotics have been shown to be superior to the monostrain probiotics[1].VSL#3 is a high-concentration probiotic preparation of eight live freeze-dried bacterial species that are normal components of the human gastrointestinal microflora, including four strains of lactobacilli, three from bifidobacterium and one streptococcus salivarius. Synbiotic 2000 is a mixture of 4 probiotics and 4 prebiotics.

In clinical practice, modification of gut flora with beneficial microbes can be achieved with either administration of adequate quantity/quality of probiotics or prebiotics (which encourage the growth of useful bacteria) or a combination of the two.

Clinical Benefits of Probiotics / Prebiotics

Acute infectious diarrhea including Rotavirus diarrhea.

Diarrhoea is the most common GI disorder worldwide. A large number of studies assessing the role of probiotics in infectious diarrhea have been done. Different studies have used different organisms and different doses, thus making it difficult for analysis. Overall, the results demonstrate that probiotics cause improvement in diarrhea either by shortening its course or decreasing the severity. In a recent meta-analysis both Lactobacillus and Saccharomyces boulardi were shown to be effective in decreasing the duration of diarrhea in children including those due to rotavirus.[2,3] Therefore they could form part of strategy to counter this ailment.

However, one cannot but notice the number of studies which show that all probiotics are not universally useful in a given locality. In a randomized clinical trial involving children aged 3-36 months, Lactobacillus Rhamnosus GG, or a probiotic mix and not Saccharomyces boulardi or Enterococcus faecium were shown to be effective[4].

Based on the current evidence, it is difficult to make a standard recommendation regarding the use of probiotics in infectious diarrhea. The consensus opinion is that probiotics are an useful adjunct to rehydration therapy in treating acute infectious diarrhea in adults and children.

Prevention of antibiotic associated diarrhea

Since antibiotics especially the broad spectrum ones cause changes in bacterial flora, the use of probiotics to counter diarrhea seems rational. There is significant favorable evidence in this regard. A recent meta-analysis suggested strong benefits of using probiotics (Lactobacillus, Saccharomyces).[5]

Prevention and treatment of Cl. Difficile infection

It was in 1970's that it was firmly established that C. difficile was the most common cause of diarrhea post antibiotic use. C. difficile causes diarrhea as a result of its toxins or even a severe enterocolitis.

There has been an increasing interest in the use of probiotics to treat C difficle associated diarrhea (CDAD) and especially in the treatment of severe and recurrent cases. There are interesting reports of treatment of CDAD with donor stools delivered via nasogastric tube or the colonoscope. This strategy is based on the presumption that repopulation of the colon with normal non-pathogenic flora may inhibit the proliferation of C. difficle.

Alteration of the gut flora with specific probiotics has been extensively investigated. In general, most case series and case reports have shown favorable results with Lactobacillus rhamnosus GG or Saccharomyces boulardii. A meta-analysis of six blinded randomized controlled trials, with a total of 354 patients, to assess the efficacy of probiotics in the treatment of CDAD[6] showed a beneficial effect with strains including S. boulardi, LGG, L plantarum and a mixture of Bifidobacterium bifidus and L acidophilus. Of the probiotic strains, only S boulardii and Lactobacillus rhamnosus GG led to a significant reduction in recurrence[7].

The consensus opinion felt that both S boulardii and LGG could be given a high recommendation in the treatment of recurrent CDAD infection. However the standard practices of using oral metronidazole or vancomycin stays as standard of care.

A positive result has been reported in the only published controlled trial assessing the efficacy of prebiotics for CDAD recurrence. Patients receiving the prebiotic oligofructose showed significantly lower incidence of recurrent diarrhea when compared to placebo. An increase in fecal Bifidobacterium in patients receiving oligofructose confirmed its prebiotic effect. Whether combining pre and probiotics in this clinical situation may provide further benefit is not currently known.

Pouchitis

Proctocolectomy with formation of an ileal reservoir (pouch) followed by anastamosis to anus is often performed for patients with ulcerative colitis(UC) or familial polyposis syndrome. Inflammation of the pouch or pouchitis is a common complication. The cumulative risk of developing pouchitis in patients who had UC may exceed 50% at 4 years following surgery. The standard therapy for pouchitis includes antibiotic therapy such as metronidazole or ciprofloxacilin.

It is now believed that bacterial stasis (leading to fecal microbial imbalance) is an important etiological factor in the development of pouchitis. Hence, altering the pouch bacteria with probiotics may play an important role in restoring the balance and preventing pouchitis. Studies have demonstrated the efficacy of probiotic mixture VSL#3 in the primary prevention or maintenance of remission of pouchitis. There is now Level I evidence to support the use of probiotics in pouchitis.[8,9] Treatment guidelines from the US and the UK include VSL#3 as a therapeutic option for the prevention of pouchitis relapse in patients with chronic pouchitis.[8]

Inflammatory bowel disease

Multiple studies of the immune system in patients with inflammatory bowel disease have demonstrated the presence of altered intestinal mucosal immunity. In humans, inflammation is present in gut containing highest bacterial concentration or areas of stasis (ileum, caecum, rectum). Disturbance of intestinal microflora (dysbiosis) may also be an element in the

environmental component causing inflammatory bowel disease. In Crohn's disease concentration of Bacteriodes, Eubacteria and Peptostreptococcus are increased whereas Bifidobacteria numbers are decreased. In ulcerative colitis facultative anaerobic bacteria are increased.[10]

The basic strategy in the treatment of IBD is aimed at suppressing inflammatory response. Modification of intestinal flora through probiotics provides the possibility of acting microbiologically as well as immunologically. Possible mechanisms of action of probiotics in IBD are.[11, 12]

a. Decreasing the secretion of pro-inflammatory cytokines IFN- Gamma, TNF Alfa, IL-12.
b. Induction of secretion of anti-inflammatory cytokine IL-10.
c. NF- Kappa B inhibition, heat shock protein induction and proteasome inhibition.
d. Induction of T cell apoptosis.
e. Interference with bacterial adhesion to epithelium.
f. Inhibition of microbial pathogen growth.
g. Increase intestinal tight junction and permeability.
h. Decomposition of luminal pathogenic antigens.

Several studies have explored the use of probiotic organisms in the treatment of Crohns disease with varied results. Subsequently, studies have focused on the role of probiotics in maintenance of remission of Crohns disease. Guslandi et al[13] demonstrated a significantly lower risk of clinical relapse in patients taking S. boulardi alongwith mesalamine as compared to those taking mesalamine alone. Other studies, however, using other strains or mixtures of strains failed to demonstrate benefit. In summary, there are varied results using varied probiotic strains in the maintenance of medically or surgically induced remission of Crohns disease. Till date, there is insufficient evidence to support or discourage the use of probiotic therapy in patients with Crohns disease except with S. boulardi results.

There are two reports on the use of prebiotics in patients with active UC showing improvement in clinical activity. Five studies have assessed the role of probiotic in active ulcerative colitis. These studies with S. Boulardii, VSL # 3 and bifidobacteria have shown encouraging results. Other studies investigating the role of probiotics in maintenance of remission of UC[14] have shown positive results in varying degrees but seem to be more promising than the experience with Crohns disease.

Overall the clinical evidence in support for use of probiotics in inflammatory bowel disease is at the most Level II and cannot yet be a standard recommendation[15]. Although studies are clearly promising, placebo-controlled, randomized, double-blind clinical trials are required to clarify the role of probiotic bacteria in the treatment of inflammatory bowel disease. The choice of probiotic bacteria, the optimal dose, mode of administration, and duration of therapy still need to be established.[12, 13]

Irritable bowel syndrome

Several controlled trials have explored the efficacy of probiotics in improving symptoms due to IBS. The most promising result comes from studies with Bifidobacterium infantis which resulted in improvement in most IBS symptoms[16]. Other trials with L. plantarum or mixture of strains also showed improvement in IBS symptoms. However, controlled trials with VSL#3 and LGG did not demonstrate any significant benefit.

Overall, the available evidence for use of probiotics in IBS is encouraging but not adequate enough to make a clear recommendation. It needs to be remembered that IBS is a complex of symptoms and further research must be directed towards specific sub-types and using different strains or mixture of strains.

Entero-colitis in premature infants

A breast-fed, full-term infant has a preferred intestine microbiota in which bifidobacteria predominate over

potentially harmful bacteria, whereas in formula-fed infants, coliforms, enterococci, and bacteroides predominate. The pattern of bacterial colonization in the premature neonatal gut is different from that in the healthy, full-term infant gut. Those infants requiring intensive care acquire intestinal organisms slowly, and the establishment of bifidobacterial flora is retarded. A delayed bacterial colonization of the gut with a limited number of bacterial species tends to be virulent. Bacterial overgrowth is one of the major factors that promote bacterial translocation. The aberrant colonization of the premature infant may contribute to the development of necrotizing enterocolitis. Breast-feeding protects infants against infection. Oligo-saccharides and glycoconjugates, natural components in human milk, may prevent intestinal attachment of enteropathogens by acting as receptor homologs. Probiotics and prebiotics modulate the composition of the human intestinal microflora to the benefit of the host. These beneficial effects may result in the suppression of harmful microorganisms, the stimulation of bifidobacterial growth, or both. In future, control and manipulation of the bacterial colonization in the neonatal gut may be a new approach to the prevention and treatment of intestinal infectious diseases of various etiologies.

Lactose intolerance

Several lines of evidence show that appropriate strains of LAB such as L thermophilus, L bulgaricus and other lactobcilli in fermented milk products can alleviate symptoms of lactose intolerance by providing bacterial lactase to the intestine. Consumption of these products may be a good way to incorporate dairy products and their accompanying nutrients into the diets of lactose intolerant individuals.

Prevention of bacterial translocation

There is considerable data in humans showing that patients who cannot take nutrients enterally have more organ failure in the ICU, poorer prognosis and a higher frequency of septicemia (in particular from bacterial species in the intestinal tract). The gut origin of sepsis is not new concept. Bacterial translocation is passage of viable resident bacteria from GI tract into the portal circulation in absence of an infective condition. The GI tract teeming with its own bacterial flora could represent a source of sepsis under certain conditions. Bacteria cross the intestinal barrier due to disruption of balance of intestinal flora leading to modification of intestinal barrier function and can cause sepsis at different sites. Bacterial species such as enteric gram –ve and gram +ve cocci are more prone to translocation whereas Lactobacilli seem to have a protective effect.

Three randomized studies using L. plantarus in patients with pancreatitis, undergoing liver transplantation or UGI surgery have been published. They suggest a beneficial role of probiotics in preventing translocation, reducing secondary infections and septic morbidity. Thus modification of intestinal bacterial flora with probiotics may be used to reduce septic morbidity in critically ill patients such as:

a. Multi-organ failure situation
b. Poor risk surgical patients
c. Acute severe pancreatitis
d. Liver failure and liver transplant
e. Intensive care patients

Diverticular Disease

Small, preliminary studies suggest an encouraging result of probiotics (E. coli Nissle and Lactobacillus Casei) for prevention of relapse of symptomatic diverticular disease. However, large double blind randomized controlled trials will be required to draw any conclusions about the utility of probiotics in diverticular disease.

H. pylori infection

Preclinical studies have shown that Lactobaccilus acidophilus has inhibitory effect on H. pylori either due to production of lactic acid or an autolysin.[17] Larger well controlled studies are needed to document the effect on eradication with use of probiotics.

Urogenital health

Probiotics may also be of use in maintaining urogenital health. Like the GI tract, the vagina is a finely balanced ecosystem. The dominant Lactobacilli normally make it too acidic for harmful organisms to survive. The system, however, can be thrown out of balance due to a number of factors e.g. antibiotics, spermicides and birth control pills. Probiotic treatment restores the microflora balance and may be helpful for some common female urogenital problems such as bacterial vaginosis, yeast infection and urinary tract infection.

Allergy

Probiotics may exert a beneficial effect on allergic reactions by improving mucosal barrier function. Probiotic such as Lactobacillus GG may be helpful in alleviating some of the symptoms of food allergy such as associated with milk protein. Probiotic consumption may thus be a means for primary prevention of allergy in susceptible individuals.

Bone mineral metabolism

There is evidence for an independent probiotic effect on facilitating mineral absorption. The underlying mechanisms are many fold: increased solubility of minerals because of increased bacterial production of short-chain fatty acids; an enlargement of the absorption surface by promoting proliferation of enterocytes; increased expression of calcium-binding proteins; improvement of gut health; degradation of mineral complexing phytic acid; release of bone-modulating factors such as phytoestrogens from foods; stabilization of the intestinal flora and ecology; stabilization of the intestinal mucus; and impact of modulating growth factors such as polyamines. Thus, probiotics may have a role in promoting bone health.

Probiotic Treatment Schedule

Limited information is available about appropriate probiotic dosing regimens. Few dose comparison studies have been undertaken. Fecal recovery of LGG after administration to adults in various doses has been studied using culture based methods. A daily dose of $> 10^{10}$ CFU was needed to ensure a reliable fecal recovery of LGG. However, fecal recovery is not an optimal outcome measure because fecal detection may not reflect clinical outcomes. Commercially available probiotic formulations generally contain $\geq 10^{6}$ CFU/g of viable organisms, but the dose of specific probiotics required for specific clinical effects are not well established.

Ambiguity Regarding Probiotics

Current probiotics have a good safety record. However, probiotic induced fungemia and bacteremia have been reported in literature and such instances have involved both immunocompetent and immunocompromised hosts.[18,19,20 & 21] When should probiotics not be used is still an unanswered question. Hence caution is required especially in neonates born prematurely or those with immunodeficiency and in immunocompromised adults. Newly developed probiotic strains should be thoroughly evaluated for safety before being marketed. Different probiotic strains can have very specific effects and their effects may vary in health and disease, in different disease states and in different age groups. Thus, clinical trial results from one probiotic strain in one population cannot be automatically generalized to other strains or to different populations. Lastly, in view of increasing use of probiotics as health supplements and therapeutic agents, clinicians need to be aware of the risks and benefits of these treatments.

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