The Microbiome-Gut-Brain Axis and Probiotics Supplementation: Implications for Dementia and Alzheimer's Disease

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Introduction

The gastrointestinal, or gut, microbiome is associated with many facets of health, including cognitive functioning. However, the link between the enteric microbiota and the central and enteric nervous systems has yet to be fully defined. Recent research has delved into describing the importance of the gut microbiota in influencing the interactions between the two, and how dysregulation of the bacteria within the intestines can lead to or affect many different health conditions. One of these conditions being the onset of dementia and Alzheimer's disease. According to the World Health Organization, dementia affects 50 million people worldwide, leading to issues with mental abilities like memory, comprehension, judgement, and orientation, and this number is only growing. While dementia is the common name for a set of declining cognitive systems, it plays a role in many diseases and conditions, the most prevalent being Alzheimer's Disease (AD), as this disease makes up 60-70% of reported dementia cases[1]. Although, there is currently no cure for AD and little is known about its disease progression, strides are being made in research surrounding understanding and possibly even delaying its onset and advancement. Some of this research is aimed at examining the differences in gut microbiota in patients with dementia and AD, along with the possible outcomes of probiotic supplementation in helping shape the dynamic of intestinal bacterial colonization.

To fully understand the research being conducted, a comprehension of the gut microbiota is first needed. The gastrointestinal microbiota is the term for the collection of microorganisms living in the digestive tract. These microorganisms include at least 1000 strains of bacteria, one-third of which is intrinsic in all people, with two-thirds being specific to the individual[2]. The intestines of a newborn are colonized by the mother and consistently evolved by the child's environment until about the age of three, when the microbiotic makeup stabilizes and changes slow, although they continue throughout life. The gut microbiota is responsible for specific physiological functions, including aiding in digestion, production of vitamins B and K, and supporting the immune system[2]. It is one of the three key players in the microbiota-gut-brain axis, by which the specific composition of microbiota and the brain communicate with one another by various routes

such as the enteric nervous system. Many factors can influence the variety of gut microbiota an individual has, such as antibiotic use, stress, and nutrition[3]. As the body ages, microbiota diversity diminishes, which can lead to dysbiosis, defined as an alteration in the microbial community resulting in the decreased diversity and number of bacteria present[2]. It is thought that this dysregulation of bacterial population can directly affect the presence of cognitive issues, namely dementia and Alzheimer's Disease, although the specific microbial communities associated with these conditions have yet to be characterized.

Dementia represents a serious loss of cognitive ability, and in the case of Alzheimer's Disease, is progressive, resulting in a long-term cognitive decline associated with the loss of neurons in the brain. This loss of neurons is characterized by microscopic changes in brain tissue, including the deposit of amyloid plaques, tau proteins, and neurofibrillary tangles[4]. It is not fully understood, though, how or why these changes occur, as examining brain tissue is not easily done in live patients. Due to this break breakdown in neurons and the connections between them, messages cannot be sent between them and to other parts of the body, leading to loss of memory and thinking skills, and eventually the inability to complete basic life tasks. These changes can occur years before the symptoms of AD and dementia appear, which typically appear later in life, making aging the number one risk factor for these conditions[5]. Some researchers are additionally studying the correlation between common risk factors for other diseases, such as high blood pressure and high cholesterol, and the role they play in increasing the risk for AD. Currently treatments for this disease surround supporting the patient in maintaining their cognitive function, managing their behavioral symptoms, and working to slow the associated symptoms[6]. How to go about delaying its onset and slowing progression is still a major question in research, however, and understanding how environmental factors, diet, and the composition of gut microbiota shape a patient's prognosis is the main focus for many.

Current Research - Microbiome Composition

Though current research in connecting the constitution of gut microbiota to dementia and Alzheimer's Disease is limited, a number of studies have begun to emerge in the past few years looking into the fecal microbiome of patients with cognitive decline, as well as the use of probiotics to alter or support the GI bacteria to offset these conditions. One of these studies conducted in Japan from March 2016 to March 2017 examined the differences between gut microbes in demented and non-demented patients using a comprehensive assessment of cognitive function including neuropsychological tests and brain magnetic resonance imaging scans. The 128 study participants, aged 68-82, were recruited from a memory clinic and divided into two groups, demented and non-demented, based on their Mini-Mental State Evaluation (MMSE) and Clinical Dementia Rating (CDR). All participants received brain imaging and donated a fecal sample to be analyzed. The microbes in the samples were then divided into subgroups: *Prevotella, Bacteroides,* Lactobacillales, *Bifidobacterium, Clostridium* cluster IV, *Clostridium* subcluster XIVa, *Clostridium* cluster IX, *Clostridium* cluster XI, *Clostridium* cluster XVIII, and 'others.' Beyond this, these groups were further defined into three enterotypes: enterotype I included *Bacteroides* at >30%, enterotype II included *Prevotella* at >15%, and enterotype III included all remaining bacteria. These groups were chosen based on the predominant bacterial genera commonly present in fecal specimens. The study found that the composition of gut microbiome did differ between demented and non-demented patients. Specifically, patients with dementia showed a higher number of microbes in enterotype III and less of enterotype I than did non-demented patients. Lactobacillales and *Bifidobacterium* were more frequent in the fecal samples of demented patients as well. Multivariable analyses were adjusted for dementia risk factors, showing that these microbial results are strongly and independently associated with dementia, and that these associations could be stronger than the traditional dementia biomarkers. This demonstrates a clear relationship between the gut microbiome and dementia in this population, however further studies need to be completed to fully understand the specific composition and differences between these enterotype groups to clarify this gut-brain connection[7].

In another study conducted to compare the microbiome of those with and without dementia due to Alzheimer's Disease, researchers set out to characterize the microbial communities within these sets of individuals, as well as examine the relationship between gut microbiota and AD pathology through biomarkers of AD found in cerebrospinal fluid. Fecal samples were collected from 25 home-dwelling patients in Wisconsin, with very mild, mild, or moderate dementia, and 25 age- and sex-matched control participants. There was additionally no significant difference between the groups in terms of ethnicity, BMI, diabetes status, or dietary intake. 16S ribosomal RNA gene sequencing was used to classify the microbial communities in both the AD and control groups, finding that at the phylum level, AD participants had decreased abundance of Firmicutes and Actinobacteria, and increased Bacteroidetes compared to the control participants. Once the researchers identified 13 genera that were differentially abundant between the AD and control groups, the relative abundance of each was compared against levels of CSF biomarkers in a subset of participants who underwent a lumbar puncture. The biomarkers reflected the amount of amyloid plaque burden, neurofibrillary tangle pathology, and overall AD pathology. Across all participants, the same trends were found in respect to relative bacterial abundance and CSF biomarkers of AD pathology. For the bacteria genera found more abundantly in AD participants, there was an increase in biomarkers related with greater AD pathology. For the genera less abundant in AD, there was decreased bacterial abundance and CSF AD biomarkers. These results mirror the results found in other studies surrounding AD, showing decreased richness and diversity, or dysbiosis, in microbial composition in those with AD compared with control. In addition, they found that levels of differentially abundant genera are linked with CSF biomarkers of AD pathology. These differences could play a role in immune activation, systemic inflammation, or even pathophysiological changes within the brain through bi-directional communication between the gut and the brain along the microbiome-gut-brain axis. However, further work needs to be completed in order to determine the cause-effect relationship between gut microbiota and the pathogenesis of AD, and discovering a potential interventional approach to restoring healthy gut microbiota composition in order to delay the onset of progression of the disease[8].

Current Research - Probiotic Supplementation

In order to prevent a deterioration of microbial diversity in the gastrointestinal tract and subsequently cognitive decline, researchers have begun to focus on the effect of probiotic supplementation on the status of patients with Alzheimer's Disease. A 2016 study conducted in Iran looked at 60 Alzheimer's Disease patients and the effect of probiotics on their cognitive function and metabolic status. The 60 participants were divided into two groups of 30, one control receiving only milk and one intervention receiving a milk and probiotic mixture daily for 12 weeks. Outcomes were assessed using MMSE measurements, along with biomarkers of oxidative stress, inflammation, and metabolic profiles commonly associated with AD. This study found that through probiotic supplementation, an improvement in MMSE score was seen in the intervention group compared to the control group, with a statistically significant difference between the two outcomes. In addition, the probiotic had favorable outcomes on the biomarkers of plasma malondialdehyde, C-reactive protein, homeostasis model of assessment-estimated insulin resistance, beta cell function, serum triglycerides, and quantitative insulin sensitivity check index in comparison with the control group. The supplementation showed no effect on other markers of oxidative stress and inflammation, fasting plasma glucose, or other lipid profiles. Through these outcomes it can be seen that probiotics may adjust brain activity through their inner workings with established gut microbiota and the bacteria's capability of producing neurotransmitters and neuromodulators in the central nervous system that play a role in the onset and progression of AD. Since the microbiota-gut-brain axis is thought to affect many actions through these nerve pathways, both dysbiosis and probiotic treatment could impact the functions of the CNS and overall cognitive ability. The hopeful outcomes of this experiment warrant further investigation into the beneficial impact on the cognitive ability of AD patients[9].

Another study completed to test the effect of probiotic supplementation on Alzheimer's Disease looked at its relationship with leaky gut, another condition that researchers are still beginning to examine. Leaky gut

syndrome can cause systemic inflammation in the body and promote neuroinflammation, which has shown to be a mechanism in the onset of AD. By studying how probiotics rebalance the gut microbiome and lessen the effects of leaky gut, the researchers were looking for a way to slow the progression in the early course of the disease. In this study, twenty outpatients from Austria with AD had serum and feces specimens analyzed both before and after supplementation with multispecies probiotics. The analysis included concentrations of biomarkers of immune activation neopterin, which has been found in elevated levels in the brains of patients with AD, and the ratio of tryptophan to its metabolite kynurenine, the production of which has shown to be accelerated in those with cognitive decline. In addition, the average concentrations of tyrosine, phenylalanine, vitamin D, and brain derived nerve growth factor (BDNF) were also measured in parallel. The fecal bacterial composition of the study participants was also compared before and after supplementation. The results showed no significant change in cognitive parameters, concentrations of tryptophan, phenylalanine, or tyrosine, but a significant increase in kynurenine was noted. This did not lead to a significant change in the kynurenine to tryptophan ratio, however could indicate the stimulation of the immune system and indicate the presence of AD. There was also a significant increase seen in neopterin and nitrite concentrations, showing an increase in immune activation associated with AD, and no change in BDNF or vitamin D levels was observed. For the bacterial composition of the fecal samples, the content of *Faecalibacterium prausnitzii* significantly increased, but the other bacteria examined had no change. Zonulin, a protein that modulates the permeability of the intestinal barrier, had levels decline during the probiotic supplementation, showing decreased intestinal permeability, or “leaky gut.” All of these results together provide evidence that supplementation with a multispecies probiotic in patients with AD influences the makeup of gut bacteria as well as tryptophan metabolism, the metabolites of which are important in neuropsychiatric status. However, the role of prebiotics and diet as drivers of gut microbiota in modulating dementia and the onset of AD must be further studied. The changes shown here point to the activation of immunologic processes with probiotic supplementation and the lessening of “leaky gut,” thought to play a role in AD. These immune processes could be beneficial or detrimental though, as the stimulation of anergic

immune cells could be helpful in protecting against cell damage leading to AD, but too intensive of activation could negatively impact gut barrier function and lead to further stimulation of neurodegenerative processes[10].

The final study reviewed concerning gut microbiota and probiotic supplementation focused solely on the use of *Lactobacillus plantarum* C-29 fermented soybean (DW2009), an anti-inflammatory probiotic, as a nutritional supplement for alleviating the cognitive impairment in individuals with mild cognitive impairment (MCI). MCI is an intermediate stage between the expected cognitive decline of normal aging and the more serious decline of dementia. By studying the effects of probiotic supplementation on those with MCI, and potentially improving their cognitive status, the researchers thought it may lead to further understanding of how to delay the onset and progression of dementia and Alzheimer's Disease. In this study, one hundred participants with MCI, aged 55-85, were divided into equal treatment groups, one receiving DW2009 and one receiving an identical placebo for a total of 12 weeks. Assessments were completed at 6 weeks and after completion, focusing on the cognitive functions of attention, working memory, and verbal memory. The association between brain-derived neurotrophic factor (BDNF) and change in cognitive function was examined as well. Cognitive function in terms of attention and memory was measured using computerized neurocognitive function tests (CNT), including a verbal learning test, auditory continuous performance test, and digit span test. In addition, fecal samples were collected from 92 of the participants at baseline and follow up in order to isolate gut microbiota DNA and examine changes in composition. The results of the experiment showed that compared to the individuals in the placebo group, those who received the DW2009 showed greater cognitive improvement by the end of the study, with attention showing a significant improvement. The change in serum BDNF levels were positively associated with change in the combined cognitive function in the treatment group, with no significant association found between BDNF levels and cognitive performance in the placebo group. In regards to gut microbiota composition, *Lactobacillus* showed a significantly increased population in the DW2009 group and not in the placebo group, but no changes were

found among *Bifidobacterium* or *Clostridium* populations in either group. Overall, these outcomes suggest that supplementation with the probiotic DW2009 increased levels of serum BDNF expression which may have mediated the cognitive enhancement. BDNF is known to modulate synaptic transmission and neuronal plasticity which protects against neuro-inflammation and neuron death in the brain. As such, higher levels of BDNF are seen here associated with higher levels of cognitive improvement in both attention and memory. The change in gut microbiota composition shows how it can be influenced by probiotic use, along with other external factors. Increasing the amount of beneficial gut bacteria may have a role in alleviating the MCI, and could play a role in the regulation of neurocognitive diseases. These results demonstrate the efficacy of DW2009 probiotic supplementation on improving cognitive function in MCI, which could in turn delay the onset and progression of dementia and Alzheimer's disease, however the mechanisms by which supplementation makes these improvements has yet to be discovered[11].

Discussion

The above described studies show that while the differences in gut microbiota have yet to be clearly defined, there are specific changes that occur in the microbiome of patients with Alzheimer's disease and dementia. More studies are needed with larger populations in order to classify the specific strains or phyla of bacteria associated with AD, as the current research is conflicting. For instance, in the above observational studies, the most prevalent bacteria found within the AD populations were very different. In the Japan study, AD patients had lower amounts of *Bacteroides* and higher amounts of 'other' bacteria than those without dementia, along with more prevalent lactobacillales and *Bifidobacterium*. The ratio of Firmicutes to Actinobacteria was also increased in this population, as has been seen in other inflammatory disease research[7]. In the AD patients from Wisconsin, though, Bacteroidetes were increased, with decreased firmicutes and actinobacteria, which is not reflective of the Japanese data[8]. These differences could be caused by differences in classification, disease progression, location, diet, or many other external or internal factors, so more research must be done to have a better wealth of data on which to base conclusions.

Additionally, with the very few studies completed on this subject, sample sizes are small and cannot be applied to the larger population. What is known about the microbial colonization of the gut, though, is the key role it plays in the immune, endocrine, and neural systems, which all may affect the onset of AD and dementia. A possible venue that the gut microbiota might take in the delay of these conditions includes the control of brain-derived neurotrophic factor (BDNF) through the production of neurotransmitters such as gamma-aminobutyric acid (GABA) and butyric acid. BDNF is known to modulate synaptic transmission and protect against neuro-inflammation and neuronal apoptosis, so increasing its expression may help protect the brain for the amyloid plaques and neurofibrillary tangles commonly seen in AD[11]. Dysbiosis of the gut, however, can cause disruption of the microbiome-gut-brain axis and cause increased inflammation and disease progression. This imbalance of gut bacteria, or "leaky gut," may lead to blood-brain-barrier permeability and ultimately neurological disorders[12]. Future research should focus on analyzing the structural differences of gut microbiota in both AD and non-AD patients. Through this, specific AD microbes may be defined and how they regulate AD pathology may be further understood.

Research into improving cognitive function and slowing the onset of Alzheimer's disease and dementia by rebalancing the gut microbiota through probiotic supplementation showed positive results in all the research examined. The Akbari et al study concluded that probiotic supplementation resulted in improved cognitive function as shown by MMSE score, as well as an improvement in some of the serum markers for metabolic status in the AD patients[9]. In looking at the relationship with "leaky gut" and AD, Leblhuber et al found that supplementation caused decreased intestinal permeability and increased immune activation consistent with the slowing of disease pathology[10]. Finally, through the supplementation with DW2009, the Hwang et al study showed improved cognitive function, as well as increased neuro-protective BDNF levels in those with mild cognitive impairment, a preceding state to AD[11]. These all show the possibilities of probiotics exerting a positive effect on cognitive health, for use as either prevention or possibly treatment for AD and dementia, though the mechanisms by which are still largely unclear. The proposed methods include the modulation of

immune reactions such as neuroinflammation, the suppression of oxidative stress through neuroprotective pathways, and the control of central nervous system function through the production of metabolites like short chain fatty acids (SCFAs)[13]. More research is necessary to define these relationships though, and which strains of probiotics have the best outcomes in the neurocognitive processes in AD and dementia patients. Given further studies resulting in similar positive outcomes, modulation of gut microbiota through probiotic supplementation or personalized diet is likely to become a treatment for neurocognitive disorders including AD[12].

Conclusion

While the research conducted on this subject is still highly unverified and needs replication, it is important to keep an eye on further advancements from the point of view of a Registered Dietitian, particularly for those working in long-term care or with the elderly population. Including probiotic supplementation as a regular part of treatment along with a multivitamin or other oral medication regimen could show great benefit to the cognitive abilities of those beginning to decline. As a future dietitian, I chose to focus on the gut microbiota and dementia as it is still so new in the research phase. For years many have looked into the role our microbiome plays in all different facets of health and I find it fascinating how it could affect our ability to think and process information properly. There are so many different factors to shaping each person's individual gut bacteria composition, and understanding how this interacts with our bodies as a whole is important in the prevention and treatment of many diseases, including neurocognitive disorders. Especially as dementia and Alzheimer's disease are on the rise, finding out more about their pathogenesis and possible methods of treatment should front and center in the medical community.

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