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Novas opções de diagnóstico e tratamento da depressão direcionadas à composição da microbiota gastrointestinal / Targeting gastrointestinal microbiota as new diagnostic and treatment options in depression

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TARGETING GASTROINTESTINAL MICROBIOTA AS NEW DIAGNOSTIC AND TREATMENT OPTIONS IN DEPRESSION

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TARGETING GASTROINTESTINAL MICROBIOTA AS NEW DIAGNOSTIC AND TREATMENT OPTIONS IN DEPRESSION

ABSTRACT

Background/Objective: Depression has been increasingly recognized as a prevalent public health problem. In order to improve the disease's diagnostic and treatment options, the genetic and environmental factors that contribute to depression pathogenesis must be further understood - a factor recently considered to be heavily influential is the composition of the intestinal microbiome. This narrative review aims to expose the current state of knowledge on the relationship between Gastrointestinal Microbiome and Depression and to outline the existing scientific evidence about the effects of probiotics, prebiotics and antibiotics supplementation in depression.

Methods: Using PubMed as the database, a research was conducted targeting articles written in English about how Gastrointestinal Microbiome relates with Depression.

Results: The gastrointestinal microbiome can affect the neuronal function through neurotransmitters, vitamins and neuroactive metabolites via the microbiota-gut-brain axis. Recent studies have pointed out a noticeable difference in the intestinal microbiota composition of patients with depressive disorder. Additionally, clinical trials have demonstrated that the supplementation of probiotics containing specific bacteria may influence the body's response to stress and may regulate some depressive and anxiety symptoms in humans. However, some studies do not support this evidence, stating that probiotics do not influence psychiatric pathology in any way.

Conclusion: Targeting microbiota composition as a diagnostic and treatment option in depression is an appealing possibility however the knowledge available today is not enough to apply those tools with certainty in clinical practice. Future studies are necessary for it to become a fully approved reality in psychiatry.

INTRODUCTION

Depression has severe implications on affected individuals' quality of life, being increasingly recognized as a public health problem. Depressive disorders are characterized by episodes of low mood and/or reduced interest in activities previously enjoyed, accompanied by physical and psychological changes. Often depression emerges in adolescence and continuing through adult life, suggesting a chronic condition with a lifelong course. It is the single most common diagnosis in psychiatry - one third of all psychiatric patients are depressed - and the main cause of disability in the world [1]. Therapeutic options mainly include antidepressants and psychotherapy but unfortunately one-third of major depression patients responds poorly to standard treatment and may become resistant [2].

Describing depression's pathogenesis beyond the acknowledged importance of genetic and environmental factors is a controversial and complex task. In fact, the first known antidepressant is actually an antibiotic, Isoniazid, which was developed as an anti-tuberculous drug but surprisingly showed positive side effects like euphoria, psychostimulation, increased appetite and improved sleep quality [3]. Interestingly, recent studies have pointed out another connection between a biologically related factor and depression - when compared to healthy individuals, patients with depressive disorder display noticeable differences in their intestinal microbiota composition. Furthermore, published works on animal models have shown that specific probiotic supplements can influence their behavior. This is possibly explained by the intestinal microbiome's influence on biological functions such as brain development, endocrine pathways, stress response and immune system maturation. A comprehensive understanding of this link may lead not only to new diagnostic tools but also to the emergence of new therapeutic options [4][1][5].

METHODS

Using PubMed as the database, a research was conducted targeting articles written in English and published during the past 5 years about the association between the human gastrointestinal microbiome and depression. The following query was used: "Gastrointestinal Microbiome"[Mesh] AND "Depression"[Mesh] AND ("2014/12/06"[PDat] : "2019/12/04"[PDat] AND "humans"[MeSH Terms] AND English[lang])". The last date of research was 04/12/2019.

The database returned a total of 52 articles. 3 were excluded for not being available for online reading. 7 articles were excluded after reading the title and abstract for not being related to the theme.

The reading of the remaining 42 articles revealed that 9 provided redundant, non-relevant or very incomplete information which was better described in other studies. The remaining 33 articles revealed 27 more with relevant information. As result, 60 articles were included in this review.

WHAT IS INTESTINAL MICROBIOTA?

Intestinal microbiota represents a symbiotic bionetwork that preserves the homeostatic balance of the human body [6]. It consists of up to 10^{18} microorganisms including bacteria, viruses, yeasts and fungi. The gut microbiome holds around 150 times more single genes than the human genome and can therefore be designated as a ‘metabolic organ’ with functions that the human body cannot execute [7].

The constitution of the intestinal microbiota´s composition begins in utero (bacteria can be found in amniotic fluid, placenta and meconium) and is afterwards influenced by the delivery method - infants born by vaginal birth are firstly exposed to maternal vaginal and fecal microorganisms while those born by cesarean section contact with their mother’s skin and hospital environment. During the first months of life the composition of the infant’s gut microbiota remains unstable until the child begins to eat solid food, around the time the

microbiome begins to resemble that of an adult [8]. Inter-individual microbiota differences in healthy adults are significant but generally the Firmicutes (species such as *Lactobacillus, Clostridium, Enterococcus*) and Bacteroidetes (species such as *Bacteroides*) phyla are represented in bigger quantities [9] while Proteobacteria, Actinobacteria, Fusobacteria, Archea and Verrucomicrobia phyla are represented in smaller quantities [7]. In adulthood, the major determinant of microbiota´s composition is diet - a high fiber and diverse diet is associated with high microbial diversity while a processed food diet is associated with limited variety. Regular aerobic exercise also promotes diversity. Drugs such as antibiotics affect microbial variety and can induce dysbiosis [10].

HOW TO STUDY HUMAN MICROBIOTA?

80% of the human intestinal microorganisms are not culturable so, in order to correctly analyze the bacterial diversity, methods that are culture-independent such as deoxyribonucleic acid (DNA) library, real-time quantitative polymerase chain reaction, denaturing gradient gel electrophoresis [6] and specially 16S Ribosomal Ribonucleic Acid (RNA) Sequencing - due to its low-cost and high-throughput sequencing instruments- have been used and are currently being further studied [11].

GUT MICROBIOME AND DEPRESSION

MICROBIOTA-GUT-BRAIN AXIS

Since patients with depression frequently have appetite disorders, metabolic disturbances, functional intestinal disorders and intestinal microbiota deviations [12], a causal relation between microbiome dynamics and depression is a valid scientific hypothesis. Furthermore, the intestinal microbiome is an increasingly recognized brain-contouring factor, acting through a biochemical signaling mechanism known as the microbiota-gut-

brain axis capable of affecting the neuronal function directly or indirectly through neurotransmitters, vitamins and neuroactive metabolites [8].

Major depression is frequently associated with chronic inflammatory comorbidities such as inflammatory bowel disease [13][14], rheumatoid arthritis, coronary heart disease and multiple sclerosis, which sustains the idea of its association with persistent low grade inflammation [15]. The cytokine hypothesis postulates that proinflammatory cytokines (IL-6 and TNF-α) are increased in depression, inhibiting the negative feedback of the hypothalamic–pituitary–adrenal axis, increasing the permeability of the blood–brain barrier, reducing the synthesis of serotonin and disturbing the glutamatergic systems, all contributing to depression genesis. On the other hand, anti-inflammatory cytokines (IL-10 and TGF-β) are decreased [12]. In fact, researchers have found that conventional anti-inflammatory agents have significant antidepressant effects and infliximab has even been shown to be effective in treating resistant depression with elevated C-Reactive Protein (CRP) levels [16][15], being CRP suggested as the strongest predictor of reduced cognitive empathy [17]. Additionally, conventional antidepressants such as Selective serotonin reuptake inhibitors (SSRIs) could have anti-inflammatory properties by affecting cytokines production [16][15]. The concept of “leaky gut” can help explain the link between how the penetration of bacteria outside the intestinal lumen may lead to systemic inflammation and subsequently play a part in the pathophysiology of depression as the loss of integrity between epithelial cells promotes the translocation of bacteria through the intestinal barrier and into the circulatory system [5]. In fact, Bruce R Stevens *et al.* highlight that gut can be considered a different target for depression and anxiety management since their study showed that there’s a correlation between human gut dysbiosis and the tripartite consensus clustering of zonulin, intestinal fatty acid-binding protein-2 and lipopolysaccharide plasma biomarkers of increased gut permeability in individuals with depression and anxiety disorders versus healthy controls [18].

It has also been demonstrated that intestinal bacteria are capable of synthesizing most of the main human brain neurotransmitters. Special relevance should be given to the Gamma-aminobutyric acid (GABA) production by Bacteroides, Parabacteroides and Escherichia species which, via vagus nerve transmission, acts in various brain regions [10][19][20]. Strandwitz *et al.* sought to further explore this modulation and determine whether Bacteroides population numbers, thought to be the major bacterial producer of GABA in the human intestine, could be associated with clinically diagnosed major depressive disorder. The study found that a relative abundance of Bacteroides levels in fecal samples was negatively correlated with brain features associated with depression [21]. In contrast, Naseribafrouei *et al.* reported that in depressed patients there was an overrepresentation of the Bacterioidales order, which contains the Bacteroides family, and the Oscillibacter genus, which produces valeric acid that resembles GABA [22].

Another neurotransmitter known to be highly involved in depression genesis is serotonin. As a matter of fact, the intestinal tract is the human body region where serotonin is found in highest concentrations due to it playing a vital role in intestinal secretion, motility and pain perception regulation [23]. Remarkably, serotonin can be not only produced by Candida, Streptococcus, Escherichia and Enterococcus species in the gut but also get its endogenous biosynthesis modulated by the microbiota since bacterial metabolic activity produces short fatty acids that can stimulate the production of neuroactive molecules such as histamine and serotonin [19][10]. It has actually been demonstrated that, in patients with depression, acetic acid and propionic acid are lowered, isocaproic acid is increased and that there is a negative correlation between acetate, propionate and Beck's depression inventory [24]. In addition to this, Szczesniak *et al.* found statistically significant correlations between depression and isovaleric acid in a cohort of 34 depressed patients [25]. Moreover, it has also been shown that bifidobacteria within the intestine can synthesize the serotonin precursor tryptophan and that administering this type of bacteria is associated with increased plasma levels of this molecule [10].

On a more specific level, recent data provided evidence that Lactobacillus helveticus R0052 affects the functioning of central nervous system neurons in the hippocampus and amygdala, Lactococcus lactis subspecies cremoris H61 modulates the activity of auditory brain stem neurons and Lactobacillus reuteri is implicated in the function of intestinal visceral nociceptive neurons [8].

MICROBIOME VARIATIONS IN DEPRESSION

Research on how microbiota changes relate to mood disorders in humans is still very poor in comparison to animal-models. Nevertheless, some valid studies have been published.

As mentioned above, Naseribafrouei *et al.*, after analyzing fecal samples from 37 patients with depression and 18 healthy controls, concluded that the Bacterioidales order was overrepresented, while the Lachnospiraceae family was underrepresentation in depressed patients [22].

Jiang *et al.* case-control study with 46 patients with major depressive disorder and 30 healthy controls showed that in depressed individuals the levels of Bacteroidetes, Proteobacteria, and Actinobacteria were increased, whereas the levels of Firmicutes was reduced. In addition, a negative correlation was observed between Faecalibacterium levels and the severity of depressive symptoms [26].

Kelly *et al.* investigation reported that patients with major depressive disorder had an high abundance of Thermoanaerobacteraceae family and Anaerofilum, Eggerthella, Gelria, Holdemania, Paraprevotella and Turicibacter genus. However, lower numbers of Prevotellaceae family and Dialister and Prevotella genus were observed [9].

Lin *et al.* case-control study with 60 patients showed that more phylum Firmicutes, less Bacteroidetes and more genus Prevotella, Klebsiella, Streptococcus and Clostridium XI were found in major depressive disorder patients. The changes of the proportion of Prevotella and Klebsiella were consistent with Hamilton depression rating scale [6].

Valles-Colomer *et al.* concluded in a study with 1,054 subjects that Faecalibacterium and Coprococcus bacteria were associated with higher quality of life indicators and that Dialister and Coprococcus were depleted in depression even after confounding effects of antidepressants were corrected. Depression diagnosis corresponded to higher prevalence of Bacteroides enterotype 2 samples [23].

Zheng *et al.* case-control clinical sampling demonstrated an abundance of Actinobacteria and lower levels of Bacteroidetes in depressed subjects. Firmicutes levels were useful to discriminate patients from healthy controls. However, no significant difference in the overall relative abundance between cases and controls was found - some members of the Firmicutes were augmented in patients while others were reduced [27].

Aizawa *et al*. case-control study with 43 patients with major depressive disorder and 57 healthy controls showed a significantly lower abundance of Bifidobacterium and Lactobacillus counts in fecal samples in the patients than the controls. However, the authors warn that the findings should be interpreted with caution once variations depending on sex, diet and medication were not fully taken into account in the analysis [28].

Chen JJ *et al*. case-control trial conducted to establish the sex differences in gut microbiota in patients with major depressive disease revealed that Bacteroidetes level was significantly decreased in male patients and the Actinobacteria level was significantly increased in female patients [29].

Kleimanet *et al*. found no significant associations between composition and diversity of gastrointestinal microbial markers and anxiety, depression, eating-related thoughts and behaviors, stress or personality scores in a large cohort of healthy adult females [30].

Ishii W. *et al*. investigated the intestinal microbiota and its impact on mental health of children with orthostatic intolerance. The mean proportion of Clostridium subcluster XIVa

and/or Enterobacteriaceae in patients was significantly higher than that in controls. Among patients, Bifidobacterium was lower in the depression group than in the non-depression group. Nevertheless, depression and anxiety showed no correlation with bacterial diversity [31].

PROBIOTICS

Probiotics are live microorganisms that, when ingested in adequate amounts, might confer health benefits to the host by interacting with his microbiota. Mechanisms revolve around restoring intestinal permeability by improving mucosal barrier function [32], competing with pathogenic bacteria for receptors on the surface of the intestinal epithelium [5] and reducing circulating levels of pro-inflammatory markers. Additionally, these probiotics are capable of neuroactive substance production and distribution, such as gamma-aminobutyric acid and serotonin that act across the brain-gut axis [33][34]. In fact, probiotics might also be able to beneficially manipulate the gut's production of peptides and hormones in a way that cannot be replicated by these compound exogenous administration [35]. They can also reduce the levels of cortisol and increase levels of oxytocin [33][34].

Through their effects on obesity, diabetes and other metabolic complications it is speculated that probiotics may improve depressive symptoms [36][37]. Evidences from animal and human studies have shown that some probiotics can reduce depression, anxiety, stress and improve cognition, raising discussion over a new class of compounds known as "psychobiotics" [34]. Clinical trials have been conducted to clarify the benefit of implementing probiotic treatment in patients with depression.

EVIDENCE OF PROBIOTIC USE IN DEPRESSION

Benton *et al*. double-blind placebo-controlled trial with random allocation of 132 healthy subjects was conducted for a 3 week period to study the impact of consuming a probiotic (Lactobacillus casei Shirota) containing milk drink or a placebo on mood and memory.

These aspects were measured at baseline, after 10 and after 20 days of consumption. The consumption of a probiotic containing milk drink improved the mood of those whose mood was initially poor, but it resulted in a slightly-poorer performance on two measures of memory [38].

Messaoudi *et al*. randomized placebo-controlled double-blind trial was conducted for 30 days to study the psychological effects of a probiotic formulation (Lactobacillus helveticus R0052 and Bifidobacterium longum R0175) in 66 healthy human volunteers. The combination decreased the global scores of Hospital Anxiety and Depression Scale and the global severity index of the Hopkins symptoms checklist [39].

Nishihira *et al*. randomized placebo-controlled double-blind trial with 238 healthy adults (109 placebo subjects and 115 test subjects) was lead for 12 weeks. Using a yogurt combining two probiotics (Lactobacillus gasseri SBT2055 and Bifidobacterium longum SBT2928) they tested the study participants immunomodulatory and stress-related functions. They quantified participants' natural killer cell activities and adrenocorticotropic hormone (ACTH) and cortisol levels. Significant improvement in anxiety/insomnia subscale was found but no change in overall General Health Questionnaire-28 scores was observed. They found elevated natural killer cell activity and reduced serum adrenocorticotrophic hormone in probiotic group [40].

Akkasheh *et al*. observed a decreased in Beck Depression Inventory total scores and significant reductions in inflammatory markers in patients with major depressive disorder taking a Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium bifidum capsule [33].

Mohammadi *et al*. randomized placebo-controlled double-blind trial with 70 healthy participants was led for 6 weeks. Subjects were randomly divided in three groups: a group

of 25 subjects receives a probiotic yogurt containing two strains of Lactobacillus acidophilus LA5 and Bifidobacterium lactis BB12 plus one placebo capsule, 25 to receive one probiotic capsule daily (Actobacillus casei 3 × 103, L. acidophilus 3 × 107, L. rhamnosus 7 × 109, L. bulgaricus 5 × 108, Bifidobacterium breve 2 × 1010, B. longum 1 × 109, S. thermophilus 3 × 108 CFU/g, and 100 mg fructo-oligosaccharide with lactose as carrier substances) plus a conventional yogurt and 20 to receive conventional yogurt plus one placebo capsule. Mental health parameters including General Health Questionnaire and Depression Anxiety and Stress Scale scores were measured. Reduced anxiety and depression scores on General Health Questionnaire-28 and Depression Anxiety Stress Scales in probiotic yoghurt or capsule group were observed. However, there was no significant improvement in the conventional yogurt group [41].

Bambling *et al*. pilot study intervention with 12 participants using a formulation combining probiotics (Lactobacillus acidophilus, Bifidobacterium bifidum, Streptoccocus thermophiles total CFU of 2 9 1010) and magnesium orotate adjuvante administered with SSRIs for treating resistant depression was conducted for 8-week then follow-up for 16 weeks. Mean changes for depression scores and quality of life in the group at the end of the 8th week were significantly improved. An intestinal anti-inflammatory response was suggested. However, patients while still on SSRI relapsed at 16th week after cessation of the test intervention [2].

Kazemi *et al*. double blind clinical trial including 110 patients with major depressive disorder was conducted for 8 weeks to compare the effect of probiotic (Lactobacillus helveticus and Bifidobacterium longum) and prebiotic supplementation on the Beck Depression Inventory versus placebo. Probiotic significantly decreased depression [42].

Pinto-Sanchez *et al*. study with 44 participants not taking any psychotropic medication with irritable bowel syndrome and mild to moderate anxiety and/or depression scores based on

self-report questionnaires - 22 patients taking the probiotic and 22 the placebo for 6 weeks - was found that the probiotic Bifidobacterium longum NCC3001 reduces depression but not anxiety scores and increases quality of life. Also, they performed functional magnetic resonance imaging that showed a reduction in response to negative emotional stimuli in amygdala and fronto-limbic regions of the brain when taking the probiotic which indicates it reduces limbic reactivity. No effect on faecal microbiome profile was observed [43].

NO EVIDENCE OF PROBIOTIC USE IN DEPRESSION

Shinkai *et al*. randomized placebo-controlled double-blind trial with 300 healthy 65 year adults was conducted over 20 weeks. Cases were given tablets containing Lactobacillus pentosus strain b240 but no effect on Profile of Mood States Scale was observed [11].

Tillisch *et al*. randomized placebo-controlled double-blind trial with 23 healthy women was conducted for 4 weeks to investigate if consuming a fermented milk product containing Bifidobacterium animalis subsp Lactis, Streptococcus thermophiles, Lactobacillus bulgaricus, and Lactococcus lactis subsp Lactis changes brain intrinsic connectivity or responses to emotional attention tasks. No changes in Hospital Anxiety and Depression Scale were detected. However, functional magnetic resonance imaging alterations in activity of brain regions that control central processing of emotion and sensation were observed [44].

Chung *et al*. 12-week double-blind randomized controlled trial was conducted to investigate the effects of Lactobacillus helveticus-fermented milk on cognition in 36 healthy over 65 years old adults. Cognitive tests and measurements of the Perceived Stress Scale and Geriatric Depression Scale-short Form were taken before and after the experiment. No effect on mood as measured by Geriatric Depression Scale Short Form was recorded and there was no effect on Perceived Stress Scale. However, there was an improvement in cognitive function in probiotic group [45].

Steenbergen *et al*. 4-week triple-blind placebo-controlled randomized trial aimed to test if a probiotic combination (Bifidobacterium bifidum W23, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, Lactobacillus casei W56, Lactobacillus salivarius W24, and Lactococcus lactis (W19 and W58)) may reduce cognitive reactivity in 20 healthy participants comparing with 20 controls receiving an inert placebo. Cases showed a significantly reduction in Leiden Index of Depression Sensitivity-Revised, indicating reduced cognitive reactivity to sad mood. Yet, no changes in Beck Anxiety Inventory or Beck Depression Inventory were observed [46].

Östlund-Lagerström *et al*. randomized placebo-controlled double-blinded clinical trial with 290 over 65 years old subjects was conducted for 12 weeks to assess the changes in level of welfare, anxiety and stress as secondary outcome (primary outcome was changes in gastrointestinal symptoms) by taking the probiotic strain Lactobacillus reuteri. No significant improvement in wellbeing, stress or anxiety was detected [47].

Kelly *et al*. reported that a 8 week randomized placebo-controlled cross-over design was conducted to determine the impact of Lactobacillus rhamnosuson stress-related behaviours. The study included 29 healthy male volunteers. No effect on anxiety or stress as measured by Beck Anxiety Inventory, Perceived Stress Scale, State Trait Anxiety Inventory was observed [48].

Romijn *et al*. randomized double-blind placebo-controlled clinical trial with 79 self-reported depressive symptoms participants not taking any psychotropic medication was lead for 8 weeks to study the impact of a probiotic preparation (combining Lactobacillus helveticus and Bifidobacterium longum) in mood, stress and anxiety. No evidence of the probiotic formulation effectiveness in treating low mood or in controlling inflammatory and other biomarkers levels was found [49].

Cepeda *et al*. survey from 2005 through 2012 included adult participants who consumed any probiotic food or supplement on any of the interview days. Subjects were classified as

depressed if Patient Health Questionnaire scores were ≥10. Unadjusted analysis suggested that subjects who consumed probiotics had lower odds of depression. After adjustment for characteristics associated with depression and probiotic exposure, the effect was attenuated and no longer significant [50].

Ng *et al*. meta-analysis of 10 randomized controlled trials found that although generally safe and pleasant, it cannot be recommended that probiotics replace antidepressant medications as the primary treatment for depressed patients [51].

PREBIOTICS

Prebiotics, which are dietary soluble fibers, stimulate the growth of beneficial commensal microbiota and can benefit both intestinal mucosa and systemic immunity as they reach the large intestine microbiota [32][19].

Wang *et al*. found that oral sesamin administration significantly attenuated depressive and anxiety-like behaviors in a long-term stress-treated mice model by inhibiting stress-induced intestinal barrier integrity damage, reducing circulating lipopolysaccharide levels, suppressing neuroinflammatory responses which might be highly related to sesamin capacity to enhance the relative abundance of Bacteroidales and S24-7 [52]. Ramnani *et al*. documented the ability of fructans derived from agave plants to increase the level of bifidobacteria and lactobacilli in fecal samples from healthy human participants [53]. On the other hand, Kazemi *et al*. conducted an 8 week double blind clinical trial with major depressive disorder patients to compare the effect of probiotic and prebiotic (galactooligosaccharide) supplementation on the Beck Depression Inventory versus placebo. While, probiotic significantly decreased depression, no changes were observed on placebo or prebiotic groups [42].

ANTIBIOTICS

Since they can significantly change the gastrointestinal microbiota, antibiotics are likely to affect the microbiota-gut-brain axis [54].

Bercik *et al*. findings indicate that microbiota alteration in adult mice after oral administration of neomycin and bacitracin combined with antifungal agent primaricin results in measurable changes in anxiety-like behaviors but neither vagotomy nor sympathectomy affected the ability of the antimicrobials to impact anxiety like behavior pointing to other unidentified mechanisms [55].

DISCUSSION

DIAGNOSTIC TOOL

The diagnosis of depression depends on subjective survey scales [6], but whether it could be based on more objective and quantifiable criteria is a matter of debate. Large-scale metagenomics studies have been conducted in order to study the neuroactive potential of microbiota, but the results' interpretation is difficult due to the lack of tools and reference databases [23]. Nevertheless, 16S Ribosomal RNA sequencing has emerged as one of the most promising and relevant methods.

Generally, in healthy adults the Firmicutes and Bacteroidetes phyla are represented in bigger quantities [9] while Proteobacteria and Actinobacteria phyla are represented in smaller quantities [7]. In major depressive disorder patients, however, these proportions might be altered: some show an overrepresentation of Firmicutes and an underrepresentation of Bacteroidetes [27][6]. On the other hand, Jiang *et al*. found an overrepresentation of Bacteroidetes and an underrepresentation of Firmicutes in these patients [26]. Zheng *et al*. found that Firmicutes abundance is useful to discriminate patients from healthy individuals [27]. Aizawa *et al*. showed a significantly lower abundance of Bifidobacterium and Lactobacillus counts in fecal samples of depressed patients [28]. In addition to this, Actinobacteria showed to be significantly increased [26][27] as well as Proteobacteria [26]. JJ *et al*. case-control trial established that Bacteroidetes level is significantly decreased in male patients and the Actinobacteria level was significantly increased in female patients [29]. Lin *et al*., highlight the importance of Prevotella and Klebsiella proportion in fecal microbial communities in the diagnosis and therapeutic monitoring of major depression disorder patients in the future [6].

The differences found between studies might be related to population size and characteristics, such as sex, age, severity of the disease and the factors that may influence the composition of the intestinal microbiome.

The causal relation between the microbiota's composition modifications and depression genesis is an aspect that is essential to further clarify. It is currently believed that the alteration in microbiota's arrangement may contribute to depression but those changes may also be induced by the depression itself. The two may occur depending on whether some particular stressors trigger changes in mood that subsequently modify intestinal microbiota or a gastrointestinal disorder produces intestinal microbiota changes that later induce to depression [56]. Further research should be conducted in order to build large databases of microbial signature associated with depression and develop more objective diagnostic criteria.

TREATMENT OPTION

The cumulative evidence suggests that probiotic supplementation may have an influence on certain cognitive functions known to determine susceptibility to mood disorders [57][58], supporting the idea that the restoration of dysfunctional intestinal microbial populations might be a viable treatment option for individuals with depression [15] or an adjuvant strategy to improve or even to prevent depression [46]. Latest research has found that traditional antidepressant therapies influence intestinal microbiota composition, possibly meaning that their antidepressant effects are partly due to their capacity of regulating the microbiota–gut–brain axis. As a matter of fact, the first antidepressant, Isoniazid, was originally used to treat Mycobacterium tuberculosis infections; the first-generation tricyclic antidepressants can inhibit the proliferation of many bacteria; SSRI antidepressants can inhibit the proliferation of Gram-positive bacteria; and ketamine can inhibit the proliferation of Staphylococcus, Enterococcus and Candida albicans. On the other hand, common antibiotics, such as Ceftriaxone and Minocycline, present some antidepressant effects [12]. Another potential use of probiotics is, since there's a diversity of microorganisms that interact with specific neuronal circuitries, the possibility of creating targeted interventions on specific neuronal functions according to patient's clinical presentation [8].

The main bacterial genera in probiotics composition used in human studies come from the Lactobacillus and Bifidobacterium genera [54]. The combination of Lactobacillus helveticus R0052 and Bifidobacterium longum R0175 decreased the global scores of Hospital Anxiety and Depression Scale in healthy human volunteers [39]. Lactobacillus helveticus and Bifidobacterium longum significantly decreased depression symptoms in major depressive disorder patients [42], but not when administered to self-reported depressive symptoms participants not taking any psychotropic medication [49]. Lactobacillus helveticus alone showed no effect on mood in healthy over 65 years old adults [45], Lactobacillus casei Shirota alone improved the mood of healthy subjects whose mood was initially poor [38], Lactobacillus gasseri SBT2055 and Bifidobacterium longum SBT2928 significantly improved anxiety/insomnia subscale in healthy adults [40], Bifidobacterium longum NCC3001 alone reduced depression scores and increased quality of life in mild to moderate anxiety and/or depression scores based on self-report questionnaires participants however no effect on faecal microbiota composition was observed [43], Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium bifidum decreased Beck Depression Inventory total scores and significantly reduced inflammatory markers in patients with major depressive disorder [33], Lactobacillus acidophilus LA5 and Bifidobacterium lactis BB12 reduced anxiety and depression scores in healthy subjects [41], Lactobacillus acidophilus, Bifidobacterium bifidum, Streptoccocus thermophiles plus magnesium orotate resistant depression patients administration significantly improved depression scores and quality of life [2], Bifidobacterium animalis subspecies Lactis, Streptococcus thermophiles, Lactobacillus bulgaricus and Lactococcus lactis subspecies Lactis didn't have impact in healthy women Hospital Anxiety and Depression Scale scores [44], Lactobacillus pentosus strain b240 administration had no effect on healthy 65 year old adults' Profile of Mood States Scale [11], Bifidobacterium bifidum W23, Bifidobacterium lactis W52, Lactobacillus acidophilus W37, Lactobacillus brevis W63, Lactobacillus casei W56, Lactobacillus salivarius W24 and Lactococcus lactis significantly reduced Leiden Index of Depression Sensitivity-Revised yet no changes in Beck Anxiety Inventory or Beck

Depression Inventory were observed in 20 healthy participants [46], Lactobacillus reuteri didn't improve wellbeing, stress or anxiety in older adults [47], Lactobacillus rhamnosuson didn't have an effect on anxiety or stress in healthy male volunteers [48].

Defining the proper probiotic dose and composition for a possible depression treatment and/or prophylaxis is one of the major challenges in this field. Some of the studies did not allow concluding which probiotic strain caused the treatment effect observed since they administered more than one probiotic at a time and variations in duration, dose and bacterial strains may influence the response to probiotic supplementation

The main prebiotics used to act on the microbiome-gut-brain axis are fructans (fructooligosaccharide, inulins, oligofructose) and glucans (gluco-oligosaccharides) [59]. Fructans derived from agave plants were capable of increasing the level of bifidobacteria and lactobacilli in healthy human participants [53] but galactooligosaccharide supplementation had no impact on Beck Depression Inventory of major depressive disorder patients [42]. Further research is essential to understand if prebiotics are useful in treating mental disorders once it would be interesting to target specific microbials to balance the microbiota in a context of depression. In addition, it is necessary to study the effects of antibiotic regimen on physiology and behaviour.

Inconsistencies in literature findings may be related to inter-study variance. As most studies were conducted in healthy or in non-clinically diagnosed participants, their results cannot be extrapolated to clinically-diagnosed patients. Furthermore, sample sizes were relatively small and there were differences in sex and age. Studies conducted in elderly populations showed no impact of probiotics administration on Mood States Scale [11], on Geriatric Depression Scale Short Form and Perceived Stress Scale [45] nor on the improvement in wellbeing, stress or anxiety [47]. No investigation was performed in younger patients. Some studies assessed subjective parameters such as low mood, irritability, anxiety and stress using different scales. Since no assays are currently able to

detect the complete microbiota profile of a given subject, this must be currently acknowledged as another important limitation in the area.

Standardized methods and control of confounding variables are required to improve future investigation [60]. Performing intra-individual comparisons - for example, by comparing acute depression with euthymic state in the same patient - would allow to minimize the effect of confounding factors in microbiota studies. Besides, since through a case-control dichotomous acquisition it is not possible to clarify the true effect of microbiome manipulation on mental pathology, and given the importance to clarify the dose response in relation to symptom severity, it might be interesting to conduct follow-up studies in this area [4]. As probiotics therapies emerge as a possible cost-effective and safe option to adjuvate, treat or even prevent mental disorders, further investigations should be carried out in groups of diagnosed patients and in younger participants, taking into account a possibly larger number of subjects and longer duration of trials [5].

CONCLUSION

The emergent idea of a different gastrointestinal microbiota composition in patients with depression and the increased susceptibility for mental disorders in patients with gastrointestinal dysbiosis suggest a potential use of probiotic and other microbiota targeted therapies in the treatment of mental disorders. However, the knowledge is currently limited and the information available nowadays is not enough to apply these assumptions with certainty in clinical practice. It is important to clarify how informative are the studies conducted to this date and to further study how gastrointestinal microbiota influences the human brain.

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NOMENCLATURE

ACTH- Adrenocorticotropic hormone

CRP- C-reactive protein

DNA- Deoxyribonucleic acid

GABA- Gamma-aminobutyric acid

RNA- Ribonucleic acid

SSRIs- Selective serotonin reuptake inhibitors

ANEXOS

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