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Alteration of Gut Microbiota in Alzheimer's Disease and Their Relation to the Cognitive Impairment

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Abstract.
Background: Dysbiosis of gut microbiota has been reported to be enrolled in the pathogenesis of Alzheimer's disease (AD). However, there is a lack of relevant studies on this topic in Egyptian patients with AD.
Objective: To investigate different species of gut microbiota in Egyptian patients with AD and correlate microbiota bacterial abundance with clinical data.
Methods: The study included 25 patients with AD and 25 healthy volunteers as age and sex-matched controls. Clinical data was taken for each patient, including medical history and examination; Mini-Mental State Examination (MMSE) and Montreal Cognitive Assessment (MoCA) were assessed for each participant. Bacterial DNA was extracted from stool, and abundance quantified via qPCR using 16S rRNA group-specific primers.
Results: *Akkermansia*, *Enterobacteria*, *Bacteroidetes*, *Bacillus cereus*, *Prevotella*, and *Clostridium* cluster IV were more abundant in the AD group than in the control group, although there was significantly less abundance of *Bifidobacterium* spp., *Firmicutes*, and *Actinobacteria* in patients with AD than in controls, whereas no such significance was found for lactic acid bacteria between both groups. Lactic acid bacteria and *Prevotella* abundance was negatively correlated with cognitive impairment ($p = 0.03$ with MMSE, and $p = 0.03$ with MoCA). *Prevotella* abundance was positively correlated with age of onset and duration of illness and negatively correlated with smoking and coronary heart disease ($p = 0.007$, $p = 0.03$, $p = 0.035$, and $p = 0.047$, respectively).
Conclusion: The current work highlighted a significant relationship between AD and gut microbiota dysbiosis. A higher abundance of *Prevotella* species and lactic acid bacteria was correlated with cognition.

Keywords: Alzheimer's disease, cognition, gut microbiota, mini-mental state examination, montreal cognitive assessment

INTRODUCTION

Alzheimer's disease (AD) is the prevalent form of dementia, accounting for 60–80% of all dementias, and ranks as the 6th most significant cause of mortality in the United States [1]. Neuropathology hallmarks of AD include extracellular amyloid-β (Aβ), senile plaques, and neurofibrillary tangles [2].

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AD is a disease that worsens with age and is becoming more common globally, especially in people older than 60. However, only a few epidemiological studies have estimated the prevalence rate of dementia, especially in Arabic countries. The first study was completed by Farrag et al., who reported a crude prevalence rate (CPR) of 4.5 of all dementias and 2.2/100 for AD [3]. Subsequently, Khedr et al. found that the CPR of all dementias was 5.07/100; the highest prevalence rate was recorded among subjects above 85 years old [4]. This recent transition in demographics places AD in a new era that requires investigation.

Gut bacteria have been shown to play a critical part in bowel and brain function regulation to enhance the initiation and progression of a variety of diseases, including Parkinson's disease, multiple sclerosis, and AD [5, 6]. The gut microbiota diversity decreases in the elderly and patients with AD. The intestinal microbiota is beneficial for human health, as it maintains the mucosal barrier integrity, inhibits adhesion of pathogens to the intestinal mucosa, produces vitamin K and short-chain fatty acids (SCFAs), and helps regulation and maturation of the immune system [7, 8].

Microbiota dysbiosis may alter or trigger AD development by increasing the gut and blood-brain barrier permeability. In addition, the gut microbiota can emit substantial amounts of amyloids and lipopolysaccharides (LPS) that can alter signaling pathways and produce proinflammatory cytokines that have a role in AD [9].

A shift in the composition of the gut microbiota has been recorded in relation to age, with lowering of certain beneficial bacteria, such as *Bacteroidetes*, *Lactobacillus*, and *Bifidobacterium* in the elderly [10, 11]. Other studies recorded that several specific bacterial groups such as *Bifidobacterium*, *Lactobacillus*, and *Faecali bacterium* could modulate inflammation of gut epithelium [12, 13].This disparity between different studies could be due to differences in gut microbiota species, criteria used for diagnosis of AD, dietary habits, or geographical distributions.

Currently, there is a lack of studies, particularly in Egypt that have directly analyzed the gut microbiotas relationship with AD. Therefore, this study aimed to assess the composition of different gut microbiota species in patients with AD and in healthy controls with matched age, sex, and education and correlate their microbiota abundance with clinical data and cognitive function.

MATERIALS AND METHODS

In this case-control study, 25 cases with AD and 25 healthy volunteers matched by age, sex, and education were enrolled. All patients with AD were recruited from Aswan University Hospital outpatient clinic from May 2020 to February 2021.

The diagnosis of AD was made using the Diagnostic and Statistical Manual of Mental Disorders-Fourth edition (DSM IV-TR) criteria [14], and the criteria of the National Institute of Neurological and Communicative Disorders Association [15]. These criteria included: the development of multiple cognitive deficits manifested by both memory impairment (anterograde amnesia) and one or more of the following: aphasia, apraxia, agnosia, and disturbance of executive functioning with intact consciousness. These cognitive deficits cause significant impairment of social and occupational functioning. The course is characterized by gradual onset and progressive cognitive decline for at least 1 year. These cognitive deficits are not due to other central nervous system disease that causing progressive dementia such as (cerebrovascular disease, psychiatric disorders, delirium, tumors, normal pressure hydrocephalus, Parkinson's disease, Huntington's disease, or subdural hematoma) [16]. Furthermore, a score of less than four on Hachinski's ischemia score [17] was used.

Patients with symptoms that could indicate another type of dementia, resulting from head injury, cerebrovascular stroke, encephalitis, metabolic disorder, previous abdominal surgery, autoimmune disease, type-1 diabetes, or irritable bowel syndrome, were excluded. Patients under treatment with antibiotics or probiotic supplements for at least one month before obtaining fecal samples were excluded. Patients taking COMT inhibitors and metformin, both of which have been found to impact gut flora, were also eliminated.

To confirm the presence of diffuse brain atrophy and rule out other causes of dementia, magnetic resonance imaging was performed on all patient brains. Clinical data, including demographic data, age of onset, duration of disease, and associated risk factors. Mini-Mental State Examination (MMSE) [18] and Montreal Cognitive Assessment (MoCA) [19] (https://www.mocatest.org), Arabic version, was assessed for each participant. The control group included 25 healthy volunteers, and this age, sex, and education matched group was used to compare gut microbiota.

Fecal sample processing and extraction

Stools were collected from patients and stored immediately after collection in sterile containers at –70°C upon arrival in the laboratory, Aswan University Hospital. Bacterial DNA was extracted from thawed stool samples using the QIAamp® DNA Stool Mini Kit (Qiagen GmbH, Germany, Cat No 12830-50), according to the manufacturer's protocol.

Bacterial abundance was quantified via qPCR using SYBR Green qPCR in a Rotorgene 3000 (Corbett Life Science, Australia) with 16S rRNA group-specific primers. The PCR reaction mixture and serial DNA dilution of typical strains were prepared according to Pirkera et al. [20].

Consent and ethical approval

Written informed consent was obtained from each participant after discussion of all aspects of the study. The study was authorized by the local ethical council of Aswan University, Egypt, with approval number 374/6/19. The study was performed in compliance with the Helsinki Declaration of 1975 and according to the ethical requirements of the institution's Committee on Human Experimentation.

Statistical analysis

IBM® SPSS® Statistics Version 26 for Windows was used to conduct the statistical analysis. Mean and standard deviation values were computed for each group in each test. Number and percent (N, percent) represent categorical variables while mean and standard deviation were used to express continuous variables (mean, SD). The chi-square test and the Fisher exact test were used to compare categorical variables, and the independent sample Mann–Whitney test was employed to analyze continuous variables. Spearman correlation was used to correlate the microbiome with the clinical data. A p-value < 0.05 was set as the significance level.

RESULTS

The fifty participants were categorized into 25 with AD and 25 healthy controls. The mean age in the AD group was 68.92 ± 7.56 years, with no significant difference to that of the control group. Females represented more than half of the participants (56%) in each group with no significant difference between the groups. Similarly, there was no significant difference regarding comorbidities, such as hypertension, diabetes mellitus, dyslipidemia, or coronary heart disease, between either group (Table 1).

Table 1
Demographic, clinical data and risk factors of Alzheimer disease patients and control groups

	Alzheimer disease group ($n=25$)	Control ($n=25$)	p
Age (yrs)			
Range	57–84	53–82	
Mean ± SD	68.92 ± 7.56	66.76 ± 8.8	0.356
Sex No. (%) Male	11 (44%)	11 (44%)	
Female	14 (56%)	14 (56%)	1.000
Education, years			
Range	0–16	1–16	0.664
Mean ± SD	9.32 ± 4.98	9.96 ± 5.37	
Smoking			
Yes	4(16%)	1(4%)	
No	21(84%)	24(96%)	0.349
Hypertension No. (%)			
Yes	6(24%)	7(28%)	0.747
No	19(76%)	18(72%)	
Diabetes mellitus No. (%)			
Yes	3(12%)	1(4%)	0.297
No	22(88%)	24(96%)	
Dyslipidemia No. (%)			
Yes	7(28%)	3(12%)	0.157
No	18(72%)	22(88%)	
Coronary heart disease No. (%)			
Yes	3(12%)	5(20%)	0.440
No	22(88%)	20(80%)	
Age of onset of illness (years)			
Range	56–80		
Mean ± SD	66.68 ± 6.47	–	–
Duration of illness (months)			
Range	12–84		
Mean ± SD	26.88 ± 17.42	–	
Mini-mental state scores			
Range	7–20	24–30	
Mean ± SD	13.4 ± 4.49	28.08 ± 1.66	**< 0.001****
Montreal cognitive score			
Range	5–18	26–30	
Mean ± SD	11.52 ± 4.27	27.76 ± 1.51	**< 0.001****

*Significant p value, **Highly significant p value.

Analysis of isolated microbiota RNA showed there were greater abundances of several species, including *Akkermansia*, *Enterobacteria*, *Bacteroidetes*, *Bacillus cereus*, *Prevotella*, and *Clostridium* cluster IV, in the AD group relative to those in the control group, although the abundances of *Bifidobacterium*, *Firmicutes,* and *Actinobacteria* spp. were significantly lower in the AD group. No significant difference between the groups was observed for the abundance lactic acid bacteria (Fig. 1).

In the AD group, there was no significant difference between males and females ($p > 0.05$), although

Table 2
Comparison between microbiota items among Alzheimer disease patients and control groups according to male & female

	Alzheimer disease group ($n=25$)			Control group ($n=25$)		
	Male ($n=11$)	Female ($n=14$)	p	Male ($n=11$)	Female ($n=14$)	p
Akkermansia	8.48 ± 1.96	7.59 ± 1.95	0.267	6.21 ± 1.99	6.97 ± 1.16	0.265
Enterobacteria	13.17 ± 4.68	11.26 ± 3.65	0.263	9.17 ± 1.31	9.37 ± 1.74	0.750
Bacteroidetes	10.49 ± 3.39	8.39 ± 3.09	0.119	5.99 ± 1.88	7.41 ± 2.07	0.089
Bacillus cereus	11.65 ± 2	11.71 ± 1.98	0.932	9.57 ± 1.31	8.85 ± 1.29	0.181
Prevotella	10.49 ± 1.35	11.23 ± 1.24	0.170	7.39 ± 1.1	8.45 ± 1.12	0.028*
Clostridium cluster IV	12.46 ± 3.74	11.51 ± 3.35	0.511	7.15 ± 0.92	8.59 ± 1.08	0.002**
Lactic acid bacteria	13.36 ± 1.7	11.81 ± 1.94	0.047*	10.63 ± 3.77	10.77 ± 4.5	0.936
Bfidobacteriumspp	9.18 ± 1.59	8.84 ± 1.8	0.620	11.21 ± 1.62	11.26 ± 1.55	0.941
Firmicutes	7.05 ± 2.02	7.23 ± 1.9	0.818	9.06 ± 1.32	8.85 ± 1.48	0.706
Actinobacter	8.84 ± 1.76	8.92 ± 1.58	0.904	12.2 ± 1.65	12.26 ± 1.52	0.929

*Significant p value, **Highly significant p value.

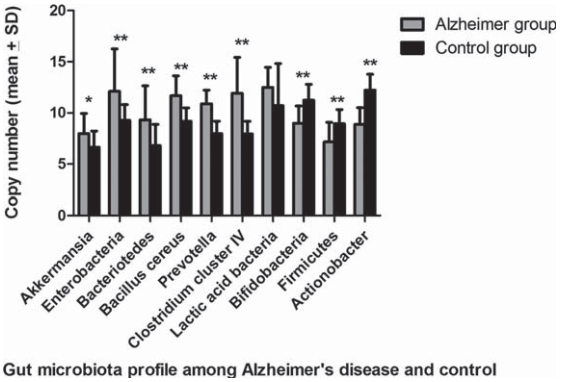

Fig. 1. The composition of the isolated microbiota in both Alzheimer's disease group and the control group.

lactic acid bacteria were significantly less abundant in females than in males in the AD group ($p=0.04$), whereas no such difference was present in the controls (Table 2). In the AD group, the MMSE score had a significant negative correlation with the abundance of lactic acid bacteria ($r=-0.43$ and $p=0.03$) but did not significantly correlate with abundances of another gut microbiota (Table 3). The abundance of *Prevotella* was negatively correlated with MoCA ($r=-0.42$ and $p=0.03$) in the AD group, indicating that a higher abundance of *Prevotella* was associated with greater deterioration of cognitive function. This correlation was not present in the control group (Table 4). In the AD group, a positive correlation was found between the abundance of *Prevotella* and age of onset and duration of illness ($r=0.52$, $p=0.007$ and $r=0.42$, $p=0.03$, respectively) in the AD group (Table 5). There was no significant correlation of microbiota with different risk factors for the AD group except for abundance of *Prevotella*, which significantly correlated with smoking ($r=0.424$, $p=0.035$). In addition, both *Akkermansia* and *Prevotella* abundance showed a significant negative correlation with coronary heart disease ($r=-0.470$, $p=0.018$ and $r=-0.401$, $p=0.047$, respectively) (Table 6).

Table 3
Relation between Mini-Mental State Examination (MMSE) and copy number of each microbiota in Alzheimer disease patients and controls

	Mini-mental state scores			
	Alzheimer disease group ($n=25$)		Control group ($n=25$)	
	r	p	r	p
Akkermansia	0.119	0.571	–0.035	0.867
Enterobacteria	0.068	0.748	–0.172	0.41
Bacteroidetes	–0.329	0.108	0.122	0.561
Bacillus cereus	0.04	0.848	0.056	0.792
Prevotella	–0.312	0.129	0.321	0.118
Clostridium cluster IV	0.306	0.137	–0.067	0.751
Lactic acid bacteria	–0.435	0.030*	0.32	0.119
Bfidobacteriumspp	–0.017	0.935	–0.301	0.144
Firmicutes	–0.304	0.139	–0.135	0.519
Actinobacter	–0.035	0.867	0.011	0.959

*Significant p value.

DISCUSSION

Microbiota dysbiosis has come to be considered an emerging risk factor for AD that can initiate an inflammatory response in the brain. The present study was performed to explore dysbiosis of the gut microbiota in Egyptian patients with AD and the relationship of this microbiota with the clinical presentation of AD and associated cognitive dysfunction.

The most abundant genera in healthy gut flora are *Bacteroidetes*, *Firmicutes, Actinobacteria*, *Proteobacteria*, and *Verrucomicrobia.* A shift in this profile has been observed in several pathological conditions, including neurodegenerative diseases [21, 22] that can affect the intestinal system. To our knowledge, this is the first study to investigate microbial dysbiosis in Egyptian patients with AD.

Table 4
Relation between Montreal cognitive score and copy number of each microbiota in Alzheimer disease patients and controls

	Montreal cognitive score			
	Alzheimer disease group ($n=25$)		Control group ($n=25$)	
	r	*p*	*r*	*p*
Akkermansia	–0.039	0.852	0.137	0.513
Enterobacteria	0.059	0.778	–0.234	0.260
Bacteroidetes	–0.276	0.182	0.007	0.974
Bacillus cereus	–0.138	0.51	0.219	0.293
Prevotella	–0.424	0.035*	0.095	0.246
Clostridium cluster IV	0.376	0.064	0.002	0.994
Lactic acid bacteria	0.255	0.218	0.295	0.153
Bifidobacterium spp	0.301	0.143	–0.159	0.448
Firmicutes	–0.108	0.608	–0.287	0.165
Actinobacter	0.011	0.959	–0.093	0.66

*Significant *p* value.

Table 5
Relationship between gut microbiota and the age of onset and duration of illness in Alzheimer disease patients

	Age of onset (yrs)		Duration of illness (months)	
	r	*p*	*r*	*p*
Akkermansia	0.101	0.632	0.009	0.966
Enterobacteria	0.106	0.615	0.183	0.382
Bacteroidetes	–0.003	0.987	0.014	0.947
Bacillus cereus	0.100	0.633	0.094	0.654
Prevotella	0.529	0.007**	0.421	0.036*
Clostridium cluster IV	0.255	0.218	0.151	0.472
Lactic acid bacteria	0.298	0.149	0.239	0.250
Bifidobacterium spp	0.224	0.281	0.022	0.918
Firmicutes	0.214	0.305	0.084	0.690
Actinobacter	0.046	0.826	0.200	0.339

*Significant *p* value. **Highly significant *p* value.

The causal relation between gut dysbiosis, blood-brain barrier permeability, pro-inflammatory cytokines, and pathogenesis of AD

We determined that a number of species, including *Akkermansia, Enterobacteria*, *Bacteroidetes*, *Bacillus cereus*, *Prevotella*, and *Clostridium* cluster IV, were significantly more abundant in the AD group than in the control group.

Akkermansia utilizes an energy source from the mucus by degrading the mucus layer. Consequently, immune cells are exposed to more microbial antigens, which raise the likelihood of inflammation [23]. Neural injury can also be potentiated by the endotoxins derived from the gut [24]. The high abundance of *Akkermansia* in patients with AD shown in this study could contribute to AD progression with greater exposure to microbial antigens and endotoxins, and subsequently more inflammation. Supporting our result, in Chinese patients with AD, *Akkermansia* abundance was found to be negatively correlated with MMSE, Wechsler Adult Intelligence Scale, and Barthel scores and a link was discovered between *Akkermansia muciniphila* and hippocampus atrophy [25, 26].

In the present study, the significantly higher abundance of *Enterobacteria* in the AD group compared with that in the control group supports the results from Wu et al. (2017) [27]. They found in their *in vivo* study in the Drosophila AD model that *Enterobacteria* increased AD progression by enhancing immune

Table 6
Correlation between different species of microbiota and risk factors of AD Lactic Bacillus acid Clostridium Bifidobacterium cereus

		Akkermansia	Lactic acid bacteria	Prevotella	Clostridium cluster IV	Bifidobacterium spp	Enterobacteria	Bacteroidetes	Firmicutes	Actinobacter	Bacillus cereus group
Smoking	*r*	0.015	–0.076	**0.424***	–0.182	–0.182	0.378	–0.008	–0.015	–0.091	–0.212
	p	0.943	0.719	0.035	0.385	0.384	0.062	0.971	0.943	0.666	0.309
Hypertension	*r*	0.345	0.052	0.065	–0.221	0.046	0.247	0.045	–0.240	0.058	–0.072
	p	0.092	0.805	0.758	0.288	0.829	0.234	0.829	0.247	0.781	0.734
Diabetes	*r*	-0.034	–0.111	0.376	0.154	0.265	0.017	–0.060	0.222	–0.137	0.103
mellitus	*p*	0.871	0.597	0.064	0.463	0.201	0.935	0.776	0.286	0515	0.626
Dyslipidemia	*r*	0.148	–0.019	0.192	–0.037	0.006	0.346	–0.068	–0.019	0.056	–0.062
	p	0.479	0.930	0.359	0.860	0.977	0.090	0.747	0.930	0.792	0.769
Coronary	*r*	**–0.470***	–0.273	**–0.401***	–0.120	0.350	–0.171	0.145	0.188	–0.034	–0.393
heart disease	*p*	0.018	0.186	0.047	0.569	0.086	0.415	0.489	0.369	0.871	0.052

*Significant *p* value.

hemocyte recruitment to the brain, thereby provoking a TNF-JNK mediated neurodegenerative process and an elevated oxidative stress status [27].

Our results regarding *Bacteroidetes* were in line with Vogt et al., who used bacterial 16S rRNA gene sequencing to denote the microbial content of stool specimens from American patients with dementia and identified an abundance of *Bacteroidetes* in comparison with that in healthy controls [28]. The authors suggested that the bacterial outer membrane LPS such as *Bacteroidetes*, enhances systemic inflammation and amyloid deposition, which in turn may contribute to or exacerbate AD pathology [28]. Similarly, another study that compared older persons without dementia or other forms of dementia discovered an increased abundance of *Bacteroides* spp., which act as markers of AD dementia [29]. Zhuang et al. found increased *Bacteroides*, *Ruminococcus*, and *Subdoligranulum*in Chinese patients with AD relative to age and gender-matched healthy controls [30]. In contrast, Li et al. observed a lower *Bacteroides* abundance in patients with AD [25]. The discrepancy between these results could be related to dietary habits and different geographic distribution.

*B. cereus*is identified as a food-borne disease. In addition, it is an opportunistic pathogen that can result in local and systemic infections, especially in immunocompromised patients [31, 32]. Several studies reported central nervous system infections caused by *B. cereus* [32–36] resulting in harmful complications [32, 37]. Bacteremia can be a risk factor for the spread to the central nervous system, particularly in immunosuppressed patients [32, 37].

Increased *Prevotella* abundance in this study was in harmony with results obtained by Guo et al. (2021), who evaluated the gut microbiome in patients with newly diagnosed AD and mild cognitive impairment and found increased *Prevotella* abundance in those with AD [38]. *Prevotella,* as a colonic commensal, can degrade plant polysaccharides and mucosal glycoproteins and also interacts with the immune system [39]. Therefore, *Prevotella* can potentiate the inflammatory responses [40] and contribute to neuroinflammation in patients with AD.

Our result regarding the Clostridium cluster is in accordance with Thursby and Juge (2017) [41]. *Clostridia* could induce neurological insult by generating a high level of noxious metabolites in the brain [42].

For the lactic acid-producing bacteria, we found no significant difference between the groups. *Lactic acid* bacteria have an anti-inflammatory role and induce regulatory T-cells to promote IL-10 production, leading to downregulation of immunity [43]. In addition, dysregulation of amino acid homeostasis induced by lactic acid-producing bacteria can contribute to AD pathogenesis [44]. Lactic acid-producing bacteria alter glutamate metabolism, which modulates GABA levels to induce neural dysfunction [45]. Similar to our finding, quantitative PCR was used to confirm the abundance of *Lactobacillus* in patients with AD [25]. In addition, Zhuang et al. found an increase *Lactobacillaceae* abundance in AD [30]. The absence of changes in lactic acid bacteria abundance in our study may be related to the small sample size. However, the significant negative correlation of lactic acid with the MMSE score recorded in the present study supports the *Lactobacillus* abundance found in patients with AD.

The second important finding in this study is the significantly lower abundance of *Bifidobacterium, Firmicutes*, and *Actinobacteria* in patients with AD compared with that in healthy controls. Our results were in accordance with Voit et al. [28] as they found that the *Firmicutes*, *Actinobacteria*, and *Bifidobacterium* phyla were less abundant in patients with AD than in controls. However, our results disagree with Li et al. [25] who found an increased abundance of *Bifidobacterium.*

The *Bifidobacterium* genusis an important and well-documented beneficial gut flora [46, 47]. Nishiwaki et al. and Stevens et al. found that *Bifidobacterium* produces lactic acid and several vitamins and lowers oxidative stress markers. Thus, the depletion of *Bifidobacterium* in patients with AD may promote oxidative stress and modulate the immune response [48, 49]. In particular, certain species of *Bifidobacterium* have anti-inflammatory properties and can lower intestinal permeability [50].

Additionally, supplementation with *Bifidobacterium* in a mouse model has been shown to decrease intestinal levels of LPS to improve the mucosal barrier [51] and decrease bacterial translocation [52]. LPS has been found to be more abundant in the brains of patients with AD [53, 54], and LPS stimulation leads to enhanced Aβ accumulation [55, 56].

In this study, the *Firmicutes* phylum as a whole was decreased in the AD group. A reduction in *Firmicutes* has been reported in patients with type 2 diabetes [57], obesity [58–60], and Parkinson's disease [5]. Reduced *Firmicutes* abundance compared with that in the control group may affect intestinal barrier integrity and immunological function in the AD group. *Firmicutes* produces SCFAs that have both

anti-inflammatory and antimicrobial properties and maintain intestinal mucosal barrier integrity [61, 62].

According to our findings, *Actinobacter* abundance was reduced in patients with AD, as shown in the Chinese AD microbiome at the phylum level [30]. Although *Actinobacter* represent a small percentage of gut microbiota, they play a vital role in maintaining gut homeostasis and degrade large polysaccharides, produce SCFAs, such as butyrate, and enhance mucin production and immune responses. These functions impact gut permeability, immune system, metabolism, and help maintain an intact gut–brain axis [63]. The low abundance of *Actinobacter* in patients with AD could disturb these functions.

In the present study AD patients have more abundance of harmful gut microbiota (*Akkermansia*, *Enterobacteria*, *Bacteroidetes*, *Prevotella*, and *Clostridium* cluster IV) and lower abundance of beneficial microbiota (*Bifidobacterium, Firmicutes*, and *Actinobacteria).*

Thus, gut dysbiosis induces the decrease of beneficial substances (such as SCFAs and hydrogen) and the increase of harmful substances (such as amyloids), which causes the intestinal mucosal barrier and blood-brain barrier to become more permeable, activates peripheral immune responses, and increases peripheral and central oxidative process levels. Thus, gut dysbiosis contributes to AD pathology progression by increasing amyloid plaque formation, and neuroinflammation,

Confirming our results, the strong correlation between the abundance of gut microbiota and cognitive functions as we observed a strong negative correlation between *Prevotella* abundance and MoCA. Consistent with our results, Guo et al. [38] found a negative correlation between *Prevotella* and the cognitive functions in patients with mild cognitive impairment. We also discovered a strong positive association between *Prevotella* abundance and the age of onset and disease duration, implying that changes in *Prevotella* abundance may be linked to aging and chronic illness. The strongest negative relationships with MMSE, Wechsler Assessment Intelligent Scale, and Barthel scores have been found in Chinese individuals with AD who had *Akkermansia* [25].

The causal relationship between gut dysbiosis, neurotransmitter, and pathogenesis of AD

On the other hand, the ability of gut microbiota to produce bioactive metabolites that regulate neurotransmitter may help to understand the pathogenesis of AD. As different bacterial genera and species (*lactobacillus and Bifidobacterium*) produce gamma-amino butyric acid (GABA) which predominant inhibitory neurotransmitter in the nervous system that regulate mood and behavioral and cognitive function [64].

Others (Escherichia, Enterococcus, Lactococcus, Lactobacillus) produce serotonin (5-HT) and dopamine [65] which are all involved in a range of mood-related, behavioral, and cognitive functions, and learning as neurotransmitters or neurotransmitter precursors [66, 67]. Lactobacillus, Bacillus produce acetylcholine as a metabolite which has main effect on cognition and memory [68]. *Bacteroides, Bifidobacterium, Lactobacillus, Clostridium*, and *Prevotella* produce SCFAs as metabolites that decrease permeability of the blood-brain barrier, promote the synthesis and secretion of neurotransmitters and hormones, reduce inflammation [69].

The relation between risk factors associated with AD and gut dysbiosis

In this study, smoking was negatively correlated with abundance of *Prevotella*. This is similar to results from Shanahan et al. and Moon et al. who demonstrated a lower abundance Prevotella in smokers [70, 71]. Chronic cigarette smoking induces dysbiosis of intestinal microbiota [72]. Huang and Shi (2019) reported an association between smoking and the diversity of microbiota. The mechanisms could be due to exposure to the bacteria in cigarettes or may be related to the immunosuppressive nature of tobacco, which impairs antimicrobial defenses [73]. However, Sabia et al. (2012) found that; in men, current smokers had a greater 10-year decline in global cognition, compared with those who had never smoked [74]. All the above results imply that smoking can induce both gut dysbiosis and cognitive decline and thus enhance dementia.

The significant negative correlation between *Prevotella* and *Akkermansia* abundance with coronary heart disease that were recorded in this study was partially consistent with Liu et al. who concluded that patients with coronary heart disease had a significantly lower abundance of *Bifidobacterium* and *Prevotella* [75]. In a recent systematic review involving coronary heart disease and risk for dementia, the authors found that coronary heart disease was prospectively associated with developing cognitive impairment or dementia [76]. The insight of causal

mechanisms or common pathways underlying the heart-brain connection is possibly due to alteration of gut microbiota.

Dementia is associated with diabetes and insulin resistance [77, 78]. Insulin resistance has been linked to decreased brain glucose metabolism and higher amyloid deposition, both of which raise the risk of AD [79, 80]. As a result, the development of insulin resistance and diabetes may represent a potential method through which microbial dysbiosis in the gut influences AD pathogenesis. The small number of diabetic patients with AD in this study may contribute to the lack of significant difference between AD patients with versus those without diabetes mellitus.

The pattern of microbiota dysbiosis is inconsistent among different studies, and this may be particularly due to the different associated risk factors that described above and other factors such as differences in dietary habits, and lifestyle habits (exercise and smoking) environmental factors, stress, obesity, and metabolic diseases, other causes of dementia, patients under treatment with antibiotics or probiotics may alter bacterial composition and diversity [81, 82]. In the present study we excluded most of these factors (other types of dementia, resulting from head injury, cerebrovascular stroke, encephalitis, metabolic disorder, type-1-diabetes, or irritable bowel syndrome) even patients under treatment with antibiotics or probiotic supplements were also excluded.

Unfortunately, we did not study other factors. For example, individuals eat a diet high in fat and sugar, which may be related to a rise in the incidence of obesity, and inflammatory bowel diseases. Sen et al. found that microbiota dysbiosis could be related to dietary changes caused by consuming a high sugar diet with a subsequent increase in gut inflammation, microglia activity, and disturbed gut–brain axis [83].

Lifestyle habit may play a role in this difference as smoking has a significant impact on gut microbiota composition, increasing *Bacteroides,* and-*Prevotella* in healthy persons [84] as previously described.

Stress, as lifestyle component, influences colonic motor activity via the gut-brain axis, which can alter gut microbiota profiles, including decreased levels of potentially helpful *Lactobacillus* [85]. Because the gut-brain axis is bidirectional, encompassing both hormonal and neuronal pathways [86], changes in gut microbiota can alter brain function, including mood [87]. Obesity is linked to high energy intake and sedentary lifestyles. Lack of exercise may significantly impact any changes in microbial populations associated with obesity. A previous study found an increase in the variety of gut microbial species in professional athletes due to exercise and the related diet [88]. Shifts in gut microbial populations occur in obese individuals and with increases in *Firmicutes* and decreases in *Bacteroidetes,* which may contribute to adiposity through increased energy harvest [60, 89, 90]. However, additional evidence suggests that alterations in microbial populations are predominantly mediated by high-fat obesogenic diets [91, 92]. Geography also has a strong bearing on the composition of gut microbial populations as supported by the Yatsunenko et al. study (2012). They found that type of fecal bacteria and their functional genes differed between individuals in the USA and in rural areas of Venezuela and Malawi [93].

Therefore, the difference in the relation between microbiota and AD in different studies could be related to several factors that alter the microbiota composition.

Limitations and recommendation of this study

The small sample size is one of the limitations of the current study. It is also a single center study conducted in Aswan Hospital which is predominantly a rural region, where the population consumes high dietary fibers which could change the microbiota composition compared to the western diet [83, 94, 95]. Therefore, multicenter studies including patients of different dietary habits and demographics will be required. In addition, the AD patients in this study were diagnosed by clinical manifestations, not by CSF AD core biomarkers or Aβ-PET. Another restriction is that other microbiota and taxa, such as fungal, were not examined. Longitudinal studies with a larger sample size diagnosed by CSF AD core biomarkers or Aβ-PET are required to confirm the diagnosis of AD current findings and further understand the impact of gut microbiota on AD. Furthermore, future controlled trials on dietary modifications targeting the gut microbiota are necessary to assess the mechanisms of diet-induced gut dysbiosis and the development of AD so that dietary-based intervention management strategies for AD can be developed.

Conclusion

The current study revealed a significantly higher abundance of *Akkermansia*, *Enterobacteria, Bacteroidetes*, *Bacillus cereus*, *Prevotella*, and *Clostridium* cluster IV and a lower abundance of *Bifidobacterium* spp., *Firmicutes*, and *Actinobacteria.* Lactic

acid bacteria and *Prevotella* were negatively correlated with cognition. The abundance of *Prevotella* species was significantly correlated with age of onset, duration of AD symptoms, smoking, and coronary heart disease.

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