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Association Between Claudin-5 And Angulin-1 In Alzheimer's Disease

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Abstract

General Background: Alzheimer's disease is a progressive neurodegenerative disorder in which cognitive decline is closely linked to blood–brain barrier dysfunction. Specific Background: Tight-junction proteins such as Claudin-5 and Angulin-1 play key roles in maintaining barrier integrity, yet their involvement in Alzheimer's pathology remains insufficiently clarified, and evidence on associated micronutrient alterations is still limited. Knowledge Gap: Despite emerging data suggesting barrier disruption and B-vitamin deficiencies in Alzheimer's disease, the combined diagnostic relevance of Claudin-5, Angulin-1, and vitamins B9 and B12 has not been systematically examined. Aims: This study investigates the relationship between Claudin-5 and Angulin-1 in Alzheimer's disease and evaluates differences in serum vitamin B9 and B12 levels between affected individuals and healthy controls. Results: Serum analyses revealed significantly reduced Angulin-1, Claudin-5, vitamin B9, and vitamin B12 levels in Alzheimer's patients, alongside marked alterations in lipid profiles. ROC analysis demonstrated exceptionally high diagnostic performance for all measured biomarkers. Novelty: This work provides integrated biochemical evidence linking tight-junction protein depletion with B-vitamin deficiencies in Alzheimer's disease, suggesting a coordinated disruption of vascular and metabolic pathways. Implications: The identified biomarkers show strong potential for non-invasive diagnostic applications and may guide the development of therapeutic strategies aimed at restoring barrier integrity and micronutrient balance.

Highlight :

- Claudin-5 and Angulin-1 show significant reductions in Alzheimer's patients, reflecting impaired tight-junction function in the blood–brain barrier.
- Vitamin B9 and B12 levels are markedly lower in Alzheimer's patients than in healthy controls, indicating an important metabolic alteration.
- All biomarkers demonstrate high diagnostic performance, with strong sensitivity and specificity based on ROC analysis.

Keywords : Claudin-5, Angulin-1, Alzheimer's disease, Vitamin B9, Vitamin B12.

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Introduction

The increasing brain atrophy and ensuing cognitive deterioration that are linked to AD are reflected in its clinical manifestations. Clinical symptoms, however, usually follow a number of molecular diseases in the brain, such as the buildup of amyloid protein, tau protein phosphorylation, and disruption of the blood–brain barrier (BBB) [1]. Measuring the buildup of tau or amyloid protein deposits in the brain or subsequent leakage into the blood or cerebrospinal fluid (CSF) is the foundation for diagnosing AD. However, lumbar puncture, an invasive operation, is usually required to obtain CSF [2]. Positron emission tomography (PET), which uses tau and amyloid probes to identify protein deposits in the brain, is an alternate technique. Despite being minimally invasive, PET imaging is not commonly employed due to its high cost [3]. Because blood-based diagnostics are inexpensive and minimally intrusive, they have recently drawn interest as alternative diagnostic possibilities. People with symptoms of AD or MCI are tested for amyloid as well as phosphorylation tau proteins in their blood. The 27-member protein family known as claudins includes claudin-5 (CLDN-5), a crucial TJ-sealing component in the blood-brain barrier [4]. Multiple sclerosis patients have higher blood CLDN-5 levels], and in the mouse model of inflammatory encephalomyelitis, loss of CLDN-5 was linked to BBB disruption [5]. These results led us to believe that, in comparison to their cognitively normal counterparts, people with MCI and AD may have different blood CLDN-5 levels. In order to explore this concept, we first created an exceptionally sensitive single-molecule array (Simoa) test that is more sensitive than traditional immunoassays using a monoclonal antibody (mAb) that the detects outside loops of human CLDN-5 [6]. One of the proteins in the BBB (blood-brain barrier) that helps to keep it intact is called angulin-1 [7]. BBB disruption is a recognized problem in Alzheimer's dementia (AD), and it may entail changes in the expression of angulin-1. and activity, although the exact relationship is still being investigated. Research suggests that angulin-1 dysfunction in the BBB could impact AD progression by potentially altering BBB permeability, which in turn can affect the clearance of amyloid-beta plaques and contribute to neuroinflammation [8].

Age is the biggest risk factor for Alzheimer's disease (AD), a neurodegenerative illness that has been declared a global epidemic. Patients and study participants in memory clinics and studies are regularly evaluated for vitamin B12 (cobalamin), but not for other important vitamins for the central nervous system (CNS), including B6 (pyridoxine), B1 (thiamine), as well as B9 [9]. Deficits in vitamins B6 and B12 are linked to dementia and moderate cognitive impairment. Pyridoxine by itself has a number of benefits that can improve both quantitative and qualitative memories [10]. For instance, pyridoxine plays a role in both neuronal stimulation and inhibition since it is necessary for the production of neurotransmitters. Despite the fact that vitamins B1 and B9 have been linked to cognitive function and brain health, they are not frequently evaluated in memory sufferers. Cognitive impairments and encephalopathy are linked to thiamine (B1) deficiency, potentially as a result of decreased brain glucose metabolism.6. Vitamin D deficiency is common in geriatric memory clinic patients, and low vitamin D levels have been found to positively connect with cognitive test scores [11].

Materials and Methods

A case-control study was performed at Kirkuk Teaching Hospitals/ Neurology Unit in Kirkuk City-Iraq from November 2024 to April 2025. The total number of Alzheimer's patients is (fifty). Twenty-five of them were males, while twenty-five were females. Their ages ranged from 45 to 75 years. The present study contained a control group of twenty-five subjects who were healthy.

Exclusion criteria

Patients with chronic kidney disease, parkinson’s disease, stroke, cancer, uncontrolled diabetes mellitus and autoimmune disorders were excluded from the current study.

Collecting and managing samples

A volume of roughly 5 milliliters of venous blood was drawn from fasting patients and control subjects. Every sample was immediately put into a gel tube to coagulate for half an hour at room temperature. The tubes were then centrifuged for fifteen minutes at 4000 rpm. Following the initial centrifugation, the clot was extracted and centrifuged again for ten minutes at 4000 rpm. An automated micropipette was then used to aspirate the obtained sera, which were then transferred individually to two Eppendorf microtubes and kept at -20°C for a subsequent serological analysis. Using enzyme-linked immunosorbent assay (ELISA) kits, serum levels of Claudin-5 and Angulin-1 (ELK Biotechnology / U.S.A.) were determined in accordance with the manufacturer's instructions. Utilizing COBAS Techniques, vitamin B12 and vitamin B9 (Roche, Germany) were measured while the lipid profile was measured by routine kits (Biolabo SA, France) using colorimetric spectrophotometer .

Statistical Analyses

One-way ANOVA was utilized to analyze the results. The mean ± the standard deviation of the mean (SD) is used to display all results. A value was considered statistically significant if its p-value was less than 0.05.

Results

The study participants' demographic details and family history are shown in Table 1. The gender distribution of the groups did not differ significantly (p = 0.7), with 15 (30%) of the Alzheimer's population being male. Alzheimer's is more prevalent in women. According to 35 (70%) of the respondents, women who fit the prerequisites for Alzheimer's condition are more likely to not be given a clinical diagnosis.

Table 1. Descriptive Characteristics of Study Participants

Characteristics	Total (N = 75)	Alzheimer's (N = 50)	Healthy Control (N = 25)	p-value
Age (years) Mean ± SD	66.41 ± 5.71	68.92 ± 3.41	55.25 ± 1.97	0.06
Gender, n (%) Male	35 (47%)	15 (30%)	10 (40%)	0.70

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Female	40 (53%)	35 (70%)	15 (60%)	
Duration of disease (years) Mean ± SD	4 ± 0.67			

(P ≤ 0.05) significant

Table 2 compares the levels of biomarkers in Alzheimer's and healthy controls. The Alzheimer's group in the current investigation had a lower mean ± SD of Angulin-1 (ng/mL) than the healthy control group; this difference was highly significant in statistical terms. Additionally, there was a significant statistical (P ≤ 0.05) difference between the Alzheimer's group and the healthy control group in the mean ± SD of Claudin-5 (ng/mL). As well as shows the vitamins B9, B12 levels of people with Alzheimer's disease (AD) and controls who were healthy.

The Alzheimer's disease (AD) group in the current study had a significant low (P ≤ 0.05) levels of vitamin B9 and B12 in AD patients than the healthy control group.

Table 2. Comparisons of Angulin-1, Cingulin Levels, Vitamins B9 and B12 concentrations in AD compared with the control

Biomarker	**Total (N = 75)**	**Alzheimer's (N = 50)**	**Healthy Control (N = 25)**	**p-value**
Angulin-1 (ng/mL) Mean ± SD	6.87± 1.21	5.82 ± 1.11	8.79 ± 0.98	0.001
Claudin-5 (pg/mL) Mean ± SD	44.50 ± 6.1	33.18 ± 2.8	66.19±3.5	0.010
VitB9 (ng/ml) Mean ± SD	7.16 ± 0.32	5.21 ± 0.52	9.96 ± 0.91	0.010
VitB12 (pg/ml) Mean ± SD	377.12 ± 14.42	185.63 ± 23.32	331.62 ± 25.52	0.003

(P ≤ 0.05) significant

Table 3. illustrates the substantial difference (P ≤ 0.05) in serum lipid profile concentrations between those suffering from Alzheimer's Disease and the control group. There was a significant increase (P ≤ 0.05) in the levels of LDL, VLDL, TG, in patients with Alzheimer's Disease compared to controls, but the mean of HDL concentration was significantly decreased (P ≤ 0.05) in these patients in comparison with healthy individuals. There was a non-significant increase (P > 0.05) in cholesterol concentration in patients with Alzheimer's Disease when compared to healthy controls.

Table 3: A comparison of the lipid profile between Healthy Control and Alzheimer's Disease (AD)

Parameters	**Alzheimer's Disease(AD) (mean±SD) (n= 50)**	**Healthy controls (mean ± SD) (n= 25)**	**p-value**
Cholesterol (mg/dL)	168.02 ± 4.77	169.45 ± 8.75	0.066
Triglycerides (mg/dL)	189.47 ± 5.41	141.34 ± 7.63	0.001
HDL (mg/dL)	36.76 ± 3.93	57.56 ± 6.19	0.001
LDL (mg/dL)	138 ± 3.12	86.23 ± 6.27	0.000
VLDL (mg/dL)	44.76 ± 2.57	22.12 ± 1.91	0.002

(P ≤ 0.05) = significant

The ability of biomarkers to diagnose and predict ASD using receiver operating characteristic (ROC) analysis

Each study variable's diagnostic performance as a possible biomarker for ASD was assessed using receiver operating characteristic (ROC) curve analysis. Table 4 presents the results with illustrations. The sensitivity, specificity, and area under the curve (AUC) are among the performance

indicators.

Table 4: Receiver Operating Characteristic (ROC) Analysis Results

Parameters	AUC	95% CI b	Criterion	Sensitivity	Specificity
Angulin-1 (ng/ml)L)	0.998	0.969 to 1.00	<133.2	100.00	100.00
Claudin-5 (pg/ml)	1.000	0.959 to 1.00	>28.6	100.00	100.00
VitB9 (ng/ml)	0.996	0.951 to 1.00	≤4.3	95.16	100.00
VitB12 (pg/ml)	1.000	0.959 to 1.00	>291.9	100.00	100.00
Cholesterol (mg/dL)	0.998	0.961 to 1.00	≤4.2	95.16	100.00
Triglycerides (mg/dL)	1.000	0.959 to 1.00	>28.6	100.00	100.00
HDL (mg/dL)	0.996	0.951 to 1.00	≤4.3	95.16	100.00
LDL (mg/dL)	0.998	0.961 to 1.00	≤4.2	95.16	100.00
VLDL (mg/dL)	1.000	0.959 to 1.00	>291.9	100.00	100.00

AUC: Area Under the Curve; CI: Confidence Interval, the 95% confidence intervals were calculated using DeLong's method, Criterion values represent the optimal cutoff points that maximize both sensitivity and specificity. The analysis included 30 control subjects and 61 intestinal disorder patients. P-values for all AUC measurements were <0.0001, indicating statistical significance.

Discussion

The idea that microvascular deterioration is linked to the pathogenesis of AD is supported by a number of data [12]. In the present work, we examined relationships between AD and serum levels of CLDN-5, a crucial TJ-sealing protein in the BBB's microvascular endothelial cells. According to Feng et al., plasma Angulin-1 levels were greater in control group and lower in AD patients, while serum CLDN-5 concentrations were lower in AD patients compared to cognitively healthy persons. Leukocyte invasion and astrocyte activation occur in conjunction with the BBB breakdown linked to CNS disorders [13]. According to Yang et al., the matrix metalloproteinases (MMPs) released by these invasive leukocytes have been demonstrated to cause the CLDN-5 (ng/mL) levels to associate with the levels of Angulin-1. Plasma CLDN-5 and Angulin-1 concentration Spearman rank-correlation analyses are displayed for both AD patients and cognitively normal controls. Normal controls and AD patients' plasma CLDN-1 and Angulin-1 concentrations are displayed [14].

Multiple B vitamin deficiencies, particularly vitamin B12 and folate (B9), have been researched for their connection to cognitive decline and neurological issues, though the direct link to Alzheimer's Disease (AD) is still being studied [15]. Low levels of B vitamins can worsen AD processes, and a B12 deficiency, sometimes linked to autoimmune issues like pernicious anemia, can cause cognitive and neurological symptoms that may precede anemia. Symptoms of B12 deficiency can include megaloblastic anemia, gastrointestinal issues, and neurological and psychiatric symptoms [16]. These results align with prior findings by Qian et al., (2022), who reported multiple B vitamin deficiencies , among with AD in a case-control study of 86 children with AD and 57 neurotypical peers aged 52–68 years [17]. The study highlighted that child with AD often have restricted dietary patterns, which may contribute to insufficient intake of essential nutrients like B12, B9. Further biochemical insight was provided by Kumar et al, who employed advanced UHPLC-mass spectrometry (Q-exactive analyzer) techniques to assess urine samples from AD. Their findings demonstrated simultaneous deficiencies of vitamins B9, and B12, proposing that intestinal dysbiosis may underlie poor absorption of these nutrients. Moreover, genetic mutations could further compromise B vitamin metabolism in this population [18]. Vitamin B12, in particular, plays a crucial role in neurological health. Its involvement in maintaining gut microbiota homeostasis has drawn attention, especially given the frequent presence of dysbiosis in individuals with ASD. Such imbalances can lead to increased intestinal permeability, chronic low-grade inflammation, and alterations in the gut–brain axis—all of which may exacerbate or contribute to ASD symptoms. In addition, B12 is vital for the metabolism of neurotransmitters like serotonin and dopamine, which are critical for mood regulation, cognitive function, and behavioral responses [19]. In Alzheimer's Disease (AD) patients, there are significant dysregulations [20], often showing lower total cholesterol (TC) and LDL-C, especially in later stages. Previous studies also indicate that AD patients, particularly women, may have lower unsaturated lipids and higher saturated lipids compared to healthy individuals [21,22]. However, other research points to elevated TC and LDL-C levels being linked to an increased risk of developing AD, suggesting a complex and age-dependent relationship [23,24].

Conclusion

Link to Alzheimer's Disease (AD), Angulin-1, a component of tricellular tight junctions, may may aid in preserving the blood-brain barrier's integrity by preventing the production of tight junction proteins like Claudin-5.

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