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ORIGINAL ARTICLE

Comparative Analysis of Oxidative Stress, Inflammatory Markers, and Neurotrophic Factors in Healthy Controls, Vascular Dementia, and Alzheimer's Dementia

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Abstract

Introduction: Dementia is a prevalent neurodegenerative condition, with Alzheimer's dementia (AD) and vascular dementia (VD) being the two most common subtypes. Despite shared cognitive symptoms, AD and VD have distinct pathophysiological mechanisms, necessitating different approaches for diagnosis and treatment. This study investigates oxidative stress markers, inflammatory cytokines, and neurotrophic factors to identify biomarkers that may differentiate VD from AD, supporting more accurate diagnosis and targeted therapies.

Methods: A total of 45 participants were grouped into healthy controls (HC), VD, and AD. Serum samples were analyzed for oxidative stress markers (TAS, TOS, OSI), thiol-disulfide balance, inflammatory cytokines (IL-1β, IL-6, TNF-α), and neurotrophic factors (GDNF). The data were statistically evaluated to compare biomarker profiles across groups and identify significant variations.

Results: AD patients exhibited significantly elevated oxidative stress markers (TOS and OSI) and disrupted thiol-disulfide homeostasis compared to VD and HC, suggesting a pronounced oxidative imbalance. Additionally, inflammatory markers (IL-1β and TNF-α) were highest in AD, indicating a heightened neuroinflammatory response relative to VD. GDNF levels were elevated in both AD and VD compared to HC, suggesting a potential compensatory neuroprotective response, although levels were higher in VD.

Discussion and Conclusion: The findings highlight oxidative stress and neuroinflammation as prominent features of AD, with VD displaying relatively lower oxidative markers. Elevated GDNF in both dementia types suggests that neurotrophic support mechanisms may play a role in counteracting neurodegeneration. Differences in thiol-disulfide balance and inflammatory cytokines between VD and AD may also reveal disease-specific mechanisms that could aid in differential diagnosis. This study identifies distinct biomarker profiles in AD and VD, emphasizing the potential for specific oxidative and inflammatory markers to differentiate these conditions. Further research may validate these findings and contribute to developing targeted therapeutic interventions for each dementia subtype.

Keywords: Alzheimer's dementia; biochemical markers; biomarkers; comparative analysis; inflammation; neurotrophic factors; oxidative stress; vascular dementia.

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Dementia, a major global health concern, includes various neurodegenerative conditions. Alzheimer's dementia (AD) and vascular dementia (VD) are the most common subtypes, accounting for the majority of cases worldwide[1]. Although AD and VD share overlapping symptoms like memory impairment and cognitive decline, they stem from distinct pathophysiological mechanisms. AD is primarily a neurodegenerative disorder marked by hallmark neuropathological features such as amyloid-beta (Aβ) plaques and neurofibrillary tangles of hyperphosphorylated tau protein, which disrupt synaptic function and contribute to neuronal loss[1,2]. VD, on the other hand, arises from cerebrovascular pathology and is the second most common cause of dementia after AD, accounting for approximately 15-20% of cases[3]. It encompasses a spectrum of conditions, including multi-infarct dementia, strategic infarct dementia, and subcortical ischemic vascular dementia, all of which involve disrupted cerebral blood flow leading to ischemic brain damage and cognitive decline.

In VD, the primary pathological processes involve damage to the small blood vessels in the brain, leading to microinfarcts, lacunar infarctions, and white matter lesions[4]. This vascular insufficiency impairs oxygen and nutrient delivery to neurons, ultimately resulting in neuronal dysfunction and cognitive impairment[5]. In contrast, AD is largely associated with neuronal death due to toxic protein aggregates, such as Aβ and tau, which trigger oxidative stress, inflammation, and synaptic loss[6]. Despite these distinct mechanisms, the overlapping clinical features between AD and VD often pose diagnostic challenges, emphasizing the need for specific biomarkers to differentiate these conditions.

Oxidative stress, inflammation, and neurotrophic factors are critical in both AD and VD progression, though underlying mechanisms differ[7]. Oxidative stress results from an imbalance between reactive oxygen species (ROS) and antioxidant defenses, contributing to pathogenesis in both dementia types. In AD, oxidative damage is often linked to Aβ accumulation and mitochondrial dysfunction, accelerating neuronal injury and cognitive decline[8,9]. Meanwhile, ischemia-related oxidative stress in VD leads to endothelial damage and inflammation, worsening cerebrovascular pathology and cognitive decline[10,11]. By analyzing oxidative stress markers such as Total Antioxidant Status (TAS), Total Oxidative Status (TOS), and the Oxidative Stress Index (OSI), this study aims to clarify oxidative stress profiles across HC, VD, and AD, potentially aiding in differential diagnosis.

Inflammation is another shared feature, yet it operates through different processes. AD is marked by microglial activation and cytokine release in response to Aβ plaques, intensifying neuroinflammation and neurodegeneration[12,13]. VD, however, experiences inflammation mainly due to vascular pathology, with ischemic damage and endothelial dysfunction leading to elevated cytokines such as IL-1β, IL-6, and TNF-α, which contribute to cognitive decline[14].

Neurotrophic factors, such as Glial Cell Line-Derived Neurotrophic Factor (GDNF), are crucial for neuronal survival and plasticity and have been implicated in compensatory mechanisms to counteract neuronal damage in both AD and VD[15]. Elevated GDNF levels in dementia patients may reflect an endogenous neuroprotective response, though the extent and implications of these elevations may vary between AD and VD, depending on the underlying pathophysiological processes[16].

We hypothesize that inflammatory cytokines (TNF-α, IL-1β), oxidative stress markers (Native Thiol [NT], Oxidative Stress Index [OSI]), and neurotrophic factors (GDNF) will be key distinguishing biomarkers between the groups. Specifically, TNF-α and IL-1β levels are expected to be significantly elevated in AD and VD compared to healthy controls (HC), with the highest levels in VD due to vascular inflammation. NT levels are anticipated to be highest in HC, moderately reduced in VD, and most disrupted in AD due to amyloid-beta-driven oxidative stress, while OSI is expected to be highest in AD, reflecting greater redox imbalance. GDNF levels are hypothesized to be elevated in both AD and VD compared to HC, with potentially higher levels in VD, reflecting its role in mitigating vascular-associated neuronal damage. These biomarkers are expected to exhibit distinct patterns across the groups, enabling differentiation of AD, VD, and HC.

By examining the roles of oxidative stress markers, inflammatory cytokines, and neurotrophic factors, this study aims to elucidate the distinct biochemical profiles of AD and VD, which could facilitate more accurate differentiation between these dementia subtypes and inform tailored therapeutic strategies.

Materials and Methods

Subjects

A priori power analysis was conducted using G*Power 3.1 to determine the minimum required sample size for detecting statistically significant differences among the three groups (HC, VD, and AD). Based on a large

effect size of 0.4 for oxidative stress and inflammatory biomarkers, a statistical power of 0.80, and a significance level of α=0.05, the estimated required sample size was 42 participants. Our study included a total of 45 participants (15 per group), meeting the required sample size to ensure sufficient statistical power. However, future studies with larger cohorts are recommended to further validate and generalize these findings. While the methods, such as ELISA, are well described, providing more detailed protocols for each biomarker would enhance the study's reliability and reproducibility.

A total of 45 participants were recruited from the University of Health Sciences, Faculty of Medicine. The sample included 15 healthy controls (6 males, mean age±SD: 61.8±5.9 years, range 55-74), 15 vascular dementia patients (6 males, mean age±SD: 63.5±6.2 years, range 56-75), and 15 Alzheimer's dementia patients (8 males, mean age±SD: 62.9±6.5 years, range 55-76). Inclusion criteria for dementia patients required a confirmed diagnosis of VD or AD based on established clinical criteria. The severity of dementia in the patient groups was classified using standardized clinical scales. In the AD group, disease severity was assessed using the Mini-Mental State Examination (MMSE), with scores ranging from 10 to 20, indicating moderate cognitive impairment. For the VD group, severity was determined using a combination of the Hachinski Ischemic Score (HIS) and MMSE. MMSE scores for this group ranged between 12 and 22, reflecting mild to moderate cognitive impairment. Healthy controls had no history of neurological or psychiatric disorders. Exclusion criteria for all groups included significant comorbidities (e.g., cardiovascular disease, metabolic syndrome), recent infections, or any conditions potentially affecting inflammatory or oxidative stress markers. Written informed consent was obtained from all participants prior to enrollment, and the study followed the ethical guidelines of the Declaration of Helsinki. The institutional ethics committee approved the study (Ethical Report number: 30.10.2024-32749).

Collection of Blood Samples

Venous blood samples (approximately 5 mL) were drawn from each participant's cubital vein under sterile conditions. To prevent clotting, samples were immediately transferred into sterile gel biochemistry tubes. They were centrifuged at 3000×g for 10 minutes using a Beckman Coulter Allegra® X-30 centrifuge to separate the serum. The resulting serum was then aliquoted and stored at -80°C to maintain analyte integrity until biochemical analyses were conducted.

Analysis of Oxidative Stress Levels

Total Antioxidant and Oxidant Status

TAS and TOS levels in serum samples were measured using a colorimetric method developed by Erel (2004, 2005).[17,18] The TAS assay determines the sample's antioxidant capacity by assessing its ability to reduce the dark blue-green radical 2,2-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) to a colorless form. Conversely, TOS evaluates oxidant capacity by measuring the oxidation of a ferrous ion complex to ferric ions, which subsequently forms a colored complex with xylenol orange in an acidic medium.

Both TAS and TOS were measured using a Rel Assay kit, following the manufacturer's protocol. TAS results were expressed as ascorbate equivalents per liter (Ascorbate Eq./L) and read at 660 nm, while TOS values were expressed in micromoles of hydrogen peroxide equivalents per liter (μmol H_2O_2 Eq./L) and read at 560 nm. Measurements were taken with a BioTek Synergy™ HTX Flash Multimode Reader. The Oxidative Stress Index (OSI), reflecting overall oxidative stress, was calculated as the TOS-to-TAS ratio and reported in arbitrary units (AU).

Thiol-Disulfide Homeostasis

Thiol-disulfide homeostasis, reflecting serum redox balance, was assessed using the colorimetric method developed by Erel and Neşelioğlu.[19] In this method, total thiol groups are measured by reducing disulfide bonds to free thiols with sodium borohydride ($NaBH_4$). Excess $NaBH_4$ is then neutralized with formaldehyde to prevent interference in the measurements. The thiol groups, including both native and total thiols, are subsequently quantified by reacting them with 5,5'-Dithio-bis(2-Nitrobenzoic Acid) (DTNB), which causes a measurable color change.

Total and native thiol levels were determined using a Rel Assay kit, adhering to the manufacturer's instructions, and results were expressed in micromoles per liter (μmol/L). Disulfide concentration was calculated as half the difference between total and native thiol levels. To further evaluate the thiol-disulfide balance and oxidative status, ratios such as disulfide/native thiol, disulfide/total thiol, and native thiol/total thiol were also calculated.

Enzyme-Linked Immunosorbent Assay (ELISA)

Serum concentrations of human glial cell line-derived neurotrophic factor (GDNF, BTLAB – E0122Hu), interleukin-1β (IL-1β, BTLAB - E0143Hu), interleukin-6 (IL-6, BTLAB - E0090Hu), and tumor necrosis factor-alpha (TNF-α,

BTLAB - E0082Hu) were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits. All assays were performed following the manufacturer's instructions to ensure consistency and accuracy.

For each sample, 40 µL of serum, 10 µL of specific antibody for the target analyte, and 100 µL of horseradish peroxidase (HRP) conjugate were added to microplate wells. After a 60-minute incubation at 37°C, wells were washed five times with 300 µL of wash solution to remove unbound components. Subsequently, 50 µL of substrate solutions A and B were added to each well to induce color development, followed by a 10-minute incubation in the dark at 37°C. The reaction was halted by adding 50 µL of stop solution to each well, and absorbance was measured at 450 nm using a BioTek Synergy™ HTX Flash Multimode Reader. Concentrations of IL-1β, IL-6, GDNF, and TNF-α were determined by comparing sample absorbance values to standard curves generated from known concentrations of each analyte.

Statistical Analysis

Statistical analyses were conducted using Jamovi (version 2.3.21) to compare biochemical markers among HC, VD, and AD groups. Data normality and homogeneity were assessed using the Shapiro-Wilk test. One-way ANOVA was performed to evaluate group differences in oxidative stress markers (TAS, TOS, OSI), thiol-disulfide balance indicators (TT, NT, DIS, %NT/TT, %DIS/TT, %DIS/NT), inflammatory markers (IL-1β, IL-6, TNF-α), and neurotrophic factors (GDNF). For significant ANOVA results, Tukey's HSD post-hoc tests were applied to identify specific group differences. Effect sizes were reported as η^2, with statistical significance set at $p<0.05$. Mean±SD values, as well as p-values and t-values for each group comparison, are presented in Table 1.

Results

Our analysis revealed statistically significant differences in oxidative stress markers, thiol-disulfide homeostasis, inflammatory cytokines, neurotrophic factors, and ischemia-modified albumin (IMA) levels across the HC, VD, and AD groups. These findings highlight distinct biochemical profiles associated with each group, potentially offering insights into disease-specific mechanisms and aiding in the differential diagnosis between VD and AD.

Oxidative Stress Markers

The HC group demonstrated significantly higher TAS levels (1.075±0.146) compared to both VD (0.949±0.149, p=0.036) and AD (0.892±0.106, p=0.002), indicating reduced antioxidant defenses in the dementia groups. TOS levels were significantly elevated in the AD group (16.185±1.187) compared to HC (10.747±2.488, p<0.001)

Table 1. Comparison of Oxidative Stress, Inflammatory Markers, and Neurotrophic Factors among HC, VD, and AD: The table presents the mean values and standard deviations (Mean±SD) of various biochemical markers in serum samples from three groups: healthy controls (HC), vascular dementia (VD), and Alzheimer's dementia (AD). Statistical significance between groups (HC vs. VD, HC vs. AD, and VD vs. AD) is provided as p-values and t-values (p (t)).

Items	HC Mean±SD	VD Mean±SD	AD Mean±SD	HC vs VD p (t)	HC vs AD p (t)	VD vs AD p (t)
TAS	1.075±0.146	0.949±0.149	0.892±0.106	0.036 (2.57)	0.002 (3.73)	0.483 (1.16)
TOS	10.747±2.488	13.546±3.08	16.185±1.187	0.007 (-3.21)	<0.001 (-6.24)	0.011 (-3.03)
OSI	10.129±2.584	14.509±3.578	18.386±2.514	<0.001 (-4.09)	<0.001 (-7.71)	0.002 (-3.62)
TT	571.058±64.154	503.686±47.961	476.934±64.586	0.009 (3.11)	<0.001 (4.34)	0.441 (1.23)
NT	432.959±43.416	254.139±46.952	196.473±34.891	<0.001 (11.6)	<0.001 (15.4)	0.002 (3.75)
DIS	69.049±42.7	124.773±33.444	140.23±38.339	<0.001 (-3.98)	<0.001 (-5.08)	0.517 (1.1)
%NT/TT	76.96±13.186	50.849±10.669	42.065±10.081	<0.001 (6.28)	<0.001 (8.39)	0.1 (2.11)
%DIS/TT	11.52±6.593	24.576±5.335	28.967±5.041	<0.001 (-6.28)	<0.001 (-8.39)	0.1 (-2.11)
%DIS/NT	16.829±11.863	52.95±24.239	75.59±32	<0.001 (-4.09)	<0.001 (-6.66)	0.036 (-2.57)
IMA	0.765±0.36	0.886±0.277	1.049±0.108	0.441 (-1.23)	0.016 (-2.9)	0.232 (-1.66)
IL1-β	445.293±49.059	574.988±14.1	709.259±9.537	<0.001 (-11.8)	<0.001 (-24.1)	<0.001 (-12.3)
IL-6	106.403±8.424	194.142±12.992	236.73±14.625	<0.001 (-19.5)	<0.001 (-29.02)	<0.001 (-9.48)
TNF-α	340.677±16.068	419.91±13.757	452.265±16.1	<0.001 (-14.1)	<0.001 (-19.91)	<0.001 (-5.77)
GDNF	0.898±0.143	1.36±0.193	1.406±0.293	<0.001 (-5.78)	<0.001 (-6.358)	0.834 (-0.576)

HC: Healthy Controls; AD: Alzheimer's Disease; VD: Vascular Dementia; SD: Standard Deviation; TAS: Total Antioxidant Status; TOS: Total Oxidant Status; OSI: Oxidative Stress Index; TT: Total Thiol; NT: Native Thiol; DIS: Disulfide; %NT/TT: Ratio of Native Thiol to Total Thiol; %DIS/TT: Ratio of Disulfide to Total Thiol; %DIS/NT: Ratio of Disulfide to Native Thiol; IMA: Ischemia-Modified Albumin; IL1-β: Interleukin-1 Beta; IL-6: Interleukin-6; TNF-α: Tumor Necrosis Factor Alpha; GDNF: Glial-Derived Neurotrophic Factor.

and VD (13.546±3.08, p=0.007), suggesting a greater oxidative burden in AD. Moreover, TOS levels were higher in AD compared to VD (p=0.011). OSI levels were highest in AD (18.386±2.514) compared to both HC (10.129±2.584, p<0.001) and VD (14.509±3.578, p=0.002), highlighting a significantly altered redox balance in AD. Additionally, OSI levels in VD were significantly higher than in HC (p<0.001), indicating an intermediate oxidative stress burden in VD (Table 1, Fig. 1).

Thiol-Disulfide Homeostasis

Significant alterations in thiol-disulfide markers were observed among the groups. TT levels were significantly reduced in the AD group (476.934±64.586) compared to HC (571.058±64.154, p<0.001), indicating a decline in thiol availability in AD. TT levels in VD (503.686±47.961) were also significantly lower than in HC (p=0.009). Similarly, NT levels were markedly lower in AD (196.473±34.891) compared to both HC (432.959±43.416, p<0.001) and VD (254.139±46.952, p=0.002). NT levels in VD were significantly reduced relative to HC (p<0.001). DIS levels in VD (124.773±33.444) and AD (140.23±38.339) were both significantly higher than in HC (69.049±42.7, p<0.001), reflecting an intermediate oxidative stress response in VD and a more pronounced response in AD (Table 1, Fig. 1).

Thiol-Disulfide Ratios

Analysis of thiol-disulfide ratios revealed significant shifts among the groups, further highlighting oxidative stress differences. The %NT/TT ratio was significantly lower in AD (42.065±10.081) compared to HC (76.96±13.186, p<0.001), and it was also significantly lower in VD (50.849±10.669) than in HC (p<0.001), reflecting decreased antioxidant potential in both dementia groups. Similarly, the %DIS/TT ratio was significantly higher in both AD (28.967±5.041) and VD (24.576±5.335) compared to HC (11.52±6.593, p<0.001), indicating an increased oxidative burden in these groups. Additionally, the %DIS/NT ratio was elevated in both AD (75.59±32) and VD (52.95±24.239) relative to HC (16.829±11.863, p<0.001), further underscoring the oxidative stress shift in VD and AD. The %DIS/NT ratio was also significantly higher in AD than in VD (p=0.036) (Table 1, Fig. 1).

Inflammatory Markers

Inflammatory cytokine levels showed significant differences among the groups. IL-1β levels were significantly elevated in AD (709.259±9.537) compared to HC (445.293±49.059, p<0.001) and VD (574.988±14.1, p<0.001). IL-1β levels in VD were also significantly higher than in HC (p<0.001), indicating a heightened inflammatory response in both dementia groups, with the highest levels observed in AD. Similarly, IL-6 levels were higher in AD (236.73±14.625) than in HC (106.403±8.424, p<0.001) and VD (194.142±12.992, p<0.001). IL-6 levels in VD were significantly elevated compared to HC (p<0.001), reflecting systemic inflammation across dementia types, with AD showing the highest levels. TNF-α levels were also significantly increased in AD (452.265±16.1) compared to HC (340.677±16.068, p<0.001) and VD (419.911±13.757, p<0.001). TNF-α levels in VD were significantly higher than in HC (p<0.001) but significantly lower than in AD (p<0.001), contributing to the pronounced neuroinflammatory environment observed in both dementia groups (Table 1, Fig. 1).

Ischemia-Modified Albumin

IMA levels were significantly elevated in the AD group (1.049±0.108) compared to HC (0.765±0.36, p=0.016). IMA levels in VD (0.886±0.277) were higher than in HC,

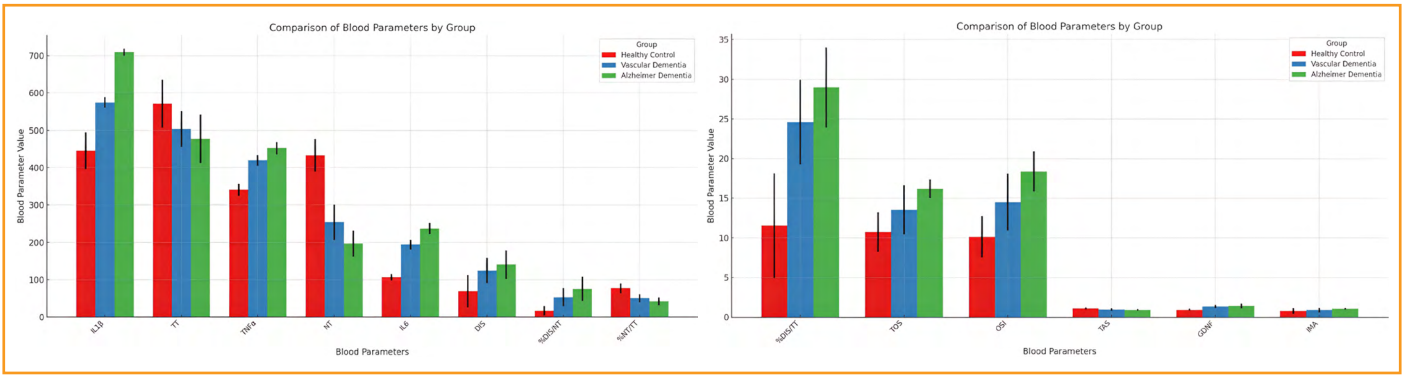

Figure 1. Comparison of Blood Biomarkers among Healthy Controls, Vascular Dementia, and Alzheimer's Dementia: The figure visualizes the mean levels of various blood biomarkers in three groups: Healthy Controls (red), Vascular Dementia (blue), and Alzheimer's Dementia (green). Biomarkers are arranged along the x-axis, with their mean concentration values displayed on the y-axis. Error bars represent the standard deviation (SD) for each measurement.

although this difference was not statistically significant (p=0.441). IMA levels in AD were significantly higher than in VD (p=0.232), reflecting hypoxia-related oxidative stress in dementia, particularly in AD (Table 1, Fig. 1).

Neurotrophic Factors

GDNF levels were significantly elevated in both AD (1.406±0.293) and VD (1.36±0.193) compared to HC (0.898±0.143, p<0.001 for both). There was no significant difference in GDNF levels between VD and AD (p=0.834) (Table 1, Fig. 1).

Discussion

Our findings highlight significant biochemical differences between VD and AD, providing insights into the distinct pathophysiological mechanisms underlying each condition. The markedly elevated oxidative stress markers in AD, particularly TOS and OSI, align with the established role of oxidative damage in AD pathogenesis. Oxidative stress is widely acknowledged as a major contributor to neurodegeneration in AD, often linked to mitochondrial dysfunction and the toxic effects of Aβ plaques, which promote ROS production and exacerbate neuronal damage[20]. In contrast, oxidative imbalance appears less pronounced in VD, suggesting that oxidative stress may be more closely associated with amyloid-driven neurodegeneration than with the vascular pathology characteristic of VD[21].

Our results indicate significant alterations in thiol-disulfide homeostasis in oxidative stress, with AD patients exhibiting lower TT and NT levels alongside elevated DIS levels. Thiol-disulfide homeostasis plays a crucial role in cellular antioxidant defense, and disruptions in this balance have been linked to various neurodegenerative diseases[22]. Reduced TT and NT levels in AD patients suggest diminished antioxidant capacity, potentially increasing neuronal vulnerability to oxidative damage. Studies have shown that lower plasma thiol levels are associated with increased oxidative stress in AD, pointing to a compromised antioxidant defense system[23]. Additionally, alterations in thiol-disulfide homeostasis have been implicated in AD pathogenesis, emphasizing the importance of redox balance for neuronal health[24]. In contrast, VD patients exhibit intermediate thiol-disulfide levels, indicating that thiol homeostasis is less disrupted in VD compared to AD. Differences in %NT/TT, %DIS/TT, and %DIS/NT ratios between groups further underscore the oxidative stress imbalance in AD, supporting the hypothesis that oxidative stress is a more prominent driver in AD's neurodegenerative mechanisms than in VD.

The observed differences in inflammatory profiles, with significantly elevated IL-1β and TNF-α levels in AD compared to HC and intermediate elevations in VD, highlight distinct underlying mechanisms in these conditions. Chronic neuroinflammation is a well-documented feature of AD, largely driven by microglial activation in response to amyloid plaques and tau pathology. This inflammatory response is believed to contribute to neurodegeneration, creating a vicious cycle of neuronal loss and further inflammation[25]. In VD, inflammation is often associated with endothelial dysfunction and vascular injury rather than direct neurodegenerative processes. The elevated IL-6 and TNF-α levels in VD suggest that inflammation in VD may stem from vascular endothelial damage, which triggers a systemic inflammatory response[26]. In AD, however, elevated IL-6 levels are closely linked to neurodegenerative processes, including microglial activation and amyloid-beta aggregation, which perpetuate a chronic inflammatory state. This neuroinflammation in AD contributes to neuronal loss and cognitive decline[27]. Thus, while both dementia types exhibit elevated inflammatory cytokines, the underlying mechanisms and implications for disease progression appear to differ significantly, with VD driven more by vascular inflammation and AD by neurodegenerative inflammation.

Our analysis also revealed increased GDNF levels in both VD and AD compared to HC. GDNF is known to support neuronal survival, promote neurogenesis, and modulate synaptic plasticity[15]. Elevated GDNF levels in dementia patients may reflect a compensatory mechanism in response to neuronal loss. Findings in the literature support this observation but reveal inconsistencies regarding GDNF levels across different tissues and disease stages. For instance, one study reported higher cerebrospinal fluid (CSF) GDNF levels in AD patients compared to HC, while serum GDNF levels were higher in HC than in AD[16]. Another study found no significant difference in serum GDNF levels between individuals with mild cognitive impairment (MCI) and AD patients[28]. These discrepancies highlight the complexity of GDNF's role in dementia and suggest that its expression may vary depending on the disease stage, severity, or tissue type being analyzed. In VD, GDNF may support neurons under ischemic or hypoxic conditions, while in AD, its effects might be insufficient to counteract progressive neurodegeneration driven by amyloid pathology[29]. Further research is needed to clarify these dynamics and evaluate GDNF's potential as a biomarker or therapeutic target in dementia.

Limitations

This study has several limitations that should be acknowledged. First, the sample size was relatively small, which may limit the generalizability of the findings and reduce statistical power. Second, participants were recruited from a single center, potentially introducing selection bias and limiting the diversity of the sample population. Third, the cross-sectional design precludes the ability to establish causal relationships or track changes over time. Future studies with larger, more diverse cohorts and longitudinal designs are needed to validate these findings and better understand the underlying mechanisms.

Conclusion

In conclusion, our study highlights the potential of biochemical markers, including TAS, TOS, OSI, TT, NT, DIS, IMA, IL-1β, IL-6, TNF-α, and GDNF, to differentiate between HC, VD, and AD. These markers offer promising insights into the distinct pathological mechanisms underlying each condition, with neurodegeneration driving AD and vascular injury contributing to VD. The distinct oxidative stress and inflammatory profiles observed in the two dementia types highlight opportunities for disease-specific diagnostic tools and therapeutic strategies. Elevated GDNF levels in both dementia types suggest a potential compensatory neuroprotective mechanism that could be harnessed in future treatments.

To further these findings, longitudinal studies with larger, more diverse cohorts are critical to understanding the temporal dynamics of these biomarkers and their relevance in disease progression. Such studies could provide robust insights into how oxidative stress markers (e.g., TAS, TOS, OSI) and inflammatory cytokines (e.g., IL-6, TNF-α) evolve over time in different dementia types. Moreover, integrating these biomarkers into clinical practice, such as through blood or cerebrospinal fluid testing, could enhance early diagnosis and enable personalized therapeutic approaches.

Future research should also explore the therapeutic potential of targeting these biomarkers. For instance, interventions aimed at modulating GDNF levels may help enhance neuroprotection, while strategies to reduce oxidative stress or inflammation could address specific pathological processes in AD or VD. Ultimately, combining biomarker-driven diagnostics with targeted treatments has the potential to significantly improve patient outcomes in dementia.

Ethics Committee Approval: The study was approved by Health Sciences University Hamidiye Scientific Research Ethics Committee Ethics Committee (No: 3274, Date: 30/10/2024).

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References

1. Kalaria RN. Neuropathological diagnosis of vascular cognitive impairment and vascular dementia with implications for Alzheimer's disease. Acta Neuropathol 2016;131:659–85.
2. Mitelpunkt A, Galili T, Kozlovski T, Bregman N, Shachar N, Markus-Kalish M, et al. Novel Alzheimer's disease subtypes identified using a data and knowledge driven strategy. Sci Rep 2020;10:1327. [CrossRef]
3. Song J, Lee WT, Park KA, Lee JE. Association between risk factors for vascular dementia and adiponectin. Biomed Res Int 2014;2014:261672. [CrossRef]
4. Kalaria RN. The pathology and pathophysiology of vascular dementia. Neuropharmacology 2018;134:226–39. [CrossRef]
5. Shabir O, Berwick J, Francis SE. Neurovascular dysfunction in vascular dementia, Alzheimer's and atherosclerosis. BMC Neurosci 2018;19:62. [CrossRef]
6. Zhang Q, Yang G, Luo Y, Jiang L, Chi H, Tian G. Neuroinflammation in Alzheimer's disease: Insights from peripheral immune cells. Immun Ageing 2024;21:38. [CrossRef]
7. Houldsworth A. Role of oxidative stress in neurodegenerative disorders: A review of reactive oxygen species and prevention by antioxidants. Brain Commun 2024;6:fcad356. [CrossRef]
8. Nunomura A, Perry G, Pappolla MA, Friedland RP, Hirai K, Chiba S, et al. Neuronal oxidative stress precedes amyloid-beta deposition in down syndrome. J Neuropathol Exp Neurol 2000;59:1011–7. [CrossRef]
9. Tamagno E, Guglielmotto M, Vasciaveo V, Tabaton M. Oxidative stress and beta amyloid in Alzheimer's disease. Which comes first: The chicken or the egg? Antioxidants (Basel) 2021;10:1479. [CrossRef]
10. Ahmad S, Yang W, Orellana A, Frölich L, de Rojas I, Cano A, et al. Association of oxidative stress and inflammatory metabolites with Alzheimer's disease cerebrospinal fluid biomarkers in mild cognitive impairment. Alzheimers Res Ther 2024;16:171.
11. Higashi Y. Roles of oxidative stress and inflammation in vascular endothelial dysfunction-related disease. Antioxidants (Basel) 2022;11:1958. [CrossRef]
12. Sobue A, Komine O, Yamanaka K. Neuroinflammation in

Alzheimer's disease: Microglial signature and their relevance to disease. Inflamm Regen 2023;43:26. [CrossRef]
13. Thakur S, Dhapola R, Sarma P, Medhi B, Reddy DH. Neuroinflammation in Alzheimer's disease: Current progress in molecular signaling and therapeutics. Inflammation 2023;46:1–17. [CrossRef]
14. Poh L, Sim WL, Jo DG, Dinh QN, Drummond GR, Sobey CG, et al. The role of inflammasomes in vascular cognitive impairment. Mol Neurodegener 2022;17:4. [CrossRef]
15. Budni J, Bellettini-Santos T, Mina F, Garcez ML, Zugno AI. The involvement of BDNF, NGF and GDNF in aging and Alzheimer's disease. Aging Dis 2015;6:331–41. [CrossRef]
16. Sharif M, Noroozian M, Hashemian F. Do serum GDNF levels correlate with severity of Alzheimer's disease? Neurol Sci 2021;4:2865–72. [CrossRef]
17. Erel O. A novel automated direct measurement method for total antioxidant capacity using a new generation, more stable ABTS radical cation. Clin Biochem 2004;37:277–85. [CrossRef]
18. Erel O. A new automated colorimetric method for measuring total oxidant status. Clin Biochem 2005;38:1103–11. [CrossRef]
19. Erel O, Neselioglu S. A novel and automated assay for thiol/disulphide homeostasis. Clin Biochem 2014;47:326–32. [CrossRef]
20. Tobore TO. On the central role of mitochondria dysfunction and oxidative stress in Alzheimer's disease. Neurol Sci 2019;40:1527–40. [CrossRef]
21. Bennett S, Grant MM, Aldred S. Oxidative stress in vascular dementia and Alzheimer's disease: A common pathology. J Alzheimers Dis 2009;17:245–57. [CrossRef]
22. Anjo SI, He Z, Hussain Z, Farooq A, McIntyre A, Laughton CA, et al. Protein oxidative modifications in neurodegenerative diseases: From advances in detection and modelling to their use as disease biomarkers. Antioxidants (Basel) 2024;13:681. [CrossRef]
23. Akunal C, Alisik M. Deteriorated thiol-disulphide and oxidized-reduced glutathione status in blood in Alzheimer's disease. Clin Biochem 2024;133:110817. [CrossRef]
24. Poimenova IA, Sozarukova MM, Ratova DV, Nikitina VN, Khabibullin VR, Mikheev IV, et al. Analytical methods for assessing thiol antioxidants in biological fluids: A review. Molecules 2024;29:4433. [CrossRef]
25. McFarland KN, Chakrabarty P. Microglia in Alzheimer's disease: A key player in the transition between homeostasis and pathogenesis. Neurotherapeutics 2022;19:186–208. [CrossRef]
26. Martins-Filho RK, Zotin MC, Rodrigues G, Pontes-Neto O. Biomarkers related to endothelial dysfunction and vascular cognitive impairment: A systematic review. Dement Geriatr Cogn Disord 2020;49:365–74. [CrossRef]
27. Heneka MT, Carson MJ, El Khoury J, Landreth GE, Brosseron F, Feinstein DL, et al. Neuroinflammation in Alzheimer's disease. Lancet Neurol 2015;14:388–405. [CrossRef]
28. Faria MC, Gonçalves GS, Rocha NP, Moraes EN, Bicalho MA, Gualberto Cintra MT, et al. Increased plasma levels of BDNF and inflammatory markers in Alzheimer's disease. J Psychiatr Res 2014;53:166–72. [CrossRef]
29. Palasz E, Wilkaniec A, Stanaszek L, Andrzejewska A, Adamczyk A. Glia-neurotrophic factor relationships: Possible role in pathobiology of neuroinflammation-related brain disorders. Int J Mol Sci 2023;24:6321. [CrossRef]