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Comparison of long non-coding RNA NEAT1 expression, P53, and anti-oxidant factors between Alzheimer's patients and healthy individuals

Sara Yarahmadi [1, 2], Leila Alidoust [3], Alia Saberi [4], Behrooz Khakpour-Taleghani [1, 4], Mohammad Rostampour [1, 2], Adele Jafari [1]*

[1] Department of Physiology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[2] Cellular and molecular research center, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[3] Department of Genetics, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran
[4] Neuroscience Research Center, Trauma Institute, Poursina Hospital, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran

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ABSTRACT

Objective(s): Several studies have reported that the lncRNA Nuclear-enriched abundant transcript 1 (NEAT1) is associated with the progression of Alzheimer's disease (AD). Oxidative stress and apoptosis also play crucial roles in the development of AD. The present study compared the serum levels of NEAT1, superoxide dismutase (SOD), glutathione (GSH), and P53 between individuals with AD and healthy controls.

Materials and Methods: Peripheral blood samples were collected from 30 AD patients and 33 healthy controls and then centrifuged to separate serum. Total RNAs were isolated, and real-time polymerase chain reaction (RT-qPCR) was applied to determine NEAT1 gene expression. Also, Enzyme-linked immunoassays (ELISA) were conducted to measure SOD and GSH as anti-oxidant factors, and p53 as an apoptosis marker.

Results: NEAT1 expression was significantly higher in AD patients than in controls ($P<0.001$). In addition, ROC analysis revealed that serum NEAT1 levels distinguished AD patients from healthy controls with 90% sensitivity and 84.85% specificity. Moreover, the anti-oxidant levels of SOD ($P<0.0001$) and GSH ($P<0.05$) were significantly decreased in AD patients, while P53 levels were significantly increased ($P<0.05$). However, no significant association was found between NEAT1 expression and the measured blood factors.

Conclusion: The serum levels of NEAT1 effectively distinguished between AD patients and non-AD people, underscoring its potential as a blood-based biomarker for the development of Alzheimer's disease.

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Introduction

Alzheimer's disease (AD), the most common dementia, is an irreversible and progressive cognitive disorder affecting the brain (1). Additionally, with the aging of populations, epidemiological data indicate a substantial increase in the prevalence of AD across various communities. Considering the AD-associated complications, including memory impairment and disability, it can impose significant financial burdens on healthcare systems. Additionally, cellular and molecular studies reveal that the primary pathology of Alzheimer's involves the deposition of beta-amyloid plaques (Aβ), neurofibrillary tangles (NFTs), gliosis, and neuron loss, alongside cerebral amyloidosis, inflammation, and major synaptic changes (2). However, numerous theories are being developed to understand the etiology of AD, including the amyloid cascade hypothesis, neurotoxicity, oxidative stress, and dysregulation of apoptosis (3-5).

Due to the progressive properties of AD, early diagnosis has consistently posed a challenge for proposed therapeutic interventions. In this regard, the clinical diagnosis and prognosis of AD have been focal points of numerous studies, underscoring the necessity of identifying molecular biomarkers in cerebrospinal fluid (CSF) and serum that signal the onset or progression of the disease (6). The advent of new sequencing technologies has led to the discovery of novel nucleotide sequences, particularly ribonucleic acids (RNA), which, although not translated into proteins, are critical for numerous cellular processes (7). These sequences, referred to as non-coding RNAs, encompass various families including microRNAs (miRNAs) and long non-coding RNAs (lncRNAs), which are categorized based on the length of their mature sequences (8, 9). Furthermore, long

***Corresponding author:** Adele Jafari. Department of Physiology, School of Medicine, Guilan University of Medical Sciences, Rasht, Iran Email: jafari.adele@gums.ac.ir, jafari.adele@gmail.com

non-coding RNAs (lncRNAs) are a class of RNA molecules exceeding 200 nucleotides in length. Although structurally similar to mRNAs, lncRNAs lack protein-coding capability. Still, they are critical for a variety of biological and cellular processes, notably the regulation of gene expression at transcriptional and post-transcriptional levels, embryonic development, and cell differentiation(10). Recent evidence suggests that lncRNAs undergo quantitative changes in both extracellular and intracellular environments during numerous pathological processes (11).

Nuclear-enriched abundant transcript 1 (NEAT1) is a long non-coding RNA that has recently been associated with the progression of AD. NEAT1 is critical for both the formation and maintenance of paraspeckles, which are dynamic nuclear bodies without membranes that influence various cellular functions, including the stress response (12). Additionally, NEAT1 is abundantly expressed in various mammalian cell types (13) and plays roles in numerous physiological and pathological processes, including corpus luteum formation, mammary gland development, cancers, viral infections, and autoimmune diseases (14). Additionally, NEAT1 acts as a crucial regulator of the cellular stress response. A study by Kukharsky *et al.* demonstrated that NEAT1 contributes significantly to stress signaling within the brain, regulating CNS functions to activate adaptive behavior in response to physiological stress (15). Despite extensive research, the precise role and function of NEAT1 in AD are not yet fully understood. For example, a study by Wang *et al.* indicated that in the early stages of AD in a genetic mouse model, NEAT1 expression levels decrease, and its down-regulation impairs Aβ clearance by neuroglial cells by reducing the expression of genes involved in endocytosis (14). Conversely, another study discovered that NEAT1 levels are significantly elevated in the plasma of patients with advanced AD (10).

Increasing evidence highlights the significant role of oxidative stress in the pathology of AD. *Oxidative stress* is defined as an imbalance between oxidation and anti-oxidant systems. Additionally, the accumulation of oxidants is driven by the buildup of free radicals, primarily reactive oxygen species (ROS) and reactive nitrogen species (RNS) (3). ROS plays a crucial role in intracellular and extracellular signaling. Anti-oxidants and other enzymatic systems that regulate cellular redox states serve as the first line of defense against ROS across all cellular environments. Key enzymes in this defense include superoxide dismutases (SOD), glutathione peroxidases, catalase, and peroxiredoxins (4). SOD enzymes regulate the levels of reactive oxygen species, thereby limiting their potential toxicity. Changes in SOD activity can lead to the production of hydrogen peroxide gradients and the subsequent activation of redox-sensitive pathways (5). Evidence suggests that oxidative stress may be critical for AD through multiple pathways (16, 17).

Additionally, apoptosis is vital to various processes, including normal cell turnover, immune function, hormone-dependent atrophy, embryonic development, and chemically induced cell death. Improper apoptosis (either too little or too much) is involved in many disorders, including neurological diseases, ischemic injury, autoimmune disorders, and many types of cancer (18). Moreover, the protein p53, the "guardian of the genome," is critical in coordinating cellular responses to genotoxic stress. Due to its significant role in cellular functions, p53 expression is regulated at multiple levels, including transcription, post-transcription, pre-translation, and post-translation. Furthermore, p53 directly interacts with pro-apoptotic factors, including Bax and Drop1, facilitating mitochondrial fragmentation (19). The assessment of p53 levels in plasma samples of Alzheimer's patients is also considered a potential biomarker for the prognosis of the disease in its asymptomatic stage (20). Therefore, in the present study, we investigated the expression of NEAT1, SOD, GSH (Glutathione), and P53 in the serum of Alzheimer's patients and compared it with that of healthy individuals. In addition, we examined whether there is a correlation between the expression of NEAT1 and other measured factors.

Materials and Methods

Patient and participant

The current study included 30 patients with AD and 33 healthy individuals without neurological disorders from the Elderly Care Center in Rasht. Before collecting samples, consent forms were provided to the patients or their companions. All of the subjects were assessed by a neurologist based on the NIA-AA criteria for Alzheimer's diagnosis. The inclusion criteria were individuals over 60 years old diagnosed with probable Alzheimer's according to the NIA-AA criteria. The exclusion criteria included individuals under 60 years old, those with a history of head trauma, stroke, motor neuron disease, neurological infections, lupus with neuropsychological manifestations, sarcoidosis, multiple sclerosis, other neurodegenerative diseases, and those with a family history of Alzheimer's in first-degree relatives. This study was approved by the local Ethical Committee (code No: IR.GUMS.REC.1402.239).

Blood samples (6 ml) were collected from all participants, incubated at room temperature for 30 min, and then centrifuged to separate serum (15 min, 4 °C, 3000 rpm). The serum was then divided into several aliquots and stored at -80 °C until subsequent analysis.

RNA extraction and screening

Total RNA was extracted using the RNX PLUS Solution (Sinaclon BioScience, Iran) according to the manufacturer's protocol. Briefly, following a series of incubation, centrifugation, and phase separation steps, the RNA was transferred to a new tube, precipitated with isopropanol, and further purified with ethanol. The RNA pellet was air-dried and dissolved in DEPC-treated water, and incubated in a water bath. The quantity and purity of the extracted RNA were determined using a NanoDrop (Thermo, USA). For qualitative RNA assessment, 1.5% agarose gel electrophoresis was employed. Suitable samples exhibit two distinct bands corresponding to 18S rRNA and 28S rRNA, without smearing, indicating no DNA contamination.

Quantified RT-PCR(qRT-PCR)

Total RNA was reverse transcribed into cDNA using the First-strand cDNA Synthesis Kit (Cinnagen, Cat. No: RT5201) according to the manufacturer's instructions. The synthesized cDNA was stored at -20 °C until use. Real-time PCR was performed using a SYBR Green-based master mix in a 10 µl reaction volume on a Corbett Rotor-Gene 6000 system. The cycling conditions consisted of an initial denaturation at 95 °C for 4 minutes, followed by 30 cycles of 94 °C for 30 seconds and 72 °C for 30 seconds. All reactions were performed in triplicate. Primer specificity

Table 1. List of primers used in this study, their annealing tempretures, and expected product length

Gene	Primer name	Primer sequence	Annealing temperature (Â °C)	Product length (bp)
NEAT1	NEAT1-F	GCTTGTTCCAGAGCCCATGAAT	60. 5	184
	NEAT1-R	AAGGCATCAATCTGCGTTGTGG		
GAPDH	GAPDH-F	TGGAGAAGGCTGGGGCTCATT	61	134
	GAPDH-R	CGTGCAGGAGGCATTGCTGAT		

was primarily assessed using melt-curve analysis at the end of each qPCR run. To provide an additional level of quality control, the final qPCR products were also subjected to agarose gel electrophoresis to visually confirm the presence of a single amplicon of the expected size and to exclude potential low-level nonspecific amplification or primer-dimer formation. Importantly, no gel-based information was used for quantification. The relative expression of NEAT1 was calculated and quantified using the comparative $2^{-\Delta\Delta Ct}$ method. GAPDH was used as a reference gene (21).

Enzyme-linked immunoassay (ELISA)

To quantify plasma levels of SOD and GSH, ELISA was used. For the SOD assay, the superoxide dismutase Kit (Zelbio GmbH, Germany) was utilized. The kit and samples were brought to room temperature before starting. Solutions were prepared as specified, and serum samples and reagents were added to the wells. The color intensity was measured at 420 nm at time zero and after 2 minutes. SOD activity was calculated based on the following formula:

SOD activity (U/ml) = (Vp-Vc)/(Vp)×100

$Vp = OD_{sample2min} - OD_{blank2min}$ $Vc = OD_{sample0min} - OD_{blank0\ min}$

Also, the GSH assay was calculated using the Glutathione Kit (Zelbio GmbH, Germany). Following similar preparation and measurement steps, the color intensity was measured at 412 nm. Finally, GSH activity was calculated based on the following formula:

GSH (mmol/l)=(OD sample-OD blank)/(OD standard-OD blank)×1 mmol/l

In addition, the P53 protein level was determined by the Human Tumor Protein 53 Kit (Padgin Teb, Co. Iran) using ELISA. Plasma samples were thawed, and standard solutions were prepared in serial dilutions. Samples and reagents were added to the wells, incubated, and washed according to the guidelines. The color intensity was measured at 450 nm within 10 min.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 9 software. The Shapiro-Wilk test was used to assess the normality of the data. The independent t-test was employed for group comparison. Data were presented as Mean ± standard error (SEM), and a significance level of 0.05 was considered for all examinations. The prognostic value of NEAT1 was evaluated using receiver operating characteristic (ROC) curve analysis and the area under the curve (AUC), with a 95% confidence interval (CI). In addition, the correlations between the variables were examined by Spearman's correlation coefficient.

Results

General clinical and demographic data of study participants

The general characteristics of AD patients and healthy subjects are summarized in Table 2. Thirty AD patients and thirty-three healthy controls were enrolled in this study. The average age of the AD and control groups was 82.67±7.2 years and 79.64±8 years, respectively. No significant differences were found between the AD patient and control groups in gender and mean age (P>0.05).

Comparison of NEAT1 expression between AD patients and healthy controls

The serum expression level of NEAT1 was evaluated by qRT-PCR and analyzed by an independent T-test. Results showed that NEAT1 expression was significantly increased (0.41 ± 0.07) compared to the control group (0.08 ± 0.01) (Figure 1A, P=0.002). In addition, to determine the biomarker potency of serum levels of NEAT1, the receiver operating characteristic (ROC) curve was conducted. The ROC curve analysis of NEAT1 expression levels successfully distinguished AD patients from control subjects with high accuracy, achieving an area under the curve (AUC) of 0.93 (95% CI: 0.88-0.99; P=0.0001). At the optimal cutoff values, the test showed a sensitivity of 90% and a specificity of 84.85%, indicating strong discriminatory power (Figure 1B).

Comparison of serum levels of SOD and GSH between AD patients and healthy controls

To determine anti-oxidant activity, the levels of SOD and

Table 2. General clinical and demographic data of study participants

Variables	AD patients (n=30)	Healthy subjects (n=33)	*P*-value
Female [no. (%)]	17 (56.6%)	17 (51.5%)	
Male [no. (%)]	13 (43.3%)	16 (48.5%)	
Age (years) (mean±SD)	82.67±7.2	79.64±8	0.36
Underlying disease [no. (%)]	17 (51%)	13 (39.4%)	
Disease duration (under/above 5 years) [no.]	15/18	-	

AD: Alzheimer's disease;

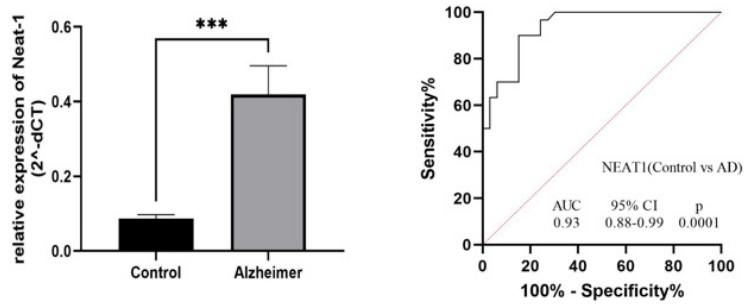

Figure 1. Expression levels of NEAT1 in Alzheimer's and healthy control groups. QRT-PCR quantified the serum level of NEAT1
A two-tailed unpaired t-test was performed. Data are presented as mean ± SEM (P<0.001) (A). The diagnostic value of NEAT1 for AD was assessed by the ROC curve (B)
NEAT1: Nuclear-enriched abundant transcript 1; ROC curve: Receiver operating characteristic curve; AUC: Area under the curve.

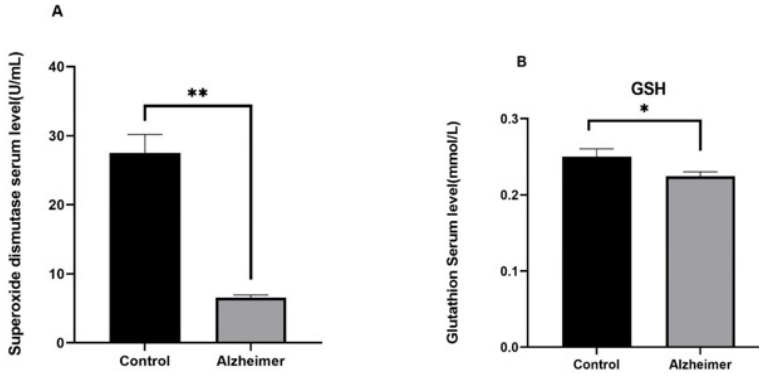

Figure 2. Serum levels of SOD (A) and GSH (B) in Alzheimer's and healthy control groups. QRT-PCR quantified the serum level of NEAT1
A two-tailed unpaired t-test was performed. Data are presented as mean ± SEM (n=33, P<0.001). SOD: Superoxide dismutase; NEAT1: Nuclear-enriched abundant transcript 1; GSH: Glutathione; RT-PCR: Real-time polymerase chain reaction

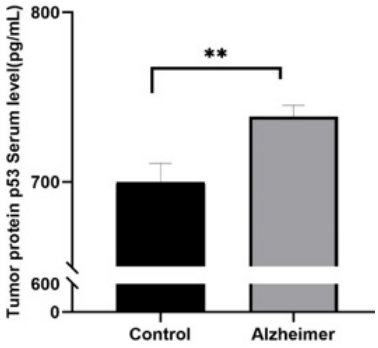

Figure 3. Serum level of P53 in Alzheimer's and healthy control groups
A two-tailed unpaired t-test was performed. Data are presented as mean ± SEM (n=33, P<0.01).

GSH were measured in serum samples using ELISA and analyzed by the independent T-test. As illustrated in Figure 2, the levels of SOD decreased in Alzheimer's patients (6.55±0.36) compared to controls (27.52±2.66; P=0.0001). In addition, the level of GSH decreased in Alzheimer's patients (0.22±0.005) compared to healthy controls (0.25±0.01; P=0.038).

Comparison of serum levels of P53 between AD patients and healthy controls

According to the independent T-test results, there was a significant elevation in p53 levels in Alzheimer's patients (738.6±6.609) when compared to the control group (699.9±11.16) (P=0.0045) (Figure 3).

Correlation of the serum level of NEAT1 with P53, SOD, and GSH

To determine if serum levels of NEAT1 were related to P53, GSH, and SOD, we employed Spearman's rank correlation coefficient. As illustrated in Figure 4, the analysis revealed no significant correlation between NEAT1 and GSH (r= 0.23, CI;

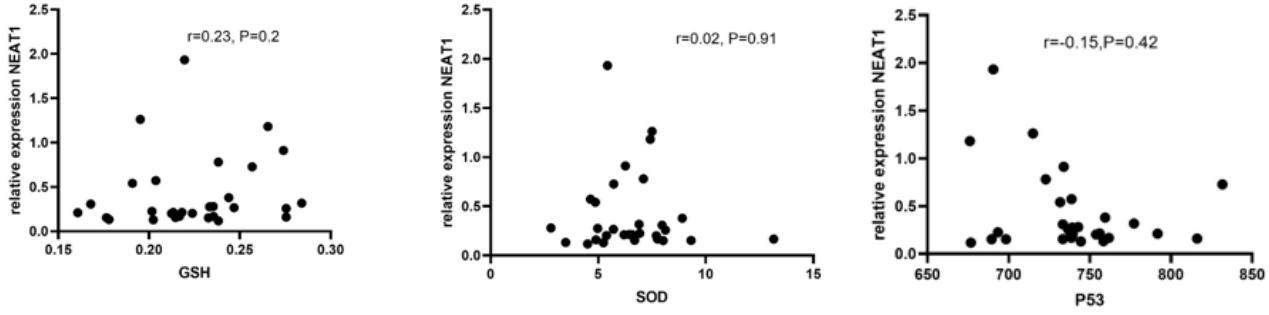

Figure 4. Correlation of the GSH, SOD, and P53 with serum levels of EEAT1 in Alzheimer's disease patients
The spearman rank test showed no significant correlation between variables. NEAT1: Nuclear-enriched abundant transcript 1; GSH: Glutathione; SOD: Superoxide dismutase

-0.14 to 0.55, *P*=0.2), SOD (r= 0.02, CI; 0.35 to 0.38, *P*=0.91), and P53 (r=-0.15, CI; -0.49 to 0.23, *P*=0.42).

Discussion

Our findings demonstrated a significant upregulation of NEAT1 expression in Alzheimer's patients compared to healthy controls. To assess the biomarker potential of NEAT1, a ROC curve was generated. The resulting area under the curve (AUC), coupled with high specificity and sensitivity values, suggests that NEAT1 may be a viable biomarker for AD. In addition, the levels of oxidative stress and apoptosis-related markers, including P53, were altered in AD patients, further emphasizing the involvement of oxidative damage and programmed cell death in Alzheimer's pathology. However, no correlation was found between NEAT1 expression and SOD, GSH, or p53 levels.

The findings reveal a notable 4.7-fold increase in NEAT1 gene expression among AD patients compared to the control individuals. Previous research has also emphasized the role of this gene in neurological disorders. Aligning with our results, Khodayi *et al.* found significantly higher expression levels of NEAT1 and BC200 in the plasma of Alzheimer's patients compared to healthy individuals, suggesting that these lncRNAs could potentially serve as biomarkers for AD (10). He *et al.* demonstrated that the expression patterns of NEAT1 and miR-27a-3p were in both the serum and cerebrospinal fluid of individuals with AD. NEAT1 levels were found to rise, while miR-27a-3p levels were observed to decline. This inverse relationship has been linked to Aβ deposition in the brains of AD patients and is believed to contribute to the progression of the disease (22). Furthermore, Huang *et al.* found that NEAT1 levels increased in the aging APP/PS1 mouse model, leading to enhanced ubiquitination and degradation of kinase 1, which inhibited autophagy signaling and caused amyloid accumulation and cognitive dysfunction (23). Simchovitz *et al.* identified elevated NEAT1 expression in the substantia nigra of patients with Parkinson's disease compared with control brains, suggesting that NEAT1 may protect cells by binding to LRRK2 in paraspeckles and acting as an LRRK2 inhibitor (24). To elucidate this phenomenon, NEAT1 can be considered a lncRNA that participates in multiple biological pathways and functions through various mechanisms, primarily the formation of paraspeckles (25). Paraspeckles regulate gene expression by sequestering edited mRNA within the nucleus, while unedited RNA is transported out to the cytoplasm (26). Additionally, lncRNAs regulate the nervous system at multiple biological levels, including epigenetic and post-transcriptional mechanisms, and contribute to the pathogenesis of AD (27). NEAT1 is critical in several conditions, including inflammation, oxidative stress, and neurodegeneration (28). NEAT1 is a sponge for miRNAs during AD, leading to PINK1 degradation, amyloid accumulation, and mitochondrial dysfunction (29). Supporting this, Pan *et al.* demonstrated that NEAT1 influences inflammation in the central and peripheral nervous systems. Their findings suggest that manipulating NEAT1 can reduce microglial activation, lipid accumulation, and inflammatory responses in clinical and preclinical studies. The NEAT1-miRNA-mRNA network plays a crucial role in the anti-inflammatory mechanism of NEAT1 regulation (30).

Moreover, our findings revealed that the serum concentrations of the enzymes SOD and GSH were significantly decreased in the Alzheimer's group compared to the control group. In this context, research conducted by Zalewska *et al.* on the saliva and plasma of Alzheimer's patients and healthy subjects found that the activity of SOD, glutathione peroxidase, and the concentration of non-enzymatic plasma anti-oxidants such as glutathione were decreased in the plasma of Alzheimer's patients compared to the control group (31). Furthermore, research by Omar and colleagues on hippocampal tissue from Alzheimer's patients found that the activities of SOD, GSH-Px, and catalase were significantly lower in Alzheimer's brains than in control brains. This reduction in catalase and glutathione peroxidase activities increases endogenous hydrogen peroxide levels (32).

Oxidative stress is suggested to play a pivotal role in AD and may even be viewed as a central factor in its pathogenesis (33). Elevated production of ROS plays a significant role in the early stages of AD, occurring before the emergence of clinical symptoms and the accumulation of amyloid-beta (Aβ) (34). One proposed mechanism by which Aβ stimulates oxidative stress involves the activation of NMDA receptors, causing calcium influx into postsynaptic neurons, which leads to mitochondrial dysfunction, increased nitric oxide synthesis, and excessive ROS production. This cascade subsequently results in macromolecular damage and neurodegeneration (35). Additionally, anti-oxidant systems are crucial in maintaining ROS balance. Also, essential endogenous anti-oxidants involved in various physiological and pathological processes include enzymatic anti-oxidants like SOD, catalase, and glutathione peroxidase (GPx), as well as non-enzymatic anti-oxidants such as glutathione, lipoic acid, bilirubin, and ferritin, which can rapidly neutralize ROS (36). The activities of these enzymes, which directly eliminate reactive oxygen species, decline with aging and in certain pathological conditions (37). Overall, Research involving both animal models and humans has revealed that oxidative stress biomarkers appear before the development of neuropathological symptoms of AD (38). Additionally, research indicates that Alzheimer's patients exhibit higher levels of peripheral oxidative stress biomarkers and diminished anti-oxidant defenses compared to healthy elderly individuals (39).

In addition, our results demonstrated that serum P53 concentration was significantly higher in patients with AD. Similarly, Kitamura *et al.* discovered that the levels of P53 in the temporal cortex of Alzheimer's patients were significantly higher than those in the control group (40). Furthermore, Ohyagi and colleagues conducted studies on the brains of guinea pigs, mice, and humans, concluding that intracellular Aβ-42 directly activates the P53 promoter, leading to P53-dependent apoptosis. Additionally, oxidative DNA damage caused nuclear localization of Aβ-42, increased P53 mRNA in primary guinea pig neurons, and elevated P53 expression in the brains of Alzheimer's mice. Notably, both Aβ-42 and p53 accumulation were observed in some neurons in transgenic mice and in human AD cases. Ultimately, intracellular Aβ-42 may induce P53-dependent neuronal apoptosis by activating the P53 promoter (41).

An analysis of NEAT1 expression in AD has revealed a significant increase in the lncRNA among Alzheimer's patients. This upregulation of NEAT1 is consistent with previous studies that have shown the involvement of this lncRNA in neurological diseases. Research indicates that NEAT1 can serve as a biomarker for Alzheimer's and plays

a role in exacerbating neuronal damage through various mechanisms, including the regulation of miR-107 and miR-27a-3p. Furthermore, increased NEAT1 expression can lead to amyloid accumulation and cognitive impairment. Additionally, the levels of the anti-oxidants SOD and GSH were significantly lower, while the level of P53 was higher in the Alzheimer's group. This finding further underscores the role of oxidative stress and apoptosis in AD.

Although NEAT1 alterations have been reported in AD, findings across studies remain inconsistent. For instance, Wang *et al.* (2019) observed decreased NEAT1 expression in early-stage AD models (42); however, such reports are limited, and current evidence is insufficient to consider NEAT1 down-regulation a consistent or generalizable feature of the disease. Instead, available data suggest that NEAT1 expression is highly context-dependent, varying according to disease stage, tissue type, and experimental model. This context-specific regulation likely explains the discrepancies observed in the literature and underscores the dynamic nature of NEAT1 involvement during disease progression.

Interestingly, despite the significant elevation of NEAT1 expression, no correlation was detected between NEAT1 and the measured systemic oxidative stress or apoptosis marker. This lack of association suggests that NEAT1 upregulation may act independently of these peripheral serum markers, possibly reflecting compartment-specific regulation, such as predominant activity within the central nervous system. Supporting this interpretation, alterations in immune regulatory molecules, such as members of the inhibitory B7 family, have been reported in AD, indicating that immune-related pathways may contribute to disease pathology independently of classical oxidative stress or apoptotic mechanisms(43).

Beyond non-coding RNAs, previous studies have highlighted that multiple blood-based molecular markers, including neurotrophin growth factors and their receptors, may reflect peripheral molecular changes associated with AD. These findings emphasize the multifactorial nature of circulating biomarkers in AD and suggest that peripheral NEAT1 alterations may represent one component of a broader molecular response rather than a direct surrogate for specific intracellular pathways (44).

Based on our results, we propose that the observed upregulation of serum NEAT1 in AD is more likely a consequence rather than a primary cause of disease pathology. Increased NEAT1 expression may reflect a compensatory or stress-responsive mechanism triggered by neuroinflammation, neuronal injury, or dysregulated cellular homeostasis. While this response may initially serve a protective or adaptive role, sustained NEAT1 elevation could potentially contribute to disease progression through modulation of inflammatory, immune, or apoptotic signaling cascades. However, due to the cross-sectional design of the present study, causal relationships cannot be established. Further, longitudinal and mechanistic investigations are required to clarify whether NEAT1 exerts protective, pathogenic, or stage-dependent effects in AD.

Our study has several limitations. First, the relatively small sample size of 30 Alzheimer's patients may limit the generalizability of the results. Second, the cross-sectional nature of the study precludes causal inference regarding the relationship between NEAT1 expression, oxidative stress, and disease progression. Additionally, the reliance on serum biomarkers may be influenced by comorbid conditions or medication use and may not fully capture central nervous system–specific molecular changes. The absence of longitudinal follow-up also limits insight into temporal changes in NEAT1 expression. Finally, the use of a single methodological approach for biomarker assessment could be strengthened by incorporating complementary techniques in future studies.

Conclusion

In summary, this study demonstrates a significant upregulation of serum NEAT1 in patients with AD, supporting its potential relevance as a peripheral biomarker. The lack of correlation between NEAT1 and systemic oxidative stress or apoptotic markers suggests that NEAT1 regulation may be independent of these pathways.

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Authors' Contributions

S Y conducted the investigation and drafted the initial version. L A conceived the study, contributed to methodology and analysis, and reviewed and edited the writing. A S helped with methodology and reviewed and edited the writing. B K supervised the project and reviewed and edited the manuscript. M R carried out the investigation and reviewed and edited the writing. A J was responsible for conceptualization, supervision, analysis, writing the original draft, and reviewing and editing the manuscript.

Conflicts of Interest

There is no conflict of interest for any of the contributing authors. The authors alone are responsible for the content and writing of the paper.

Declaration

During manuscript preparation, the authors used ChatGPT, a generative AI tool, to assist with grammar and language refinement. All scientific content, including concepts, data analysis, interpretations, and conclusions, was generated and validated solely by the authors without the use of AI for content creation.

References

1. Lyketsos CG, Carrillo MC, Ryan JM, Khachaturian AS, Trzepacz P, Amatniek J, *et al.* Neuropsychiatric symptoms in Alzheimer's disease. Alzheimers Dement 2011; 7:532-539.
2. Guo T, Zhang D, Zeng Y, Huang TY, Xu H, Zhao Y. Molecular and cellular mechanisms underlying the pathogenesis of Alzheimer's disease. Mol Neurodegener 2020; 15:40.
3. Wang X, Shen C, Zhu J, Shen G, Li Z, Dong J. Long noncoding RNAs in the regulation of oxidative stress. Oxid Med Cell Longev 2019; 2019:1318795.
4. Karihtala P, Soini Y. Reactive oxygen species and anti-oxidant mechanisms in human tissues and their relation to malignancies. Apmis 2007; 115:81-103.
5. Wang Y, Branicky R, Noë A, Hekimi S. Superoxide dismutases: Dual roles in controlling ROS damage and regulating ROS signaling. J Cell Biol 2018; 217:1915-1928.
6. Yao Z, Wang H, Yan W, Wang Z, Zhang W, Wang Z, *et al.*

Artificial intelligence-based diagnosis of Alzheimer's disease with brain MRI images. Eur J Radiol 2023; 165:110934.
7. Kimura T. Non-coding natural antisense RNA: Mechanisms of action in the regulation of target gene expression and its clinical implications. Yakugaku Zasshi 2020;140:687-700.
8. Gibbons A, Udawela M, Dean B. Non-coding RNA as novel players in the pathophysiology of schizophrenia. Noncoding RNA 2018; 4:11.
9. Wang J, Zhu S, Meng N, He Y, Lu R, Yan G-R. ncRNA-Encoded peptides or proteins and cancer. Mol Ther 2019; 27:1718-1725.
10. Khodayi M, Khalaj-Kondori M, Hoseinpour Feizi MA, Jabarpour Bonyadi M, Talebi M. Plasma lncRNA profiling identified BC200 and NEAT1 lncRNAs as potential blood-based biomarkers for late-onset *al*zheimer's disease. Excli J 2022; 21:772-785.
11. Li M, Zhao Y, Li H, Deng X, Sheng M. Application value of circulating LncRNA in diagnosis, treatment, and prognosis of breast cancer. Funct Integr Genomics 2023; 23:61.
12. Prinz F, Kapeller A, Pichler M, Klec C. The Implications of the Long Non-Coding RNA NEAT1 in Non-Cancerous Diseases. Int J Mol Sci 2019; 20:627.
13. Taiana E, Ronchetti D, Todoerti K, Nobili L, Tassone P, Amodio N, *et al.* LncRNA NEAT1 in paraspeckles: A structural scaffold for cellular DNA damage response systems? Noncoding RNA 2020; 6:26.
14. Wang Z, Zhao Y, Xu N, Zhang S, Wang S, Mao Y, *et al.* NEAT1 regulates neuroglial cell mediating Aβ clearance via the epigenetic regulation of endocytosis-related genes expression. Cell Mol Life Sci 2019; 76:3005-3018.
15. Kukharsky MS, Ninkina NN, An H, Telezhkin V, Wei W, Meritens CR, *et al.* Long non-coding RNA Neat1 regulates adaptive behavioural response to stress in mice. Transl Psychiatry 2020; 10:171.
16. Forman HJ, Zhang H. Targeting oxidative stress in disease: Promise and limitations of antioxidant therapy. Nat Rev Drug Discov 2021; 20:689-709.
17. Butterfield DA, Halliwell B. Oxidative stress, dysfunctional glucose metabolism and Alzheimer disease. Nat Rev Neurosci 2019; 20:148-160.
18. Elmore S. Apoptosis: a review of programmed cell death. Toxicol Pathol 2007; 35:495-516.
19. Chang JR, Ghafouri M, Mukerjee R, Bagashev A, Chabrashvili T, Sawaya BE. Role of p53 in neurodegenerative diseases. Neurodegener Dis 2012; 9:68-80.
20. Abate G, Fowler CJ, Martins RN, Memo M, Uberti D. Unfolded P53 as a novel lead biomarker for Alzheimer's disease diagnosis: Biomarkers (non-neuroimaging)/plasma/serum/urine biomarkers. Alzheimer's & Dementia 2020; 16:e042920.
21. Pfaffl MW. A new mathematical model for relative quantification in real-time RT-PCR. Nucleic Acids Res 2001; 29:e45.
22. He L, Chen Z, Wang J, Feng H. Expression relationship and significance of NEAT1 and miR-27a-3p in serum and cerebrospinal fluid of patients with Alzheimer's disease. BMC Neurol 2022; 22:203.
23. Huang Z, Zhao J, Wang W, Zhou J, Zhang J. Depletion of LncRNA NEAT1 Rescues Mitochondrial Dysfunction Through NEDD4L-Dependent PINK1 Degradation in Animal Models of Alzheimer's Disease. Front Cell Neurosci 2020; 14:28.
24. Simchovitz A, Hanan M, Niederhoffer N, Madrer N, Yayon N, Bennett ER, *et al.* NEAT1 is overexpressed in Parkinson's disease substantia nigra and confers drug-inducible neuroprotection from oxidative stress. Faseb j 2019; 33:11223-11234.
25. Pisani G, Baron B. NEAT1 and Paraspeckles in Cancer Development and Chemoresistance. Noncoding RNA 2020; 6:43.
26. Klec C, Prinz F, Pichler M. Involvement of the long noncoding RNA NEAT1 in carcinogenesis. Mol Oncol 2019; 13:46-60.
27. Ma N, Tie C, Yu B, Zhang W, Wan J. Identifying lncRNA-miRNA-mRNA networks to investigate Alzheimer's disease pathogenesis and therapy strategy. Aging (Albany NY) 2020; 12:2897-2920.
28. Li Y, Fan H, Ni M, Zhang W, Fang F, Sun J, *et al.* Targeting lncRNA NEAT1 hampers alzheimer's disease progression. Neuroscience 2023; 529:88-98.
29. Srinivas T, Mathias C, Oliveira-Mateos C, Guil S. Roles of lncRNAs in brain development and pathogenesis: Emerging therapeutic opportunities. Mol Ther 2023; 31:1550-1561.
30. Pan Y, Wang T, Zhao Z, Wei W, Yang X, Wang X, *et al.* Novel insights into the emerging role of Neat1 and its effects downstream in the regulation of inflammation. J Inflamm Res 2022; 15:557-571.
31. Zalewska A, Klimiuk A, Zięba S, Wnorowska O, Rusak M, Waszkiewicz N, *et al.* Salivary gland dysfunction and salivary redox imbalance in patients with Alzheimer's disease. Sci Rep 2021; 11:23904.
32. Omar RA, Chyan YJ, Andorn AC, Poeggeler B, Robakis NK, Pappolla MA. Increased expression but reduced activity of anti-oxidant enzymes in Alzheimer's disease. J Alzheimers Dis 1999; 1:139-145.
33. Bai R, Guo J, Ye XY, Xie Y, Xie T. Oxidative stress: The core pathogenesis and mechanism of Alzheimer's disease. Ageing Res Rev 2022; 77:101619.
34. Ghafouri-Fard S, Safari M, Taheri M, Samadian M. Expression of linear and circular lncRNAs in Alzheimer's disease. J Mol Neurosci 2022; 72:187-200.
35. Kamat PK, Kalani A, Rai S, Swarnkar S, Tota S, Nath C, *et al.* Mechanism of oxidative stress and synapse dysfunction in the pathogenesis of Alzheimer's disease: Understanding the therapeutics strategies. Mol Neurobiol 2016; 53:648-661.
36. Nocella C, Cammisotto V, Pigozzi F, Borrione P, Fossati C, D'Amico A, *et al.* Impairment between oxidant and anti-oxidant systems: Short- and long-term implications for athletes' health. Nutrients 2019; 11:1353.
37. Andersen HR, Jeune B, Nybo H, Nielsen JB, Andersen-ranberg K, Grandjean P. Low activity of superoxide dismutase and high activity of glutathione reductase in erythrocytes from centenarians. Age Ageing 1998; 27:643-648.
38. Nunomura A, Perry G, Aliev G, Hirai K, Takeda A, Balraj EK, *et al.* Oxidative damage is the earliest event in Alzheimer disease. J Neuropathol Exp Neurol 2001; 60:759-767.
39. Charisis S, Ntanasi E, Yannakoulia M, Anastasiou CA, Kosmidis MH, Dardiotis E, *et al.* Plasma GSH levels and Alzheimer's disease. A prospective approach.: Results from the HELIAD study. Free Radic Biol Med 2021; 162:274-282.
40. Kitamura Y, Shimohama S, Kamoshima W, Matsuoka Y, Nomura Y, Taniguchi T. Changes of p53 in the brains of patients with Alzheimer's disease. Biochem Biophys Res Commun 1997; 232:418-421.
41. Ohyagi Y, Asahara H, Chui DH, Tsuruta Y, Sakae N, Miyoshi K, *et al.* Intracellular Abeta42 activates p53 promoter: A pathway to neurodegeneration in Alzheimer's disease. Faseb j 2005; 19:255-257.
42. Wang Z, Zhao Y, Xu N, Zhang S, Wang S, Mao Y, *et al.* NEAT1 regulates neuroglial cell mediating Aβ clearance via the epigenetic regulation of endocytosis-related genes expression. 2019; 76:3005-3018.
43. Sabaie H, Tamimi P. Expression analysis of inhibitory B7 family members in Alzheimer's disease. 2023; 38:2563-2572.
44. Asadi MR, Gharesouran J, Sabaie H, Zaboli Mahdiabadi M, Mazhari SA, Sharifi-Bonab M, *et al.* Neurotrophin growth factors and their receptors as promising blood biomarkers for Alzheimer's disease: A gene expression analysis study. Mol Biol Rep 2024; 51:49.