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ORIGINAL ARTICLE

Neurotrophin growth factors and their receptors as promising blood biomarkers for Alzheimer's Disease: a gene expression analysis study

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Abstract

Background Alzheimer's disease (AD) is a multifaceted neurological ailment affecting more than 50 million individuals globally, distinguished by a deterioration in memory and cognitive abilities. Investigating neurotrophin growth factors could offer significant contributions to understanding AD progression and prospective therapeutic interventions.

Methods and results The present investigation collected blood samples from 50 patients diagnosed with AD and 50 healthy individuals serving as controls. The mRNA expression levels of neurotrophin growth factors and their receptors were measured using quantitative PCR. A Bayesian regression model was used in the research to assess the relationship between gene expression levels and demographic characteristics such as age and gender. The correlations between variables were analyzed using Spearman correlation coefficients, and the diagnostic potential was assessed using a Receiver Operating Characteristic curve. NTRK2, TrkA, TrkC, and BDNF expression levels were found to be considerably lower (p-value < 0.05) in the blood samples of AD patients compared to the control group. The expression of *BDNF* exhibited the most substantial decrease in comparison to other neurotrophin growth factors. Correlation analysis indicates a statistically significant positive association between the genes. The ROC analysis showed that *BDNF* exhibited the greatest Area Under the Curve (AUC) value of 0.76, accompanied by a sensitivity of 70% and specificity of 66%. *TrkC*, *TrkA*, and *NTRK2* demonstrated considerable diagnostic potential in distinguishing between cases and controls.

Conclusion The observed decrease in the expression levels of *NTRK2*, *TrkA*, *TrkC*, and *BDNF* in AD patients, along with the identified associations between specific genes and their diagnostic capacity, indicate that these expressions have the potential to function as biomarkers for the diagnosis and treatment of AD.

Keywords Alzheimer's Disease · Neurotrophin growth factors · Biomarkers · Peripheral blood · Gene expression · Bayesian regression · ROC curve analysis

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Introduction

Alzheimer's disease (AD) is a neurodegenerative disorder that exhibits a gradual regression in cognitive function and memory impairment [1]. As per the World Health Organization (WHO) report, the prevalence of dementia is observed in excess of 55 million individuals worldwide, wherein AD accounts for 60–70% of the cases [2, 3]. According to projections, the present incidence of AD in the United States is roughly 5.8 million, with the figure expected to climb to 14 million by 2050 [4]. The condition is also connected with a considerable economic load, with the total expenditure on care in the United States predicted to reach $305 billion in 2020 [4]. AD is a complicated and multifaceted condition, with many genetic and environmental variables influencing its development and course [5]. Mutations in the *APP*, *PSEN1*, and *PSEN2* genes, which are involved in the formation and processing of amyloid-beta protein, are among the recognized genetic risk factors for AD [6]. Other genetic variants, such as the *APOE 4* allele, have been linked to a higher chance of developing AD [7]. Head injury, cardiovascular disease, and prolonged stress have all been associated with an elevated probability of AD [8]. Given the probable significance of neurotrophin growth factors in neuroprotection and neuroplasticity, more research into the role of neurotrophins in AD may give valuable insights into its aetiology and prospective treatment options.

Neurotrophin growth factors, such as nerve growth factor (*NGF*), brain-derived neurotrophic factor (*BDNF*), neurotrophin-3 (*NTF3*), neurotrophin-4 (*NTF4*), and their receptors, have been linked to the aetiology of AD [9, 10]. These growth factors are essential for the survival, development, and normal functioning of neurons in the central nervous system (CNS) and peripheral nervous system (PNS) [11]. The tropomyosin receptor kinase (Trk) family is a vital neurotrophin receptor that helps control cell survival, development, and synapse plasticity [12]. For example, the *TrkA* receptor attaches to *NGF* and regulates neuronal survival and differentiation in the PNS, while the *TrkB* and *TrkC* receptors bind to *BDNF* and *NTF3/4*, respectively, and control CNS neuronal viability, development, and synapses' plasticity [13, 14]. Research has indicated that in AD, there are changes in how neurotrophins and Trk receptors are expressed and signaled, resulting in compromised neuroprotection, neuroplasticity, and cognitive function. For example, *BDNF* and *TrkB* reduced levels have been documented in the AD patient's hippocampus and cortex, which may lead to synaptic impairment and cognitive decline [15, 16]. The levels of pro*NGF* are elevating in AD [17], although *NGF* production is intact [18], and Bruno et al. revealed that there is an interfere with in the processing of pro*NGF* to physiologically active m*NGF*, and a probable increased breakdown of m*NGF* [19]. Hence, additional research on the function of neurotrophins and their receptors in AD could offer significant knowledge regarding the development of this ailment and possible remedial objectives [20, 21]. Testing the levels of neurotrophins and their receptors in the blood of individuals with AD may give a practical, non-invasive, and affordable technique for identifying, monitoring, and predicting disease development. Moreover, this methodology could prove to be advantageous in evaluating the efficacy of treatments that rely on neurotrophic factors.

The purpose of this research is to study into the potential of neurotrophin growth factors and their receptors as biomarkers for AD by assessing their expression levels in peripheral blood. Examining neurotrophin levels in the blood may offer valuable insights into the development and progression of AD and potential therapeutic targets, given their crucial role in neuronal survival, development, and synaptic plasticity and their altered expression and signaling in AD. The current study aims to bridge the existing gap in understanding concerning the involvement of neurotrophins in AD and to facilitate the development of economic and non-invasive approaches for diagnosing and treating AD.

Materials and methods

Samplings of human peripheral blood

This research used a cross-sectional strategy to gather blood samples from 50 AD patients and 50 healthy controls. The aforementioned study obtained ethical approval from Tabriz University of Medical Sciences under the code IR.TBZMED.REC.1401.711. The neurologists at Imam Reza Hospital in Tabriz gathered patients who met the diagnostic and statistical manual of mental disorders-V (DSM-V) [22] criteria for AD. During case selection, it was determined whether the patients met the inclusion criteria by reviewing their medical records, which included information on the patients' disease period, educational background, heritage, as well as history of other diseases and how long they persisted, narcotics used, and mental state assessment findings. Individuals must be at least 65 years old and have no mental or neurological disorders other than AD to be eligible for the research. Participants who had current or previous medical conditions of diabetes, ongoing or persistent infectious illnesses, thyroid abnormalities, neoplastic disorders, kidney and hepatic insufficiency or failure, inflammatory pathologies or those receiving anti-inflammatory medications, metabolic diseases or abnormalities, recent acute ischemic heart problems, cerebrovascular incidents, habitual alcohol consumption, or who had received corticosteroid treatment

in the prior eight weeks were not permitted to participate in the study. The Mini-Mental State Examination (MMSE) was used to measure cognitive performance. Each and every participant was approached before their involvement in the research, and those who were capable of providing informed consent willingly did so. In situations where people suffering from AD were incapable of giving their consent due to cognitive impairment, written consent was acquired from their legal guardians after they were provided with detailed information about the study's objectives and methods. Subsequently, a volume of 5 ml of peripheral blood was obtained from every contributor and transferred into tubes treated with EDTA for subsequent analysis. Each participant also provided demographic and medical information, which was kept for further study.

mRNA expression analysis of neurotrophin growth factors and their receptors

The extracted RNA was used to generate complementary DNA (cDNA) using the GeneALL cDNA synthesis kit, as directed by the manufacturer. For future investigation, the cDNA was stored at -20 °C. To evaluate the expression levels of neurotrophin growth factors and their receptors, quantitative polymerase chain reaction (qPCR) was performed utilizing Step OnePlusTM Real-Time PCR and the RealQ Plus2x Master Mix (Ampliqon, Odense, Denmark). Table 1 shows the primer sequences used in reverse transcription and qPCR, and the qPCR tests were done in duplicate. To standardize mRNA levels, internal controls such as ubiquitin C (*UBC*) were used. The evaluation of the RNA's quality and quantity was conducted using the NanoDrop instrument, developed by Thermo Scientific (Wilmington, DE, USA). To eliminate DNA contamination, the extracted RNA was processed with DNase I using the Hybrid-RTM Blood RNA purification kit (GeneALL, Seoul, South Korea).

Table 1 List of primers used in this study

Gene name	Primer sequences (5'-3')	Product size	Tm
NTRK2	Forward primerACAGTCAGCTCAAGCCAGACAC Reverse primerGTCCTGCTCAGGACAGAGGTTA	134 nt	61
NGF	Forward primerACCCGCAACATTACTGTGGACC Reverse primerGACCTCGAAGTCCAGATCCTGA	123 nt	61
BDNF	Forward primerGGAAAACTTGGGAGGCGGAAT Reverse primerTCTCACCTGGTGGAACCATT	234 nt	60
TrkA	Forward primerCACTAACAGCACATCTGGAGACC Reverse primerTGAGCACAAGGAGCAGCGTAGA	122 nt	60
NTF3	Forward primerCAAGCAGATGGTGGACGTTAAGG Reverse primerTCGCAGCAGTTCGGTGTCCATT	136	61
TrkC	Forward primerCCGACACTGTGGTCATTGGCAT Reverse primerCAGTTCTCGCTTCAGCACGATG	140	61
NTF4	Forward primerGCAAGGCTGATAACGCTGAGGA Reverse primerCCTGGGCATCAGCGGTCAATG	135	61
UBC	Forward primerCAGCCGGGATTTGGGTCG Reverse primerCACGAAGATCTGCATTGTCAAGT	72	60

QPCR statistical analysis

The R software and GraphPad Prism 8 (Graph-Pad Software, Inc., San Diego, CA, USA) were utilized for the purpose of conducting statistical analysis and data visualization. The major purpose of the research was to examine the levels of *NTRK2*, *NGF*, *TrkA*, *NTF3*, *TrkC*, *NTF4*, and *BDNF* in patients with AD and healthy controls. The acquisition of the normal or Gaussian distribution of the values was accomplished by means of the execution of the Shapiro-Wilk test, a statistical technique that is extensively utilized in the determination of the normality of a particular dataset. Once the confirmation of a normal distribution was obtained, the next step entailed the scrutiny of the variations in gene expression levels between the two groups, a comparison that was performed through the utilization of a t-test. P-values that were computed, and results that were less than 0.05, fold change (FC) $> +1.5$, and FC < -1.5 were deemed significant. The research assessed not only the changes in gene expression across groups but also between men and women. Spearman correlation coefficients were calculated to assess the correlations between the variables in the research. Finally, a receiver operating characteristic (ROC) curve was developed to assess the diagnostic capability of the genes.

Results

BDNF shows the most significant decline in expression compared to other neurotrophin growth factors

In blood samples from controls and AD patients, the study examined the levels of gene expression associated with neurotrophin, provided in Table 2, and the relative expressions in case and controls are shown in Fig. 1. *NTRK2*,

Table 2 Relative expression of genes in total, females, and males AD patients and healthy controls

Gene name	Parameters	ΔCt means		Up/Down	1/Fold Change	*P*-Value
		Cases	Controls			
NTRK2	Total	-2.952	-3.956	Down	2.008	< 0.0001****
	Female	-2.942	-3.650	Down	1.633	0.0269*
	male	-2.968	-4.415	Down	2.724	0.0004***
NGF	Total	-1.798	-1.768	NS	0.979	0.908
	Female	-1.535	-1.803	NS	1.204	0.3969
	male	-2.226	-1.715	NS	0.701	0.2527
TrkA	Total	-3.268	-4.312	Down	2.066	< 0.0001****
	Female	-3.239	-4.203	Down	1.953	0.0025**
	male	-3.316	-4.475	Down	2.237	0.0025**
NTF3	Total	-3.296	-2.986	NS	0.806	0.1532
	Female	-3.306	-2.867	NS	0.737	0.1260
	male	-3.279	-3.165	NS	0.924	0.7353
TrkC	Total	2.476	1.458	Down	2.028	< 0.0001****
	Female	2.442	1.437	Down	2.008	0.0008***
	male	2.532	1.490	Down	2.061	0.0074**
NTF4	Total	-1.700	-1.692	NS	0.994	0.9753
	Female	-1.545	-1.697	NS	1.111	0.6477
	male	-1.953	-1.685	NS	0.830	0.5264
BDNF	Total	2.61	1.032	Down	2.985	< 0.0001****
	Female	2.73	0.87	Down	3.623	< 0.0001****
	male	2.41	1.26	Down	2.212	0.0221*

*Significant *p*-value < 0.05, **significant *p*-value < 0.01, *** significant *p*-value < 0.001, ****significant *p*-value < 0.0001, NS: not significant.

TrkA, *TrkC*, and *BDNF* expression levels were substantially downregulated (*p*-value < 0.05) in AD patients compared to controls. *NGF*, *NTF3*, and *NTF4*, on the other hand, did not exhibit significant variations in expression levels between the two groups. The statistical analysis also showed that men had much higher downregulated *NTRK2* expression than females, as well as *TrkA* and *TrkC* expression levels. In contrast to healthy controls, both male and female AD patients had considerably lower levels of *BDNF* expression. The fold variations in the expression levels of the relevant genes also revealed that *BDNF* was the neurotrophin-related gene that was most substantially downregulated (2.985-fold), followed by *NTRK2* (2.008-fold), *TrkC* (2.028-fold), and *TrkA* (2.066-fold).

Correlation analysis

Utilizing correlation coefficient data, the relationship between these genes was assessed and provided in Table 3. The results demonstrate a significant positive association between the expression of *NTRK2* and age (0.339*, *p*-value = 0.016), as well as between *NTRK2* and other genes such as *BDNF* (0.613****, *p*-value < 0.0001), *NGF* (0.543****, *p*-value < 0.0001), *TrkA* (0.717****, *p*-value < 0.0001), *TrkC* (0.686****, *p*-value < 0.0001), *NTF3* (0.598****, *p*-value < 0.0001), and *NTF4* (0.531****, *p*-value < 0.0001). Additionally, *NTF4* had a strong positive connection with *BDNF* (0.511****, *p*-value < 0.0001), *NGF* (0.648****, *p*-value < 0.0001), *TrkC* (0.522****, *p*-value < 0.0001), and *NTF3* (0.640****, *p*-value < 0.0001), but not with age (*p*-value = 0.566). The results demonstrate that *NTF3* exhibits noteworthy positive associations with *BDNF* (0.528****, *p*-value < 0.0001), *NGF* (0.550****, *p*-value < 0.0001), *TrkA* (0.482****, *p*-value < 0.0001), and *TrkC* (0.547****, *p*-value < 0.0001). Conversely, no significant correlation was observed between *NTF3* and age, as evidenced by a p-value of 0.412. Additionally, it was observed that *TrkC* exhibited noteworthy favorable associations with *BDNF* (0.511****, *p*-value < 0.0001) and *NGF* (0.379**, *p*-value = 0.007); however, no significant correlation was found with age (*p*-value = 0.619). The study found that *TrkA* and *NGF* did not exhibit a statistically significant correlation with age (*p*-value = 0.253 and *p*-value = 0.072, respectively). However, these genes demonstrated a significant positive correlation with other genes, namely *BDNF*, *NTF3*, and *NTF4*.

ROC curve analysis

The ROC analysis results, including the area under the curve (AUC), sensitivity, specificity, 95% confidence interval (CI), cut-off, and P-value, are presented in Table 4 and are shown in Fig. 2 and in combination in Fig. 3. The results showed that *BDNF* had the highest AUC of 0.76, with a sensitivity of 70% and a specificity of 66%. The optimal

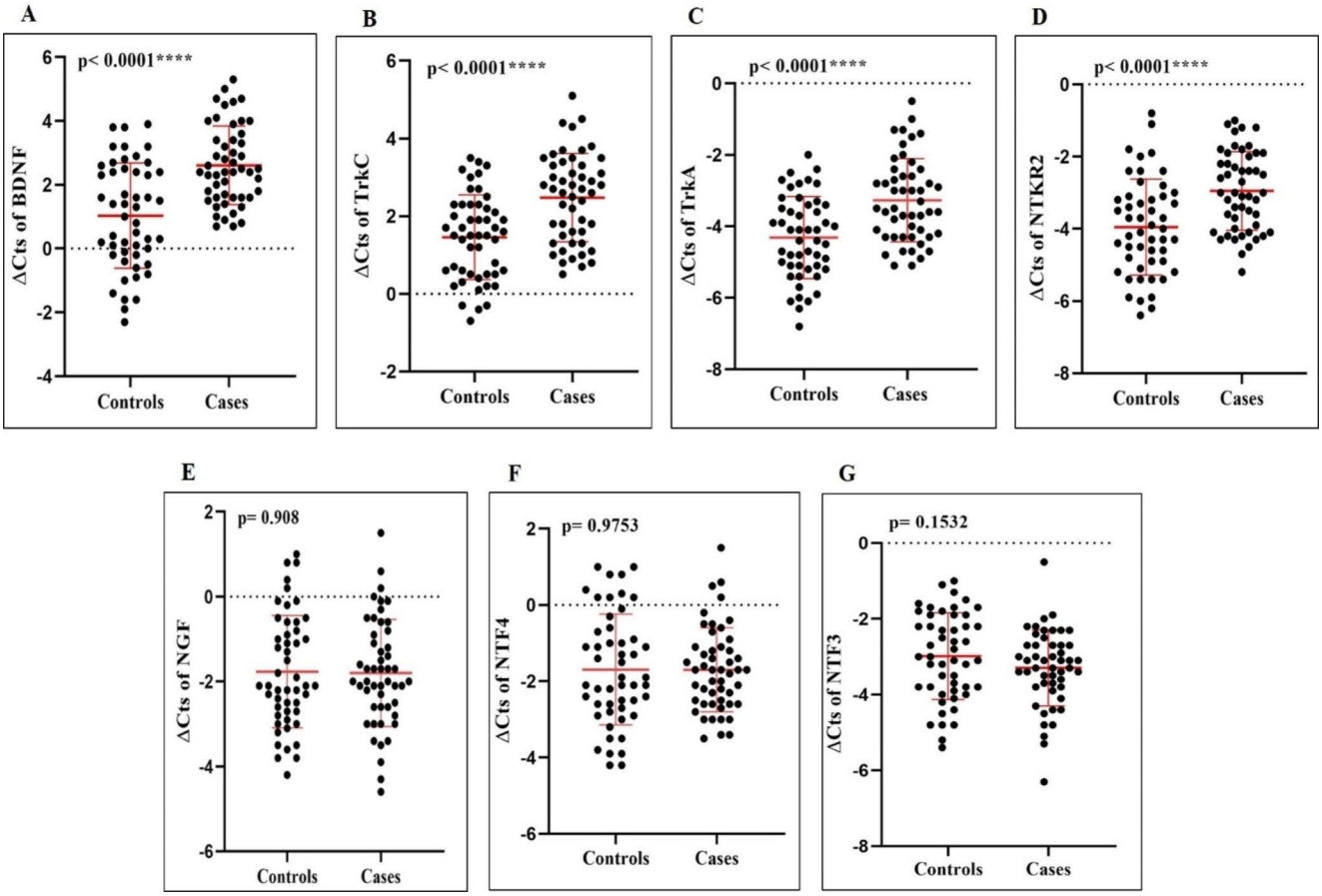

Fig. 1 The test covered an assessment of the relative expression of (**A**) BDNF, (**B**)Trkc, (**C**)TrkA, (**D**)NTRK2, (**E**)NGF, (**F**)NTF4, and (**G**) NTF3 in both the patient and control groups. The gene expression levels of each sample were normalized in relation to UBC expression, serving as the reference gene. The relative expression of the transcripts was ascertained by employing the t-test and the formula 2^-ΔΔCt. Black dots demonstrate values, and the means of expression levels range are shown in cases and controls

Table 3 Pairwise correlation between expression levels of genes in cases group

	Age	BDNF	NGF	TrkA	TrkC	NTF3	NTF4
NTRK2	0.339*	0.613****	0.543****	0.717****	0.686****	0.598****	0.531****
	0.016	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001
NTF4	− 0.083	0.511****	0.648****	0.400**	0.522****	0.640****	
	0.566	< 0.0001	< 0.0001	0.004	< 0.0001	< 0.0001	
NTF3	0.119	0.528****	0.550****	0.482****	0.547****		
	0.412	< 0.0001	< 0.0001	< 0.0001	< 0.0001		
TrkC	− 0.072	0.511****	0.379**	0.546****			
	0.619	< 0.0001	0.007	< 0.0001			
TrkA	0.165	0.500****	0.519****				
	0.253	< 0.0001	< 0.0001				
NGF	0.257	0.584****					
	0.072	< 0.0001					
BDNF	0.058						
	0.690						

cut-off point for *BDNF* was more significant than 1.75, and the P-value was less than 0.0001. *TrkC* had an AUC of 0.73, with a sensitivity of 62% and a specificity of 72%. The study determined that the optimal cut-off point for *TrkC* was found to be greater than 2.15, with a statistically significant P-value of less than 0.0001. The AUC for *TrkA* was found to be 0.72, accompanied by a sensitivity of 66% and a specificity of 66%. The statistical analysis revealed that the optimal cut-off point for *TrkA* was found to be greater than − 3.85, with a P-value of less than 0.0001. The AUC for

Table 4 The results of ROC curve analysis of genes in the separation of cases and controls

Gene Name	AUC	Sensitivity%	Specificity%	95% CI	Cut off	P-Value
BDNF	0.76	70	66	0.665 to 0.852	> 1.75	< 0.0001
TrkC	0.73	62	72	0.636 to 0.829	> 2.15	< 0.0001
TrkA	0.72	66	66	0.626 to 0.821	> -3.85	< 0.0001
NTRK2	0.72	70	60	0.623 to 0.821	> -3.65	< 0.0001
Model	0.77	74	64	0.680 to 0.860	> 0.46	< 0.0001

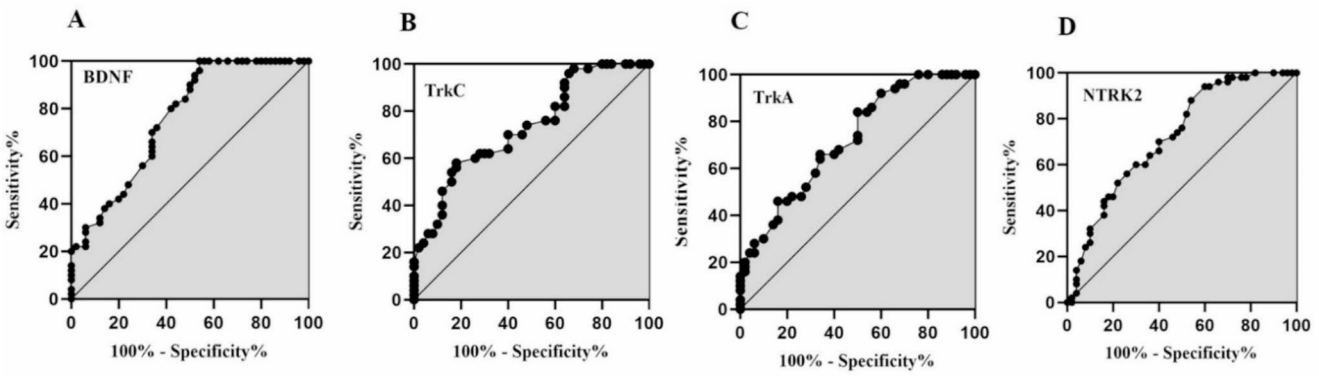

Fig. 2 The ROC curve analysis shows the diagnostic power of genes in separating cases and controls. **A-D.** BDNF, TrkC, TrkA, and NTRK2 transcript levels displayed diagnostic power of 0.76, 0.73, 0,72, and 0.72, respectively

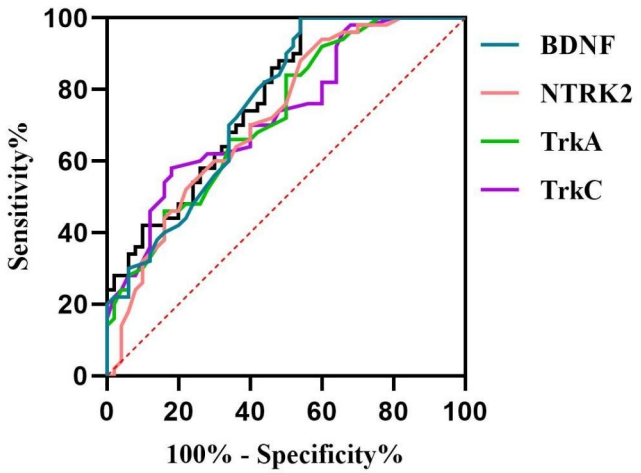

Fig. 3 ROC curve for the combination of expression levels of BDNF, NTRK2, TrkA, and TrkC.

NTRK2 was determined to be 0.72, while the sensitivity and specificity were found to be 70% and 60%, respectively. The study findings indicate that the *NTRK2* optimal cut-off point was observed to be greater than − 3.65, with a statistically significant P-value of less than 0.0001. The predictive model exhibited an area under the ROC curve (AUC) of 0.77, accompanied by a sensitivity of 74% and a specificity of 64%. The model's optimal cut-off point was found to be greater than 0.46, and the P-value was determined to be less than 0.0001. In summary, the findings indicate that *BDNF*, *TrkC*, *TrkA*, *NTRK2*, and the model exhibit considerable diagnostic capacity in distinguishing between individuals.

Discussion

AD is a neurodegenerative disorder that impacts a significant number of individuals globally, as reported by previous studies [23]. The condition is distinguished by the demise of neurons, malfunction of synapses, and decline in cognitive abilities [24]. These changes can be attributed to modifications in the expression of genes that participate in neurotrophin signaling [16, 25]. Neurotrophin signaling has been linked to sustaining cognitive function and is essential for neuronal survival, development, and synaptic plasticity [26]. To gain a greater understanding of the pathogenesis of AD, we investigated changes in the gene expression of several neurotrophin signaling regulators. With possible diagnostic and therapeutic implications, our results provide insightful information about the molecular pathways driving AD.

AD has been the subject of extensive research on *BDNF* dysregulation and changed expression [27, 28]. When compared to other neurotrophin growth factors, our research found that *BDNF* expression had significantly decreased. *BDNF* demonstrated a moderate to high capacity for separating cases from controls. *BDNF*-reduced levels of expression have been seen in the brain [16], cerebrospinal fluid [29], serum [30], and peripheral blood [31], which indicates systemic dysfunction in AD [32]. Dysregulated *BDNF* levels have been linked to impaired brain neuronal function and disruption of the peripheral immune system [33]. The augmented presence of bacterial DNA in the bloodstream,

known as BB-DNA, has the potential to facilitate inflammation in both the peripheral and neuro regions, thereby playing a role in the development of cognitive dysfunction [34]. It has been recorded that during the initial stages of AD, an escalation in BB-DNA concentration is directly proportional to elevated plasma *BDNF* levels, implying an effort to counteract the condition [34]. In the previous study we conducted, the reduced expression of *BDNF* was obtained both in the peripheral blood and the brain tissue of AD patients, which resulted in the conclusion that the expression of *BDNF* in the brain can be influenced by the *BDNF/TTP/miR-16-5p* regulatory axis [31].

BDNF is a critical factor in neurons' development, nourishment, and survival [35]. In the AD background, BDNF deficiency is related to tau phosphorylation, Aβ plaque, neuroinflammation, and neuronal death [36]. The impairment of the plasticity of neurons and trophic assistance due to reduced *BDNF* signaling is believed to be a contributing factor to the synaptic dysfunction and neuronal loss that is associated with AD [37]. In this regard, gene delivery of *BDNF* through liposomes in transgenic mice led to a notable reduction of approximately two times in plaque load within the cortex region and approximately three times in the hippocampus region, thereby demonstrating significant enhancement [38]. When *BDNF* is stimulated, *TrkB* and phosphatidylinositol 3-kinase (*PI3K*) signalling are activated, which results in tau dephosphorylation [39, 40]. Aβ impairs *BDNF* signalling by dysregulating the glutamatergic *NMDAR/Ca2+/calpain* signalling cascade [41]. Aβ can lessen the dendritic atrophy and neuronal death, it causes by upregulating *BDNF* through the extracellular directed kinases/cyclic AMP response element-binding protein (*ERK/CREB*) signalling pathway [42]. suppressing *BDNF* antisense RNA may greatly increase *BDNF* expression, decrease Aβ-induced neurotoxicity, and improve cell survival [43]. Furthermore, *BDNF* has been identified as a plausible therapeutic target and a diagnostic indicator for AD [31, 44].

The Tropomyosin receptor kinase B (*TrkB*) protein is encoded by the *NTRK2* gene and works as a neurotrophin receptor, regulating neuronal development, viability, and synaptic plasticity [45]. According to Chen et al., there is a notable correlation between *NTRK2* and AD, as indicated by the genetic variations of *NTRK2* [46]. In addition, Devi et al. revealed that decreased *TrkB* does not impact β-amyloidosis but increases the development of hippocampal mnemonic and signalling dysfunctions in early AD [47]. Furthermore, Wang and colleagues have documented that the absence of *BDNF/NTRK2* undeniably plays a role in the development of AD-like pathologies in wild-type mice [36]. However, in a recent investigation, Iglesias and colleagues have reported the absence of any variations in NTRK2 mRNA expression when comparing healthy individuals (n = 9) and AD patients (n = 7) in blood buffy coat specimens [48]. Our results show that the levels of *NTRK2* expression among individuals with AD are much lower than in the control group. This conclusion is consistent with prior research that has shown a decline in *TrkB* mRNA levels in both the central nervous system and peripheral blood in individuals with AD [47, 49]. The literature reports that the dysregulation of *TrkB* signaling leads to decreased neuronal plasticity, progressive brain degeneration, and cognitive impairments [50]. The reduced expression of *NTRK2* in individuals with AD suggests that it may serve as a promising diagnostic marker for early disease detection, and this has the potential to enable timely intervention and delay cognitive decline.

TrkA plays a fundamental role in regulating the survival and development of cholinergic neurons, which are the primary neuronal subtype that undergoes degeneration in AD [51]. *TrkA* and *p75* neurotrophin receptor (*p75NTR*) are two receptors that *NGF* interacts with and which are known to mediate conflicting biological effects, namely cell death and survival, in specific conditions [52]. Pro-survival *TrkA* expression is decreased in AD, which may facilitate *NGF* binding to *p75NTR* and subsequently lead to cholinergic dysfunction [53]. Additionally, pro*NGF* levels rise extracellularly when AD pathology is present because deficiencies in *NGF* metabolism impair the processing of pro*NGF* to mature *NGF* [52]. Pro*NGF* interacts with *p75NTR* selectively to cause neurodegeneration [52]. Moreover, the pathogenesis of AD is represented by a considerable degeneration of cholinergic neurons in the basal forebrain, which is closely linked to the observed cognitive decline [54]. *TrkA* expression reduction could have an integral part in the demise of cholinergic neurons found in AD patients, hence exacerbating cognitive function impairment [55]. The significance of cholinergic neurons originating from the nucleus basalis of Meynert concerning memory is underscored by the observation that the targeted degeneration of these neurons occurs in AD and is a contributing factor to the memory impairment displayed by individuals with AD [54, 56]. Notably, Xima et al. revealed that using ultrasound for delivering a *TrkA* agonist may help retain these neural connections while preserving against AD-related pathologies. *TrkA* agonists have the ability to trigger and stimulate pathways that promote neuronal development and survival [57].

In this regard, Crispoltoni et al. observed that *TRKA* expression was heightened in individuals with mild cognitive impairment and mild AD, while it was reduced in those with severe AD, as indicated by the analysis of peripheral blood monocytes throughout the course of the disease [58]. Furthermore, Chen et al.'s findings, which demonstrated the downregulation of *TRKA* in peripheral leukocytes in AD,

provide further substantiation for the involvement of *TRKA* in monocytes during the progression of AD [59]. The observation of reduced expression levels in affected individuals has highlighted the significance of *TrkA* in the pathogenesis of AD. The findings indicate that there were notable changes in the levels of *TrkA* expression, which may suggest its potential dysregulation role in AD development. The present investigation involved assessing the *TrkA* expression levels and their associations with other genes implicated in neurotrophin signaling. The findings of our analysis suggest a significant positive correlation between *TrkA* and genes involved in neurotrophin signaling pathways, including *BDNF*, *NTF3*, and *NTF4*. This implies that there are complex connections between these genes in AD development. *TrkA*'s diagnostic potential implies that it could serve as a reliable biomarker for the timely detection of AD, which could facilitate prompt intervention and potentially postpone cognitive deterioration.

The *TrkC* receptor, a member of the neurotrophin receptor family, is essential for modulating synaptic plasticity, cellular survival, and differentiating in both the central and peripheral nervous systems [11]. The present investigation has noted a noteworthy reduction in the levels of *TrkC* expression among individuals with AD in comparison to the control group, and this implies the absence of a crucial mechanism in the progression of the disease. Prior research has detected modifications to *TrkC* signaling in diverse neurodegenerative conditions, such as AD, indicating its alleged involvement in the pathogenesis of the disease [60]. In this context, Gonzalez et al. demonstrated the prevention of cholinergic neuron atrophy in an advanced clinical stage of AD by targeting *TrkB* and *TrkC* receptors with small compounds [61]. *TrkA* expression is generally restricted to basal forebrain cholinergic neurons (BFCN), while *TrkB* and *TrkC* are found not only in BFCNs but also in a considerable percentage of neuronal populations afflicted by AD. These neuronal groupings make up BFCN target locations such as the hippocampus, amygdala, and some cerebral areas [62–65]. Additional research is necessary to fully clarify the exact mechanisms that underlie alterations in *TrkC* expression in AD. As reported in our study, the observed changes in *TrkC* expression levels in AD may have implications for the development of innovative therapeutic interventions for this condition.

The results of our correlation analysis demonstrate significant positive correlations between the expression levels of *NTRK2* and other genes involved in neurotrophin signaling, including *BDNF*, *NTF3*, *NTF4*, and *TrkC*. The findings suggest that the disruption of intrinsic neurotrophin signaling in AD may play a role in the alterations detected in the levels of *NTRK2* expression. The potential consequences of *NTRK2* downregulation include reduced neuronal plasticity, cognitive deficits, and gradual deterioration of brain structure and function.

The study also assessed the gene expression levels of *NGF*, *NTF3*, and *NTF4*. However, no significant alterations were observed in cases compared to controls. The results indicate that alterations in the expression of genes related to neurotrophin signaling pathways in AD may not be evenly distributed among all genes and may be inconspicuous. Furthermore, it is imperative to consider that alterations in gene expression within the whole blood may not precisely mirror changes in expression within brain tissue.

Limitations

The study is subject to certain constraints, such as the utilization of blood instead of brain tissue, a relatively small sample size, the lack of longitudinal data, and the possibility of medication use or comorbidities having an impact. Subsequent investigations may extend the scope of this inquiry by scrutinizing a more comprehensive range of genes or complete pathways and clarifying the mechanisms underlying these modifications to devise more efficacious interventions.

Conclusions

The present study specifies insights into the aberrant modulation of gene expression profiles implicated in neurotrophic factor signaling cascades in AD. According to the findings, *BDNF* expression levels have decreased significantly, suggesting that it might be used as a diagnostic marker for early AD detection. Furthermore, the investigation reveals that alterations in the expression of *NTRK2*, *TrkA*, *TrkC*, and *BDNF* were detected in individuals with AD in contrast to those without the condition, suggesting that these genes could potentially have a significant impact on the development of AD. The study's limitations, such as the sample size and the utilization of blood instead of brain tissue, emphasize the necessity for forthcoming inquiries to enhance the study's credibility and applicability. Conducting additional research to investigate the genes mentioned above or pathways can facilitate the creation of interventions with greater efficacy and yield novel understandings regarding the mechanisms that underlie the pathogenesis of AD. In general, the results of the study can augment our comprehension of AD pathogenesis and contribute toward the formulation of diagnostic and therapeutic approaches for AD.

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Author contributions MRA, MZM and JG and HS performed the experiment. ZSF analyzed the data. SAS, MR and MSB wrote the draft and revised it. MT and AS designed and supervised the study. All the authors contribute equally and read the submission.

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Declaration

Competing interests The authors declare no competing interests.

Ethics approval and consent to participate All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. Informed consent forms were obtained from all study participants. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences. All methods were performed in accordance with the relevant guidelines and regulations.

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