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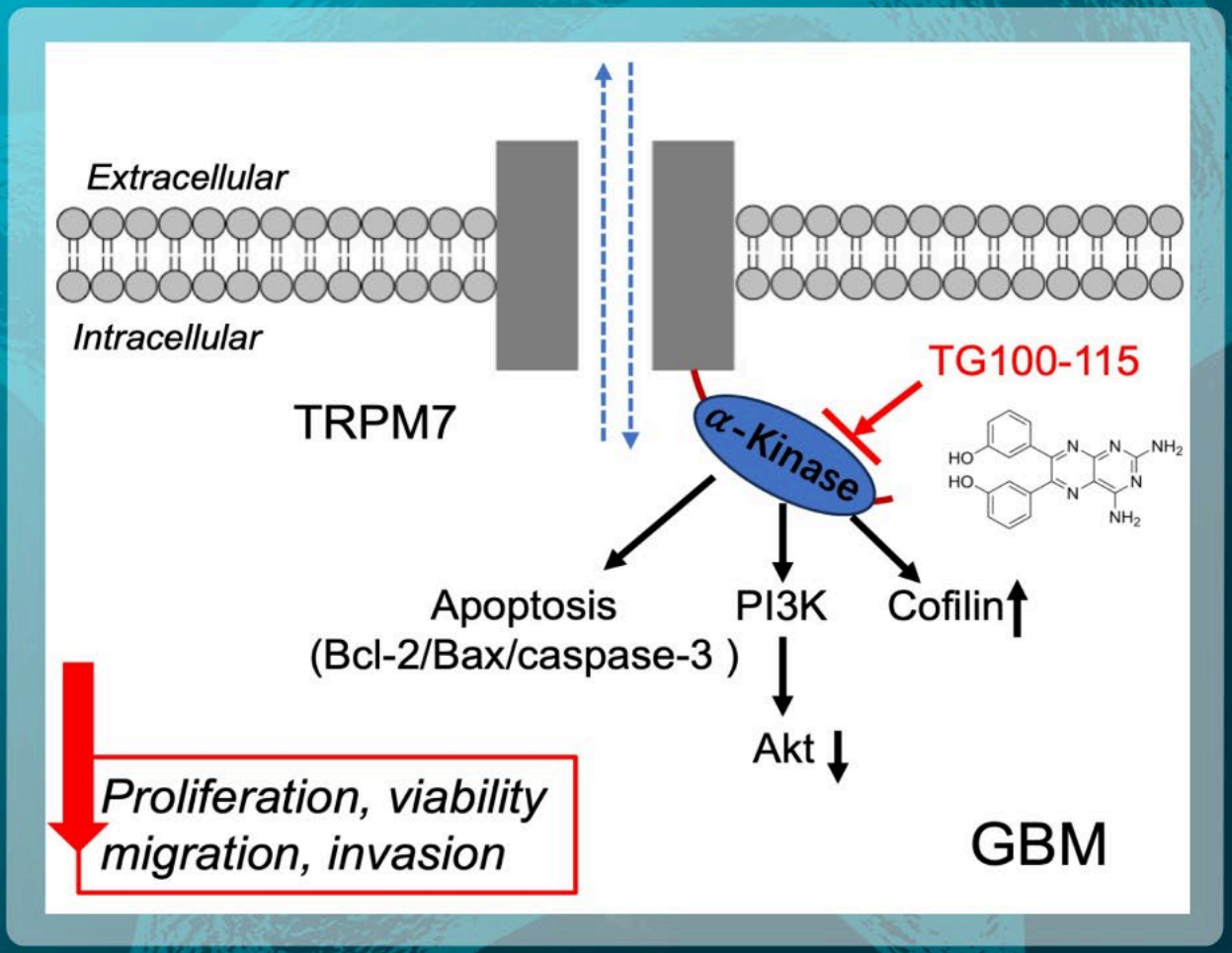

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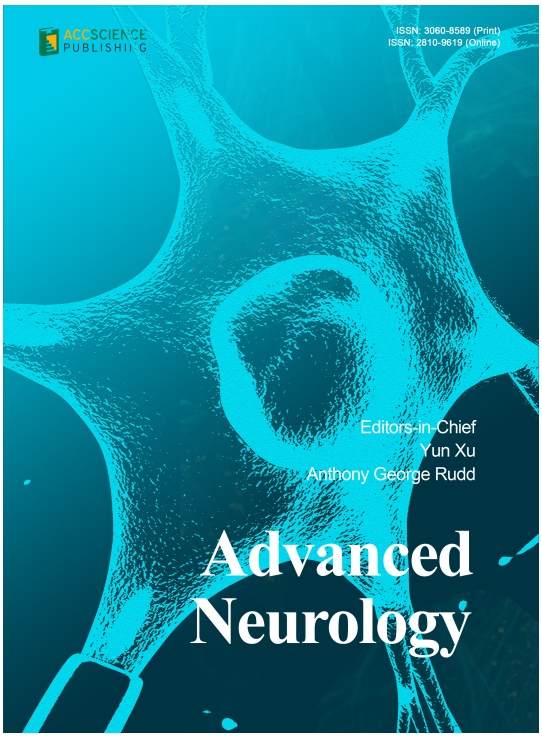

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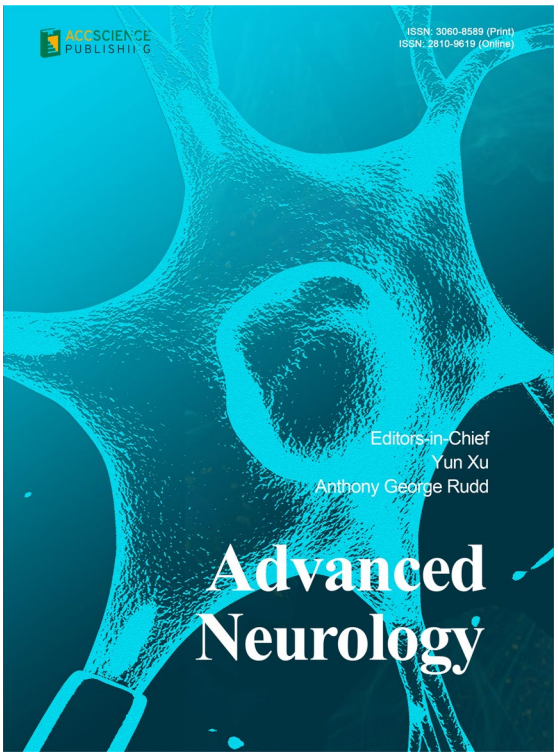

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REVIEW ARTICLE

A narrative review on the role of stem cell therapy in stroke treatment: Current advances, mechanisms, and future prospects

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Abstract

Stroke is a neurological condition characterized by blood vessel obstruction, leading to localized loss of brain function and associated neurological manifestations. Fatal cases of stroke are underrepresented in case–control studies compared to incident cases attributed to common causes. Hemorrhagic and ischemic strokes make up the primary classifications of stroke. This review describes the most recent findings on stem cells and how they can be used to treat stroke in people with serious illnesses. It highlights the potential of stem cells in stroke rehabilitation and gives an overview of their various forms and mechanisms of action. Stem cells promote stroke recovery through diverse pathways, depending on the cell type employed. Recent advancements in cell therapy for stroke include different stem cell sources, application methods, modification of culturing media and stem cells *ex vivo*, stem cell-derived extracellular vesicles, and 3D bioprocessing methods. Pre-clinical and clinical trials are important in assessing the effectiveness of the therapy, as the effect varies among individuals. Data were collected from different trials, highlighting the need for further advancements in stem cell therapy to improve its outcomes for treating stroke.

Keywords: Stroke; Stem cells; Mesenchymal stem cells; Clinical trials; Bioprocessing methods

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1. Introduction

Stroke is a neurological disorder and the second leading cause of mortality and infirmity around the world. Ischemic stroke, accounting for about 80% of all stroke cases, in which blood vessels get occluded by the formation of clots that interrupt cranial blood flow. This clogging reduces the blood flow to the brain, leading to necrosis – cell death along with cellular degradation and loss of neuronal function.[1] Hemorrhagic stroke contributes to about 15% of stroke cases but possesses the highest mortality rate. It is caused by the rupture of cranial blood vessels due to prolonged stress, along with both internal and external injuries, leading to hemorrhage and blood loss. Hemorrhagic stroke is further classified into two types: intracerebral, caused by ruptured blood vessels due to increased

cranial blood pressure, and subarachnoid hemorrhage, where blood accumulates in the subarachnoid space in the brain due to the injury at cerebral aneurysm.[2] A schematic representation of the detailed classification of stroke and its pathophysiology is described in Figure 1. Another type of stroke with an unknown etiology is cryptogenic stroke, which also contributes to approximately 15% of total stroke cases. Since its exact pathophysiology is not clear, it is relatively common and should receive more attention in clinical research and among neurologists.[3] Rapidly emerging signs and symptoms of stroke highly affect normal brain functions and become a leading cause of death. Individuals suffering from chronic hypertension, congestive heart failure, and other diseases such as hyperlipidemia and diabetes are highly susceptible to stroke. A significant number of stroke-related deaths are linked to a history of cardiovascular problems or modifiable risk factors, including alcohol intake, smoking, ischemic cardiac attack, and genetics.[4] The recovery period after suffering from stroke is divided into several phases: hyperacute phase (within 24 h), acute phase (up to 7 days), early sub-acute phase (up to 3 months), late sub-acute phase (up to 6 months), and chronic phase (more than 6 months).[5] Stroke therapy mainly focuses on the restoration of normal blood flow and alleviation of stroke-associated symptoms, including the removal of embolism and neurological damage. Numerous animal models have been developed to assess the effects of various herbal, synthetic, and semisynthetic molecules on stroke, with many of them already marketed. Negative animal models are also available for investigating the resistance mechanism of stroke, and ongoing studies are exploring novel interventions for the management of stroke.[6] In addition, stem cell-based therapies are emerging as novel therapeutics for managing disease severity and suppressing disease progression. Stem cells are unspecialized cells that can differentiate into any other types of cells and exist in both embryonic and adult forms. Recent studies based on embryonic stem cells (ESCs) and mesenchymal cells have gained high attention for their effectiveness against stroke, which indicates their potential in different aspects such as cellular regeneration, proliferation, remodeling of neural circuits, and maintaining energy production by modulating cellular functions.[7] Nowadays, neovascularization is another interesting area of research, where stem cells promote angiogenesis in several animal models of stroke.[8,9] This review will illustrate a comprehensive overview of stem cell-based therapeutic approaches, emphasizing both established procedures and recent advancements in the methodologies, and will conclude with future perspectives on mitigating the various consequences arising from stroke.

2. Epidemiology

Clinically, stroke is defined as rapid blood loss due to ischemia or hemorrhages that affect population health, and its prevalence rate is gradually increasing in developed countries. About 70% of stroke cases are reported in low-to-middle-income countries. In the current era, stroke is considered a global burden because of its continuously growing incidence, prevalence, and

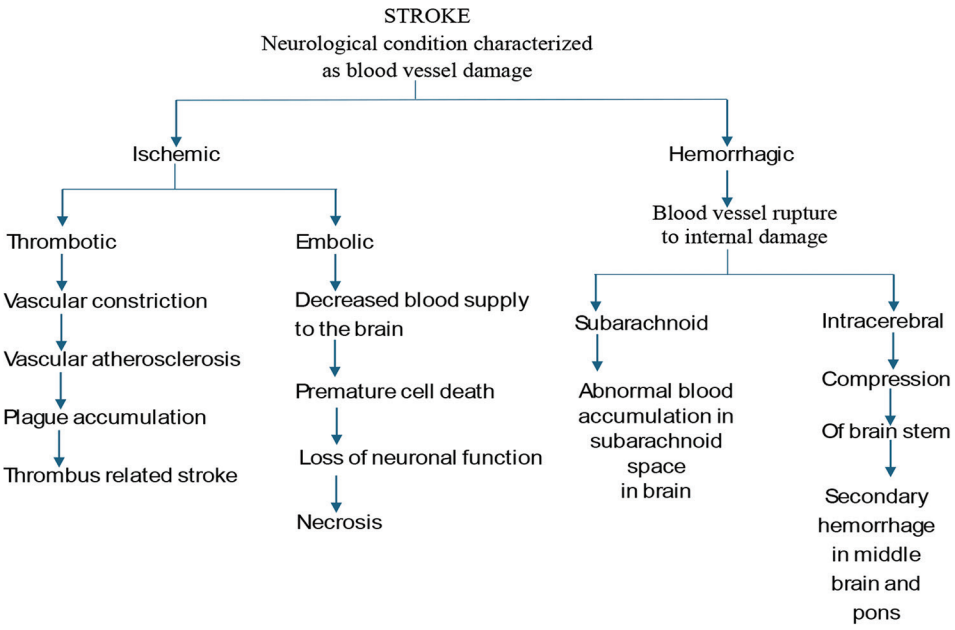

Figure 1. Pathophysiology of stroke

mortality rates. According to the recent data, the estimated number of reported annual stroke cases in India is 150 – 152/100,000 individuals, with an estimated incidence of about 1,175,778 cases.[10] Numerous systemic studies have been published with demographic and epidemiological data, and regular updates are provided on the websites of various regulatory bodies. Regulatory authorities should be concerned regarding this serious health issue and should implement healthcare policies and allocate resources for better management of stroke to reduce the global burden.[11,12]

3. Stem cells

Stem cells are a group of specialized, undifferentiated cells that have the potential to differentiate into any other cell types and play a key role in the formation of various tissues and organs. They are present at all stages of life, such as embryonic, fetal, and adult stages, where they produce differentiated cells that develop into tissues and organs. The ability of self-proliferation, differentiation, and the potential to form colonies are the most essential characteristics of stem cells. According to their capacity for differentiation, stem cells can be divided into five categories: totipotent or omnipotent, pluripotent, multipotent, oligopotent, and unipotent.[13] Based on these properties, stem cells are distinguished from other cell types. For instance, ESCs originated from zygotic blastocyte cells and possess a high ability to self-proliferate and differentiate into tissue-specific cells, a characteristic lacking in the other tissues. A diagram illustrating the ability of stem cells to proliferate extensively is shown in Figure 2.

Totipotent cells (TCs) are the least identifiable cells involved in the development of a mammalian embryo, from forming the placenta to giving rise to a fully developed zygote. TCs of a fertilized egg develop into both embryonic and extraembryonic tissues during early differentiation, giving rise to two main layers: The inner cell mass (ICM) and the trophectoderm.[14] Pluripotent stem cells are another cell type derived from the ICM, play a role in the formation of somatic cells along with three germ layers – ectoderm, endoderm, and mesoderm – from which all tissues and organs originate.[15] Multipotent stem cells (MSCs) are specialized cells that can differentiate into particular cell types, but they possess lower differentiation ability than pluripotent stem cells. MSCs can give rise to many cell types within a single germ layer and are predominantly present in most vital organs. These cells can develop into tissues including adipose tissue, bone, cartilage, and muscle from the mesoderm. Recently, MSCs have been differentiated into ectoderm-derived neural tissue. For example, a hematopoietic stem cell can differentiate into several types of blood cells, and upon further differentiation, it may become a typical oligopotent cell. The most prevalent type of MSCs can give rise to bone marrow, adipose tissue, and bone. In addition, oligopotent stem cells can further differentiate into other cell types. For example, a single myeloid stem cell can generate only white blood cells but not red blood cells (RBCs).[16] On the other hand, unipotent stem cells (USCs) are known for their limited differentiation potential and unique dividing properties. USCs can self-renew into a single cell and generate a single lineage. They are similar to mass stem cells, which exclusively produce adult muscle cells.[17] At present, induced pluripotent stem cells (iPSCs) are reprogrammed to display pluripotency similar to that of ESCs, which are being investigated for their potential. With this development, ethical issues

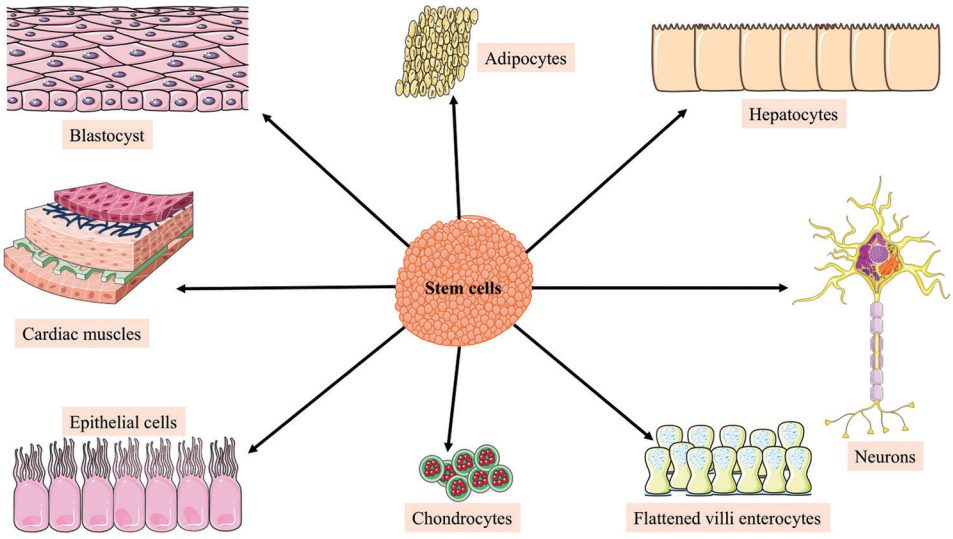

Figure 2. Stem cells regeneration

surrounding the use of ESCs are addressed and new opportunities for individualized regenerative therapy and disease modeling are created.[18] Recent interventions for treating neurodegenerative diseases, such as Alzheimer's Disease (AD), Parkinson's Disease (PD), Amyotrophic Lateral Sclerosis (ALS), and traumas, require a better understanding of the mechanisms of stem cells and their capacity for neuronal differentiation.[19-21]

4. Stem cell therapy in stroke patients

Stem cell therapy is considered a revolution in the management of stroke, overcoming the limitations of conventional medications. Nowadays, stem cell therapy is becoming a promising alternative management and acts as regenerative medicine for attenuating the disease severity, through its diverse mechanisms, including tissue regeneration, neuroprotection, anti-inflammatory actions, and the induction of host brain plasticity.[22] Various types of stem cells originate from different sources are under investigation in different animal models of stroke to assess their potency and efficacy. The potential mechanisms of stem cells in the recovery of stroke are discussed in Table 1.

4.1. Stem cell therapy in stroke with other comorbid conditions

Stem cell transplantation therapy is an effective technique for stroke treatment. Here, we present recent advances in stem cell research for comorbid conditions, which may have

Table 1. Pharmacological actions of different types of stem cells in stroke management

Type of stem cells	Abbreviations	Pharmacological action in stroke treatment	References
Bone marrow stem cells	BMSCs	They can express angiogenic and arteriogenic cytokines, which migrate to infarct areas and secrete various neurotrophic factors, such as BDNF, GDNF, CNTF, VEGF, PDGF, and NAP-2, enhancing the neuronal differentiation in the damaged area to promote healing.	23
Embryonic stem cells	ESCs	ESCs are primarily pluripotent cells that have a high capability for differentiating into multiple types of cells. They can also repair neuronal circuits, promote angiogenesis, and regenerate new tissues in the infarct area.	24
Endothelial progenitor cells	EPCs	During a stroke, these cells migrate to the damaged area of the blood vessels from the bone marrow and trigger blood vessel remodeling, neurogenesis, and angiogenesis. In addition, they also improve the rate of cerebral blood flow due to the blood vessel remodeling process and reduce infarct volume in stroke	25
Hematopoietic stem cells	HSCs	They can readily differentiate into RBCs and other lymphoid cells, which helps reduce the actual infarct size and causes vascular remodeling.	26
Human umbilical cord stem cells	HUCBCs	HUCBCs possess the maximum differentiation ability into any cell types, such as neurons and astrocytes. They can also migrate to the site of injury, reduce the infarct area, and improve the damaged tissue.	27
Induced pluripotent stem cells	iPSCs	These cells are also able to show pluripotency by improving the neuronal cell differentiation. They also enhance the local short-term sensorimotor recovery mechanism, and thus reduce the infarct size and lesions.	28
Mesenchymal stem cells	MSCs	MSCs show multipotency, as they possess the ability to differentiate into various cell types as needed. They migrate to the damaged site, show immunomodulatory and trophic effects, and suppress the apoptotic pathway, while also promoting angiogenesis and vascular remodeling. In addition, they induce cellular proliferation endogenously, which helps reduce infarct volume and heal the damaged area.	29
Mononuclear cells	MNCs	These cells are used in the sub-acute and acute phases of stroke, and possess high potential for immediate transplantation due to their high differentiation ability.	30
Neural stem/precursor cells	NSCs	Neural stem cells differentiate into various types of neural cells, showing multipotency. They also maintain the blood brain barrier integrity by regulating the tight junction cells and ensuring proper cellular adherence. Precursor cells are also help reduce neuroinflammation, promote neurogenesis and angiogenesis, and vascular regeneration.	31
Olfactory ensheathing/glial cells	OECs	Surrounding the olfactory neurons, OECs secrete neurotrophic factors that will further potentiate neuronal regeneration. They also help to scavenge pathogens, thus reduce inflammation at the infarct area.	32

Abbreviations: BDNF: Brain-derived neurotrophic factor; GDNF: Glial cell line-derived neurotrophic factor; CNTF: Ciliary neurotrophic factor; VEGF: Vascular endothelial growth factor; RBCs: Red blood cells; PDGF: Platelet-derived growth factor; NAP-2: Neutrophil-activating peptide 2; ESCs: Embryonic stem cells.

a synergistic effect when administered following a stroke. Stem cell transplantation is a potential new technique, but it may also exacerbate existing comorbidities. Several comorbid disorders associated with stroke have been clinically described, and their incidence is rapidly increasing.[33] Some of the clinically significant comorbid conditions include diabetes and hypertension.

4.1.1. Stem cell therapy in diabetic stroke

Diabetes is a metabolic disorder, while stroke is a cerebrovascular disease. However, diabetes-induced vascular damage and an increased inflammatory milieu are likely to contribute to poor post-stroke outcomes. Diabetic stroke patients have an increased pathogenic cascade, and interventions that benefit non-diabetic stroke patients may not always be effective for diabetic stroke patients.[34] As a result, there is a pressing need to develop stroke therapies specifically for diabetics. Exosome therapy may be a better choice for diabetic stroke, with some preclinical studies showing promising results.[35] Stem cell-based therapy for stroke is a promising treatment approach with a long therapeutic window. These therapies enhance endogenous central nervous system repair and neurorestorative mechanisms, including angiogenesis, neurogenesis, vascular remodeling, and white matter remodeling. They also control inflammatory and immunological responses both locally and systemically.[36]

4.1.2. Stem cell therapy in hyperlipidemia-induced hypertensive stroke

Atherosclerosis (AS) is the leading cause of cardiovascular and cerebrovascular disorders, with lipid buildup in the vessel wall serving as the primary marker of AS.[37] While statins are currently used to reduce lipids and low-density lipoprotein (LDL) levels in AS, the cure rate remains low. As a result, there is an urgent need to develop new therapeutic approaches, making stem cells a focus of extensive research. Stem cells are a type of cell that retain the ability to differentiate into various cell types and tissues. Stem cell transplantation techniques have shown efficacy in the treatment of other diseases.[38]

The treatment for hyperlipidemia-induced hypertension with stem cells involves transplanting healthy cells into damaged blood arteries, tissues, and organs. MSCs are the most effective option among all accessible cell types.[39] MSCs are processed and cultured to promote cell differentiation and treat diseases. They have been investigated as a promising therapeutic target for various diseases, including myocardial infarction, acute lung injury, acute renal failure, ischemia, and others, due to their ability to differentiate into multiple cell lineages, such as mesodermal and myogenic lineages.[37,40-42] Furthermore, the immunomodulatory abilities of MSCs are becoming more widely recognized. Several studies have studied the ability of MSCs in affecting both innate and adaptive immune responses.[43,44] MSCs inhibit dendritic cell differentiation and maturation by reducing the expression of co-stimulatory molecules and pro-inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-12 (IL-12), while increasing the production of anti-inflammatory cytokines, including transforming growth factor-β (TGF-β) and interleukin-10 (IL-10). This indirectly suppresses T-cell proliferation and contributes to the healing process.[45,46] In addition, MSCs can directly limit T-cell proliferation by inducing cell cycle arrest in all subsets, resulting in a quiescent state and decreased proliferation. In mice, MSC therapy not only modulates inflammatory responses but also significantly lowers dyslipidemia.[47,48] Given the critical role of inflammation and immunomodulation in the onset and progression of atherosclerosis, MSC transplantation has been extensively studied as a therapeutic approach to treat atherosclerosis or hyperlipidemia-mediated stroke.

5. Recent advancements in stem cell therapy for stroke

5.1. Different stem cell sources

Stem cells can be isolated and cultured after being extracted from the human body, and although they can hypothetically be collected from any type of tissue, adipose tissue is a main source of stem cells. As a therapy for neurodegenerative diseases, the implementation of neuronal stem cells is evolving as a promising approach for reestablishing normal brain function.[49] At present, a few types of stem cells are available for mitigating the onset and progression of these diseases.[50] BMSCs are most often utilized for treating ischemic stroke, and several preclinical studies also support the efficacy of MSC transplantation as an alternative therapeutic approach. MSCs are also intended to exert neuroprotective actions by secreting various neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), ciliary neurotrophic factor (CNTF), glial cell line-derived neurotrophic factor (GDNF), vascular endothelial growth factor (VEGF), platelet-derived growth factor (PDGF), and neutrophil-activating protein-2 (NAP-2), thereby promoting neuronal cell differentiation.[51] Umbilical cord MSCs are isolated from Wharton's jelly and perivascular fluid of the umbilical cord and have shown superior differentiation potential, minimal immune rejection, and selective neuroprotective, neurogenic, and angiogenic properties.[52]

5.2. Stem cell application method

Stem cell number delivered to the target site and potential side effects can be significantly influenced by the method

of stem cell administration. The potential for stem cells to become lodged in blood-filtering organs is a significant problem in the systemic infusion of stem cells (first-pass effect). To prevent this, different delivery methods are used, along with techniques to reduce lung adhesion and improve the homing of systemically administered cells. Venous access is preferable to arterial access for stroke recovery as arterial access can cause blockage, leading to stroke.[53] Apart from intravenous administration, alternative methods like intrathecal injections enable the direct delivery of stem cells into the cerebrospinal fluid (CSF), avoiding obstacles like the blood-brain barrier (BBB) and guaranteeing that a greater number of cells reach the central nervous system (CNS). For more precise targeting of certain vascular areas, intra-arterial delivery via catheter can also be used. However, there are risks associated with this technique, including the possibility of arterial blockage. In addition, more sophisticated methods for delivering stem cells specifically to affected regions while reducing systemic exposure have been made possible by advancements in catheter technology. The use of scaffolds and biomaterials, which offer localized support for stem cell engraftment and retention at the site of damage, is another emerging strategy.[54] Growth factor or cytokine preconditioning methods can also be used before injection to increase cell survival rates and functional outcomes after transplantation. Overall, enhancing therapeutic efficacy and reducing side effects in stem cell therapy requires the optimization of administration techniques. Ongoing research aims to investigate novel delivery methods and refine current protocols to improve patient outcomes in regenerative medicine applications.[55]

5.3. Modification of the culture media and stem cells *ex-vivo*

Telomere shortening leads to decreased telomerase activity, impaired cellular secretions, and altered interactions with the environment, which may contribute to the reduced activity of progenitor cells. Many culture expansion techniques, such as genetic modification of cells, pretreatment with trophic factors, isolation and utilization of functional subpopulations of stem cells, and modulation of intracellular signaling, can reduce senescence while increasing MSC proliferation, survival, and nutritional support.[56] The medium used in stem cell cultivation determines the eventual result. Instead of using human serum, platelets, or xenogeneic fetal bovine serum, lysates allow MSCs to grow and rejuvenate quickly without adversely affecting the immunophenotype.[57] Recent studies indicate that maintaining stem cell identity and promoting differentiation requires the use of a specified serum-free medium supplemented with certain growth factors. iPSCs and NSCs are two examples of stem cell types whose self-renewal has been demonstrated to be supported by the addition of basic fibroblast growth factor (bFGF). In addition, balancing self-renewal and differentiation through the optimization of oxygen content and cytokine combinations in the culture environment can have a substantial impact on stem cell fate decisions.[58] Along with these adjustments, continuous perfusion systems in bioreactors can improve waste removal and nutrient supply, which will increase cell viability and growth rates in comparison to conventional static cultures.[59] A dynamic culture environment is necessary for rapidly multiplying stem cells to meet their metabolic requirements. In addition, MSC-derived extracellular vesicles (EVs) have become crucial mediators in tissue regeneration and cell communication because they contain bioactive chemicals that can alter recipient cell activity. Overall, improving culture methods and medium composition is essential to unlocking stem cells' therapeutic potential in regenerative medicine.[60] Researchers can improve the effectiveness of stem cell treatments for a range of tissue repair and regeneration applications by addressing the drawbacks of conventional culture techniques and creating ideal settings for specific stem cell types.[61]

5.4. Extracellular vesicles generated by stem cells

An innovative and therapeutically viable cell-free therapy strategy, which avoids many of the drawbacks of direct cell transplantation, utilizes EVs produced by stem cells. This approach may represent a paradigm shift in regenerative medicine by using secretions such as trophic factors, cytokines, and chemokines, which are generated by paracrine signaling in exosomes and microvesicles. Vascular occlusion, which results in tumor growth and infarction, is a key factor in human ischemic or hemorrhagic strokes.[62] In addition, soluble substances, such as EVs produced by MSCs are essential for tissue healing. EVs produced from stem cells are enriched with bioactive substances such as proteins, lipids, and microRNAs (miRNAs), which play an essential role in promoting cellular communication and regeneration. In contrast, EVs derived from stem cells transport more complex cargos compared to those from other cellular sources, which enhances their therapeutic potential. One of the important factors for their effectiveness is their ability to cross the BBB to deliver therapeutic drugs directly to the CNS.[63] Bioactive substances encapsulated within EVs improves their stability and bioavailability, shielding them from deterioration while circulating in the body. Furthermore, the distinct makeup of EVs produced from stem cells enables them to provide regenerative benefits comparable to those of their parent cells, while reducing the risks

associated with live cell treatments, such as cancer and immune rejections. Recent research indicates that these EVs can trigger endogenous repair processes in various tissues, promote angiogenesis, and modulate inflammatory responses. The targeting capabilities of modified EVs can also be improved by customizing them to carry specific medicinal compounds. This adaptability makes stem cell-derived EVs attractive options for therapeutic use in the treatment of various diseases, such as tissue damage, neurodegenerative disorders, and stroke.[64]

5.5. The use of 3D bioprocessing methods

Many attempts have been made to develop new 3D bioprocesses that can accelerate the clinical application of stem cell research. Apart from the numerous pharmacological and genetic preconditioning methods already discussed, the biological characteristics of stem cells can be improved by using 3D bioprocessing methods that physically mimic the natural *in vivo* environment.[65] Gradually, it is becoming evident that multiple passages during culture expansion utilizing traditional tissue culture flasks change the natural phenotype of MSCs. As a result, MSC phenotypes and unique properties can be effectively preserved by forming an MSC collection that mimics true 3D interactions between adjacent cells or the extracellular matrix. However, techniques for growing stem cells in 3D bioprocesses require further investigation. While cells close to the outer layer remain viable, necrosis occurs in the center of the cell mass due to the restricted transport of nutrients, oxygen, and metabolic waste caused by conventional static culture conditions.[66]

6. Preclinical studies with stem cells in stroke

In recent years, increasing experimental proof has highlighted the potential of stem cells for stroke treatment. Many types of stem cells are effective and safe at both pre-clinical and clinical levels for treating various neurological disorders. Preclinical approval of stem cells for stroke treatment was significant. Numerous studies have evaluated stem cells based on several parameters, including cell type, cell number, dosage, route of administration, safety, and efficacy.[67,68] MSCs are the most extensively researched and utilized stem cells for stroke management. Among the tissue sources of MSCs, bone marrow and adipose tissue are the most prevalent and well-researched, with bone marrow being the earlies identified source.[69] Most preclinical studies have investigated various aspects of stem cell transplantation for stroke using autologous BM-MSCs. Other studies have documented the use of MSCs derived from adipose tissue, umbilical cord, placenta, and other sources. Surface marker profiling is utilized to characterize MSCs for transplantation, including the presence of markers such as CD29, CD44, CD73, CD90, and CD105, and the absence of markers such as CD34, CD45, CD14, and human leukocyte antigen (HLA) class II. The cell number, dosage, and route of administration are other important factors to be considered in pre-clinical investigations. MSCs are transplanted using various methods, including intravenous, intranasal, and intra-arterial delivery of $1 \times 10^6 - 8 \times 10^6$ cells.[70] Although transplanted MSCs have been shown to adapt to their new surroundings and differentiate into neurons, astrocytes, and oligodendrocytes following intravenous and intranasal administration, concerns remain regarding the impact on MSC migration in the brain. The ability of stem cell transplantation to achieve key therapeutic outcomes such as tissue regeneration, enhanced angiogenesis, neovascularization, improved blood-brain barrier (BBB) function, and functional restoration remains controversial. More studies are needed to develop definitive cell therapies for stroke.[71] The outcomes of stem cell therapy in stroke are discussed in Figure 3.

7. Clinical trial of stem cell therapy in stroke patients

Stroke has become the leading cause of long-term disability, morbidity, and mortality, significantly reducing the quality of life. Neuroinflammation, a key component of the pathophysiology of various forms of cerebrovascular diseases, is also associated with stroke. A thorough search of clinical trials on stroke uncovered 56 studies investigating cerebrovascular stroke using regenerative medicine, including both autologous and allogeneic cell therapies.[72] Most of these cells were obtained from bone marrow, adipose tissue, spinal cord, umbilical cord, and mesenchymal tissue. An observer-blinded, open-label clinical study was carried out to assess the long-term safety and effectiveness of autologous MSCs. Following MSC transplantation, patients who received MSC treatment showed clinical improvement, which was correlated with both the degree of involvement of the subventricular area in the lateral ventricle and blood levels of stromal cell-derived factor 1 (SDF-1). The frequency of comorbidity was similar to that of control group.[73] A single-blind controlled Phase I/II study that included patients with middle cerebral artery (MCA) stroke was conducted. At 5 – 9 days post-stroke, autologous BM-MNCs were administered as a therapy (Figure 4). An increase in plasma concentration of neural growth factor was observed, and no adverse effects were reported during the 6-month follow-up, except for two individuals who experienced partial seizures after 3 months. According to the study's findings, administering BM-MNCs intravenously is both beneficial and safe.[74] Patients with MCA infarction participated in a randomized, single-

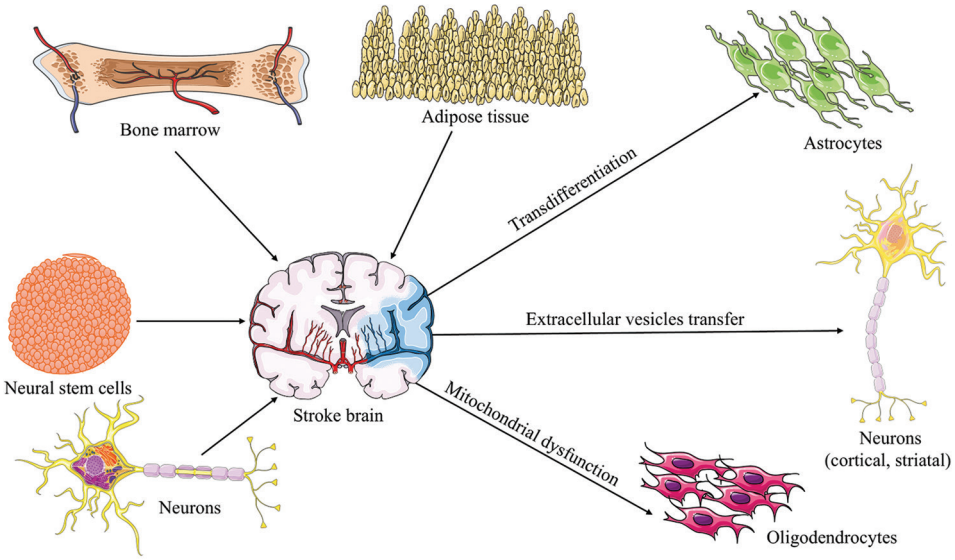

Figure 3. Stem cell therapy in stroke

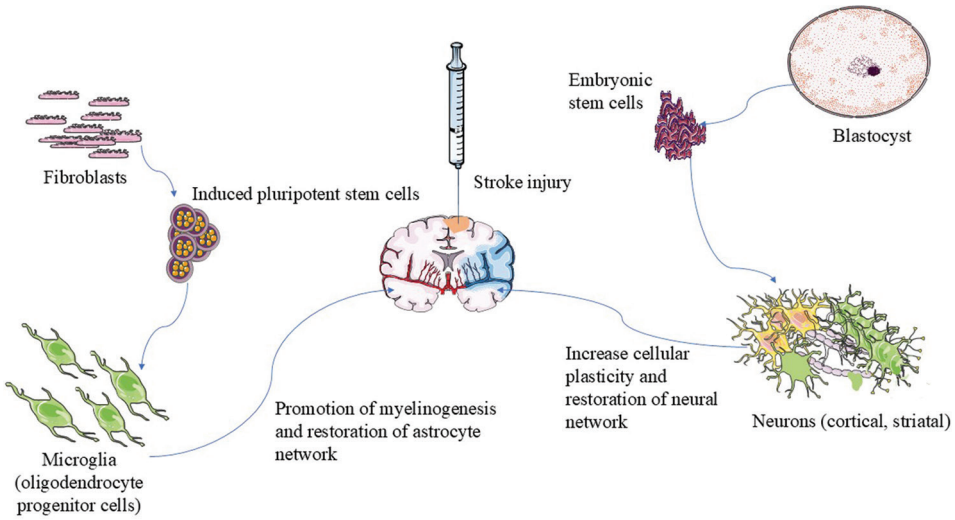

Figure 4. Mechanism of action of stem cell therapy in stroke

blind, controlled study, which was based on the findings of a preclinical investigation employing peripheral blood stem cells (PBSCs). Before stereotaxic transplantation of immunoselected PBSCs included in the trial, patients who fulfilled the study eligibility criteria received granulocyte-colony stimulating factor (G-CSF) subcutaneous injections for five consecutive days.[75] Neither the study method nor the follow-up caused any adverse events. Clinical outcomes were assessed by tracking changes based on NIHSS, ESS, EMS, and MAR scores from baseline to 12 months in both the PBSC-treated and control groups. This study also provided significant evidence that PBSCs are effective in reversing stroke-related motor deficits, remodeling the injured corticospinal tract (CST), and restoring activity from the brain to the extremities as mentioned.[76] According to other findings, intravenous administration of BM-MNCs is both beneficial and safe. A single-arm Phase I trial employing autologous BM-MNCs in acute ischemic stroke showed promise as a novel experimental approach that could assist extend the therapeutic window for ischemic stroke patients. No adverse events (AEs) were discovered following transplantation.[77,78] NSI-566 primary adherent neural cells, isolated from a human fetal spinal cord, were tested for safety and durability in a Phase I trial at a second site. The effectiveness of implanting human spinal cord-derived neural stem cells at three distinct doses into the peri-infarct region of stable stroke patients was demonstrated in a patient cohort study. The bulk of

stem cell-derived tissue is composed of interneurons and glial cells, which promote regeneration and serve as connections between regenerated brain fibers. This may explain the observed effects.[79] Following intravenous BM-MSCs delivery, a group of patients in a randomized multicenter Phase II study demonstrated outstanding safety in subacute ischemic stroke. Paracrine BM-MSCs function in patients with persistent ischemic stroke. The BM-MNCs of each patient were characterized with CD34+ markers, which recent studies have shown are also present on cancer stem cells (CSCs). BDNF, GDNF, IGF-1, and VGEF are secreted by BM-MNCs and may help prevent motor neuron degeneration. These findings suggest that administering BM-MNCs to stroke patients is both safe and convenient. In another open-labeled prospective Phase I clinical trial, acute ischemic stroke patients received a single intravenous infusion of allogeneic human umbilical cord blood cells within 3 – 10 days of the stroke. Graft-versus-host disease and hypersensitivity were assessed during patient visits at 3, 6, and 12 months after umbilical cord blood (UCB) infusion.[80,81] Taken together, these studies highlighted the promise of stem cell treatment as a promising option for improving outcomes in stroke patients. However, they also emphasize the significance of continued research to optimize procedures for safety and efficacy. Future research will be essential for developing standardized treatment plans and for comprehending how different types of stem cells affect long-term recovery following cerebrovascular events.[82] Some trial databases are referenced, and study outcomes are discussed in Table 2.

8. Future perspectives

As more studies and ongoing trials on stroke are reported, regenerative medicine continues to demonstrate increasing promise and effectiveness. Neurophysiological evidence suggests that stem cells and their mechanisms play a role in restoring the function of the brain. There are various methods in labeling cells, including simple incubation with transfection agents and magneto suboperation. These methods are optimal for monitoring cells in the middle coronary artery occlusion (MCAO) animal model of stroke. However, magnetic resonance (MR) cell tracking using superparamagnetic iron oxide nanoparticles (SPIO) still needs clinical validation.[94] Although stem cells have shown great promise in stroke treatment, many issues remain to be resolved soon. Recent research aims to cure strokes by exploring various types of stem cells, including stem cells modified using polymeric materials. However, the use of programmable stem cells raises ethical concerns that need to be sorted out by regulatory bodies. There are restrictions on the number of neural progenitor cells (NPCs) for transplantation when they are cultured *in vitro*.

Table 2. Clinical trials of stem cell therapy in stroke

Country/Region, year	Sample size	Cell types	Interventional types	Route of administration	Dose	Time point from onset of infusion	Primary outcomes	References
Spain, 2014	20/20	Adipose tissue	Allogenic MSCs from adipose tissue	Intravenous	1 million/kg body weight	2-week post-stroke	mRS, NIHSS, MRI, biochemical markers	83
South Korea, 2010	16/36	Posterior iliac crest	MSCs, conventional	Intravenous	50×10^6	7 days	mRS	84
Spain, 2012	10/10	Posterior superior iliac crest	BM-MNCs, conventional treatment	Intra-arterial	159×10^6	Mean 5 – 9 days post occurrence of stroke	NIHSS, BI, mRS	85
China, 2019	9	Cell derived from human fetal spinal cord	NSI-566, primary adherent neural stem cell	Intracerebral injection	Cohort A=1.2×10^7 B=2.4×10^7 C=7.2×10^7	Mean 496 days	CT, MRI, PET, DTI	86
India, 2014	60/60	Posterior iliac crest	BMSCs	Intravenous	280.5×10^6	7 – 30 days	NIHSS, BI	87
USA, 2018	10/10	Allogenic umbilical cord	Umbilical cord blood infusions	Intravenous	3.34×10^6	3 – 9 days	mRS	88
India, 2013	20/20	Post superior iliac crest	MNC+MSCs	Intravenous	50 – 60×10^6	3-months – 2 year-post-stroke	BI, FMA	89
India, 2012	12/12	Posterior iliac crest	BM-MNCs, conventional treatment	Intravenous	50 – 60×10^6	3 months – 12 months post-stroke	FMA, BI	90

(Contd...)

Table 2. ***(Continued)***

Country/Region, year	Sample size	Cell types	Interventional types	Route of administration	Dose	Time point from onset of infusion	Primary outcomes	References
Taiwan region, 2014	15/15	Peripheral cells	CD34+	Intracranial	$3 – 8\times110^6$	6 months – 5 years	NIHSS, ESS, mRS	91
China, 2014	15/15	PBSCs	PBSCs	Stereotactic	$3 – 8\times10^6$	6 months – 5 years	NIHSS, mRS	91
USA, 2019	25/30	Bone marrow harvest	BM-MNCs	Intravenous	10 million cells/kg b. wt.	1 – 3 days	mRS, NIHSS	92
Egypt, 2016	12/18	Posterior iliac crest	BM-MNC	Intra-arterial	1×10^6	2 – 4 weeks	NIHSS, mRS, BI	93

Abbreviations: MSCs: Mesenchymal stem cells; mRS: Modified Rankin scale; NIHSS: National Institutes of Health Stroke Scale; MRI: Magnetic resonance imaging; BM: Bone marrow; MNCs: Mononuclear cells; BM-MNCs: Bone marrow-mononuclear cells; BI: Barthel index; CT: Computed tomography; PET: Positron emission tomography; DTI: Diffusion tensor imaging; FMA: Fugl–Meyer assessment; BMSCs: Bone marrow stem cells; b. wt.: Body weight; ESS: European stroke scale.

This problem can be addressed with MSCs, as they possess multipotency and a high regeneration capacity. However, it is still too early to conclude whether MSC therapy can improve outcomes in stroke cases.[95] A recent meta-analysis in the field of cardiology showed that adult bone-marrow transplantation when compared to conventional therapy, improved infarct size, remodeling, and left ventricular function in individuals with ischemia-related cardiovascular disease.[96] This result was based on data from 50 studies involving 2625 patients. These studies utilized echocardiograms and included long-term follow-ups for patients receiving stem cell transplants. The first effective stem cell treatment in hematology – hematopoietic stem cell transplantation – took 60 years to develop.[97] Therefore, developing important and innovative treatments will necessitate ongoing collaboration between researchers and medical professionals involved in clinical trials. The basic principles of stem cell therapy will be better understood through further advancements in both laboratory research and clinical settings, ultimately improving the therapeutic efficacy of cell-based treatments for stroke cases.

9. Conclusion

Despite stem cell treatment for stroke shows great promise, significant problems still need to be fixed to fully harness its potential. In addition to improving delivery methods and developing protocols to ensure patient safety and efficacy, the present study aims to enhance cell survival and integration into ischemic brain tissue. Further understanding of the therapeutic potential and mechanisms of action of various types of stem cells is expected to lead to better treatment outcomes for stroke patients. Together, basic research and clinical practice will be crucial in overcoming present challenges and transforming these findings into effective therapies, ultimately improving the quality of life and recuperation of stroke survivors. Furthermore, reaching this objective will require research into innovative strategies. By investigating cutting-edge treatments, including neuroprotective drugs, robotic rehabilitation, and individualized treatment programs, we can better address the various needs of stroke patients and maximize their chances of recovery. Collaboration among researchers, physicians, and patients will pave the way for translating these developments into effective therapeutic applications, which will transform the future of stroke rehabilitation.

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Conflict of interest

The authors declare that they have no competing interests.

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Availability of data

Data are available upon reasonable request.

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REVIEW ARTICLE

Artificial intelligence in epilepsy education

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Abstract

The emergence of artificial intelligence (AI) has revolutionized the landscape of epilepsy education and management by providing innovative solutions to the challenges of diagnosis, treatment, and patient care. This review evaluates the multifaceted role of AI in epilepsy, focusing on its impact on early diagnosis, seizure prediction, and the development of personalized treatment plans. AI tools, including machine learning algorithms and neural networks, have demonstrated significant promise in enhancing diagnostic accuracy and identifying epileptic patterns. This study explores various AI-driven educational platforms designed to improve the knowledge and skills of healthcare professionals, patients, and caregivers in managing epilepsy. Moreover, AI applications in wearable devices and mobile health platforms facilitate real-time monitoring and patient engagement, ultimately improving quality of life. However, integrating AI into clinical practice presents several challenges, including the need for large and high-quality datasets, interdisciplinary collaboration, data privacy, and ethical considerations. This review highlights these barriers while suggesting uniform protocols and frameworks for efficiently translating AI technologies into clinical practice. It underscores AI's transformative potential in epilepsy care and education, advocating for ongoing research and collaborative efforts among technologists, clinicians, and educators, while emphasizing the importance of user-friendly design, regular assessments, and ethical considerations to maximize AI's impact in this critical field.

Keywords: Epilepsy education; Artificial intelligence; Epilepsy

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1. Introduction

Epilepsy is a neurological disorder characterized by unprovoked, recurrent seizures stemming from abnormal electrical activity in the brain.[1] Affecting millions globally, epilepsy arises from a complex interplay of genetic and environmental factors that disrupt the balance between excitatory and inhibitory brain signals, leading to hyperexcitability

and seizure activity.[2] With diverse manifestations across patients, diagnosing and managing epilepsy poses unique challenges, often requiring specialized approaches for personalized treatment.[1,2]

In the United States, approximately 3.4 million individuals are affected by epilepsy, with 150,000 new cases diagnosed annually.[3] This condition spans all ages, from children to older adults, and imposes significant medical, emotional, social, and financial challenges for patients and their families.[4] Major risk factors include traumatic brain injury, genetic predispositions, neurodevelopmental issues, stroke, and central nervous system infections.[5] Diagnosis commonly involves reviewing medical history, conducting physical exams, performing neuroimaging, and primarily utilizing electroencephalography (EEG) to assess brain activity.[6] However, accurately interpreting EEG data requires advanced expertise, creating barriers to timely diagnosis. While genetic testing can provide insights in specific cases, its accessibility and cost remain limiting factors.[7]

Artificial intelligence (AI) is increasingly advancing epilepsy care by automating EEG analysis, detecting subtle seizure patterns, and facilitating faster, more accurate diagnoses, especially in settings with limited specialized expertise.[8] AI-driven tools also have the potential to predict seizure patterns, enabling patients to take proactive safety measures.[9] In addition, AI-powered applications, wearable devices, and telehealth services are revolutionizing epilepsy care by improving access to personalized support tailored to individual needs.[10]

This study explores how AI implementation can enhance epilepsy education by improving EEG interpretation skills, supporting patient self-management, and creating innovative learning pathways for neuro physicians while highlighting the collaborative efforts of multiple institutions in this field. Moreover, the study examines the ethical considerations of AI integration, such as transparency, bias mitigation, and privacy protection, aiming to maximize its benefits while addressing potential limitations in clinical practice.[1-10]

2. Epilepsy: The impact and importance of early diagnosis and treatment

The International League Against Epilepsy (ILAE) defines epilepsy as a disease of the brain characterized by any of the following conditions: at least two unprovoked (or reflex) seizures occurring more than 24 h apart, one unprovoked (or reflex) seizure with a probability of further seizures similar to the general recurrence risk (at least 60%) after two unprovoked seizures over the next 10 years, or a diagnosis of an epilepsy syndrome.[11,12] Epilepsy affects approximately 65 million individuals worldwide, with around 80% of cases occurring in developing regions (World Health Organization [WHO], 2020).[13] Epilepsy can be classified into various types based on the nature and origin of the seizures,[14] primarily categorized into generalized and focal seizures.[13] Understanding these classification are crucial for effective diagnosis and treatment, highlighting the need for continuous research and refinement of diagnostic criteria.[12] Early diagnosis and treatment are crucial for improving the quality of life for those affected by epilepsy and for preventing complications associated with this chronic neurological disorder.[13,14] Advanced technology, particularly AI, is transforming the diagnosis and management of epilepsy by providing new tools to detect and predict seizures with increasing accuracy.[15] The prevalence of epilepsy varies globally, impacting 0.6 – 1.0% of the population, with higher rates observed in developing regions (0.7 – 1.2%) compared to developed regions (0.5 – 0.8%).[16] This disparity highlights the urgent need for enhanced diagnostic and treatment resources in under-resourced areas, where epilepsy care is often limited or unavailable (WHO, 2020).[1] Diagnosis typically involves a detailed history, semiology examination, and electrophysiological methods to identify the epileptic focus and classify the type of seizures.[10,11] Treatment primarily includes long-term use of antiseizure medications (ASMs) to reduce seizure frequency or achieve remission, with careful monitoring for adverse reactions.[8] The primary goal in conventional epilepsy management is to control seizures, minimize side effects, and improve quality of life.[8,9] This includes accurate diagnosis using EEG and magnetic resonance imaging (MRI) to classify seizure types and guide treatment choices.[8,9] First-line treatment typically involves ASDs, like carbamazepine or valproate, often using monotherapy to minimize interactions.[10,11] For drug-resistant cases, surgical interventions or alternative treatments, such as vagus nerve stimulation and ketogenic diets, may be applied.[10,11] Lifestyle adjustments and psychosocial support, alongside regular monitoring, are crucial for effective ongoing management and treatment sucess.[8-11]

3. Challenges in epilepsy education and awareness

Epilepsy education faces numerous challenges, including inadequate knowledge and awareness, as well as significant disparities in care and educational needs.[17] One of the primary issues is the poor knowledge and awareness about epilepsy among patients, which hampers effective self-management and diminishes the quality of life.[14] This lack of awareness extends to the public and healthcare professionals, contributing to the under-recognition

of seizures and delays in diagnosis and treatment.[16] In educational settings, there is a pressing need for well-designed teaching units on epilepsy that encompass basic knowledge, neuronal processes, and first aid measures.[18] However, challenges such as accommodating students with epilepsy in the classroom, ensuring adequate preparation time, and preventing anxiety among students must be addressed.[15] In addition, children and adolescents with epilepsy often face emotional, cognitive, and social challenges that impact their school experience, leading to learning difficulties, such as dyslexia, spelling disorders, and dyscalculia.[17] For healthcare professionals, particularly pediatric neurologists, significant challenges include integrating guidelines into practice, identifying epilepsy events, incorporating genetic testing, and transitioning from pediatric to adult care – all of which require targeted educational interventions.[1] Moreover, people with epilepsy in low- and middle-income countries face substantial barriers to quality care, including language, economic, and technological barriers, that further complicate educational efforts.[16] Addressing these multifaceted challenges requires a comprehensive approach that includes public education, specialized training for healthcare providers, and tailored educational programs in schools to improve the overall management and quality of life for individuals with epilepsy.

Epilepsy education for neurologists faces several challenges, as highlighted by various studies. A significant issue is the limited evidence base for treating seizures in adults with neurodevelopmental disorders, complicating the management of epilepsy in this population.[1] This challenge is exacerbated by higher rates of physical and psychiatric comorbidities, polypharmacy, and neuropsychiatric side effects of medications, necessitating a nuanced treatment approach that lacks robust Level 1 evidence.[18] In addition, while achieving seizure freedom is a primary goal, many neurologists lack confidence in optimizing the dosing of ASMs in combination therapies, as evidenced by a continuing medical education (CME) certified activity that showed only a minimal educational effect in this area.[19] Another barrier is the underutilization of epilepsy surgery, despite its recognition as a valid early intervention.[19] Many physicians refer patients for surgery only after the failure of multiple ASMs, often due to an overestimation of surgical risks and inadequate healthcare resources.[20] Furthermore, there is a gap in pre-service and ongoing education about epilepsy, including first-aid measures and neuronal processes. Enhancing this education could significantly improve the quality of life for individuals with epilepsy if better integrated into medical education.[15] These multifaceted challenges underscore the need for comprehensive, evidence-based educational programs and resources to better equip neurologists in effectively managing epilepsy.[19]

Epilepsy awareness faces numerous challenges globally, significantly impacting the quality of life for individuals with epilepsy.[20] Misconceptions and stigma are rampant, with epilepsy often misunderstood as a psychiatric or contagious disease in various regions, further complicating access to appropriate care and treatment. Stigma manifests in several forms – internalized, interpersonal, and institutional – creating significant barriers for patients and preventing them from seeking help and fully participating in society.[21] In addition, barriers to care are intensified by economic, language, and technological challenges, particularly in low- and middle-income countries where 80% of epilepsy patients reside.[16] These barriers lead to delays in diagnosis and treatment, resulting in many individuals with epilepsy not receiving optimal seizure control or access to necessary surgical options.[16] Furthermore, awareness and research on epilepsy-related deaths, such as sudden unexpected death in epilepsy (SUDEP), are still evolving, highlighting the need for more comprehensive practices among health professionals to enhance prevention and epidemiological surveillance.[22] Overall, enhancing epilepsy awareness requires a multifaceted approach involving education, policy reform, and community support to dismantle the barriers faced by these patients. Figure 1 illustrates the distribution of publications across various regions globally, highlighting efforts in AI-driven epilepsy education

4. Transforming education with AI: Personalized learning, enhanced instruction, and professional development in healthcare

AI is transforming the educational landscape by enhancing learning, teaching, and administration methods.[23] AI-powered adaptive learning mechanisms personalize education by constructing tailored educational pathways based on student performance data, which enhances overall engagement and academic achievements.[23] AI technologies, such as ChatGPT, have opened new possibilities for educational practices, allowing teachers to create study materials, presentation media, and evaluation tracks with greater ease and efficacy while facilitating personalized learning tailored to each student's unique needs.[24]

AI applications in education, such as chatbots, learning analytics, and intelligent tutoring systems, enable data-driven decision-making and streamline administration, although they also raise ethical dilemmas.[25] In general, AI in education presents new opportunities to improve learning outcomes for students, preparing them to succeed

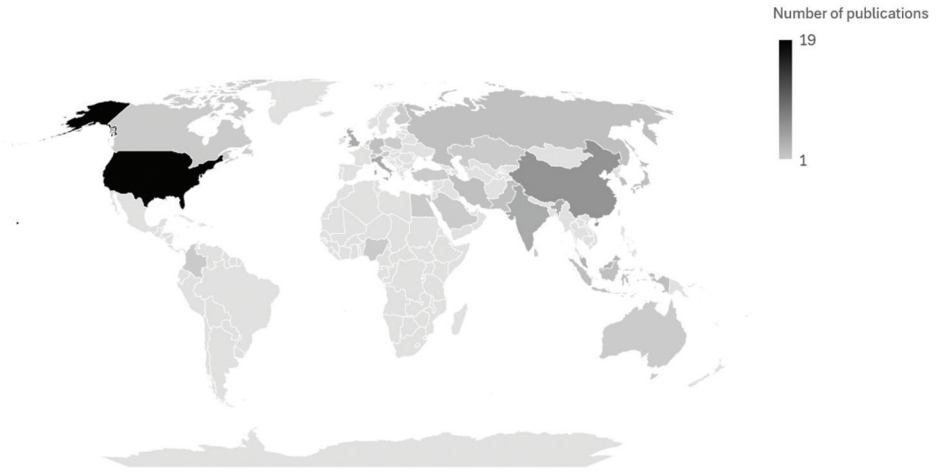

Figure 1. Distribution of artificial intelligence-driven epilepsy education publications across various regions globally

in the digital age.[23,25,26] AI-enabled personalized learning systems offer a potentially transformative educational strategy that utilizes advanced technology to benefit individual students.[27] Online education with AI assistants provides customized recommendations for monitoring performances, suggesting relevant topics, and structuring individual learning programs. This approach addresses the drawbacks of traditional systems while fostering the development of academic and soft skills.[28] AI-based recommendation algorithms have proven effective in personalizing teaching content, improving student outcomes, and increasing satisfaction by recommending appropriate content based on multi-dimensional information, such as students' interests and learning histories.[29] AI is increasingly integrated into CME and training, offering significant potential to enhance learning outcomes and professional development for healthcare providers. AI's ability to answer CME quiz questions with high accuracy was demonstrated by a study where AI completed 90% of quizzes with an 86% accuracy rate, underscoring its capability in this domain.[30] Integrating AI into postgraduate training can enhance personalized learning, provide real-time feedback, and assist in decision-making processes for healthcare professionals. Beyond CME, AI's role in medical education includes delivering personalized learning experiences and improving practical skills, as evidenced by improved outcomes in training labs and other educational settings.[31,32]

5. Empowering epilepsy management through mobile health applications and gamification

There is relative success in knowledge enhancement, medication compliance, and overall seizure control through mobile applications and gamification for patient education in epilepsy.[33] The development of mobile health applications is increasing, particularly to support chronic disease management, with a strong focus on epilepsy patients. These applications cover various aspects, including stress management, drug side effect monitoring, patient education, and adherence promotion.[33] They utilize simple games and role-playing activities centered around epilepsy, delivering essential information through interactivity and storytelling, which fosters better user engagement and encourages a respectful, tolerant community toward epilepsy.[34]

These digital tools empower patients to take control of their healthcare by allowing them to share health records and emotional states with neurologists while receiving close support from dedicated care managers.[35] For instance, the mobile application – ThinkNinja for Epilepsy – has provides cognitive behavioral therapy interventions to enhance the quality of life and mental health of individuals with epilepsy and anxiety, thereby improving overall well-being for those with comorbid anxiety or depression.[35] Other applications, such as Epilepsy Journal, Seizure Tracker, Helpilepsy, and Seizure First Aid, help monitor seizures, record medication intake, offer emergency treatment advice, and adjust dosage regimens. This functionality reduces risks associated with ASMs and promotes better patient compliance.[36]

6. Revolutionizing epilepsy education: AI-powered simulation-based learning (SBL) and training modules for healthcare professionals

Integrating AI into SBL will transform the training of healthcare professionals in epilepsy education.[37,38] Management of epilepsy is highly individualized, making

it essential for AI to fine-tune and customize learning pathways; without this personalization, achieving mastery and timely interventions may prove difficult.[39] AI tools, like ChatGPT, can aid in enabling simulation-based training by providing realistic and interactive scenarios that mimic practical epilepsy cases, thereby improving diagnostic accuracy and decision-making.[40] The incorporation of AI in SBL can also address the challenge of low-frequency, high-risk events, such as pediatric seizures, by allowing healthcare workers to practice and refine their skills repeatedly in a controlled environment.[40] This approach not only enhances technical and cognitive skills but also boosts self-efficacy and confidence among providers, leading to improved performance in simulated settings.[40]

It has been reported that AI-based automated seizure detection systems exhibit remarkable capabilities in developing models for accurate diagnosis and interpretation of EEG patterns, significantly reducing the risk of human error.[41] These systems can analyze vast amounts of EEG data within very short periods, consistently identifying subtle patterns that may elude human detection, thereby enhancing the overall efficacy of the diagnostic process.[41] With AI support, the likelihood of misdiagnosis is significantly reduced, as these systems are trained to identify specific patterns associated with epileptic seizures, resulting in more consistent outcomes.[41] The integration of AI within the SBL framework aligns with the broader trend of using intelligent tutoring systems and role-plays involving active agents that engage learners' sense of responsibility.[42] Continuous improvement of AI-driven SBL methods, incorporating feedback from both learners and educators, could greatly benefit healthcare professionals' training in epilepsy management, ultimately enhancing patient outcomes and healthcare delivery.[38,43] These advancements are expected to significantly improve epilepsy education for medical professionals by providing personalized, effective, and accessible learning experiences through these training modules.[41]

The ILAE Academy served as a structured online learning venue featuring self-paced, interactive modules based on competency-led curricula in epileptology, catering to both entry and proficiency levels.[44,45] On this case-based platform, AI can enhance the educational experience through intelligent tutoring systems and individualized feedback, alongside relevant clinical practice and an online EEG and MRI reader complemented with live-tutored courses for CME credits.[46] AI's capabilities in pattern recognition and data-driven decision-making can significantly improve the precision of disease diagnosis and treatment – which is vital in epilepsy management.[44] Furthermore, AI is being used for seizure forecasting, predicting possible seizure occurrences based on historical data and current EEG readings, thereby allowing patients and caregivers time to plan accordingly.[45] Figure 2 shows the flowchart diagram illustrating the steps in EEG-based epilepsy management using machine learning.

7. Comparative analysis of machine learning models for epilepsy detection using EEG data

AI is increasingly transforming epilepsy management through applications that encompass diagnostic support, seizure prediction, treatment personalization, and patient education.[46] Each AI model offers distinct advantages tailored to different aspects of epilepsy care.[47] For instance, convolutional neural networks (CNNs) have become a critical tool in processing EEG data, enhancing the precision of seizure classification.[46,47] Similarly, recurrent neural networks (RNNs) are well-suited for predicting seizures due to their capability to interpret time-sequential data, which is critical in assessing patterns in EEG recordings.[46,47]

Below is a comparative analysis of various AI models frequently applied in epilepsy management, highlighting

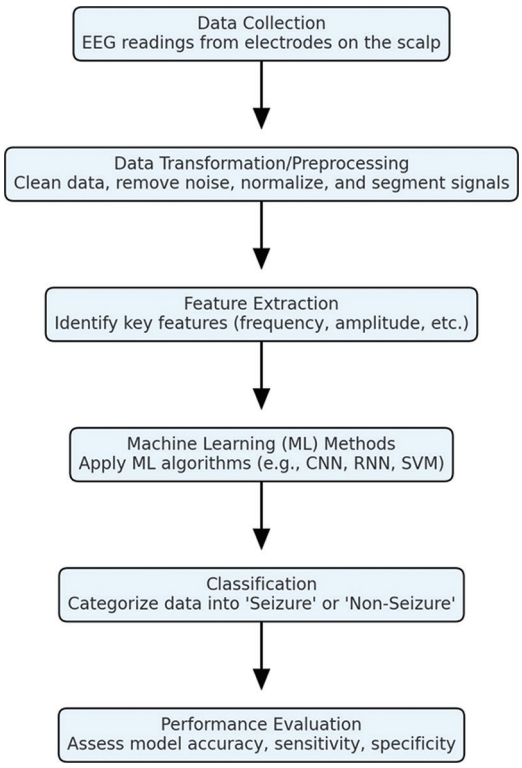

Figure 2. Electroencephalography-based epilepsy detection workflow using machine learning
Abbreviations: CNN: Convolutional neural network; EEG: Electroencephalography; RNN: Recurrent neural networks; SVM: Support vector machines.

their primary applications, strengths, and limitations. This table aims to give a concise overview of each model's role and applicability in addressing the unique challenges presented in epilepsy care. Table 1 shows the characteristic features of different AI applications in epilepsy management.

Several studies have explored machine learning models for detecting epileptic seizures based on EEG data, achieving varying degrees of accuracy, sensitivity, and specificity.[48-56] Hannun *et al.*[45] developed a CNN for seizure detection, achieving an accuracy of 95.6%, sensitivity of 93.2%, and specificity of 96.5%.[47] However, when compared with the lightweight triscale yielding-CNN model by Yang *et al.*,[48] which attained an accuracy of 99.9% on the SWEC-ETHZ dataset, Rajpurkar's CNN shows slightly reduced accuracy while still being a practical approach for seizure detection.[45] Similarly, Mirowski *et al.*[49] applied support vector machines (SVM) for heart rate-based seizure detection and obtained an accuracy of 91.4%, with a sensitivity of 89.2%, and a specificity of 92.5%.[49] This was lower than the results reported by Pedersen *et al.*,[50] who also used SVM with relative energy features from discrete wavelet transform, achieving a higher accuracy of 98%.[50]

In a different approach, Balta[51] used a random forest classifier for early seizure detection on EEG data, achieving an accuracy of 90.8%, sensitivity of 88.5%, and specificity of 91.9%.[51] In contrast, Huang[52] achieved over 96% accuracy using a random forest with intracranial EEG data, showing that model performance can vary substantially across different datasets.[52] Pascual *et al.*,[53] used a long short-term memory (LSTM) model to capture temporal dependencies within EEG signals, reporting an accuracy of 94.2%, sensitivity of 92.1%, and specificity of 95.2%.[53] In comparison, Zhao *et al.*[56] implemented a residual bidirectional LSTM (ResBiLSTM) model, achieving up to 100% accuracy in binary classification, indicating a marked improvement over Zhang's approach, particularly in managing temporal dependencies.[56] Lastly, Mbiazi *et al.*[57] deployed a RNN model for seizure prediction, reporting an accuracy of 92.1%, sensitivity of 90.5%, and specificity of 93.3%, which demonstrates solid predictive capability, although it falls short of the advanced accuracy levels achieved by models like ResBiLSTM.[56]

These studies collectively illustrate the strengths and limitations of various machine learning models in epilepsy detection.[49-57] Although CNN and LSTM models consistently demonstrate high sensitivity and specificity, their performance can vary based on dataset characteristics and model refinements.[49-51] Models, like ResBiLSTM, show that integrating bidirectional temporal processing can enhance accuracy, especially in binary classification.[56] The effectiveness of each model appears linked to specific factors, such as EEG data type, temporal analysis, and feature extraction techniques, underscoring the importance of selecting models based on the dataset and clinical application requirements.[56,58] This comparative analysis highlights the promise of refined neural network architectures, particularly for achieving near-perfect accuracy in seizure detection. Table 2 shows an overview of AI models currently utilized for epilepsy diagnosis and prediction.

8. Leading institutions in AI epilepsy research

Institutions worldwide are making significant strides in applying AI technologies to improve epilepsy management.[58-60] The National Institute of Neurological Disorders and Stroke is at the forefront, conducting pioneering research in CNNs to enhance seizure prediction capabilities.[58-60] The Epilepsy Foundation is actively funding projects focused on AI-driven seizure detection to introduce innovative solutions into clinical practice.[58,59] At the University of California, Los Angeles, researchers are developing RNN-based diagnostic tools that promise earlier and more accurate epilepsy detection.[58,59] The University of Oxford is advancing AI-powered EEG analysis, which is essential for precise seizure monitoring.[60]

Table 1. Comparative analysis of AI applications in epilepsy management by type of model and purpose

AI model	Primary application	Advantages	Limitations
CNN[45]	EEG signal classification	High accuracy in pattern recognition	Computationally intensive
RNN[45,46]	Seizure prediction and temporal analysis	Suitable for time-series data	Requires large datasets
SVM[47]	Feature-based EEG classification	Effective for small datasets	Limited flexibility with complex data
Decision trees[47]	Patient outcome prediction	Easy interpretability	Prone to overfitting in high-dimensional data
Transfer learning[48]	Adaptation to specific patient groups	Reduces the need for extensive training data	Requires pre-trained, similar domain models
Reinforcement learning[47,48]	Personalized treatment and adherence	Dynamic adaptation to patient needs	Complex to train and validate in healthcare

Abbreviations: CNN: Convolutional neural network; EEG: Electroencephalography; RNN: Recurrent neural networks; SVM: Support vector machines.

Meanwhile, Harvard Medical School is working on LSTM-based models for real-time seizure prediction, which are vital tools for mitigating risks associated with SUDEP and enhancing patient safety.[61] Collectively, these institutions are shaping the future of epilepsy care by creating accessible, accurate diagnostic tools that significantly improve patient outcomes globally.[58-61] Table 3 highlights the contributions of these institutions in advancing AI's role in epilepsy management.

9. Case studies and practical implementations

Various research efforts have underscored the utility of AI in epilepsy outreach and the central goals within this field.[57] AI models for detecting and forecasting epileptiform EEG patterns and seizures have yielded significant outcomes in clinical applications.[58] For instance, the AI-based model mjn-SERAS exhibits notable sensitivity and specificity in early seizure detection using customized mathematical models for individualized patient care through EEG analysis.[59] Research highlights specific AI applications in epilepsy, focusing on seizure detection, prediction, and localization.[59] Jeon *et al.*[60] demonstrated a deep-learning model for identifying epileptiform discharges in self-limited epilepsy, achieving high specificity and sensitivity.[60] This model reduced false positives, thus aiding accurate early diagnosis.[60] Similarly, Altaheri *et al.*[61] applied a deep learning-based EEG analysis method that enhanced detection rates and simplified the identification of complex seizure patterns, improving real-time clinical interventions.[61] Another study that incorporated predictive models using EEG and electrocardiogram (ECG) data achieved high performance in seizure prediction, facilitating timely interventions and comprehensive patient monitoring.[63] These examples illustrate the advancement of engineering science in utilizing AI to enhance epilepsy diagnosis, streamline clinical workflows, and support informed decision-making in patient care.[60-63]

10. Barriers to AI adoption in healthcare and education

Despite the need to integrate AI into teaching, learning, and healthcare, several challenges must be addressed to facilitate better integration.[54,55] Concerns include a lack of technical proficiency in medical schools, often stemming from inaccurate descriptions and volunteered information given by generative intelligence models.[61] In addition, issues related to data privacy, moral and legal aspects, compatibility issues, and complexities in human-AI communication hinder the integration of AI in

Table 2. Key findings of machine learning models for detecting epileptic seizures based on electroencephalogram

Authors	Year	Type of study	Results (Statistical values)
Hannun *et al.*[47]	2019	Deep neural network for arrhythmia detection	ROC AUC: 0.97, F1 Score: 0.837
Zhou *et al.*[48]	2024	Heartbeat classification using CNNs and transformer	Accuracy: 99.4%
Siddiqui *et al.*[49]	2020	Review of machine learning classifiers for seizure detection	Overview of classifiers and features
Yang *et al.*[50]	2022	An AI system for clinical seizure recognition	High sensitivity and specificity
Mirowski *et al.*[51]	2019	Comparison of SVM and CNN for seizure prediction	High accuracy, CNN slightly better

Abbreviations: AUC: Area under the curve; CNN: Convolutional neural network; LSTM: Long short-term memory; RNN: Recurrent neural network; ROC: Receiver operating characteristic; SVM: Support vector machine.

Table 3. Contributions of different institutions in advancing artificial intelligence in epilepsy management

Institution	Location	Key focus areas in epilepsy AI	Selected contributions
Mayo Clinic	USA	EEG signal analysis, seizure prediction, patient monitoring	Mayo Clinic AI Lab on Epilepsy
Boston Children's Hospital	USA	Pediatric epilepsy, EEG monitoring, and AI-driven educational resources	AI-EEG study for early seizure diagnosis
University College London	UK	EEG and MRI imaging analysis, epilepsy surgery decision support	UCL AI for epilepsy diagnostics
Seoul National University	South Korea	Machine learning for seizure classification, patient adherence strategies	Epilepsy AI Lab, Seoul National University
Indian Institute of Science	India	Deep learning for EEG analysis and epilepsy care in low-resource settings	Epilepsy AI in developing regions
WHO Collaborating Centre on Epilepsy	International	Ethical and social aspects of AI in epilepsy global access to AI diagnostics	WHO Report on AI in Epilepsy[62]

Abbreviations: AI: Artificial intelligence; EEG: Electroencephalography; MRI: Magnetic resonance imaging; UCL: University College London; UK: United Kingdom, USA: United States of America; WHO: World Health Organization.

healthcare interventions.[63] Healthcare professionals must develop digital skills, understand legal and ethical issues, and enhance their eHealth literacy to promote the safe and efficient integration of AI.[64] Furthermore, language barriers in online medical education can be mitigated through AI-generated multilingual educational materials, ensuring global harmonization of healthcare practices and adherence to digital health standards.[64]

11. Potential negative aspects of AI in epilepsy management

AI systems can inadvertently perpetuate biases in training data, leading to diagnostic inaccuracies for certain demographics.[65] Research by Theodore *et al.*[65] highlights that biases in seizure data may result in inequitable epilepsy care for underrepresented groups.[65] In addition, using vast medical data in AI raises privacy concerns.[65] Timan and Mann[64] emphasize that strict data protection protocols are essential to prevent misuse and maintain patient trust in AI-driven treatments.[64] Many AI models operate as "black boxes," making it difficult to trace decision-making pathways.[64] Wang *et al.*[66] stress the need for transparent AI systems with defined accountability to ensure safe management in epilepsy care.[67] Excessive reliance on AI could erode clinicians' judgment and diagnostic skills.[67] Shoeibi *et al.*[67] suggest that balancing AI assistance with clinical experience is crucial to retaining essential skills for managing complex epilepsy cases.[67] Routine use of AI may diminish critical thinking, affecting clinicians' ability to manage epilepsy effectively without AI support.[67] Continuous training is recommended to mitigate skill erosion.[68] Assigning responsibility for AI-driven errors is complex.[68] Robust frameworks are necessary to address accountability, especially in cases where AI misdiagnoses epilepsy-related events.[62] The use of AI may also depersonalize patient interactions, potentially impacting patient satisfaction.[62] Evidence-based data supports the necessity of human oversight in AI-augmented care to preserve empathy and trust,[62] as epilepsy's intricate management often requires personalized approaches that AI alone may not fully address.[62] Recent guidelines suggest AI should support, not replace, individualized care.[62] Ongoing audits and ethical standards are essential to prevent unintended harm.[62,69] Timan and Mann[64] advocate for regular evaluations to ensure AI aligns with ethical healthcare practices.[64,66] Comprehensive regulations are vital to ensure the safety, reliability, and fairness of AI applications in healthcare.[65] Recent studies underscore the need for robust national and international guidelines to effectively govern the use of AI technologies in clinical settings.[64-69]

12. Ethical and regulatory challenges in AI-driven epilepsy management and education: Balancing innovation with patient-centered care

The integration of AI in epilepsy management and education raises several ethical and regulatory concerns that are essential to address for equitable and effective patient care.[65] A major issue is bias and fairness; AI models can inadvertently propagate biases embedded within the training data, potentially causing disparities in treatment outcomes across diverse demographics.[64] For instance, an AI system trained on data representing a particular demographic may underperform for underrepresented groups, thereby amplifying existing healthcare inequalities.[66] This emphasizes the need for diverse data representation to prevent unequal treatment and foster fairness in AI-driven healthcare applications.[66] Privacy and data security are also critical considerations, especially given the sensitive nature of patient data managed by AI in healthcare.[66] In epilepsy diagnosis, vast amounts of labeled datasets containing personal information are required, posing risks to personal data privacy.[66] Breaches in data privacy could lead to identity theft, discrimination, and diminished trust in healthcare systems.[66] Furthermore, data transmitted over networks, such as EEG recordings, are vulnerable to cyber-attacks.[66] However, innovative strategies, like encrypted EEG data classification using advanced algorithms and CNNs, show promise in enhancing data security and privacy.[67] This highlights the importance of robust measures to safeguard patient privacy and ensure data security in AI-based epilepsy care.[67] Informed consent is fundamental for patient autonomy; individuals must understand how their data will be used and the potential benefits and risks of AI interventions.[58] However, obtaining informed consent can be challenging due to the complexity of AI technologies, which may hinder patients' ability to fully comprehend the implications of AI-driven care.[58] Transparent communication and clear consent processes are necessary to uphold patient rights and build trust.[58] Transparency and accountability in AI systems are also crucial.[53] Many AI models function as "black boxes," making it difficult to interpret how specific conclusions are reached.[53] This lack of transparency can obscure accountability, especially when adverse outcomes arise.[59] Regulatory frameworks that mandate transparency and define responsibility among healthcare providers, AI developers, and system manufacturers are essential to address these challenges effectively.[59] Over-reliance on AI in clinical settings can lead to "de-responsibilization," where healthcare professionals may experience a decline in critical thinking and clinical judgment.[59] If professionals become overly dependent on AI tools, they may lose vigilance in

assessing the multifaceted aspects of epilepsy, potentially missing nuances critical to patient care.[66] Striking a balance between AI assistance and human expertise is necessary to prevent the erosion of clinical judgment.[66] In addition, skill degradation is a risk; reliance on AI for routine tasks can weaken healthcare providers' ability to perform essential clinical functions independently.[67] Continuous training is needed to ensure professionals retain their expertise and remain prepared for situations where AI tools may be unavailable or malfunctioning.[68]

Ethical issues in AI deployment encompass fairness, privacy, safety, transparency, and explainability.[64-67] In the pharmaceutical industry, the use of AI in drug discovery has raised risks and ethical concerns, particularly regarding patient care outcomes and data privacy.[67] Addressing these ethical challenges requires AI systems to be transparent, accountable, fair, and genuine, especially in educational settings where AI's role is growing.[68] The inclusion of AI in medical training programs should focus on its complementary role to traditional learning methods, reinforcing critical thinking and clinical judgment skills among neurophysicians and other healthcare professionals.[68] The impact of AI on patient experiences and the patient-provider relationship is another crucial consideration.[68] As AI assumes a larger role in decision-making, patients may perceive that decisions are increasingly driven by algorithms rather than human judgment, potentially weakening the personal connection and empathy central to healthcare.[68] A human-centered approach that integrates AI insights with personalized care is essential for maintaining trust and compassion in patient interactions.[68] Furthermore, epilepsy management requires personalized care plans that combine AI-generated insights with clinical expertise to meet the complex needs of each patient.[68]

To ensure the ethical use of AI in epilepsy care, continuous monitoring and adherence to ethical standards are imperative.[64-68] Establishing regulatory frameworks, conducting regular audits, and implementing ethical safeguards can help prevent unintended harm while ensuring that AI systems align with healthcare's ethical principles.[64-68] By recognizing and proactively addressing these ethical and societal considerations, stakeholders can maximize AI's potential to improve epilepsy care while safeguarding patient welfare.[64-68] This requires ongoing dialogue among healthcare providers, patients, AI developers, and policymakers to navigate challenges and leverage the benefits of AI effectively.[64-68]

13. Future research directions and emerging trends

Future research directions and trends in AI for epilepsy education include using natural language processing (NLP) to analyze clinical and textual data within epilepsy care.[66] NLP tools are advancing to better interpret unstructured data from patient records, notes, and academic literature, facilitating more streamlined and accessible epilepsy information. Deep learning models, including few-shot learning, metric learning, and capsule neural networks, have shown promise in tackling data scarcity and enhancing diagnostic accuracy in epilepsy.[67] These enable robust pattern recognition even with limited datasets, which is particularly beneficial for rare and complex seizure disorders.

EEG signal processing remains a critical challenge in these applications, particularly in noise removal and feature extraction. Ongoing efforts aim to design AI prediction tools with high sensitivity and specificity to improve real-time seizure prediction, which could enable preemptive interventions and enhance patient quality of life.[68] For example, the development of mobile applications for epileptic seizure detection based on EEG signals demonstrates the importance of user-friendly tools that leverage advanced algorithms for practical uses.[62]

One area of ongoing exploration is the integration of multimodal data, such as EEG, ECG, and imaging data, to further refine seizure prediction algorithms. The potential for wearable technology and mobile health applications is being leveraged to support continuous monitoring and allow real-time data collection.[68] Such devices can potentially improve patient autonomy by providing early warnings and individualized feedback based on AI-driven analysis. Simultaneously, techniques for baseline removal in EEG signal processing are paving the way for more accurate and subject-independent emotion classification.[69]

Collaborative frameworks are also evolving to foster data sharing and enhance epilepsy research across institutions.[70] For example, federated learning enables institutions to collaborate on AI models without sharing sensitive patient data directly, thereby protecting privacy while advancing collective knowledge.[70] These frameworks are essential for scaling AI research and ensuring that AI models are trained on diverse datasets that reflect a broader patient population.[70] Ultimately, as AI in epilepsy diagnosis and management progresses, collaboration with healthcare providers and regulatory bodies is vital to ensure that novel models or devices meet clinical standards, maintain patient safety, and support personalized patient care.[70] With these advancements, AI can continue to evolve as a transformative tool in epilepsy treatment and diagnosis, helping to bridge gaps in healthcare access, refine precision medicine approaches, and improve outcomes for epilepsy patients worldwide.[71]

14. Conclusion

The role of AI in epilepsy management is undeniably transformative, offering unprecedented opportunities for improving patient outcomes. AI technologies have shown remarkable efficacy in early diagnosis, seizure prediction, and personalized treatment, which are critical for enhancing the quality of life for individuals with epilepsy. Integrating AI in wearable devices and mobile health platforms has facilitated continuous monitoring and patient engagement, contributing to more effective and timely interventions. However, the implementation of AI in clinical practice presents challenges. Issues related to data privacy, ethical considerations, and the need for rigorous clinical validation must be addressed to realize the potential of AI in epilepsy care. Future research should focus on overcoming these barriers and exploring innovative AI applications to further advance epilepsy management. Collaborative efforts among researchers, clinicians, and technology developers are essential to harness the full potential of AI, ultimately leading to better health outcomes for individuals with epilepsy.

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ACCSCIENCE PUBLISHING

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REVIEW ARTICLE

Depression and its major risk factors in India: A narrative review

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Abstract

Major depressive disorder is a complex neurological condition marked by persistent sadness, hopelessness, and loss of interest in daily activities for at least 2 weeks. It significantly affects mental, emotional, and physical health and is a major contributor to global disease. In India, rising depression rates have demonstrated the necessity of better understanding its causes for improved interventions and public health policies. This review comprehensively explores the diverse factors contributing to the increasing prevalence of depression in India. An unstructured survey of research articles and a detailed literature review were conducted using electronic databases, including Google Scholar, PubMed, Springer, and Elsevier. The findings reveal that in India, depression stems from a combination of external and internal risk factors. External factors such as cultural norms and gender roles heavily influence societal perceptions of mental health and individual psychological experiences. Other significant external contributors include educational stress, unemployment, occupational pressure, and challenges from rapid urbanization. Meanwhile, internal factors including genetic predisposition and epigenetic mechanisms play a critical role in individual susceptibility to depression. These biological factors interact with environmental stressors to shape the onset and progression of depression. Based on the findings, rising depression rates in India necessitate targeted efforts to address modifiable factors, such as stigma, work stress, and mental healthcare access, while recognizing genetic influences. A comprehensive strategy that integrates policy reforms and community initiatives is crucial for reducing the burden of depression in India.

Keywords: Major depressive disorder; Healthcare in India; Depression risk factors; Mental health; Public health policy; Culture; Gender

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1. Introduction

The story of evolution is more than a tale of life's journey from simple beginnings to complex forms; rather, it is a never-ending adventure of adaptation and improvement. For humans, this journey goes beyond physical changes to encompass how we tackle new challenges, innovate, and redefine what it means to be human. Each step forward makes us stronger and more capable. However, amid humanity's progress, stress plays a crucial role, shaping lives in ways we often do not realize. Stress arises from several sources, which can be broadly categorized into physical and mental tiredness.[1] Physical fatigue is a sign from our body to slow down and usually affects our muscles and energy levels. However, mental fatigue caused by stress can alter our perception and impact every aspect of our lives, reflecting the close association among evolution, stress, and daily experiences. Notably, stress and fatigue, although related, are not the same. Stress involves a response to demands or threats, whereas fatigue is a state that can arise from various causes, including stress.[2]

Major depressive disorder (MDD), commonly known as depression, is a complex condition affecting millions of people worldwide. According to the World Health Organization (WHO) (https://www.who.int/news-room/fact-sheets/detail/depression), depression is characterized by a range of symptoms (Figure 1) that persist for most of the day, nearly every day, for a period of at least 2 weeks. This condition often remains unnoticed amid daily life activities, reflecting its complexity and the careful consideration needed to address it.

According to statistical estimates, globally, approximately 280 million people suffer from depression, comprising about 3.8% of the world's population. The prevalence rate is around 5.0% for adults and rises to 5.7% among those aged over 60 (Institute for Health Metrics and Evaluation | (healthdata.org). In particular, Greece, Palestine, and Spain have recorded some of the highest rates, ranging from 4,462.98 to 5,024.51 cases/100,000 people. Developed countries such as the United States, Canada, Japan, and Russia also report significant figures (VizHub – GBD Compare (healthdata.org) (Figure 2). Moreover, since the onset of the COVID-19 pandemic, cases of MDD have increased by 27.6% globally, comprising approximately 53.2 million new cases and bringing the total prevalence to 3,152.9 cases/100,000 people in 2020. The pandemic also resulted in 49.4 million disability-adjusted life years (DALYs) globally in the same year.[3] DALY is a measure of overall disease burden, expressed as the number of years lost due to ill health, disability, or early death.

Stress is the main culprit behind this increase in cases of depression. As mentioned previously, stress can result from different factors (Figure 1), including limited educational opportunities, financial hardship, restricted personal

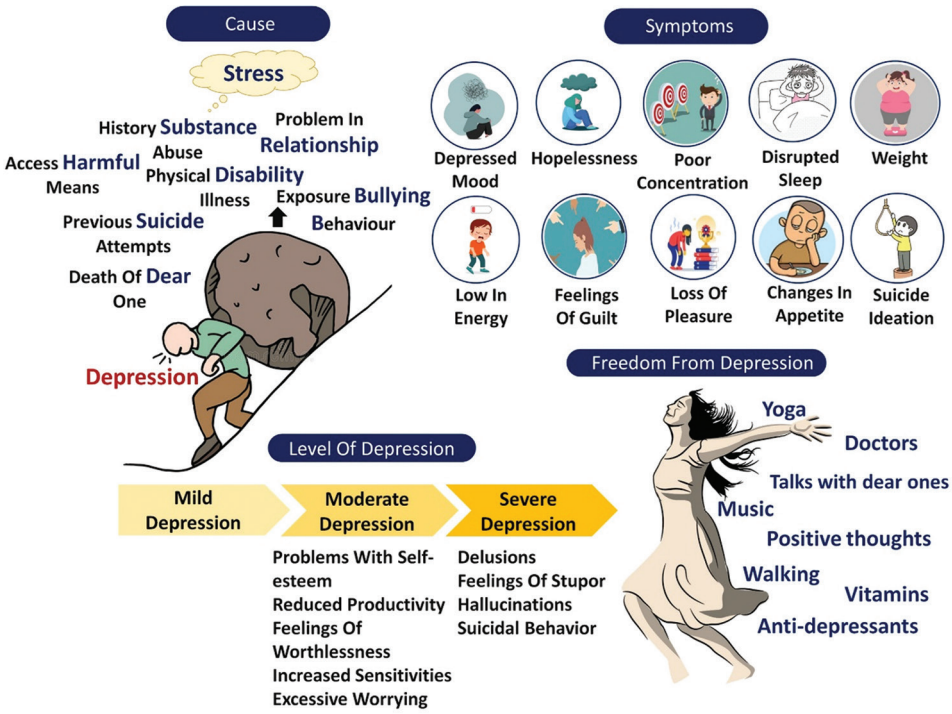

Figure 1. Depression infographics. Image created by authors.

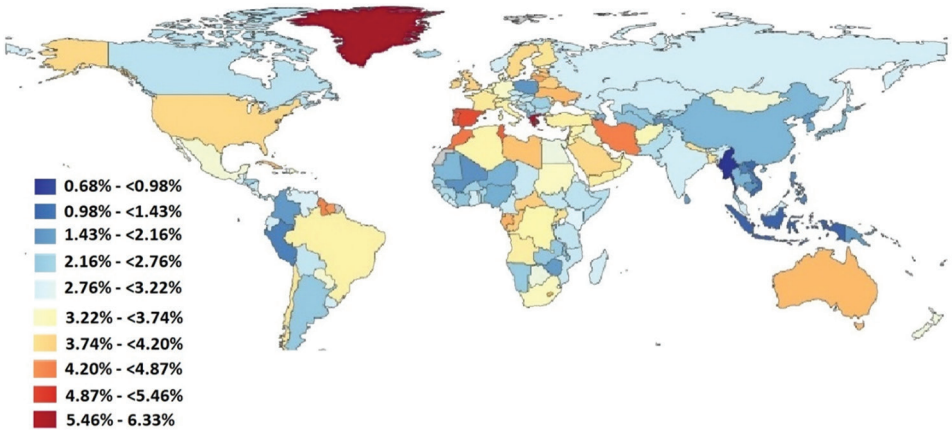

Figure 2. Prevalence of depression adjusted for gender and age

freedoms, early marriage practices, domestic violence, and inadequate family support.[4] These factors can significantly influence the prevalence of depression, especially in developing and/or low-income countries such as India.

2. Materials and methods

An unstructured literature review was conducted to survey research articles across electronic databases, namely, Google Scholar, PubMed, Springer, and Elsevier. Various keywords were employed, including "genetic association," "serum association," "epigenetics," "cultural impacts on depression," "gender gaps," "societal outlooks," "traditional viewpoints," "the stigma surrounding mental health," "cultural standards," "gender expectations," "cultural variations," "customary behaviors," and "depression prevalence in the Indian population."

The selection process employed linguistic filters to focus on articles published in English. Primary research articles were prioritized, and secondary sources such as meta-analyses and systematic reviews were excluded. In addition, studies with incomplete or partially accessible data were omitted to ensure integrity. Extensive measures were taken to eliminate any undesirable traits (e.g. incomplete or missing data, studies that are not in English, poor methodological quality, inconsistencies, errors, duplicate or redundant data, unverified or non-peer-reviewed sources, and non-generalizable findings in the included data. The study surveyed a substantial body of the recent literature, with data validity independently verified by the authors. Their collective effort involved a thorough review of articles from multiple electronic databases, ensuring a comprehensive analysis of the topic.

3. Results

MDD, commonly called depression, is a complex neurological condition characterized by symptoms such as hopelessness, lack of concentration, insomnia, fatigue, a loss of pleasure, appetite changes, and suicidal thoughts, which persist for most of the day for at least 2 weeks (Figure 1) (Depression- WHO). The main cause for the increased risk of depression in recent years is stress, which can include occupational, physical, family, or relationship, and educational stress (Figure 1). Mental health represents a significant concern in emerging and developing nations and is influenced by various context-specific factors including economic status, educational infrastructure, access to proper nutrition, and overall developmental initiatives. India serves as a pertinent case study for such concerns, given its substantial population (1,380,004,385), which positions it as the second most populous country after China (1,439,323,776) (Worldometer).

3.1. Prevalence of depression in India

The 2019 Global Burden of Disease study highlights specific Indian states facing higher rates of depression. Tamil Nadu, for instance, recorded the highest prevalence at 4.45%, followed by Haryana at 3.7%, Andhra Pradesh at 3.53%, and Maharashtra at 3.48% (Institute for Health Metrics and Evaluation | (healthdata.org). This regional variation emphasizes the need for a nuanced understanding of depression that is tailored to India's diverse sociocultural context.

3.2. Tools for assessing depression in the Indian population

"Diagnostic questionnaires" for evaluating depression, commonly known as "depression assessment questionnaires," are a primary tool for assessing depression before clinical intervention. A critical review of the literature revealed that research groups have used a variety of questionnaires, including the hamilton depression rating scale (HAM-D),[5-9] the diagnostic and statistical

manual of mental disorders,[8,10,11] the Montgomery–Åsberg depression rating scale,[12] and the center for epidemiologic studies depression scale,[13] with each scale having different sensitivity and specificity (Tables 1 and 2). Moreover, the review revealed that no specific scale has been developed for use in India. However, several widely used scales have been linguistically translated, standardized, and validated for the Indian context, and show promising reliability (Tables 1 and 2).

To understand the increasing prevalence of depression in India, researchers have used various methods to identify the contributing risk factors.

3.3. Risk factors

A disease's risk factors refer to any attribute, characteristic, or exposure that increases the likelihood of developing the disease. These factors can stem from various sources and are broadly categorized as external and internal. External factors encompass a range of indicators such as lifestyle factors, behavioral patterns, and physiological states. Meanwhile, internal factors also play a significant role and include genetic predispositions and epigenetic modifications. Risk factors contribute to the overall probability of disease occurrence and vary in impact depending on individual circumstances and interactions with other factors.[14] In preventive medicine and public health, recognizing and identifying a disease's risk factors is crucial as it enables targeted interventions to mitigate risk, promote health, and enhance outcomes.

3.3.1. External or environmental factors

Stress is recognized as a pivotal external risk factor for MDD, and it is defined by the WHO as "a state of worry or mental tension caused by difficult situations" (Stress-WHO.int). From a physiological standpoint, stress encompasses "any change that triggers physical, emotional, or psychological strain or pressure."[15] As an intrinsic aspect of the human condition, stress arises from various sources such as traumatic events, disappointments, or feelings of unease; therefore, it impacts everyone to some degree. Critically, people's response to stress can profoundly affect their overall well-being.

There are two distinct types of stress: Eustress and distress. Eustress, which is often viewed as a positive motivator, can promote improved performance. In contrast, distress acts as a negative force that can overwhelm individuals and lead to exhaustion, despair, and impaired functionality.[16] Among the Indian population, this review reveals different sources of distress that deeply affect people's lives, focusing on gender, academic stress, occupational stress, and unemployment.

Culture encompasses a group's collective beliefs, customs, and behaviors. It shapes traditions, language, arts, and social norms that define identities and interactions within communities, evolving and varying widely across societies.[17] As a significant factor linked with depression, understanding the role of culture in depression in the Indian context is crucial. Research indicates that "cultural influences" significantly impact gender roles, behaviors, and societal expectations, contributing to disparities in mental health and achievement between genders.[18] For example, women, influenced by cultural norms, are 2 times more likely to experience depression than men.[19] Moreover, gender disparities contribute to a pronounced increase in depression rates among girls during adolescence.[20,21]

Depression is influenced by longstanding societal expectations regarding gender roles and limited emotional and financial support in familial and marital contexts.[22] In particular, in the context of marriage, child marriages reveal a complex issue that is deeply rooted in cultural and societal norms. Despite legal protections, India harbors the highest number of child brides in the world, with 223 million girls married before adulthood, comprising approximately a third of the world's total (Ending-Child-Marriage.pdf (unicef.org). Annually, approximately 1.5 million girls marry under the legal age of 18 (Ending-Child-Marriage.pdf (unicef.org), reflecting the deep-seated cultural practices perpetuating this phenomenon. Recent studies have emphasized the profound and negative consequences of early marriage, including heightened risks of depression, abuse, and adverse health outcomes.[23,24] This practice also correlates with the increased odds of tobacco use among women who were married as children, underscoring broad disparities in health outcomes.[25] Addressing child marriage requires challenging cultural norms and societal structures through legal, social, and educational interventions to protect and empower young girls. Stressful experiences such as seeking healthcare, education, employment, and domestic violence disproportionately affect women, impacting their mental well-being. Gender-specific norms shape factors such as upbringing, education, relationships, and broader social influences, contributing to differing life experiences for young women compared with men.[19,26,27]

In the cultural context of students, depression manifests with symptoms such as restlessness, anxiety, and cognitive difficulties, which are often linked with personal factors such as perceived failure. Specifically, depression reflects a significant economic disparity among Indian university students: those from rural and socioeconomically disadvantaged backgrounds often endure heightened feelings of despair due to limited financial resources, exacerbating feelings of low self-worth and insecurity.[28] These

Table 1. Prevalence of depression among students in India

No.	Author and year	State	Age group	Tools	Number of participants	Prevalence of depression (%)	Results
1	Sarkar *et al*., 2012[60]	Jharkhand	9th – 12th grade children	K-SADS-PL	1,851	3.13	Adolescent depression was deemed a distinct diagnostic entity affecting a significant number of teenagers.
2	Sandal *et al*., 2017[74]	Chandigarh (Punjab)	9th – 12th grade children	DASS	470	65.53	The prevalence of DAS was high among adolescents in Chandigarh. There was a need for the early diagnosis to prevent other nascent psychiatric disorders.
3	Jayashree *et al*., 2018[115]	Mangaluru, (Karnataka)	University students	BDI	201	48.8	Depression was high among university students.
4	Kumar *et al*., 2016[120]	Mysore (Karnataka)	Medical students	DASS	332	37.3	Medical students' distress influenced professional development and adversely impacted academic performance, contributing to academic dishonesty, substance abuse, and attrition from medical school.
5	Kumar *et al*., 2012[154]	Karnataka	Students >13 years	BDI	400	71.25	Depression was most prevalent among medical students. Broad screening and psychiatric counseling were deemed necessary.
6	Jha *et al*., 2017[155]	Bihar	9th – 12th grade children	BDI	1,412	49.2	Older teenagers were more depressed. Depression was statistically significantly associated with gender and religion.
7	Mishra *et al*., 2018[156]	Uttar Pradesh	9th – 12th grade children	CDI	200	14.5	Depression was more prevalent among teenagers living in joint families.
8	Kumar *et al*., 2017[157]	Puducherry	1st – 5th year medical students	BDI	444	48.4	Depression was common among medical students and significantly associated with stress. Coping mechanisms and relationship improvement were recommended for reducing depressive symptoms.
9	Singh *et al*., 2017[158]	Chandigarh (Punjab)	9th – 12th grade children	PHQ-9	542	40.1 (MDD: 7.6 Other: 32.5)	There was a need to modify home and school environments to reduce the risk of depression among teenagers.
10	Kaur *et al*., 2014[159]	Amritsar (Punjab)	18 – 24-year-olds	PHQ-9	200	16.5	Family-related and academic factors were common risk factors for depression among university students.

(Cont'd...)

Table 1. ***(Continued)***

No.	Author and year	State	Age group	Tools	Number of participants	Prevalence of depression (%)	Results
11	Raghunathan *et al*., 2019[160]	Thiruvananthapuram (Kerala)	22-year-olds	PHQ-9	364	26.9	Romantic break-ups were independent risk factors for depression among university students.
12	Verma *et al*., 2014[161]	Raipur (Chhattisgarh)	16-year-olds	CES-D	321	19	Among the various factors examined for association with depression among teenagers, the statistically significant factors identified were working mothers, students staying away from home, poor relationships with family, and self or parental dissatisfaction with academic achievement. Peer pressure also had a significant association.
13	Yadav *et al*., 2016[162]	Jhansi (Uttar Pradesh)	1st – 4th year medical students	DASS	330	57	Risk factors outside academic stressors can predispose medical students to psychological morbidity.
14	Sharma *et al*., 2015[163]	Bhopal (Madhya Pradesh)	21-year-old medical students	TDEQ	440	31	A substantial proportion of medical students had ongoing psychiatric problems that were associated with multiple social, behavioral, and educational factors.
15	Vankar *et al*., 2014[164]	Karamsad (Gujarat)	Medical students	PHQ-9	331	64	High stigma existed among medical students about the causation of depression, and students discriminated against peers based on the presence of depression.
16	Nezam *et al*., 2020[165]	Patna (Bihar)	Professional students	BDI	3,100	47.78	There was an alarming prevalence of depression among students pursuing professional courses.
17	Arun *et al*., 2022[166]	Puducherry	Medical students	PHQ-9	425	13.9	One-fourth of medical students had depression.
18	Shukla *et al*., 2019[167]	Lucknow (Uttar Pradesh)	10 – 19-year-old girls	KADS	2,187	39.7	Adolescent girls were at high risk for depression.

Abbreviations: K-SADS-PL: Schedule for affective disorders and schizophrenia for school-age children; DASS: Depression anxiety stress scales; DAS: Depression, anxiety, and stress; BDI: Beck depression inventory; CDI: Children's depression inventory; PHQ-9: Patients health questionnaire; MDD: Major depressive disorder; CES-D: Center for epidemiological studies depression scale; TDEQ: Theoretical depressive experiences questionnaire; KADS: Kutcher adolescent depression scale.

cultural perspectives highlight broader issues, as cultural norms such as the caste system and the global economic challenges faced by ethnic minorities lead to increased vulnerability to depression among those from marginalized communities. Understanding and addressing these cultural and socioeconomic influences is crucial in the development of effective interventions and support systems, particularly for vulnerable groups such as university students in India.

Moreover, cultural backgrounds significantly influence perceptions of depression, with traditional Indian views

Table 2. Candidate depression-related genes within Indian demographics

No.	Gene	SNPs	Tool (s)	Case group/ control group	Location	Type of study	Relationship	Results	Author and year
1	*SLC6A4*	5-HTTLPR	HDRS-17	102/None	Puducherry	DR	✓	SLC6A4 was associated with a favorable treatment response to fluoxetine in MDD patients in Southern India.	Manoharan *et al*., 2016[5]
2	*APOE*	ApoE4	HDRS	31/31	Karnataka	RA	✓	Elderly Indians with an ApoE4 allele had a×4.7 higher risk of developing depression.	Sureshkumar *et al*., 2012[6]
3	*SLC6A4*	5-HTTLPR, STin2 VNTR	HDRS	125/None	Tamil Nadu	DR	✓	The 5HTTLPR polymorphism and the SLC6A4 intron 2 polymorphisms were associated with the treatment response, with the l/l genotype and 12-copy allele showing a tendency toward better SSRI treatment, respectively.	Ramesh *et al*., 2022[7]
4	*SLC6A4*	5-HTTLPR-	DSM-IV-TR; HDRS	57/None	Kashmir	DR	✓	The serotonin transporter gene polymorphism may influence the effectiveness of SSRI treatment in depressive disorders, irrespective of clinical variables.	Margoob *et al*., 2008[8]
5	*BDNF*	Val66Met,	HDRS	104/106	West Bengal	RA	✓	CYP2D6 deletion significantly contributes to the severity and stress factor in MDD patients in contrast to the *BDNF* gene.	Haldar *et al*., 2022[9]
	CYP2D6	P4502D6					X		
6	*TPH 2*	C11993A	DSM-IV-TR	60/40	Kashmir	RA	X	The PH 2C *11993 A* gene was not found to be associated with MDD and anxiety disorder in the Kashmiri population.	Mushtaq *et al*., 2014[10]
7	*MTHFR*	C677T	DSM-IV-TR	15/15	Uttar Pradesh	RA	X	A non-significant difference between the cases and control may be because of the small sample size.	Upasana *et al*., 2014[11]
8	*HTR1A*,	rs6295	DSM-IV-TR; MADRS	55	New Delhi	DR	X	No significant association was found between the SNPs analyzed and responses to escitalopram, although a significant association was observed between the side effects of memory loss with rs6311.	Basu *et al*., 2015[12]
	HTR2A	rs6311 and rs6313					X		
	SLC6A4	5-HTTLPR					X		

(Cont'd...)

Table 2. ***(Continued)***

No.	Gene	SNPs	Tool (s)	Case group/ control group	Location	Type of study	Relationship	Results	Author and year
9	*SLC1A3 and BDNF*	C3590T, C869G, and G196A	CES-D	108/205	Uttar Pradesh	RA	✓	SLC1A3 C35A90T was a predisposition factor for stress and depression in an Eastern Indian population, whereas SLC1A3 G869C and BDNF G196A were not.	Ghosh *et al.*, 2020[13]
10	*MTR*	A2756G	BDI-II	808/None	New Delhi	RA	✓	Only the MTR *A2756G* gene polymorphism was significantly associated with mild depression, but not moderate or severe depression.	Dahal *et al.*, 2022[98]
		A66G					X		
11	*CBS*	844ins68					X		
12	*MTHFR*	C677T	BDI-II	271/557	Haryana	RA	X	The MTHFR *C677T* gene polymorphism was not associated with depression in the present study.	Kaur *et al.*, 2021[99]
13	*MTHFR*	C677T	PHQ-9	93/210	New Delhi	RA	X	The T allele of the MTHFR *C677T* gene polymorphism was associated with an increased risk of depression and anxiety disorder, although this was not significant.	Saraswathy *et al.*, 2019[100]
14	*DAT*	TaqA1	GDS	30/30	Lucknow	RA	X	No significant association was observed for either variant.	Mahdi *et al.*, 2022[101]
		VNTR					X		
15	*LEP*	D7S1875	None	133/136	Rajasthan	RA	✓	*LEP* gene variants may be related to depression and associated comorbidities like hypertension	Kapoor *et al.*, 2009[168]
16	*TPH*	A779C	DSM-IV TR	140/160	Kashmir	RA	✓	The TPH 1 *A779C A* gene was found to be associated with MDD in the Kashmiri population.	Mushtaq *et al.*, 2016[169]
17	*SLC6A4*	5-HTTLPR-	HDRS-17; MADRS; CGI	148/None	Karnataka	DR	✓	Results suggest that 5HTTLPR and rs-25531 polymorphisms can influence escitalopram treatment response in depressive patients in a South Indian population, with LL genotypes and LALA haplotypes being the predictors of better treatment response.	Mandal *et al.*, 2020[170]
18	*VDBP*	rs7041	EPDS	330/330	Puducherry	RA	✓	Rs4588 and rs7041 were associated with lower circulating serum vitamin D levels, which may lead to PPD susceptibility.	Pillai *et al.*, 2022[171]
		rs4588					✓		

(Cont'd...)

Table 2. ***(Continued)***

No.	Gene	SNPs	Tool (s)	Case group/ control group	Location	Type of study	Relationship	Results	Author and year
19	*ACE*	I/D	BDI-II	75/120	Haryana	RA	P	In conclusion, the findings of this study suggest that the D allele of the *ACE I/D* gene polymorphism poses a potentially reduced risk of depression among North Indian adults.	Sharma *et al*., 2024[172]
20	*BDNF*	Val66met	DSM-V; HDRS	50/None	Tamil Nadu	DR	✓	Baseline serum levels of hsCRP and BDNF predicted the response to SSRIs in MDD, and Val/Val patients responded better when compared to patients carrying the Met allele.	Ramesh *et al*., 2021[173]
21	*5HT 2A*	−1438 A/G	None	31/40	Kashmir	RA	✓	−1438 A/G single-nucleotide polymorphism in the promoter region of the *5HT-2A* gene was associated with anxiety and depressive disorders	Mushtaq *et al*., 2014[174]

Abbreviations: SNPs: Single-nucleotide polymorphisms; HDRS-17: Hamilton depression rating scale, 17 items; DR: Drug response; ✓: Relationship found; MDD: Major depressive disorder; HDRS: Hamilton depression rating scale, 21 items; RA: Risk association; SSRI: Selective serotonin reuptake inhibitor; DSM-IV-TR: Diagnostic and statistical manual of mental disorders, fourth edition, text revision; X: No association found; MADRS: Montgomery-Åsberg depression rating scale; CES-D: Center for epidemiologic studies depression scale; BDI-II: Beck depression inventory II; PHQ-9: Patient health questionnaire; GDS: Geriatric depression scale; CGI: Clinical global impression scale; EPDS: Edinburg postnatal depression scale; P: Protective risk; DSM-V: Diagnostic and statistical manual of mental disorders, fifth edition.

often differing from Western interpretations. A study comparing students from Northern India and the United States emphasized the role of social support and practices such as yoga and meditation as effective coping strategies in India.[29] These findings emphasize the need for culturally sensitive approaches that integrate traditional practices and strengthened social support systems to address depression in countries such as India.

Based on this understanding of the cultural influences shaping women's lives in India and their correlation with depression, exploring the diverse factors that substantially contribute to the high prevalence of depression among Indian women is crucial.[30] Women, often considered as the backbone of future generations, are frequently overlooked during their adolescence, the crucial transition from childhood to adulthood (11 – 19 years). This period is marked by rapid physical and mental changes, leading to an increase in stress and a number of health challenges including depression.[31,32] In particular, menstruation-related psychosocial issues are notable, impacting girls' lives in various ways. In this regard, common issues include pre-menstrual syndrome, menorrhagia (heavy or prolonged bleeding), sleep disturbances, body pain, and headaches.[33] Dysmenorrhea, which is characterized by painful menstrual cycles, is particularly stressful, causing considerable pain, limitations in one's ability to engage in daily activities, and increased school absenteeism.[34]

Subsequently, the transitioning from unmarried to married life results in significant changes, especially considering India's entrenched dowry system.[35] This system entails the transfer of payments such as capital, durable goods, and real estate from the bride's family to the groom's as a prerequisite for marriage. Despite the Dowry Prohibition Act, implemented in 1961 to curb dowry-related issues, cases continue to be reported at concerning levels (The Dowry Prohibition Act. 1961 28 of 1961 | Ministry of Women and Child Development|IN|bfult (wcd.nic.in). According to a report from the Statista Research Department, in 2022, nearly 6,400 dowry-related deaths were reported, a decrease from approximately 8,500 cases in 2014 (India: Dowry deaths 2022|Statista). Although this decline reflects increased awareness and legal enforcement,

the substantial number indicates that challenges remain in fully eradicating the dowry system. Notably, dowry-related abuse is significantly associated with depression among women.[36-38] Thus, despite the Dowry Prohibition Act's aim of safeguarding women from the financial and emotional strains associated with the dowry system, cultural and societal pressures, coupled with economic disparities, often perpetuate the practice.

Another notable contributor of stress among women in India is particularly evident during their reproductive years, with infertility emerging as a major issue. In India, the infertility rate has risen from 22.4% in 1992 – 93 to 30.7% in recent years and is a significant contributor to stress.[39] Infertile women report considerable conflicts with their husbands and families, with the prevalence of intimate partner violence (IPV) among these women being 50%.[40-42] Studies have indicated a significant link between infertility in women and experiencing IPV, which can include physical and sexual abuse, stalking, and emotional mistreatment by partners. These traumatic experiences are often associated with increased rates of depression.[40-42] Notably, 32% of married women in India report experiencing some form of spousal violence, with physical, emotional, and sexual violence being the most frequently reported 28%, 14%, and 6%, respectively).[43]

Other than infertility, after pregnancy, women can experience depression, largely categorized into two types: antenatal depression (AD) and postpartum depression (PPD). AD represents the onset of depressive symptoms during pregnancy, whereas PPD is experienced after giving birth.[44] In particular, PPD significantly impacts the mother-infant relationship and the child's development.[45] Factors such as cesarean births, health complications for the infant, difficulties in nursing, and psychological and obstetric challenges significantly impact maternal mental health, particularly in less developed countries. In particular, stillbirths, defined by the WHO as losing the baby before or during delivery, are profoundly devastating events. Approximately 1 million stillbirths occur annually across the world, equivalent to 1 every 16 s; this staggering statistic highlights the immense global burden of this phenomenon. In this regard, Swain *et al*. highlighted psychosocial risks such as stressful life events, family psychiatric history, domestic abuse, financial strain, and the lack of spousal support during delivery. Therefore, identifying vulnerable mothers before they give birth is crucial for effective interventions.[46]

With respect to statistical estimations, in a cross-sectional, community-based study conducted in South Indian, George *et al.* observed an incidence rate for AD of approximately 16.3% m,[47] an increase from a previous study conducted in 2002 (11%).[48] Meanwhile, a study conducted in North India (specifically, New Delhi) indicated a 6% risk of PPD after utilizing the Edinburgh postnatal depression scale.[49] Another community-based cross-sectional study conducted in Bihar showed a 23.9% prevalence of AD,[50] similar to the findings of Patel *et al*. in a study conducted in Western India (specifically, Goa), which reported an incidence of PPD of 23%.[44] These studies clarify the potential risk factors that strongly correlate with an increased risk of depression, including financial strain and income, the birth of a daughter, relationship difficulties with the spouse's parents.[44,48,50,51] a history of miscarriages or stillbirths, martial conflicts, non-arranged marriages,[47] and a nuclear family structure.[49] AD and PPD critically affect the growth of a child and are thus considered as risk factors for malnutrition in children aged 6 – 12 months[52] and are also associated with low birth weight (LBW) among infants, with female infants presenting a greater likelihood of LBW.[53]

The desire for a male child is a common one in India for a number of reasons, such as patrilineal inheritance systems, patrilocal marriage frameworks, dowry, and the reliance among older parents on their sons.[54] The risk factor of "not living in a joint family system" reflects a distinct sociocultural norm in India compared with Western countries. Living in a joint family may decrease the chance of PPD due to support from family member, however, many women avoid living in a joint family due to extra work and responsibilities.[48,55] Gopalakrishnan *et al.*[56] discovered a significant correlation among newlywed females – strengthened bonds with their husbands and mothers-in-law are associated with a significant reduction in depressive symptoms. Crucially, these insights emphasize the influential role of mothers-in-law in the association between spousal relationships and depressive symptoms.

Finally, widowhood can be a predictor of depression; widows face significant mental health challenges, although longer-term widows exhibit stability or slight improvements. Over 3 years, both groups (recent and longer-term widows) experienced unintentional weight loss, with minimal and inconsistent variations in health habits and physical health modifications. Married women generally show better physical, emotional, and health behaviors than their widowed counterparts.[57] Meanwhile, Jangam *et al*. compared women with unipolar depression (UD) who sought therapy with healthy women, observing that women with UD had significantly higher rates of emotional maltreatment during childhood than healthy women.[58]

In conclusion, depression has profound effects on women in India, affecting not only individuals themselves

but also their families and society. To address the unique challenges of the cultural context, it is crucial to empower women through education, legal reforms, and stronger enforcement of dowry laws, striving to reverse entrenched cultural norms that contribute to stress and therefore depression among women.

Academic stress is a common issue that affects students worldwide at different levels of education[59-64] due to the high expectations from parents, teachers, and students themselves.[65,66] Multiple studies have discussed the high depression rates among Indian students across various grades and academic streams (Table 1). Students at medical colleges, especially those in their final year, showed a notably higher risk of depression due to uncertainties about the future and concerns about job placements.[67] In contrast, students in the humanities and social sciences generally experience lower stress levels.[59]

Increased depression rates among high school students were found to be linked to diverse stressors, including crowded classrooms, examinations, a lack of resources,[68] pressure to select a subject after the tenth grade, the university admissions process, and adjusting to university expectations.[69] An alarmingly high prevalence of depression, anxiety, and stress symptoms has also been found among university students.[70] Leaving home to study in distant locations can exacerbate stress and depression as students adapt to new lifestyles and cultures.[71] Various factors, such as lower grades, increased tuition fees, negative feedback from parents and teachers, discrepancies between expected and actual grades, socioeconomic status, high aspirations, competition, examinations, and poor study habits, can contribute to stress among pre-university students, further exacerbating these risks.[63,72-74] In addition, language barriers, including the dominance of English in multilingual societies like India, can hinder understanding, expression, and academic performance, leading to frustration, isolation, and increased stress and anxiety.[63,75] Gender disparities also contribute, with girls and women experiencing higher stress levels from academic pressure and examinations.[64]

Stress significantly impacts students' health at all levels, altering vital parameters such as pulse rate, blood pressure, sleep, and eating habits.[76] These changes highlight the urgent need to address academic stress, demonstrated by the alarming suicide rates among students in India.[65] As part of this effort, the Union Government has introduced the New Education Policy 2020 (NEP-2020). NEP-2020, based on the principles of access, equity, quality, affordability, and accountability, was built to foster holistic development, critical thinking, creativity, and problem-solving skills. Mental health initiatives such as counseling, stress management workshops, and supportive academic environments were incorporated to further promote student well-being (National Education Policy, Government of India, Ministry of Education).

After graduation, a significant factor that can increase the risk of depression is unemployment. To grasp its impact on mental health, it is important to understand what unemployment entails. According to *The Economic Times* of India, unemployment occurs when individuals are actively seeking jobs but are unable to find employment (The Economic Times (Indiatimes.com). Among individuals aged 15 and above, the unemployment rate significantly dropped in May 2024, falling to 7%, the lowest recorded since September 2022. This was a marked improvement compared with April 2024, when the unemployment rate was at 8.1% (CMIE). Unemployment seriously harms people's mental health, especially affecting young people. Studies have consistently highlighted the link between unemployment and depression. For example, in Kashmir, Bhat and Joshi observed that unemployed youth face greater mental health challenges compared with their employed peers.[77] Likewise, Buvneshkumar *et al.* identified a strong connection between unemployment and depression among older adults in Tamil Nadu.[78] Sengupta and Benjamin reinforced these findings in Punjab: In a study of 3,038 older adults, they noted higher rates of depression among the unemployed.[79] Verma and Mishra also reported a clear association between unemployment and increased depression, emphasizing the widespread impact of unemployment on mental health across age groups.[80]

Largely influenced by the COVID-19 pandemic, India's unemployment rate increased markedly between 2019 and 2021. During this period, economic uncertainties, job losses, and social isolation heightened vulnerability to depression. One's employment status also impacts their mental health through occupational stress factors such as workload, job insecurity, and lack of control.[81] A 2022 meta-analysis confirmed the link between unemployment and MDD, reporting an odds ratio of 2.06. Notably, the risk was higher in men (odds ratio: 2.27) than in women (odds ratio: 1.62).[82]

Based on the above, given the significant impact of unemployment on mental health across various age groups in India, future efforts to address mental health should focus on targeted interventions and policies to address unemployment. Research must explore how unemployment affects diverse demographic groups, including youth and older adults, with longitudinal studies offering insights into its long-term effects. Addressing structural factors such as education, skill development, entrepreneurship,

and job creation is essential for reducing unemployment rates across the country. Moreover, gender-sensitive strategies are vital, considering the significant impact of unemployment on men's risk for MDD. By integrating research studies, policy actions, and community-based programs, India can effectively address the complex link between unemployment and mental health, fostering societal well-being.

Occupational stress refers to the physical, emotional, and mental strain individuals experience due to work-related factors. As mentioned previously, common causes include heavy workloads, tight deadlines, long hours, job insecurity, interpersonal conflicts, and organizational pressures. In India, cultural, societal, and economic factors often intensify workplace stress, with professionals in sectors such as education, healthcare, law enforcement, and information technology (IT) facing distinct challenges.[83] Rapid modernization, technological advancements, and sociopolitical changes further add to these stressors. The symptoms of occupational stress range from physical issues such as tiredness and headaches to psychological effects such as anxiety, sadness, and burnout.[84]

Research on professions such as teaching, policing, nursing, class-IV jobs, banking, and software engineering reveals a concerning trend of chronic stress in these fields, often leading to depression.[85-90] Educators often face excessive workloads, limited career growth opportunities, and interpersonal conflicts within their institutions. The responsibility of shaping young minds and adapting to changing educational demands can further contribute to elevated stress levels.[91] Meanwhile, police officers face high operational demands and organizational pressures, leading to heightened stress. Their exposure to risky situations, traumatic events, and strict performance expectations leave law enforcement professionals vulnerable to mental health issues, including depression.[92] Nurses, especially those working in high-pressure settings such as intensive care units, also experience significant stress. Inadequate compensation and conflicts related to patient care add to their challenges, increasing the risk of developing depressive symptoms.[93] Meanwhile, Darshan *et al*. reveal the vulnerability of software engineers to occupational stress stemming from demanding work schedules and job insecurity.[94] Community health workers face stressors related to underparticipation and low status, highlighting the need for targeted interventions to protect their mental well-being and prevent depression.[95] MDD also occurs in career fields beyond those listed above. High rates of depression (60.5%), anxiety (47%), and stress (36.5%) are observed among cab drivers, likely influenced by poor working conditions and stressors on the roads.[96] The study underscores the need for addressing mental health among those working in customer service jobs like driving by providing specific support services and improving their working conditions.[96] Meanwhile, another study showed a higher prevalence of mental health issues among non-healthcare professionals compared with healthcare workers, suggesting the importance of psychological counseling to manage stress levels in both groups.[97] Pandya *et al*. conducted an in-depth review of the current literature on overall workplace mental health interventions specifically within the Indian context.[83]

Many jobs can be stressful. Therefore, it is especially important to help employees stay mentally strong and happy while working. By understanding the causes of occupational stress and providing appropriate support to employees, companies can make sure their employees are happier. In this context, rapid action is crucial because occupational stress factors can lead to depression. Therefore, it is imperative to find ways to make work less stressful and protect workers' health.

3.3.2. Internal factors

Genetic factors play a significant role in the risk of depression. As phenotypes, including depression, are determined by genes, certain genetic changes can increase the risk of specific disorders. Depression is an example of polygenic inheritance, where multiple genetic changes can contribute to its development (GeneCards.org). In this regard, a common change is a single-nucleotide polymorphism, which acts as a genetic marker that influences how likely someone is to develop certain diseases. To determine the risk of depression in the Indian population, multiple candidate gene association studies (CGAS) (Table 2) have been conducted to measure genetic risk factors for depression in different parts of India (Figure 3A). These studies are quick, cost-effective, and have identified several variants across different regions of the genome. Many of these variants have been linked with a higher risk of depression. These include *LEP* (D7S1875); *MTR* (A2756G); *TPH* (A779C); *APOE* (ApoE4); brain-derived neurotrophic factor (*BDNF*) (Val66Met); 5-HT 2A (−1438 A/G); *SLC1A3* and *BDNF* (C3590T, C869G, G196A); and *VDBP* (rs7041, rs4588). In addition, variants of numerous genes contributed to determining the impact of drugs on treating depression (Table 2).

However, no significant association was found between other common variants and the risk of depression, including *MTR* (A66G); *CBS* (844ins68); *CPY2D6* (P4502D6, TPH2 (C11993A); *DAT* (TaqA1 and VNTR); and *MTHFR* (C677T).[9-11,98-101] Of the reviewed literature, three studies have attempted to explore the association

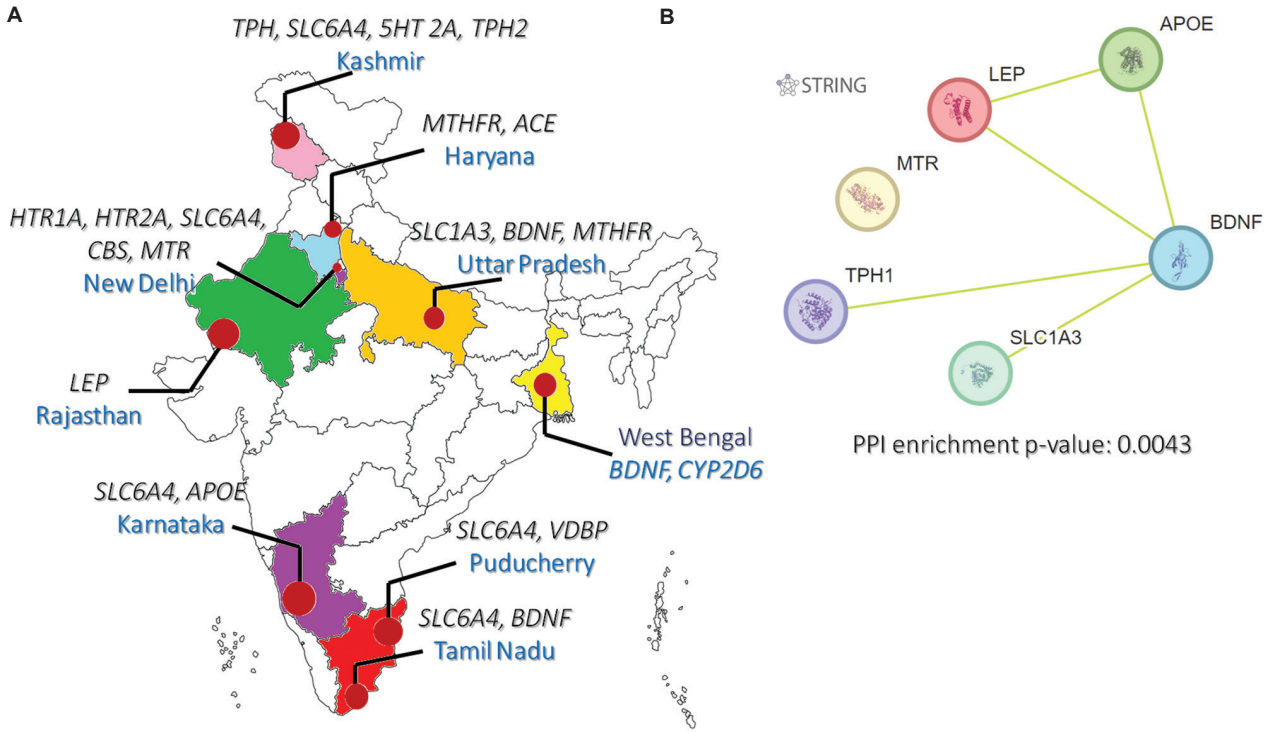

Figure 3. Genetic variants in India. (A): Map of India representing the genetic variants found across states. (B): PPI network downloaded from the string database.

of the C677T variant of *MTHFR* with depression[11,99,100]; however, an association could not be found. This suggests two possibilities: either there truly is no association or the sample sizes in these studies may have been too small to detect significant associations. To overcome such challenges, a meta-analysis that involves pooling data across studies could provide a more robust answer. Therefore, this area warrants further investigation.

Understanding how diseases progress involves finding the most connected gene or point in the network of genes and proteins. Changes to peripheral genes can affect more central genes.[102] To determine which genes are the most significantly associated with the studied gene, the researchers used String v11, a tool for protein interaction (PPI) that collects data from various online databases (String-db. Org/). Metascape was used for quality control and association analysis (Metascape). The researchers focused on terms with a $p < 0.01$, a minimum count of 3, and an enrichment factor >1.5. After utilizing String v11, a PPI was created (Figure 3B). The PPI showed *BDNF* to have a maximum number of edges ($n = 4$) and a PPI enrichment $p < 0.0043$. In addition, *BDNF* was also found to be strongly associated with migraines, along with suicide ideation and severe depression (Figure 4). Epigenetics, which involves mechanisms such as histone modification, CpG island methylation, and RNA interference, has revolutionized contemporary understandings of genetic disorders, especially neurological ones.[103] These modifications are crucial for research on depression treatments, neuroplasticity, and antidepressant responses, and may serve as biomarkers. Early-life stress can induce epigenetic changes, increasing vulnerability to depression. Although much research has focused on the relationship of DNA methylation, histone modifications, and micro RNAs (miRNAs) with depression, research on long non-coding RNAs is ongoing, emphasizing the need for further investigation into their interactions with miRNAs and target messenger RNAs.[104]

Notably, there is a paucity of epigenetic research focused on the Indian population. A study by Prabu *et al.* found that Indian patients with Type 2 diabetes with depression had elevated levels of miRNA-128 and cortisol, a reduced BDNF, and shorter telomeres, indicating significant neuroendocrine changes.[105] In addition, Rawat *et al.* discovered a trend toward decreased global DNA methylation in Indian individuals with depression, particularly those with suicidal ideation, although this trend did not reflect statistical significance.[106]

Although current studies are not solely focused on depression[105] and some findings have been statistically non-significant,[106] they highlight the critical need for broader

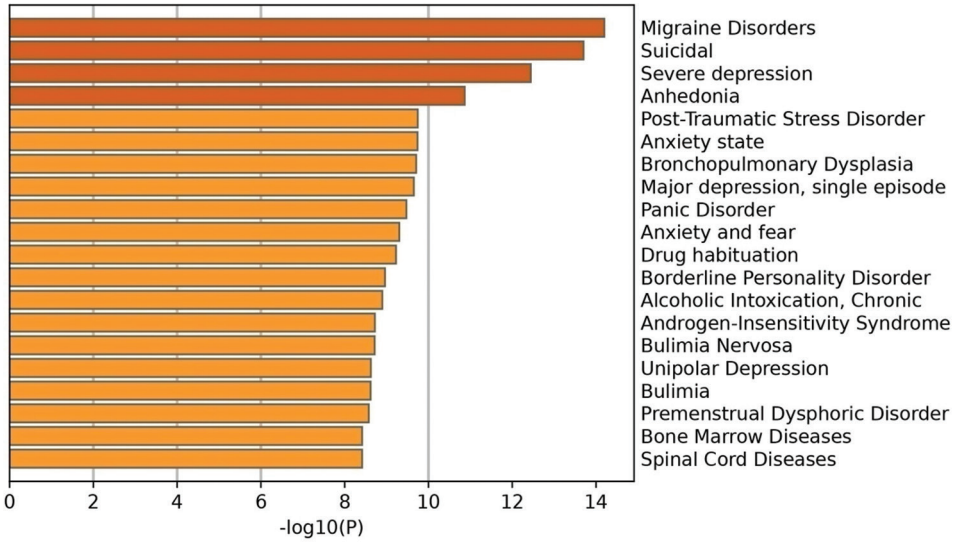

Figure 4. Significant associations observed between brain-derived neurotrophic factor and diseases (Metascape)

research to uncover the diverse epigenetic mechanisms in depression in the Indian population. These findings illustrate the complex role of epigenetic mechanisms in depression and the necessity for more targeted epigenetic studies within this demographic.

Understanding the serum markers associated with depression is crucial as they offer insights into the physiological and biochemical changes underlying the disorder. By studying these markers, researchers can unravel the complex mechanisms involved in MDD, potentially leading to improved diagnostic tools and treatment strategies. Numerous research groups have explored various serum markers among the Indian population to reveal a plethora of potential markers that may correlate with depression.[107] [Mar]kers of depression among Indians include lower serum total cholesterol (TC) and low-density lipoprotein (LDL)-cholesterol levels, along with elevated S100B protein levels.[108] However, researchers found no significant correlation between lipid levels and depressive symptoms measured using Patient Health Questionnaire-9 in Haryana.[107] Other studies, such as those by Varambally *et al*. and Khalid *et al*., underscored the role of markers such as BDNF and TC as potential predictors of depression severity.[109,110] Moreover, recent investigations in Puducherry by Dhiman *et al*. emphasized the importance of vitamin B12 levels in PPD, revealing differences in biomarker profiles between affected and unaffected women.[111]

Numerous additional studies collectively demonstrate the diverse landscape of serum markers implicated in depression, offering valuable avenues for further exploration and clinical application in understanding and managing this pervasive mental health condition. These investigations have spanned various regions in India and employed a range of diagnostic tools and biochemical techniques to examine markers such as cytokines (interleukin [IL] 1 beta, IL-2, IL-6, tumor necrosis factor), metabolic indicators (glucose, cholesterol, triglycerides), and oxidative stress markers (C-reactive protein, prolidase). It has also been observed that the mean serum kynurenine level is significantly higher in individuals with depression and suicidal attempts (464.05 ± 89.11 ng per milliliter [ng/mL]) compared with those with depression without suicidal attempts (420.78 ± 69.66 ng/mL).[112] The association of serum markers highlights the complexity of the depression mechanisms. Studies contribute uniquely by elucidating how these markers correlate with both depressive symptoms and disease progression (Table 3).

3.4. Comorbidity and depression

Disease-related stress can be easily understood after understanding the comorbidity, which is another great thing of concern that needs to be thought about and is defined as the presence of two or more chronic conditions at the same time.[113] A plethora of research (Table 4) has shown that the prime disorder that was found to be comorbid with depression is anxiety, which critically affects every age but specifically in adolescents[114-119] studying in a different range of professional courses.[120,121] This is because students have a lot of educational stress,[16,67] which negatively impacts their lives. Headache, including migraine, which is known to be the second leading cause of disability, has also been found to be comorbid with depression (Figure 5).[113,122]

Table 3. Serum markers and association with depression

No.	Author and year	Location	Diagnostic tool (s)	Case group/ control group	Serum marker (s)	Technology used	Results
1	Mulchandani *et al*., 2023[107]	Haryana	PHQ-9	None	TC; LDL; HDL; TG	TC	No association was found between lipids and depressive symptoms.
2	Patra *et al*., 2014[108]	New Delhi	HDRS	30/30	TC; LDL; HDL; TG	Phosphotungstate-MgCl2 method	TC and LDL-cholesterol levels were found to be significantly lower in depressed individuals.
3	Varambally *et al*., 2013[109]	Karnataka	HDRS	43/24	BDNF	Sandwich ELISA	BDNF was significantly lower in patients with depression.
4	Khalid *et al*., 1998[110]	Lucknow (UP)	HDRS	28/28	TC; LDL; HDL; TG	None	Total serum cholesterol was the most important predictive variable of the severity of depression.
5	Dhiman *et al*., 2021[111]	Puducherry	EPDS	217/217	Total Vit-B12; MMA; 5-methyl THF; Hcy; Holotc; SAM; 5-HTT; cB12	ELISA	Women with probable PPD had lower vitamin B12 levels and higher MMA and 5-methyl THF levels. No differences were observed in Hcy, Holotc, or SAM levels.
6	Arora *et al*., 2019[175]	New Delhi	BDI-II; HDRS	42/42	S100B	Sandwich ELISA	Levels of serum S100B were significantly elevated in patients with MDD.
7	Saldanha *et al*., 2009[176]	Maharashtra	BDI	40/40	Serotonin	ELISA	The correlation between serotonin levels before and after the treatment and between the rating scales did not reveal a significant association.
8	Chaudhary *et al*., 2022[177]	Haryana	BDI-II	252/251	FBG; TC; TG; HDL; VLDL	Spectrophotometry	The distribution of individuals with varying blood glucose levels (normal, low, high) did not significantly differ between the non-depressed and depressed categories. However, the control group had significantly more individuals with high TC and high LDL compared with the case group.
9	Narayan *et al*., 2014[178]	Tamil Nadu	HDRS	40/40	Hcys	CLIA	A significant association was found between total Hcys levels and depression in younger women, specifically correlating with the severity of depression.
10	Mathew *et al*., 2013[179]	Kerala	PHQ-9	100/None	FBS	None	FBS values were significantly higher in depressed individuals.
11	Pallavi *et al*., 2015[180]	New Delhi	DSM-IV, BDI-II	77/54	IL-1β; IL-2; IL-6; IL-10; TNF-α; IFN-γ; TGF-β1; IL-17A	ELISA	Depressed adolescents had significantly higher levels of IL-2 and IL-6. The female population skewed the IL-6 results among MDD patients.

(Cont'd...)

Table 3.*(Continued)*

No.	Author and year	Location	Diagnostic tool (s)	Case group/ control group	Serum marker (s)	Technology used	Results
12	Muthuramalingam *et al*., 2016[181]	Puducherry	DSM-IV	55/42	TNF-α; IL-6; TGF-B	ELISA	Drug-naïve depressed individuals demonstrated significantly raised baseline levels of TNF-α and IL-6 but no difference in levels of TGF-β.
13	Kaithwas *et al*., 2023[182]	MP	None	70/70	Vit-D	None	Although nearly two-thirds of the depressed participants had either insufficient/ deficient levels, the difference was not statistically significant.
14	Kale *et al*., 2014[183]	Maharashtra	BDI	40/30	TC, LDL, TG, VLDL	CHOD-PAP Method	Low levels of TC, LDL, TG, and VLDL were significantly linked to depression. Screening for depression in patients with low lipid levels was deemed crucial for timely intervention.
15	Gupta *et al*., 2024[184]	Haryana	HDRS; BDI-II	200/None	Lipid profile	None	Higher LDL-cholesterol levels were linked to increased depression risk, whereas HDL cholesterol offered protective benefits.
16	Sandeep Varma *et al*., 2024[185]	Karnataka	MADRS	30/30	L-acetyl carnitine	ELISA	A significant strong negative relationship was found between MADRS scores and acetylcarnitine levels, indicating that lower acetylcarnitine levels are associated with more severe depression.
17	Baradia *et al*., 2018[186]	UP	HDRS	44/44	Glucose; total protein; albumin; SGPT; SGOT; TSH; Vit-B9; Vit-B12	CLIA	Most patients with clinical depression had a Vit-B12 deficiency.
18	Ullas Kamath *et al*., 2019[187]	Karnataka	ICD-10; GHQ-28	24/20	AChE; PON1; Copper	Spectrophotometry and Colorimetry	AChE, PON1, and copper levels were significantly greater in patients with moderate depression.
19	Singh *et al*., 2011[188]	Kanpur	DSV-IV; ICD-10	100/40	Mg++; Ca++; Na+; K+	Flame Photonetery	An imbalance in the level of serum electrolytes, especially of Ca and Mg, occurred in accordance with the severity of depression.
20	Mishra *et al*., 2018[189]	MP	HDRS	25/25	CRP	Latex-enhanced Immunoturbidimetry	Late-onset depression was associated with higher levels of CRP.
21	Jeenger *et al*., 2018[190]	Rajasthan	BDI	52+33/50	CRP; BDNF; IL-2	ELISA	FED and RDD were associated with lower BDNF and higher IL-2 levels, with no difference in CRP.

(Cont'd...)

Table 3.*(Continued)*

No.	Author and year	Location	Diagnostic tool (s)	Case group/ control group	Serum marker (s)	Technology used	Results
22	Ray *et al*., 2024[191]	Odisa	ICD-10; HDRS	50/50	Vit-D; cortisol	ECLIA	The study found a negative correlation between MDD and Vit-D levels, with no significant change in serum cortisol levels, suggesting a link between Vit-D deficiency and MDD.
23	Verma *et al*., 2017[192]	UP	DSM-IV	80/80	Prolidase; antioxidants; total oxidants	None	The study found higher SPA and oxidative stress in patients with MDD, suggesting a potential correlation with MDD progression and its pathogenesis.

Abbreviations: PHQ-9: Patient health questionnaire-9; TC: Total cholesterol; LDL: Low-density lipoprotein; HDL: High-density lipoprotein; TG: Triglycerides; HDRS: Hamilton depression rating scale; MgCl2: Magnesium chloride; ELISA: Enzyme-Linked immunosorbent assay; UP: Uttar Pradesh; EPDS: Edinburgh postnatal depression scale; Vit-Bl2: Vitamin Bl2; MMA: Methylmalonic acid; THF: Tetrahydrofolate; Hcys: Homocysteine; Holotc: Holotranscobalamin; SAM: S-adenosyl methionine; 5-HTT: Serotonin transporter; cBl2: Combined indicator of Vitamin Bl2 status; PPD: Postpartum depression; MDD: Major depressive disorder; BDI-II: Beck depression inventory II; S100B: S100 calcium-binding protein B; FBS: Fasting blood sugar; DSM-IV: Diagnostic and statistical manual of mental disorders, fourth edition; IL: Interleukin; TNF-α: Tumor necrosis factor-alpha; IFN: Interferon; TGF: Transforming growth factor; MP: Madhya Pradesh; Vit-D: Vitamin D; BDI: Beck depression inventory; FBG: Fasting blood glucose; VLDL: Very low-density lipoprotein; CLIA: Chemiluminescence immunoassay; CHOD-PAP Method: Cholesterol oxidase-peroxidase aminoantipyrine method; MADRS: Montgomery-Åsberg Depression Rating Scale; SGPT: Serum glutamic pyruvic transaminase; SGOT: Serum glutamic oxaloacetic transaminase; TSH: Thyroid-stimulating hormone; Vit-B9: vitamin B9; ICD-10: International classification of diseases, tenth revision; GHQ-28: General health questionnaire-28; AChE: acetylcholinesterase; PON1: Paraoxonase 1; Mg++, Ca++, Na+and K+: Magnesium ions, calcium ions, sodium ions, potassium ions; CRP: C-reactive protein; FED: First-episode depression; RDD: Recurrent depressive disorder; ECLIA: Electrochemiluminescence immunoassay; SPA: Serum protease activity.

In a comprehensive exploration of the intersection between physical health and mental well-being, several studies have shed light on the profound impact of certain medical conditions on individuals' psychological states. A study conducted by Thakur *et al*. in North India focused on women with breast cancer, utilizing the Hamilton Depression 17-item Rating Scale. The findings revealed a striking 47.05% rate of depression among this population, underscoring the substantial psychological burden associated with a breast cancer diagnosis.[123] Similarly, leprosy, a neglected tropical disease, not only poses physical challenges but also contributes to stigma and discrimination in low- and middle-income countries. Govindasamy *et al*. identified an association between leprosy and mental health issues, particularly depression and anxiety, emphasizing the need for a holistic approach to address both dimensions of health.[124] Psoriasis, a psychocutaneous disorder, has also been the subject of research revealing a noteworthy association with mental health concerns. A study conducted by Lakshmy *et al*. demonstrated substantial comorbidity between psoriasis and conditions such as depression and anxiety. This finding indicates that individuals grappling with psoriasis not only contend with the visible and physical aspects of the skin disorder but also face an elevated risk of experiencing concurrent mental health challenges.[125] Finally, emotional stress was identified as a factor worsening acne, negatively impacting the quality of life, self-esteem, and mood of affected individuals.[126] These studies illustrate the intricate relationship between mental and physical health, specifically in conditions with dermatological manifestations.

Another important and common chronic metabolic condition, diabetes, is significantly comorbid with depression and anxiety.[127-129] Among patients with diabetes, depression has been strongly linked to various related factors, including low income, living in urban areas, being unmarried, using insulin therapy, and conditions such as retinopathy and ischemic heart disease. Anxiety is significantly predicted by diabetic complications such as neuropathy, retinopathy, and cardiovascular disorders (e.g., ischemic heart disease).[130,131] Irritable bowel syndrome is another very common gastrointestinal dysfunction that is comorbid with depression and anxiety disorders.[118] Oral mucosal or submucous fibrosis is also significantly comorbid with anxiety and depression.[132,133] Gender disparity was found to be comorbid between depression and obsessive-compulsive disorder, with depression being more common in women.[134] In recent decades, drug-

Table 4. Comorbidity association with different diseases in the Indian population

No.	Author	Year of publication	State	Disease	Depression assessment tools	Results
1	Sarkar *et al*., 2016[60]	2016	West Bengal	Acne	MINI-5	MDD was relatively higher in people with acne.
2	Sahoo and Khess 2010[114]	2010	Jharkhand	Anxiety	DASS-21, MINI	Depression, stress, and anxiety are present in up to a quarter of all young adults.
3	Kabra and Nadkarni, (2013) [118]	2013	Maharashtra	IBS	HDRS	The prevalence of depression was high in the population with IBS.
4	Banerjee *et al*., 2008[119]	2008	Chandigarh	AMD	GDS; SCID-CV	Depression was a major problem in people with AMD.
5	Ghogare and Saboo[122]	2019	Central India	TTH	HDRS	TTH was highly associated with depression and was more frequent in married people.
6	Thakur *et al*., 2019[123]	2019	Uttar Pradesh	Breast cancer	ICD-10	Depression is commonly associated psychiatric morbidity in patients diagnosed with breast cancer.
7	Lakshmy *et al*., 2015[125]	2015	Puducherry	Psoriasis	PHQ-9	Patients suffering from psoriasis were more prone to depression.
8	Weaver and Hadley 2011[127]	2011	Delhi	Diabetes mellitus	HSC-25	Females with diabetes were more prone to depression.
9	Rajput *et al*., 2016[128]	2016	Haryana	Diabetes mellitus	HDRS	People suffering from diabetes mellitus showed a high incidence of depression, especially women.
10	Aminu *et al*., 2017[129]	2017	Karnataka	Diabetes mellitus	PHQ-9	The prevalence of depression was high among diabetic patients.
11	Karpha *et al*., 2022[130]	2022	West Bengal	Diabetes mellitus	PHQ-9	Depression and anxiety are highly prevalent among diabetic patients.
12	Suresh *et al*., 2015[132]	2015	Karnataka	Oral mucosal diseases	HADS	People with depression had more oral lesions than the normal control population.
13	Cherian *et al*., 2014[134]	2014	Karnataka	OCD	MINI-5	Women with OCD had a higher rate of depression than men with OCD.
14	Srinivasan *et al*., 2021[135]	2021		DR-TB	PHQ-9	Patients became depressed when not able to recover from TB after multiple treatments.
15	Dayal *et al*., 2022[136]	2022	Maharashtra	Primary glaucoma	HADS	Patients suffering from glaucoma also showed symptoms of anxiety and depression.
16	Mathew *et al*., 2013[179]	2013	Kerala	Diabetes mellitus	PHQ-9	Patients suffering from diabetes were more prone to depression.
17	Joseph *et al*., 2013[193]	2013	Mangalore	Diabetes mellitus	PHQ-9	A high proportion of depression was found among patients with Type 2 diabetes mellitus.
18	Siddiqui *et al*., 2014[194]	2014	New Delhi	Diabetes mellitus	PHQ-9	Depression was highly prevalent in patients with Type 2 diabetes mellitus.
19	Raval *et al*., 2010[195]	2010	Punjab	Diabetes mellitus	PHQ-9	MDD was highly prevalent in people with diabetes.
20	Negi *et al*., 2014[196]	2014	Bihar	COPD	PHQ-9	One-fifth of patients with COPD suffered from depression.

(Cont'd...)

Table 4. *(Continued)*

No.	Author	Year of publication	State	Disease	Depression assessment tools	Results
21	Das *et al*., 2013[197]	2013	West Bengal	Diabetes mellitus	DSM-IV	Depression was highly prevalent in patients with Type 2 diabetes mellitus and led to a lower quality of life.
22	Balhara and Sagar, 2011[198]	2011	New Delhi	Diabetes mellitus	PHQ-9	Depression was highly prevalent in patients with Type 2 diabetes mellitus.

Abbreviations: MINI-5: Mini international neuropsychiatric interview, Version 5; MDD: Major depressive disorder; IBS: Irritable bowel syndrome; HDRS: Hamilton depression rating scale; AMD: Age-related macular degeneration; GDS: Geriatric depression scale; SCID-CV: Structured clinical interview for DSM-IV Axis I disorders, clinical version; TTH: Tension-type headache; ICD-10: International classification of diseases, tenth revision; PHQ-9: Patient health questionnaire-9; HSC-25: Hopkins symptoms checklist-25; HADS: Hospital anxiety and depression scale; OCD: Obsessive-compulsive disorder; DR-TB: Drug-resistant tuberculosis; COPD: Chronic obstructive pulmonary disease; DSM-IV: Diagnostic and statistical manual of mental disorders, fourth edition.

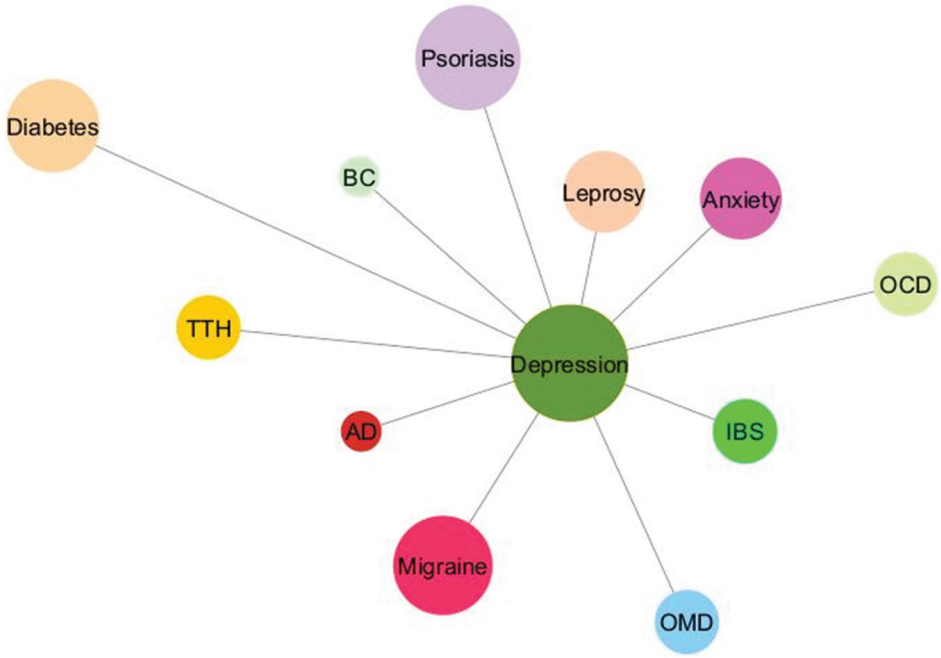

Figure 5. Depression comorbidity association network

resistant tuberculosis (TB) has become another significant concern. It is generally categorized into two types: multidrug-resistant and extensive drug-resistant TB. In North India, Srinivasan *et al*. discovered that psychological problems such as depression and anxiety are common among patients with chronic illnesses who also have TB.[135] In a cross-sectional prevalence study, Dayal *et al*. examined 200 patients attending a glaucoma outpatient clinic at a prestigious eye hospital in Pune, India. Their investigation revealed that the severity of the disease was strongly correlated with elevated scores on the Hospital anxiety and depression scale. This suggests that as the severity of glaucoma worsens, individuals may experience higher levels of anxiety and depression.[136] Academic stress has meanwhile been linked with oral health issues, with higher perceived stress scores correlating with increased cases of caries (the decay and crumbling of a tooth).[137]

These studies illustrate the importance of recognizing and addressing mental health in the overall management of medical conditions to ensure comprehensive and effective patient care.

4. Discussion

4.1. Consequences of depression

Depression can have profound consequences across various aspects of an individual's life.[26] It significantly impairs one's quality of life, diminishing their ability to engage in daily activities, maintain relationships, and pursue personal and professional goals. Depressive symptoms often strain interpersonal relationships, leading to conflicts with family, friends, and coworkers. Meanwhile, social withdrawal and isolation exacerbate feelings of loneliness and alienation.[138] In the workplace, depression can impair cognitive abilities and decision-making, resulting in reduced productivity, absenteeism, or even job loss,[139,140] which can cause financial strain due to increased healthcare expenses and loss of income.[141] Individuals may even turn to alcohol or drugs as a coping mechanism, which can worsen the situation and lead to addiction.[142] Research has also shown that wives of alcoholic men are particularly stressed and more likely to experience symptoms of depression,[143] highlighting the intricate links between mental health, substance use, and social dynamics.

Apart from their social life, individuals with depression often exhibit changes to their brain structure, such as the shrinking of the hippocampus, the part of the brain that governs memory creation and recall. In addition, a study found that individuals with depression had an increased thickness in the left inferior parietal gyrus, a brain area involved in processing emotions and thoughts.[144] Furthermore, there is a concerning link between depression, brain structure, and the risk of attempting suicide.[145] Researchers have observed that people who have attempted suicide due to depression tend to have lower levels of gray matter in the ventral striatum, a region associated with motivation and reward, which may contribute to feelings of hopelessness.[146,147]

As discussed above, chronic depression is also associated with various physical health problems, including cardiovascular issues, weakened immunity, sleep disturbances, and chronic pain, highlighting the interconnectedness of mental and physical well-being.[127-129,148] Severe depression significantly increases the risk of suicidal thoughts and behaviors, necessitating rapid recognition and intervention to ensure safety.[149] It has also been observed that mean serum kynurenine levels are significantly higher in patients with depression and suicidal ideation compared with those without such ideation.[112] Despite India's 2019 suicide rate ranking of 49 globally at 14.04 attempts/100,000 people, the country consistently reports the highest number of suicides each year.[150,151] Persistent feelings of worthlessness and self-blame can erode an individual's self-esteem and confidence in the long-term, hindering their ability to assert themselves, pursue goals, and engage in social activities.[152] These effects highlight the deep interconnection between mental health, stress, and overall well-being, emphasizing the urgent need to address mental health issues and reduce the burden of suicide in the country. The multidimensional consequences of depression require a comprehensive, individualized framework, integrating psychotherapy, medication, and support from mental health professionals and loved ones.[153] Early intervention is crucial for mitigating the impact of depression on an individual's life.

In conclusion, understanding the factors that exacerbate the risk of depression is crucial. This review highlighted the involvement of a range of factors that can broadly be categorized into two categories: internal and external factors. Internal factors, such as genetic variants, epigenetic changes, and serum marker levels, play a foundational role in determining an individual's predisposition to depression. Meanwhile, external factors include an array of influences such as lifestyle and environmental conditions and can significantly amplify this predisposition among individuals. While we cannot alter our genetic makeup, we can take proactive steps to minimize the impact of environmental factors, thereby reducing the overall risk of depression (Figure 6). This approach empowers us to control the factors we can influence, paving the way for better mental health outcomes.

4.2. Strengths, limitations, and future perspective

The study's strengths lie in its comprehensive examination of research on MDD in India, covering prevalence, diagnostic tools, environmental stressors, genetic factors, comorbidities, and their implications. It emphasizes the unique impact of depression on Indian women, given the influence of gender-specific factors. Advanced methodologies, such as CGAS, can enrich our understanding of genetic contributions to depression. Nevertheless, this study emphasizes the continued need for a deeper understanding of depression in India, given its potential impact on the nation's economy, education, and overall development.

This review has several limitations. The sole reliance on existing research might introduce a lag in capturing real-time changes, and the failure to account for regional variations within India could limit the generalizability of the findings. While the review acknowledges the complexity of addressing the cultural differences that shape mental health perspectives, it provides limited suggestions for specific interventions.

Depression has far-reaching consequences, affecting quality of life, relationships, and work productivity, emphasizing the urgent need for effective interventions.

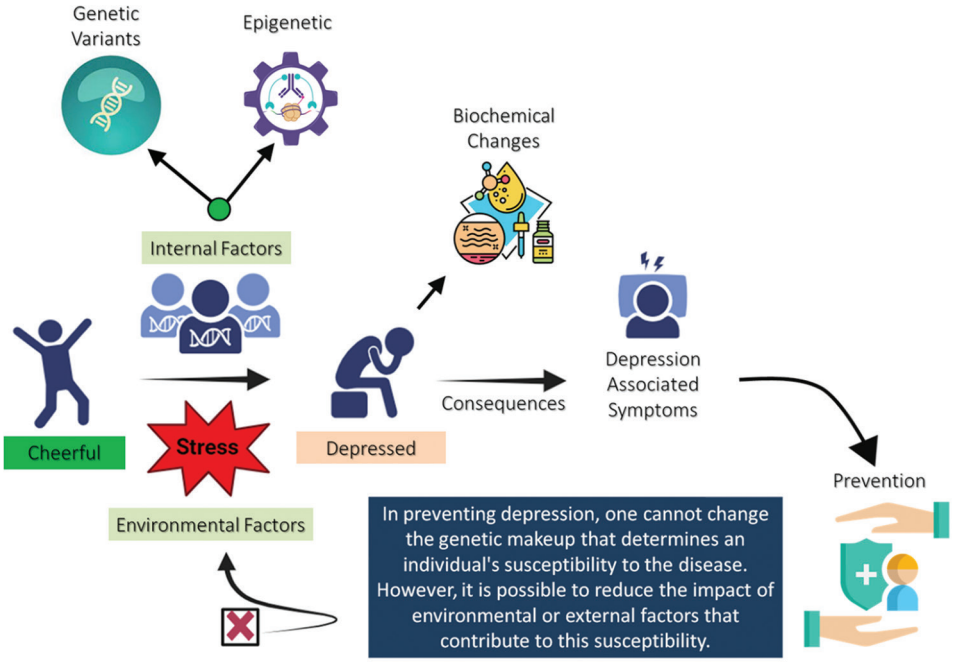

Figure 6. Flow diagram showing the causes and prevention of depression

In particular, prioritizing mental health services and workplace programs is essential. It is crucial to adopt integrated healthcare approaches that address mental as well as physical health, along with community support and early intervention strategies. Policymakers must implement national mental health strategies and increased funding, particularly in a country with high suicide rates such as India. Collaborative efforts involving healthcare providers, policymakers, employers, and communities are crucial for addressing the multifaceted impacts of depression. Furthermore, recognizing the importance of linguistic diversity and ensuring equitable access to education in students' preferred languages would be vital steps toward fostering an inclusive learning environment. By doing so, an environment can be created in which all students feel valued, understood, and empowered to reach their full potential. This commitment would not only enhance educational outcomes but also promote social cohesion and contribute to the overall well-being of Indian society.

5. Conclusion

This review has clarified the intricate landscape of MDD research in India, recognizing this complex neurological condition as being influenced by diverse causes, including professional, individual, and interpersonal factors. Considering these findings, it advocates for the implementation of a comprehensive, culturally aware mental health strategy. This strategy should prioritize specific interventions and programs targeting the multifaceted factors contributing to the high rates and negative consequences of depression in India.

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Conflict of interest

The authors declare that they have no conflicts of interest.

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Availability of data

All the data analyzed in the present study are downloaded from online databases such as PubMed and Web of Science.

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ORIGINAL RESEARCH ARTICLE

The most significant brain regions implicated in olfactory dysfunction in Parkinson's disease

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Abstract

Olfactory dysfunction is observed in over 95% of patients with Parkinson's disease (PD). This study examines the relationship between gray matter volume (GMV) and olfactory impairment in a cohort of 182 subjects, including PD patients and healthy controls (HCs). Using the Iran Smell Identification Test, which is a standardized 24-item olfactory identification assessment, to evaluate the olfactory performance, PD patients were divided into two groups (scores ranging from 0 to 18 indicate olfactory dysfunction, while scores from 19 to 24 indicate normal olfaction): those with normal smell (PD-NS, n = 23) and those with smell disorders (PD-SD, n = 69). Differences in GMV were analyzed using voxel-based morphometry. Statistical analysis was conducted using SPSS 26. The results revealed that the PD-NS group exhibited reduced GMV in the right thalamus and the left parahippocampal gyrus compared to the HCs. Furthermore, the HC group demonstrated no statistically significant olfactory dysfunction. In contrast, the PD-SD group showed significant decreases in GMV in the right entorhinal cortex and both the right and left hippocampus compared to both the HC and PD-NS groups. These findings indicate that PD patients experience more severe olfactory dysfunction in hippocampal regions than the HC group, likely attributed to the initial pathological loss of gray matter in both the right and left hippocampus.

Keywords: Parkinson's disease; Smell; Gray matter; MRI; Brain volumetry

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1. Introduction

Parkinson's disease (PD) is a chronic and progressive neurodegenerative condition that predominantly affects the dopamine-producing neurons in the substantia nigra, resulting in the development of motor symptoms.[1] PD is acknowledged as a multifaceted condition that impacts various systems and is marked by a range of both motor and non-motor symptoms, including deficits in olfaction. Among the non-motor symptoms

associated with PD, olfactory dysfunction is one of the most prevalent.[1-3]

Studies have shown that over 95% of individuals with PD experience a notable decline in olfactory function, which can have a significant impact on their daily lives. Unfortunately, the importance of olfaction in enhancing the quality of life and the capacity to derive pleasure is frequently overlooked.[4,5] Onset age, therapeutic intervention, treatment duration, and disease severity do not have an impact on olfactory dysfunction.[1,6,7]

Neural responses to olfactory stimuli are transmitted from the nasal epithelium to the olfactory bulb, then to the olfactory cortex along with its primary connections within the brain.[8,9] The anterior olfactory nucleus, olfactory tubercle, pyriform cortex, amygdala, and entorhinal cortex comprise the olfactory cortex, each receiving input from neurons in the olfactory bulb. The pyriform cortex, divided into anterior and posterior segments, serves as the primary output region for projections from the olfactory bulb. Odorant identity is encoded in the anterior pyriform cortex, while the posterior pyriform cortex encodes odor quality.[10] The entorhinal cortex is connected to the hippocampus, and its dysfunction can have an impact on the ability to remember and distinguish between different odors.[11,12] The orbitofrontal cortex represents a significant olfactory pathway extending beyond the olfactory cortex. Despite its importance, the precise function of this brain region in olfaction remains unclear. Studies have linked it to tasks such as distinguishing between different odors, recognizing smells, and regulating olfactory attention.[13-15] Lewy bodies have been observed in the olfactory bulb, entorhinal cortex, and pyriform cortex during post-mortem examinations of individuals with PD.[14,15]

The present study aims to investigate the relationship between olfactory dysfunction and gray matter volume (GMV) using voxel-based morphometry (VBM). VBM is capable of examining and assessing the structural alterations in brain areas affected by neurodegenerative disorders, such as dementia, PD, and multiple sclerosis.[16-18] The software (Computational Anatomy Toolbox [CAT], Structural Brain Mapping Group, Germany) was initially suggested and adopted as the norm by Ashburner and Friston[19] VBM automatically assesses the volume of each voxel within the segmented tissues to detect differences that suggest gray matter (GM) atrophy or localized alterations in white matter (WM) density. Consequently, VBM remains impartial regarding structural changes in specific brain regions, circumventing subjective variations introduced by the artificial delineation of regions of interest. It offers an objective and thorough assessment of anatomical changes throughout the entire brain, capable of detecting even minor variations in brain volume or density.[20-23]

The goal of this study is to explore the relationship between olfactory dysfunction and GMV in PD, with a focus on comparing individuals with and without olfactory dysfunction.

2. Materials and methods

2.1. Subjects

This study was conducted on 182 subjects, including 92 patients with PD (23 with normal smell [PD-NS] and 69 with smell disorder [PD-SD]) and 90 healthy controls [HCs] from July 29, 2024, to December 13, 2024. A 3-week interval separated the first study visit and the follow-up visit. Informed consent was obtained from all subjects.

The exclusion criteria were as follows: individuals diagnosed with various neurological conditions (e.g., stroke), Parkinsonism syndromes (e.g., progressive supranuclear palsy, multiple system atrophy, and uncommon motor and non-motor symptoms), psychological disorders (e.g., severe depression), individuals for whom magnetic resonance imaging (MRI)'s results were contraindicated, and cases in which any form of artifact on MRI hindered accurate volumetric analysis.

The HCs did not exhibit any neurological conditions, such as stroke, brain tumors, or severe mental disorders. Furthermore, they reported no cognitive complaints.

To control for potential confounding effects of age, gender, and educational level on the outcomes, participants in the HC group were meticulously matched with those in the PD groups based on these covariate variables. To minimize potential confounders, such as cognitive status and medication, the cognitive status of the patients was evaluated using the Montreal Cognitive Assessment, and no significant differences were found between the two groups. In addition, only the *L*-dopa-carbidopa combination treatment was used for the patients in this study, and the effect of this variable is similar in both groups.

2.2. Olfactory assessments

In the present study, the Iran Smell Identification Test (ISIT), a standard 24-item odor identification test, was used to evaluate the olfactory performance of PD and HC groups according to cultural adaptation. A score of 0 to 18 in this test indicates a smell disorder, while 19 to 24 indicates normal smell.[24] Baseline demographic and clinical data, including age, gender, educational level, and disease duration, were documented. To develop the ISIT, researchers adapted the University of Pennsylvania Smell Identification Test (UPSIT) for the Iranian population.

Given Iran's diverse ethnicities, the goal was to identify odors familiar to all Iranians. A group of 90 students from various cultural backgrounds residing in Tehran dormitories was asked to review the 40 odors included in the UPSIT and identify those most familiar. To ensure better linguistic comprehension, the original UPSIT was translated into Farsi, and participants were asked to suggest local odors commonly encountered in different regions of Iran. The development of ISIT involved the following steps:

(i). Identifying and replacing unfamiliar odors.
(ii). Compiling a preliminary list of 40 odorants, which included both natural and synthetic options, while also producing fragrance microcapsules when necessary.
(iii). Designing scratch-and-sniff stickers by mixing microcapsules with varnish ink and printing them using a silk screen printer on sticker paper.

A pilot study was then conducted with 43 participants (23 females and 20 males, aged 20 – 40) using this initial version of ISIT. The pilot study aimed to identify any deficiencies in the procedure and to select the most appropriate odors among the 40 items and their alternatives.[24]

2.3. VBM pre-processing

Preprocessing analysis for VBM was performed using the CAT12 toolbox[25] on high-resolution T1-weighted structural images, acquired from all patients on a 1.5 Tesla MRI scanner (Avanto, Siemens Healthineers, Germany), within the Statistical Parametric Mapping (SPM12) framework developed by the Department of Imaging Neuroscience Group (http://www.fil.ion.ucl.ac.uk/spm). This analysis was conducted using MATLAB R2023b software (CAT, Structural Brain Mapping Group, Germany). Initially, the anatomical images were segmented into GM, WM, and cerebrospinal fluid using the unified segmentation module.[26] After segmentation, the GM images were normalized to the Montreal Neurological Institute (MNI) standard space using the diffeomorphic anatomical registration through exponentiated lie algebra (DARTEL) algorithm.[27] Following the affine and non-linear registration of the GM templates in MNI space, the images were modulated to preserve the relative GMVs post-spatial normalization. The resulting GM images were then smoothed with a Gaussian kernel featuring a full width at half maximum of 10 mm. In summary, a VBM analysis comprises the following steps:

(i) Pre-processing: T1 images are normalized to a template space and segmented into GM, WM, and cerebrospinal fluid. The pre-processing parameters can be adjusted through the module "Segment Data." After the pre-processing is finished, a quality check is highly recommended. This can be achieved through the modules "Display slices" and "Check sample." Both options are located in the CAT12 window under "Check Data Quality." Furthermore, quality parameters are estimated and saved in XML files for each dataset during pre-processing. These quality parameters are also printed on the report PDF page and can be used in the module "Check sample." Before inputting the GM images into a statistical model, the image data needs to be smoothed.
(ii) Statistical analysis: The smoothed GM images are input into a statistical model. This requires building a statistical model (e.g., *t*-tests, analysis of variance (ANOVAs), and multiple regressions). This is done by the standard SPM modules "Specify 2nd Level" or preferably "Basic Models" in the CAT12 window, covering the same function but providing additional options and a simpler interface optimized for structural data. The statistical model is estimated. This is done with the standard SPM module "Estimate" (except for surface-based data, where the function "Estimate Surface Models" should be used instead). If total intracranial volume (TIV) is used as a confound in a model to correct for different brain sizes, it is necessary to check whether TIV reveals a considerable correlation with any other parameter of interest, and rather uses global scaling as an alternative approach. After estimating the statistical model, contrasts are defined to get the results of the analysis. This is done with the standard SPM module "Results".[25,26]

2.4. MRI data acquisition

All patients underwent MRI scans using a 1.5T MRI scanner. The choice of a 1.5T scanner is based on its widespread availability and effectiveness in clinical settings for a variety of neurological assessments. This model has been well-studied and is known for providing high-quality images while maintaining patient comfort.

The scans were performed utilizing the HE1_4 coil element, specifically designed for this scanner. Coil elements play a crucial role in MRI examinations, as they determine the sensitivity of the MRI equipment to the magnetic fields produced by the scanned tissues. The HE1_4 coil element is optimized for head imaging, resulting in an improved signal-to-noise ratio and enhancing the clarity and resolution of the images captured. This optimization is essential for accurately visualizing fine anatomical details and potential pathological changes in brain structures.

To ensure patient comfort and minimize movement during the scanning process, all participants were instructed to lie supine on the MRI table. This position helps in achieving a stable and reproducible imaging setup,

which is critical for consistent results across all scans. In addition, earplugs were provided to reduce the effects of excessive scanner noise, which often reaches levels that can be uncomfortable for patients. The loud sounds produced during MRI scanning can lead to anxiety and involuntary movements, potentially compromising image quality. By using earplugs, we aimed to enhance patient comfort and cooperation during the procedure.

To further enhance stability during the MRI session, firm foam pads were used around the patient's head. These pads served to restrict movement and help maintain the participants' heads in a fixed position, which is vital for acquiring high-quality images. Even slight head movements can result in image blurring, making it difficult to analyze the resultant data accurately. The use of these foam pads, therefore, contributes significantly to the overall quality of the imaging process.

The MRI sequences utilized in this study included T1-weighted images, which are particularly effective for assessing anatomical structures within the brain. T1-weighted imaging is optimal for visualizing GM, WM, and the overall structure of the brain. The imaging parameters for the T1 sequences were set to a voxel size of 1.2 mm × 1.0 mm × 5.5 mm, which allows for a balance between spatial resolution and acquisition efficiency. This voxel size enables the differentiation of various brain tissues while ensuring that the scan duration remains manageable for patients.

The repetition time was set to 426 ms, and the echo time was maintained at 8.7 ms, settings that are widely recognized in the literature for producing high-quality T1-weighted images. These parameters were carefully selected to optimize the contrast between different types of brain tissue, enhancing the visibility of anatomical details essential for both clinical assessment and research analysis.

In summary, the meticulous attention to the MRI acquisition protocol, including the choice of scanner, coil element, patient positioning, noise reduction strategies, and specific imaging parameters, underscores our commitment to obtaining high-quality imaging data that is essential for subsequent analyses and interpretations.

2.5. Statistical analysis

Statistical analysis was performed using version 24.0 of the Statistical Package for the Social Sciences (SPSS, IBM Corporation, USA) software. The paired- and independent-samples *t*-test were utilized to assess the difference in means between two distinct groups. The utilization of one-way ANOVA allowed for the examination of the discrepancies in GMV across the different PD groups and the comparison of average ISIT test scores. Categorical variables were analyzed through the chi-squared test. The Spearman correlation coefficient was employed to assess the association between the variables. A $p<0.05$ was deemed statistically significant.

3. Results

3.1. Characteristics of the participants

The analysis included a total of 23 PD-NS, 69 PD-SD, and 92 HCs. The demographic and clinical profiles of these three groups are detailed in Table 1. Age and gender exhibited no significant differences between the HC and PD groups ($p>0.05$). No significant difference was observed in disease duration, medication administration status, educational level, and disease severity (between PD-NS and PD-SD) ($p>0.05$). The results of the ISIT tests exhibited strong and significant differences between the means of the HC and PD groups. The ISIT scores for HC, PD-NS, and PD-SD, respectively, are 21.50, 19.50, and 11.40 ($p<0.001$).

The present study also compared the mean scores for drug types, family history (PD patients with first-degree relatives affected by the disease), first sign of the disease, smoking status, accommodation status, the Montreal Cognitive Assessment, blood types, and weights among the PD groups, revealing no significant differences between the groups ($p>0.05$).

3.2. Comparison of the GMV between the PD patient with normal smell group and the HC group

The comparison of the mean GMV in the brain of the participants in the PD-NS and HC groups demonstrated a significantly decreased volume within the right thalamus and parahippocampal gyrus of the PD-NS group compared to the HC group (Table 2 and Figure 1).

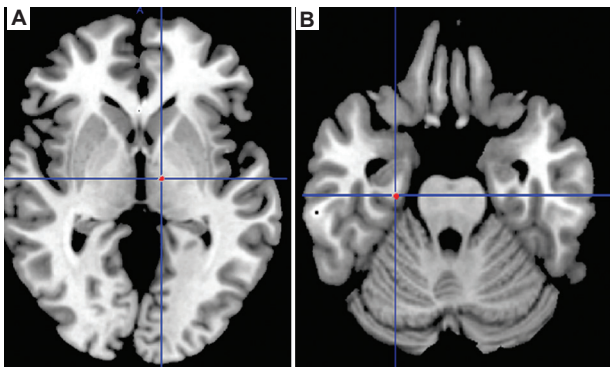

Figure 1. The magnetic resonance imaging scanning of the brain of the participant from the Parkinson's disease patient with normal smell (PD-NS) group. (A) Right thalamus. (B) Left parahippocampal gyrus. The red dots indicate the Montreal Neurological Institute coordinates and a significant reduction in gray matter volume in the PD-NS's brain compared to the healthy control group's brain.

Table 1. Clinical and demographic characteristics of the healthy control (HC), Parkinson's disease patient with normal smell (PD-NS), and PD patients with smell disorder (PD-SD) groups

Characteristics	PD-NS (*n*=23)	PD-SD (*n*=69)	HC (*n*=90)	p-value
Age (years)	61.09±10.35	62.32±10.76	60.83±10.07	0.445[a]
Gender (male/female)	14/9	40/29	53/37	0.432[b]
Educational level (years)	4.18±1.42	3.30±1.22	3.31±1.49	0.390[a]
Disease severity[d]	2.62±1.03	3.17±1.05	-	0.036[c]
Disease duration (years)	4.87±3.16	5.47±3.01	-	0.550[c]
Medication administration status[e]	1.25±0.32	1.18±0.38	-	0.552[c]
ISIT scores	19.50±1.16	11.40±1.30	21.50±1.16	<0.001[a]*
Type of used drugs (*L*-dopa-carbidopa)[f]	21/2	64/5	-	0.439[b]
Pharmacological treatment (years)	4.1±1.12	4.3±1.19	-	0.419
Smoker (+/-)	3/20	4/65	-	0.772[b]
Accommodation status (metropolis/town)	8/15	19/50	-	0.390[b]
Family history (+/-)[g]	2/21	7/62	1/89	0.590[b]
First sign of the disease[h]	[i]	[i]	-	0.213[b]
MoCA scores	24.34±1.26	24.07±1.63	-	0.370[c]
Blood types	[i]	[i]	[i]	0.872[b]
Weight	[i]	[i]	[i]	0.654[b]

Notes: Data are expressed as mean±standard deviation or number; *p<0.05; [a]One-way ANOVA; [b]Chi-squared test; [c]Independent samples t-test; [d]This is the disease severity based on the Modified Hoehn and Yahr Scale; [e]This is the number of medications the patients are taking; [f]*L*-dopa-carbidopa combination treatment of 250/25 mg or 100/25 mg one tablet every 4 h; [g]PD patients who have first-degree relatives affected by PD; [h]The onset of the first symptoms of PD with tremor, rigidity, or bradykinesia. For the clinical presentation of PD at the onset of the disease, all patients exhibited asymmetric tremors in the upper limbs, and at the time of enrollment, they presented with tremors, rigidity, and bradykinesia, scoring between 1 and 3 on the Hoehn and Yahr scale. [i]Data not presented in this table due to the presence of multiple categorical values.
Abbreviations: ANOVA: Analysis of variance; ISIT: Iran Smell Identification Test; MoCA: Montreal Cognitive Assessment.

Table 2. Comparison of the mean gray matter volume between the Parkinson's disease patient with normal smell (PD-NS) group and the healthy control (HC) group

Brain region	PD-NS (*n*=16)	HC (*n*=90)	*p*-value
Right thalamus	5.35±1.39	5.86±1.50	0.021*
Left parahippocampal gyrus	3.27±1.01	3.99±1.10	0.009*

Notes: Data expressed as mean±standard deviation; Statistical comparisons were conducted using paired-samples *t*-test; *p<0.05.

3.3. GMV between the PD patient with normal smell group and the PD patient with smell disorder group

The comparison of the mean GMV between the brains from the PD-NS group and those from the PD-SD group demonstrated a significantly decreased volume within the right entorhinal cortex, right hippocampus, and left hippocampus of the PD-SD group compared to the PD-NS group (Table 3 and Figure 2).

3.4. Comparison of the GMV between the PD patient with smell disorder group and the HC group

The comparison of the mean GMV between the brains from the HC group and those from the PD-SD group demonstrated a significantly decreased volume within the right entorhinal cortex, right hippocampus, and left hippocampus of the PD-SD group compared to the HC group (Table 4 and Figure 2).

4. Discussion

The present study investigated the structural changes of the cortical and subcortical regions in PD with and without olfactory dysfunction compared to the HC group. The findings revealed that individuals with PD and olfactory dysfunction had a reduction in the volume of both the right and left hippocampi and entorhinal cortex in comparison to those in the PD-NS and HC groups. In addition, the PD-NS group demonstrated a decrease in the volume of the right thalamus and left parahippocampal gyrus. These results align with findings from previous studies.[28-30]

As a primary olfactory cortex, the entorhinal cortex is vital for processing olfactory signals originating from the olfactory bulb.[31] Lewy bodies initially emerge in the olfactory nerves. Notably, they are also present in the primary olfactory cortex in the early phase of PD. Their presence may contribute to olfactory dysfunction.[32-34] In addition, it serves as a "gateway" for sensory information

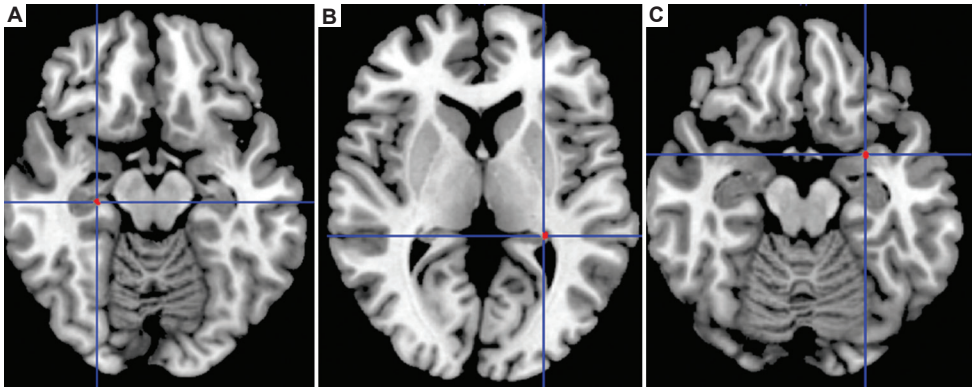

Figure 2. The magnetic resonance imaging scanning of the brain of the participant from the Parkinson's disease patient with smell disorder (PD-SD) group. (A) Left hippocampus. (B) Right hippocampus. (C) Right entorhinal cortex. The red dots indicate the Montreal Neurological Institute coordinates and a significant reduction in gray matter volume in the PD-SD's brain compared to the PD patient with normal smell group's brain.

Table 3. Comparison of the mean gray matter volume between the Parkinson's disease patient with normal smell (PD-NS) group and the PD patients with smell disorder (PD-SD) group

Brain region	PD-NS (*n*=23)	PD-SD (*n*=69)	*p*-value
Right entorhinal cortex	2.25±1.00	1.91±1.02	<0.001*
Left hippocampus	2.33±1.10	1.84±1.08	<0.001*
Right hippocampus	2.36±1.17	1.85±1.20	<0.001*

Notes: Data are expressed as mean±standard deviation or number; Statistical comparisons were conducted using paired-samples *t*-test; *$p<0.05$.

Table 4. Comparison of the mean gray matter volume between the Parkinson's disease patient with smell disorder (PD-SD) group and the healthy control (HC) group

Brain region	PD-SD (*n*=69)	HC (*n*=90)	*p*-value
Right entorhinal cortex	1.91±1.01	2.19±1.11	<0.001*
Left hippocampus	1.84±1.04	2.36±1.45	<0.001*
Right hippocampus	1.85±1.40	2.15±1.05	<0.001*

Notes: Data are expressed as mean±standard deviation; Statistical comparisons were conducted using paired-samples *t*-test; *$p<0.05$.

from various cortical areas to access the hippocampus. Recent research indicated that the direct circuit from the lateral entorhinal cortex to the hippocampal CA1 is essential for olfactory associative learning.[35] In this study, the GMV of the entorhinal cortex in the PD-SD group was lower than that in the other groups. These findings align with pathological research suggesting that the entorhinal cortex may be one of the earliest brain regions affected by PD. Another finding of this study is that both the right and left hippocampi exhibited a significant positive correlation with olfactory dysfunction in PD patients. This indicates that a reduction in bilateral hippocampal volume is associated with olfactory deficits in these individuals. Selective hyposmia in PD exhibits a stronger correlation with hippocampal dopamine innervation than with that of the amygdala, ventral striatum, or dorsal striatum, as evidenced by diffusion tensor imaging. These results suggest that the mesolimbic dopamine innervation of the hippocampus may play a crucial role in the development of selective hyposmia in PD.[36] Recent studies on the hippocampus emphasize that this structure may play a more significant role in olfaction.[37,38]

Based on the findings of the present study, one can infer the role of the structural network between the entorhinal cortex and the amygdala in PD patients.[23] The entorhinal cortex and the hippocampus have significant connections with each other, and a distortion in these connections may disrupt the olfactory process in the patients. Furthermore, the reduction in brain structure volume can be an important factor alongside the decrease in WM volume, ultimately leading to functional impairments.[23,39,40]

Neuroimaging research has demonstrated that pathological alterations during aging initiate in the amygdala and hippocampus, particularly in the parahippocampus and entorhinal cortex, which play a crucial role in the recognition and identification of olfactory stimuli.[41] Research showed that a decrease in olfactory function, occurring independently of cognitive decline, was associated with reductions in the volumes of the left hippocampus and left parahippocampus.[42] Another study demonstrated that a decrease in the volume of the entorhinal cortex was not directly linked to a reduction in olfactory function. Instead, the reduction in entorhinal cortex volume correlated with a decrease in the volume of the parahippocampus, suggesting that the entorhinal cortex influences olfactory ability indirectly through its relationship with the parahippocampus.[41,43,44] Based on the studies at the WM and cellular levels,[45] as well as the

structural findings in this study, we conclude that the structural connectivity between the entorhinal cortex and parahippocampal regions plays a crucial role in olfactory function. In PD patients, the reduction of volumes in these areas may have a more significant impact on the olfactory dysfunction.

Another factor that may influence a VBM study is the type of treatment that can influence the morphologic features of the VBM. For example, the study by Donzuso *et al.*[46] investigated whether a neuroanatomical substrate might underlie the development of long-duration responses using structural MRI and VBM analyses. Their study showed that some cortical structural changes may predispose individual patients to developing long-duration responses to *L*-dopa. Based on the results of the present study, it can be concluded that the type of treatment, along with other factors, such as olfactory dysfunction and the presence of cognitive impairment (mild/moderate/severe), may influence the morphologic features of the VBM.[23] Furthermore, the brain regions exhibiting reduced volume in PD patients undergoing *L*-dopa treatment, with or without olfactory dysfunction, differ from those affected in PD patients with cognitive impairment. Future studies should focus on the effects of medication on brain volumetric results in PD patients. Accounting for comorbid disorders, such as cognitive impairment and olfactory dysfunction, may offer valuable insights.

Moreover, the interplay between pharmacotherapy and neuroanatomical changes may be further elucidated by investigating the underlying biological mechanisms influenced by *L*-dopa treatment. Research indicates that neuroinflammation and dopaminergic signaling alterations can affect neuroplasticity and neurodegeneration in PD.[47-49] Studies employing advanced imaging techniques, such as VBM and diffusion tensor imaging, can offer critical insights into how *L*-dopa influences brain volumetric outcomes in PD, particularly regarding its impact on neural structure and the association with cognitive and olfactory function.[50,51] In addition, the incorporation of machine learning algorithms in volumetric analysis could facilitate the identification of subtle morphometric changes that traditional VBM may overlook. Such approaches could enhance our understanding of the differential effects of treatment modalities on brain structure and function, ultimately leading to more personalized therapeutic strategies for PD treatment.

This study encountered limitations. The MRI scanning was conducted at 1.5 Tesla. Acknowledging the limitations of 1.5T MRI, higher-field strengths in 3T or 7T MRI can significantly enhance sensitivity. The increased magnetic field strength improves the signal-to-noise ratio, leading to greater image clarity and the ability to detect smaller lesions. This could provide more in-depth results by examining cortical thickness and comparing it with brain volume.

5. Conclusion

PD patients exhibited more severe olfactory dysfunction in the hippocampal regions compared to the HC group. This may be attributed to the initial pathological loss of GM in both the right and left hippocampi.

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Conflict of interest

The authors declared that they have no competing interests.

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Methodology: Naser Moradi, Siamak Shahidi, Bahareh Zaker Harofteh, Mohammad Ahmadpanah
Writing – original draft: Naser Moradi
Writing – review & editing: Naser Moradi

Ethics approval and consent to participate

Ethical approval was obtained from Hamadan University of Medical Sciences (ethical approval: IR.UMSHA.REC.1401.1053). All participants in our study were adults, and each provided written informed consent before participation.

Consent for publication

Written informed consent was obtained from all participants to publish their data.

Availability of data

Data are available through the corresponding authors upon reasonable request.

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ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

Association between traumatic brain injury and depression stratified by veteran status: Findings from the National Health Interview Survey

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Abstract

Globally, traumatic brain injury (TBI) is one of the major causes of morbidity and mortality, with increased incidence reported among veterans. In this study, we explored the relationship between TBI and subsequent screening of depressive symptoms, with further analysis stratified by veteran status. For this study, the National Health Interview Survey data for 2023 was used, which was conducted among 29,522 non-institutionalized U.S. adults aged 18 and older. The patient health questionnaire-2 was used to screen for depression. Self-reported incidence of lifetime TBI was documented. From a Chi-square test, a significant association was observed between TBI and depression (p<0.05), with TBI more commonly being reported among veterans compared to non-veterans. Our regression model indicated that, when adjusted for sociodemographic and health variables, TBI was associated with 1.80 times higher odds of depression among the whole sample population (adjusted odds ratio [aOR] = 1.80; 95% confidence interval [CI] 1.61 – 2.02, p<0.05). When stratified by veteran status, veterans with TBI had 2.92 times higher odds of depression (aOR = 2.92; 95% CI 2.05 – 4.14, p<0.05). Compared to the whole general population, veterans with a brain injury history have higher odds of depression, identifying them as a key group in prioritizing depression management in the United States population.

Keywords: Concussion; Depression; Head injury; Mental health; Military; Traumatic brain injury; Service member; Veteran

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1. Introduction

Depression ranks fourth in the list of diseases causing the most death and disability, only behind other serious medical conditions such as cardiac disease, stroke, and acquired immunodeficiency syndrome.[1] Traumatic brain injury (TBI) is defined as an injury caused to the brain by an outside force such as blows, jolts, or a forceful bump. TBI, at any point in life, can be a risk factor for future psychiatric complications including depression and anxiety. TBI is usually classified into three broad categories – mild, moderate, and

severe based on clinical presentations, which at times are non-specific and non-predictive of future disease course.[2] Nevertheless, TBI is the principal cause of mortality and morbidity among people under the age of 45 years globally.[3] Despite major advancements in TBI treatment and long-term management, a significant number of people still suffer from long-term sequelae. According to prior research, in 2014 alone, the United States (U.S.) documented 2.53 million emergency department visits due to some form of TBI along with 288,000 hospitalizations and 56,800 subsequent deaths.[4] TBI is cited as one of the most common injuries with lifetime prevalence of 22% across a population.[5] It is important to note that not all cases of TBIs are formally diagnosed in the hospital setting. This number excludes military veterans who are thought to be at an elevated risk of suffering from TBI.

There is support that psychological symptoms associated with TBI, such as depression and anxiety, are alleviated within 12 weeks of injury.[6] Recently, researchers have been discussing about a more transient nature of depression following acute injury that re-emerges as a chronic medical condition later in life.[7] In line with recent thoughts, new evidence illustrated that approximately 25 – 50% of people suffer from depression of varying severity within 1 year of a TBI, 60% within the first 7 years, and the heightened risk persists even after 50 years of the initial injury.[8] In another study, researchers recorded a 40% prevalence of depression along with a 7.5 relative risk among participants, irrespective of TBI severity.[9]

In a previously conducted study, it was demonstrated that veterans are 31% more likely to suffer from major depression at any point in their life, which is significantly higher than the general population.[10] In a subsequent study, increased frequency of depressive episodes among veterans was also linked to higher rates of suicide.[11] This is compounded by the fact that veterans usually have worse health outcomes compared to the general population.[12] Veterans who were deployed overseas were twice more likely to get diagnosed with depression compared to others.[13] While veterans have a higher odds of suffering life threatening injuries, previous studies indicate that, just like the general population, most of the brain injuries suffered by veterans are mild in nature.[14] Another interesting aspect is the assumption that mode of injury might be an important indicator of higher prevalence of depression among military veterans. In a recent study, researchers found that veterans with blast or non-blast injuries had quite similar post-traumatic outcomes.[15] This highlights the complex causal mechanism of depression among veterans. While frequent TBI contributes significantly to depression, other factors such as living away from family, multiple combat missions, job stress, and cultural differences might also contribute to higher prevalence of depression among veterans.[16]

Previous studies have identified a relationship between the incidence of TBI and diagnosis of subsequent mental health disorders among the general population. For this research, we are focusing our attention solely to TBI and depression. There remains limited research examining this association among veterans, especially using a large nationally representative U.S. sample. Therefore, in our present study, we analyze the association between TBI and depression, stratifying this relationship by veteran status.

2. Materials and methods

2.1. Data source

We retrieved data of 29522 participants from the National Health Interview Survey (NHIS) administered by the Centers for Disease Control and Prevention (CDC) National Center for Health Statistics in 2023. Starting in 1957, the NHIS is one of the largest datasets in the U.S. that annually collects data on a broad range of health topics across the country using a geographically clustered sampling technique through face-to-face interviews with non-institutionalized civilians.

2.2. Depression

The dependent variable was depression, measured using the two-item patient health questionnaire (PHQ-2), a subset of the larger PHQ-8 scale used to screen for depression.[17] Participants were asked during the past 2 weeks whether they have been bothered by the following problems – "little interest or pleasure in doing things" and "feeling down, depressed, or hopeless." Scores ranged from 0 ("not at all") to 3 (nearly every day). Based on scores, results are categorized as either "positive" or "negative," with a score of 3 or higher being identified as "positive" for depression screening. Despite the shorter scale, the diagnostic performance is comparable to longer scales. The PHQ-2 has been previously tested to have a sensitivity of 87% and a specificity of 78% for major depressive disorder.[18]

2.3. TBI history

The independent variable was a history of TBI. Participants were asked, "In your lifetime, have you ever had a significant head injury or concussion?." This question was asked to only adults 18 years and older who did not experience any loss of consciousness or head injury symptoms within the past 12 months. Responses were either "yes" or "no."

2.4. Veteran status

Sample adults aged 18 and older were asked if they had ever served on active duty in the U.S. Armed Forces,

military reserve, or the National Guard. Responses were either "yes" or "no."

2.5. Covariates

Several sociodemographic variables and chronic diseases were included as covariates in the regression model. Age was continuous and top-coded to 85+ years. Sex was categorized as either "male" or "female." Race and ethnicity were categorized into four categories – "White," "African American," "Hispanic," and all other races into the "other" category. Education was coded into "below high school," "12th grade, GED, or equivalent," "associate degree," "bachelor's degree," and finally "postgraduate degree (Master's/PhD/Professional)." General health was categorized into five categories from poor to excellent. Participants were asked whether, at any point in their lives, they were diagnosed with hypertension, cardiovascular disease, cancer, and diabetes. These chronic conditions were also adjusted for as covariates. All variables with the "unknown," "refused," or "not ascertained" entries were coded as missing values.

2.6. Data analysis

A Chi-square test was performed to evaluate the association between TBI and depression. For continuous variables, a t-test was used, and for categorical variables chi-square test was employed. For the multiple logistic regression, a model was constructed using the whole sample population adjusting for sociodemographic and chronic diseases. Subsequently, the regression was performed again stratified by veteran status. When testing for multicollinearity, no individual variance inflation factor (VIF) value was >10, and the average VIF value of all variables was 2.75, indicating no violation of this assumption. All the analyses in this research were conducted with two-tailed tests using IBM Statistical Package for the Social Sciences Statistics version 29, with $p<0.05$ indicating statistical significance.

3. Results

3.1. Sample characteristics

From a total of 28,623 participants, 1,988 (6.7%) were screened as positive for depression based on the PHQ-2 (Table 1). Among the study participants, 66% (n = 19,495) identified themselves as White, followed by 10.9% as African American (n = 3,216), 15% as Hispanic (n = 4,418), and 8.1% as others (n = 2,393). Regarding education, 40.1% (n = 1,853) of study participants had a high school diploma or equivalent and formed the largest group in the education category. People who reported to have at least a bachelor's degree were the second largest group (23%, n = 6,792). Regarding general health, more than half of the participants (54%) reported their health status as either "excellent" or "very good," with only 3.8% (n = 1,111) reported their health status as "poor." Regarding the history of comorbidities, 37.6% (n = 11094) were reported to be diagnosed with hypertension, followed by 6.2% (n = 1,833) with cardiovascular disease, 12.7% (n = 3762) with cancer, and finally 11.2% (n = 294) of participants were diagnosed with diabetes. Among the participants, 18.7% (n = 5,533) reported to have suffered TBI at least once in their lifetime. From the Pearson Chi-square test, a significant association was observed between positive results in depression screening and TBI across the whole sample ($\chi^2[^1 = 171.20$, $p<0.05$) (Table 1).

3.2. Multiple logistic regression

In our multiple logistic regression model, a statistically significant association was observed between TBI and depression (Table 2). When adjusted for sociodemographic and chronic disease variables, participants with a TBI history had 1.80 times higher odds of depression compared to participants with no brain injury record (adjusted odds ratio [aOR] = 1.80; 95% confidence interval [CI] 1.61 – 2.02, $p<0.05$).

When stratified by veteran status, adjusting for other covariates, veterans with a brain injury history had 2.92 times higher odds of depression compared to veterans without TBI history (aOR = 2.92; 95% CI 2.05 – 4.14, $p<0.05$) (Table 2). On the other hand, non-veterans had 1.68 times higher odds of depression compared to non-veterans without TBI history (aOR = 1.68; 95% CI 1.49 – 1.90, $p<0.05$).

4. Discussion

This study probed the relationship between TBI and depression among the whole sample as well as veterans. In both the bivariate test and multiple regression models, a significant association was observed between TBI and depression among the whole sample. In addition, it was found that the magnitude of the TBI-depression association was higher among veterans compared to non-veterans, with 2.9 increased odds versus 1.7 increased odds, respectively. In comparison, a recently published meta-analysis revealed that the odds of depression were highest at 4.1 1 to 2 years after the injury, but decreased to 3.2 after 10 years of the original injury. Interestingly, the researchers did not find any statistically significant difference between post-TBI depression and injury etiology.[5] While the researchers included studies conducted on military personnel in their meta-analysis, no specific odds ratio for depression among veterans was provided.

A recently published meta-analysis on depression prevalence among veterans worldwide demonstrated that veterans, due to their unique nature of work, sleep

Table 1. Sample characteristics stratified by veteran status and depression

Variable	Whole sample		Veteran		Non-veteran	
	Depression (*n*, %)	No depression (*n*, %)	Depression (*n*, %)	No depression (*n*, %)	Depression (*n*, %)	No depression (*n*, %)
TBI history						
No	1180 (5.4)	20602 (94.6)	93 (12.9)	77 (4.6)	1098 (5.5)	18890 (94.5)
Yes	564 (10.2)	4941 (89.8)	629 (87.1)	1596 (95.4)	469 (9.9)	4290 (90.1)
Age, mean±SD	51.84±18.86	53.22±18.52	59.06±16.53	64.80±16.13	51.05±18.94	52.17±18.36
Sex						
Male	848 (6.5)	12239 (93.5)	177 (7.9)	2054 (92.1)	656 (6.2)	9986 (93.8)
Female	1139 (7.3)	14392 (92.7)	26 (9.2)	256 (90.8)	1092 (7.3)	13906 (92.7)
Race and ethnicity						
White	1289 (6.8)	17719 (93.2)	145 (7.3)	1836 (92.7)	1127 (6.7)	15658 (93.3)
African American	273 (9)	2766 (91)	31 (11.5)	239 (88.5)	233 (8.7)	2448 (91.3)
Hispanic	282 (6.6)	3991 (93.4)	17 (9.8)	156 (90.2)	259 (6.5)	3753 (93.5)
Other	144 (6.3)	2159 (93.7)	10 (11.2)	79 (88.8)	130 (6.0)	2037 (94.0)
Education status						
Below high school	247 (10.2)	2179 (89.8)	6 (7.3)	85 (93.4)	235 (10.3)	2052 (89.7)
12th grade/GED/ Equivalent	982 (8.6)	10489 (91.4)	121 (10.7)	1005 (89.3)	841 (8.3)	9294 (91.7)
Associate degree	259 (7)	3445 (93)	33 (8.2)	369 (91.8)	223 (6.9)	3030 (93.1)
Bachelor's degree	323 (4.9)	6298 (95.1)	29 (5.6)	489 (94.4)	291 (4.8)	5722 (95.2)
Postgraduate degree	164 (3.8)	4111 (96.2)	13 (3.5)	358 (96.5)	148 (3.8)	3698 (96.2)
General health status						
Excellent	137 (2.3)	5732 (97.7)	13 (3.7)	342 (96.3)	121 (2.2)	5288 (97.8)
Very good	361 (3.7)	9302 (96.3)	26 (3.4)	737 (96.6)	328 (3.7)	8436 (96.3)
Good	568 (6.7)	7876 (93.3)	51 (6.1)	784 (93.9)	507 (6.8)	6964 (93.2)
Fair	543 (15.2)	3041 (84.8)	57 (13.8)	356 (86.2)	476 (15.3)	2632 (84.7)
Poor	378 (36.1)	670 (63.9)	56 (38.9)	88 (61.1)	316 (35.8)	566 (64.2)
Hypertension	910 (8.4)	9866 (91.6)	121 (8.9)	1232 (91.1)	771 (8.3)	8519 (91.7)
Cardiovascular disease	190 (10.7)	1588 (89.3)	40 (12)	293 (88)	147 (10.3)	1280 (89.7)
Cancer	258 (7)	3407 (93)	35 (6.3)	523 (93.7)	221 (7.2)	2839 (92.8)
Diabetes	354 (11.1)	2832 (88.9)	49 (11.1)	393 (88.9)	299 (11.1)	2404 (88.9)

Abbreviations: SD: Standard deviation; TBI: Traumatic brain injury.

deprivation, exertion, and nutritional deficiency, often develop psychological disorders later in their lives.[19] This is often compounded by TBI suffered by veterans on active duty as many of them suffer from post-traumatic stress disorder, cognitive deficits, and suicidal thoughts.[20] Affected persons might suffer from other symptoms alongside depression including chronic pain and loss of employment.[21] In addition, an increased incidence of brain cancer was reported among veterans suffering from moderate-to-severe head injury.[22]

The higher odds of depression and incidence of TBIs reported among veterans contribute to a major public health issue that may have debilitating effects both on the healthcare system and the personal lives of the affected individuals. This requires urgent intervention from public health organizations both among the general population and veterans. Most of the cases of depression among veterans are resolved after psychotherapy and pharmacological management. Cognitive behavioral therapy and interpersonal therapy help people manage negative thoughts and suicidal ideation, as well as help developing coping skills.[23] Patients unresponsive to psychological therapy are often treated with antidepressants and mood stabilizers.[24] At present, available antidepressants are highly effective, reducing symptoms by more than 50%

Table 2. Association between TBI history and depression stratified by veteran status

Variable	Whole sample aOR (95% CI), p	Veteran aOR (95% CI), p	Non-veteran aOR (95% CI), p
TBI history	1.80 (1.61 – 2.02), <0.05	2.92 (2.05 – 4.14), <0.05	1.68 (1.49 – 1.90), <0.05
Age	0.98 (0.97 – 0.98), <0.05	0.97 (0.96 – 0.98), <0.05	0.98 (0.977 – 0.984), <0.05
Sex			
Female	Reference	Reference	Reference
Male	0.79 (0.71 – 0.87), <0.05	0.71 (0.42 – 1.18), >0.05	0.78 (0.70 – 0.87), <0.05
Race and ethnicity			
White	Reference	Reference	Reference
African American	1.15 (0.98 – 1.34), >0.05	1.62 (0.99 – 2.66), >0.05	1.10 (0.93 – 1.30), >0.05
Hispanic	0.75 (0.64 – 0.88), <0.05	1.09 (0.58 – 2.02), >0.05	0.73 (0.62 – 0.86), <0.05
Other	0.92 (0.75 – 1.12), >0.05	1.63 (0.73 – 3.62), >0.05	0.89 (0.72 – 1.09), >0.05
Education status			
Below high school	Reference	Reference	Reference
12th grade/GED/Equivalent	1.00 (0.84 – 1.19), >0.05	1.54 (0.57 – 4.14), >0.05	0.96 (0.80 – 1.14), >0.05
Associate degree	0.81 (0.65 – 1.0), <0.05	1.00 (0.34 – 2.94), >0.05	0.80 (0.64 – 0.99), <0.05
Bachelor's degree	0.75 (0.62 – 0.92), <0.05	0.98 (0.34 – 2.82), >0.05	0.74 (0.60 – 0.91), <0.05
Postgraduate degree	0.64 (0.51 – 0.81), <0.05	0.75 (0.24 – 2.36), >0.05	0.64 (0.50 – 0.81), <0.05
General health status			
Excellent	0.04 (0.03 – 0.05), <0.05	0.04 (0.02 – 0.10), <0.05	0.04 (0.03 – 0.05), <0.05
Very good	0.06 (0.05 – 0.08), <0.05	0.04 (0.02 – 0.08), <0.05	0.06 (0.05 – 0.08), <0.05
Good	0.12 (0.10 – 0.14), <0.05	0.08 (0.05 – 0.14), <0.05	0.12 (0.10 – 0.15), <0.05
Fair	0.31 (0.26 – 0.36), <0.05	0.19 (0.11 – 0.32), <0.05	0.32 (0.26 – 0.39), <0.05
Poor	Reference	Reference	Reference
Hypertension	1.05 (0.93 – 1.19), >0.05	1.08 (0.73–1.60), >0.05	1.04 (0.91 – 1.18), >0.05
Cardiovascular disease	1.08 (0.89 – 1.31), >0.05	1.14 (0.69 – 1.19), >0.05	1.07 (0.87 – 1.33), >0.05
Cancer	0.82 (0.70 – 0.97), <0.05	0.73 (0.45 – 1.19), >0.05	0.85 (0.71 – 1.02), >0.05
Diabetes	0.98 (0.84 – 1.14), >0.05	1.09 (0.69 – 1.71), >0.05	0.96 (0.82 – 1.14), >0.05

Abbreviations: aOR: Adjusted odds ratio; TBI: Traumatic brain injury.

within 8 weeks after treatment initiation. However, some cases advance to chronic conditions and require extensive management straining the already fragile healthcare system.[25] Nurses, medics, corpsmen, and primary healthcare providers should be included in a holistic treatment approach where education and monitoring would be considered as integral components. Any suspicion of depression among veterans with recent TBI should be addressed with utmost caution.[26] Previous research demonstrated that females are more likely to get diagnosed with depression compared to males.[27] For future research, it remains to be seen whether gender of veterans has any interacting effect on the association between TBI and depression.

From a public health perspective, our study offers important contributions in shaping intervention strategies for people with a history of TBI. This is even more impactful for veterans who are more prone to TBI due to the nature of their prior service and subsequent depression diagnosis. Nonetheless, the ramifications of depression are not solely confined to the health of the affected individual as it has ripple effects for the society more broadly due to increased healthcare utilization.[28] The robust association between TBI and positive screening for depression among veterans in later life underscores the necessity for implementing targeted mental health screening for vulnerable individuals. Since the incidence of depression among veterans are higher than the general population, routine screening after each TBI incident might alleviate the risk up to a certain extent.[29] This would improve long-term outcomes and reduce the burden on already scarce healthcare resources. While the PHQ-2 is a useful screening tool for depression among individuals, the longer and more robust PHQ-9 could also provide more data and clarity while screening for depression in high-risk groups.[30]

Veterans are a unique subpopulation with cumulative exposure to multiple stressors including blast injury, long overseas deployment, and other psychological stressors, that coupled with TBI may exacerbate depression among veterans.[31] Veterans Affairs initiatives such as telepsychiatry and trauma informed therapy could lessen the psychological impacts post-TBI and reduce depression incidence among veterans.[32,33]

Policy makers and health care providers should consider implementing longitudinal surveillance due to the unpredictable and often late-onset nature of depressive symptoms post-TBI. Patients should be monitored over a long period of time, and health records should be stored electronically.[32,33] There should be a policy where the Department of Defense, Department of Veterans Affairs, and public health agencies relating to veteran health should operate on a common platform where they can share data across providers to ensure continuity of care and identification of each high-risk individual.[34,35]

At the federal level, the government should allocate sufficient funds for treatment and prevention of TBI associated depression. At present, there is a shortage of staff and services geared toward minimizing post-TBI sequelae among affected individuals.[36] Non-governmental organizations dealing with mental health issues should also come forward and include post-TBI depression prevention in their organizational framework. While treatment and management of post-TBI depression warrants significant resources, efforts should also be directed toward formulation and implementation of preventive strategies. Approaches such as an evidence-based resilience training, a post-deployment debriefing program, and a peer support network could be adopted to reduce post TBI morbidity in addition to traditional psychotherapies such as CBT.[37] Furthermore, a large scale destigmatization program should be conducted among both military individuals and civilians regarding mental health treatment, as stigmatization is hitherto a significant barrier to accessing care.[38] Finally, people of color as well as individuals from socioeconomically disadvantaged backgrounds traditionally face significant hurdles while accessing mental health care. Ensuring equal opportunity and equity of access for all the people would be a key determinant while seeking successful outcomes at the population level.[39]

There are several limitations to our study. First, the two-item questionnaire used to screen for depression is not a clinical depression diagnosis among study participants. This is originally part of a larger PHQ-8 questionnaire that also commonly screens for depression. While the two-item questionnaire was tested for sensitivity and specificity, further evaluation is required to reach a definitive conclusion regarding the presence and severity of depression among study participants. Second, all the data included in the analysis are self-reported and cross-sectional, making them susceptible to information or recall bias. For example, participants were asked about whether they had suffered significant brain injury or concussion in their lifetime. Injuries sustained in early life might not be reported properly, and the definition of "significant brain injury" might be open to interpretation. Finally, brain injuries were reported on a lifetime basis but excludes recent TBI incidents, along with source and severity of the injury. Further research is warranted to investigate how these TBI characteristics may influence mental health. Nonetheless, measurement of lifetime TBI history strengthens our findings by lending temporality to our analysis of the association between TBI and depression. In addition, the cross-sectional nature of the study design does not allow us to establish causality between TBI and depression, even though TBI is often associated with increased prevalence of mental health issues and health resource utilization.[40] Despite these limitations, we analyzed a large nationally representative U.S. sample and applied a rigorous analysis adjusting for many possible covariates that may confound the association between TBI and depression.

5. Conclusion

Our study sheds light on the association between TBI and depression in the general population, as well as veterans. The findings of our study reveal that veterans with head injuries are more susceptible to depression compared to the general population. This establishes veterans as priority groups for future intervention programs aimed at reducing the burden of depression among the U.S. population.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors
Formal analysis: Md Maruf Ahmed Molla
Investigation: Md Maruf Ahmed Molla
Methodology: All authors
Writing–original draft: Md Maruf Ahmed Molla
Writing–review & editing: All authors

Ethics approval and consent to participate

This project was determined to be exempt from approval by the Institutional Review Board (IRB) of SUNY Upstate Medical University. Ethical review and approval were waived for this study because it meets IRB exemption category #4(i) – identifiable private information or identifiable biospecimens used in this study are publicly available. Patient consent for this study was not required due to the approval of IRB exemption. There was no potential for harm to subjects in the database and the data were used without identifying information.

Consent for publication

Not applicable.

Availability of data

This study uses public data, which may be obtained through the CDC and is publicly available at: https://www.cdc.gov/nchs/nhis/documentation/2023-nhis.html

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ACCSCIENCE PUBLISHING

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ORIGINAL RESEARCH ARTICLE

The role of high-density lipoprotein and Vitamin D in exercise-induced neuroprotection in epileptic rats

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Abstract

Previous studies have reported the neuroprotective effects of exercise in epilepsy. Hence, this study aimed to investigate the potential mechanisms underlying these neuroprotective effects by examining changes in high-density lipoprotein (HDL), low-density lipoprotein (LDL), and Vitamin D levels. Wistar rats were divided into four groups: sham, pentylenetetrazole (PTZ), exercise (EX), and PTZ + EX. Seizures were induced in the PTZ and PTZ + EX groups through intraperitoneal administration of PTZ (35 mg/kg) every other day for 1 month. The EX and PTZ + EX groups underwent daily exercise sessions (30 min/session) for 1 month. Serum levels of Vitamin D, HDL, and LDL were measured, and neuronal damage was assessed in the hippocampus and somatosensory cortex. The PTZ + EX group showed a significant increase in serum HDL and Vitamin D levels compared to the PTZ group. Histological analysis revealed a significant increase in neuronal damage in the PTZ group compared to the sham group. In contrast, both PTZ + EX and EX groups demonstrated a reduction in neuronal damage compared to the PTZ group. These findings suggest that HDL and Vitamin D may contribute to the neuroprotective benefits of exercise. Furthermore, exercise could potentially serve as a complementary strategy to counteract Vitamin D and HDL deficiencies associated with long-term antiepileptic drug use.

Keywords: Exercise; Vitamin D; Lipoproteins; Hippocampus; Somatosensory cortex; Epilepsy

1. Introduction

Regular physical activity has been widely acknowledged for its extensive health benefits, supporting the optimal function of various bodily systems, including the skeletal,

endocrine, cardiovascular, and nervous systems.[1] In recent years, growing attention has been directed toward the neurological effects of exercise, particularly for its potential anticonvulsant properties in individuals diagnosed with epilepsy.[2-4]

This growing interest is supported by emerging evidence suggesting that physical exercise can modulate neuronal excitability, reduce oxidative stress, and enhance the brain's resilience to seizures.

The brain is one of the most cholesterol-rich organs in the human body, containing nearly 25% of the body's total cholesterol despite comprising only about 2% of the body's weight.[5] This high cholesterol content is essential for the brain's structure and function. Lipids, especially cholesterol and phospholipids, play a critical role in the development and maintenance of the central nervous system (CNS).[6] They are necessary for various neural processes such as myelination, synaptogenesis, signal transduction, and neuronal membrane integrity maintenance. Disruptions in lipid metabolism within the CNS have been implicated in the onset and progression of various neurological disorders, including Alzheimer's disease, ischemic stroke, traumatic brain injury, and epileptic seizures.[7] These metabolic disruptions may impair neuronal function and contribute to neurodegeneration by affecting membrane fluidity, inflammatory responses, and synaptic transmission.

Furthermore, recent studies have revealed that during glutamate-induced excitotoxicity – a major pathological mechanism in various CNS diseases – cholesterol synthesis and catabolism exhibit stage-specific dysregulation across the progression of neural injury.[8] These alterations might affect neuronal survival, plasticity, and the brain's capacity for repair, suggesting that lipid homeostasis is a crucial factor in the brain's response to injury or disease.

One potential mediator of neuroprotection is high-density lipoprotein (HDL), which has emerged as an important factor in brain health. The beneficial effects of regular exercise, a balanced diet, weight control, and smoking cessation on brain function might be partly attributed to their role in modulating HDL levels.[9] Individuals suffering from dyslipidemia, particularly those with reduced HDL or elevated low-density lipoprotein (LDL) levels, appear to be at a higher risk of developing neurodegenerative diseases.[10] HDL has demonstrated multiple neuroprotective properties, including enhancement of synaptic maturation, support of synaptic plasticity, an increase in hippocampal volume, and anti-inflammatory and antioxidant effects.[11] In contrast, LDL has been shown to suppress the proliferation of adult hippocampal neural precursor cells.[12] This suggests that an imbalance in lipoproteins not only affects vascular health but may also impair neurogenesis and cognitive functions by disrupting critical metabolic processes in the brain.

Vitamin D, a fat-soluble vitamin, is another important factor contributing to CNS function, with established roles in brain development and neuroprotection.[13] Vitamin D deficiency has been associated with various neurological and psychiatric disorders, including multiple sclerosis, Alzheimer's and Parkinson's diseases, autism spectrum disorders, schizophrenia, and cerebrovascular disease.[14,15] A well-documented correlation also exists between antiepileptic drug use and decreased serum Vitamin D levels, which might exacerbate bone fragility, immune dysfunction, and neuronal vulnerability in affected individuals.[16] Consequently, patients undergoing antiepileptic therapy should be regularly monitored for Vitamin D levels and supplemented accordingly.

Adequate Vitamin D levels are essential not only for skeletal health but also for maintaining optimal nervous system function. Vitamin D contributes to neuroprotection by exerting anti-inflammatory and antioxidant effects, enhancing neuronal growth and survival, and modulating neurotransmitter synthesis and release.[17] It has been suggested that Vitamin D may play a role in mitigating seizure-related neuronal damage and enhancing the brain's ability to recover following seizure attacks.

In this study, we investigated the potential role of exercise in modulating lipid metabolism, specifically HDL and LDL levels, and Vitamin D status as key mechanisms underlying neuroprotective effects in chemical models of epilepsy. By exploring these pathways, we aimed to better understand how physical activity might contribute to CNS resilience and recovery following epileptic seizures.

2. Methods

2.1. Animals

Male Wistar rats weighing between 250 – 300 g (Animal house of Iran University of Medical sciences, Iran) were accommodated in the animal house of Iran University of Medical Sciences within a controlled environment free of specific pathogens, with a 12-h light and dark cycle at a temperature of 21 ± 2°C. The rats were acclimated for a week before the start of the study. All animal procedures were conducted in accordance with the ethical guidelines approved by the Research Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (IR.IUMS.REC.1400.863). We divided the animals into four groups ($n = 6$ in each group):

(i) Sham: Rats were administered saline through intraperitoneal injections every other day for 4 weeks, without any exercise
(ii) Pentylenetetrazole (PTZ): Seizures were induced

through PTZ injections using the same protocol as the sham group

(iii) Exercise (EX): Rats underwent exercise 5 days/week for 4 weeks without any injections

(iv) PTZ + EX: Rats received PTZ injections on the same schedule as the PTZ group and exercised 5 days/week for 4 weeks.

Chemical kindling through intermittent injection of PTZ is a well-established experimental model of epilepsy.[18] Repeated sub-convulsive injection of PTZ over a month induces tonic-clonic seizures resembling those in epileptic subjects.[18] Accordingly, the PTZ kindling model was employed for this study. PTZ (35 mg/kg) was injected every other day for 4 weeks to induce generalized seizures in the rats.

2.2. Exercise protocol

In the exercise groups, after acclimation to the treadmill, rats underwent the following protocol: 5 min at 10 m/min, 5 min at 15 m/min, 5 min at 20 m/min, and 9 min at 25 m/min. All exercises were performed at 0° inclination to maintain moderate intensity. The first and the last 3 min of the session served as warm-up and cool-down periods, respectively, at 8 m/min.[19,20]

2.3. Blood sample collection and biochemical analysis

Rats were anesthetized through intraperitoneal injection of a mixture comprising xylazine (5 mg/kg) and ketamine hydrochloride (50 mg/kg). Blood samples were subsequently obtained from the left ventricle using the cardiac puncture protocol (Paulose and Dakshinamurti, 1987). The blood samples (2 – 3 mL) were collected and centrifuged at 1,500 × g for 10 min at 4°C. The clear serum was stored at −20°C for the measurement of HDL, LDL, and Vitamin D levels. The total serum levels of HDL and LDL were determined using an enzymatic colorimetric method with the commercial DiaSys Kit (Diagnostic System GmbH, Germany), according to manufacturer's instruction.

Total serum level of 25-hydroxy Vitamin D was measured using the Enzyme-Linked Immunosorbent Assay kit (CAT. NO. MBS261766, MyBioSource, United States) following the manufacturer's instructions.

2.4. Tissue preparation and cell counting

Saline, followed by 4% paraformaldehyde (PFA), was perfused transcardially. Subsequently, the brains were carefully dissected and stored in a solution of 4% PFA for a week at 4°C. Paraffin-embedded tissue blocks were prepared, and serial coronal sections were obtained from regions 1.8–3.3 mm posterior to the bregma. Each section, with a thickness of 8 μm, was meticulously prepared using a microtome (DS920, Did Sabz, Iran). Ten pairs of sections were selected from each animal and stained with toluidine blue. The hippocampal regions and the somatosensory cortex were assessed under light microscopic (C-P8, OPTIKA, Italy). The images were taken using a 40× objective lens. The number of dark cells per 1 mm^2 was counted using the Infinity software (Infinity1, Lumenera, Canada).

2.5. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM) and analyzed using the one-way analysis of variance and Tukey's *post hoc* test. Statistical analyses were conducted using the Statistical Package for the Social Sciences (SPSS version 23, IBM, United States), with a significance level set at $p<0.05$.

3. Results

3.1. The serum level of HDL

The statistical analysis revealed a significant increase in serum level of HDL in the PTZ + EX group compared to the PTZ and EX groups (Figure 1, $p<0.05$). The mean ± SEM (mg/dL) was 52 ± 0.8 in the sham, 50 ± 0.55 in the PTZ, 51 ± 0.36 in the EX, and 55 ± 0.7 in the PTZ + EX groups. There was no significant difference between the EX and sham groups.

3.2. The serum level of LDL

The statistical analysis indicated no significant differences in the level of LDL across all experimental groups. The mean ± SEM (mg/dL) was 81.3 ± 0.82, 79.8 ± 0.48, 80.3 ± 0.55, and 81.5 ± 0.63 in the sham, PTZ, EX, and PTZ + EX groups, respectively (Figure 2).

3.3. The serum level of Vitamin D

The statistical analysis showed that the serum level of Vitamin D significantly increased in the EX and PTZ + EX groups compared to the PTZ group (Figure 3, $p<0.05$). The mean ± SEM (ng/mL) of the sham, PTZ, EX, and PTZ + EX groups were 18.1 ± 0.1, 17.2 ± 0.43, 19.1 ± 0.31, and 19.2 ± 0.18, respectively. There was no significant difference in the EX and PTZ + EX groups compared to the sham group.

3.4. Dark neurons assessment

We calculated the number of damaged neurons in the hippocampal and cortical areas (Figure 4). The mean number of dark neurons increased significantly in the PTZ group compared to the sham group in the cornu ammonis (CA)1, CA3, and cortex areas. Furthermore, the mean

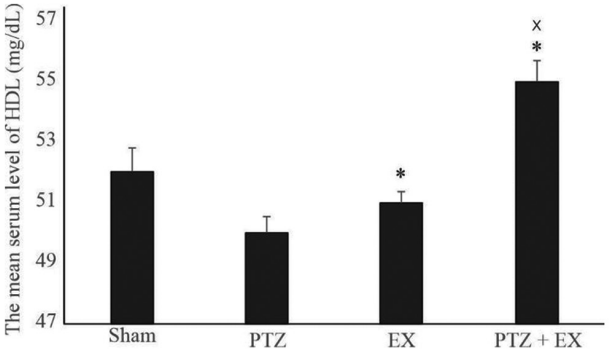

Figure 1. Serum levels of high-density lipoprotein in rats across different experimental groups
Notes: *indicate $p<0.05$ compared to PTZ group; X indicates $p<0.05$ compared to Sham group.
Abbreviations: EX: Exercise; HDL: High-density lipoprotein; PTZ: Pentylenetetrazole.

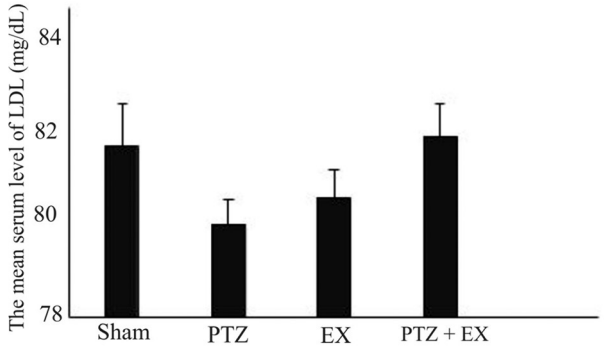

Figure 2. Serum levels of low-density lipoprotein in rats across different experimental groups
Abbreviations: EX: Exercise, LDL: Low-density lipoprotein, PTZ: Pentylenetetrazole.

number of dark neurons significantly decreased ($p<0.001$) in the CA1, CA3, and cortex areas in the EX and PTZ + EX groups compared to the PTZ group. Dark neurons number significantly decreased in the cortex area of the EX and PTZ + EX groups compared to sham group ($p<0.05$ and $p<0.001$, respectively).

In the CA1 area, the mean number of dark neurons (neurons/mm^2) was 0.34 ± 0.03 in the sham group, 0.90 ± 0.1 in the PTZ group, 0.28 ± 0.02 in the EX group, and 0.51 ± 0.06 in the PTZ + EX group. In the CA3 area, the mean number of dark neurons was 0.39 ± 0.06, 1.17 ± 0.06, 0.34 ± 0.1, and 0.53 ± 0.07 in the sham, PTZ, EX, and PTZ + EX groups, respectively. In the cortex area, the mean number of dark neurons was 0.18 ± 0.01 in the sham group, 0.78 ± 0.09 in the PTZ group, 0.38 ± 0.04 in the EX group, and 0.51 ± 0.03 in the PTZ + EX group.

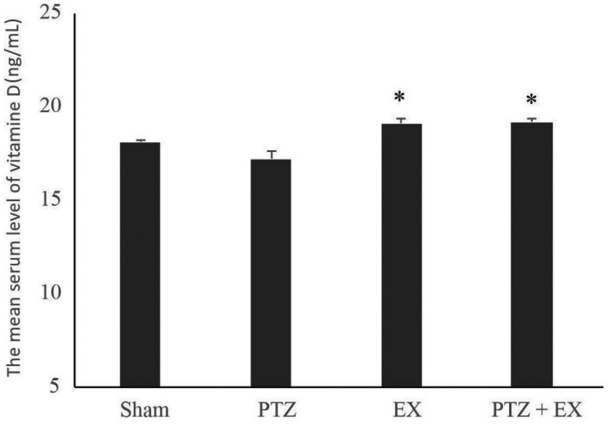

Figure 3. Serum levels of Vitamin D in rats across different experimental groups
Note: Statistical significance ($p<0.05$) is denoted by an asterisk (*) above the error bars.
Abbreviations: EX: Exercise; PTZ: Pentylenetetrazole.

4. Discussion

4.1. Exercise modulated cholesterol metabolism in epileptic conditions

We observed an increase in serum levels of HDL in rats that underwent exercise simultaneously with seizure induction. HDL cholesterol is known as the good cholesterol due to its role in reducing the risk of cardiovascular disease.[21] Higher levels of HDL may also contribute to reducing the risk of seizure onset and improving seizure control.[22]

Physical activity has been shown to modulate cholesterol metabolism, and regular exercise has been associated with increased HDL levels.[23] Exercise has also been reported to stimulate the production of enzymes involved in the transport of HDL to the liver for reutilization, resulting in an overall increase in HDL concentrations.[24]

Our findings show that exercise alleviates HDL deficiency caused by epilepsy. These results suggest that exercise might have a modulatory effect on HDL metabolism under epileptic conditions and contribute to a reduction in seizure severity. In addition, multiple studies have demonstrated that long-term use of certain antiepileptic drugs can lead to elevated levels of total cholesterol and LDL in the blood.

Given these findings, it is highly recommended that clinicians routinely monitor the lipid profiles of individuals undergoing long-term antiepileptic treatment. Antiepileptic drugs might alter lipid metabolism, and the resulting dyslipidemia may contribute to the development of cardiovascular and metabolic disorders.[25] By closely tracking lipid levels, healthcare providers might better

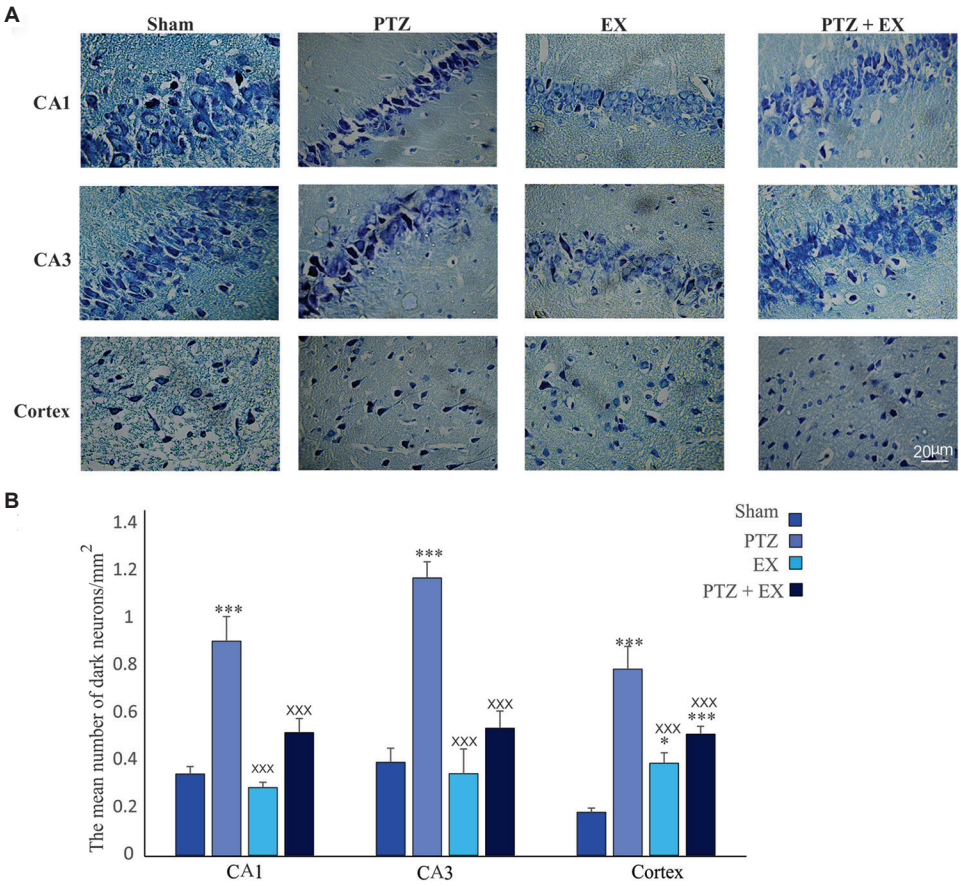

Figure 4. Histological assessment of dark neurons across different experimental groups. (A) Light-microscopic photos of toluidine blue staining in the cornu ammonis (CA)1, CA3, and cortex areas. Scale bar: 20 μm, magnification: 40×. (B) The bar graphs indicate the mean number of dark neurons in the experimental groups. The area of each field was calculated, after which all dark neurons within the defined area were counted. The number of dark neurons per 1 mm^2 was then determined.
Notes: * and *** indicate $p<0.05$ and $p<0.01$ compared to sham group, respectively; XXX indicates $p<0.001$ compared to PTZ group.
Abbreviations: CA: Cornu ammonis; EX: Exercise; PTZ: Pentylenetetrazole.

assess the overall health of epilepsy patients and adjust treatment plans accordingly.

For all the aforementioned reasons, exercise is suggested as a highly beneficial strategy for the effective management of epilepsy. Exercise can regulate lipid metabolism, improve cardiovascular health, and support overall well-being. Incorporating exercise as part of a comprehensive treatment plan may significantly enhance the quality of life for individuals with epilepsy and serve as a cost-effective adjunct to pharmacological interventions.[26,27]

4.2. Exercise improved Vitamin D deficiency

Our findings show that exercise increased serum Vitamin D levels in both epileptic and non-epileptic rats. Previous research has reported an association between epilepsy and Vitamin D deficiency.[28] In a study involving 13 patients with pharmacoresistant epilepsy, intake of Vitamin D_3 was associated with a reduction in seizure frequency of up to 40%.[28] An extensive retrospective study of 524 individuals found evidence of Vitamin D deficiency in patients with epilepsy.[29] Similarly, a comprehensive cross-sectional study involving 244 epileptic children living in tropical regions demonstrated a high prevalence of Vitamin D deficiency.[30] It is important to note that Vitamin D deficiency has also been identified as one of the side effects of antiepileptic drugs, with long-term treatment using carbamazepine and valproate shown to decrease Vitamin D levels in epileptic patients.[31] Although the mechanistic pathway was not investigated in our study, Vitamin D has been suggested to play a critical role in lipid metabolism. It has been reported that Vitamin D supplementation reduces total cholesterol and LDL levels while increasing HDL levels.[32] For example, a trial reported that administering 50,000 IU of Vitamin D improved lipid and insulin metabolism in infertile women.[33] Another study showed a high ratio of triglycerides to HDL in obese children with low Vitamin D levels compared

to normal children.[34] Moreover, a significant correlation was observed between inadequate Vitamin D levels and adverse lipid metabolism in obese pediatric patients.[35] Among male adolescents, those deficient in Vitamin D exhibited lower serum HDL levels compared to those with sufficient Vitamin D.[36] Our findings indicate that regular physical exercise may have a significant beneficial impact on the lipid profiles, potentially by modulating Vitamin D deficiency.

These findings are particularly relevant in the context of neurological health, as both lipids and Vitamin D are crucial in maintaining brain function and protecting neurons from damage. Exercise-induced regulation of lipid metabolism, especially the increase of HDL, might contribute to reducing neuroinflammation, oxidative stress, and neuronal damage involved in the pathophysiology of epilepsy.[37] In parallel, our findings suggest that exercise could be an effective strategy for mitigating the adverse effects of Vitamin D deficiency, a condition that is widely observed in individuals with epilepsy.

Furthermore, physical activity might play an important role in the prevention and management of Vitamin D deficiency in individuals undergoing long-term treatment with antiepileptic drugs.[38] Long-term administration of antiepileptic drugs interferes with the metabolism of Vitamin D, leading to suboptimal serum levels and potentially exacerbating bone fragility, immune dysfunction, and neuronal vulnerability.[38] By enhancing the synthesis of Vitamin D through increased sun exposure and optimizing overall metabolic health, exercise might help to counteract some of the negative effects of antiepileptic drugs. These dual effects – improving lipid profiles and restoring Vitamin D levels – suggest that exercise could be a simple and accessible therapeutic intervention for individuals with epilepsy, enhancing their overall well-being and potentially improving their neurological outcomes.

4.3. Neuroprotective effect of exercise

Epileptic seizure attacks have been reported to induce neural cell damage. To assess the neuroprotective effects of certain supplements, such as *Nigella sativa* and astaxanthin, researchers have commonly used the PTZ kindling models.[39,40] This model has been shown to cause neural cell damage in various brain regions.[41,42]

Dark cells are considered a valid histological marker for assessing neuronal damage in the brain.[43] In this study, we observed a significant decrease in the number of dark cells in epileptic rats that underwent physical activity. These findings indicate that exercise may have a moderate neuroprotective effect by improving neural cell survival in epileptic rats.

Seizure attacks are known to activate apoptotic pathways in various brain areas.[44,45] Both intrinsic and extrinsic factors contribute to the regulation of the apoptosis signaling pathway. Activation of the Vitamin D receptor has been shown to decrease the level of cleaved caspase-3 following traumatic brain injury,[46] and the expression of Vitamin D receptor in the hippocampus plays an important role in cell survival through the calcium buffering system.[47] In addition, pretreatment with Vitamin D_3 has been reported to improve mesencephalic neuron survival in the pathological condition induced by 6-hydroxydopamine administration.[48] Vitamin D is also known to protect the hippocampus through its anti-inflammatory and antioxidant properties. The anti-inflammatory properties of Vitamin D reduce neuronal loss and the risk of dementia and Alzheimer's disease by modulating pro-inflammatory cytokines such as interleukin 6 and tumor necrosis factor alpha in the hippocampus.[49] In addition, Vitamin D_3 has been shown to protect neurons by reducing oxidative stress factors such as catalase enzyme.[50]

Further evidence on the neuroprotective role of HDL derives from *in vitro* studies involving astrocytes, the most abundant glial cells in the CNS, which play a critical role in maintaining neuronal homeostasis and responding to injury. It has been demonstrated that the incubation of astrocytes with HDL isolated from human plasma significantly enhances their viability in the presence of toxic concentrations of divalent copper ions in the culture medium.[51]

This finding is particularly noteworthy because copper, while essential in trace amounts for enzymatic activity, becomes neurotoxic at high levels and contributes to oxidative stress, mitochondrial dysfunction, and inflammatory factors commonly implicated in seizure pathology and neurodegenerative diseases.[51] HDL, through its antioxidant property and lipid-transporting capacity, may protect against this toxicity, thereby preserving astrocyte function under stress conditions.[52]

In contrast, prolonged exposure to elevated levels of LDL, particularly in the oxidized form, has been associated with detrimental effects on the brain. Studies have shown that chronic high LDL concentrations aggravated the deposition of beta-amyloid in brain tissue, a process central to the pathogenesis of Alzheimer's disease.[53] Furthermore, LDL has been implicated in compromising the integrity of the blood–brain barrier, a selectively permeable structure that protects the brain from circulating toxins and immune cells. Damage to the blood–brain barrier might permit the infiltration of pro-inflammatory mediators, further exacerbating neuronal damage.[54]

Moreover, astrocytes subjected to oxygen-glucose deprivation – a well-established *in vitro* model that

simulated ischemic injury – exhibited enhanced survival when incubated with apolipoprotein M-associated HDL.[55] This specific HDL subtype is known to exert superior antioxidant and anti-inflammatory effects, which might support cellular repair and functional recovery. Collectively, these findings suggest that HDL not only supports vascular health but also plays a direct protective role in the survival of the glial cells and the CNS integrity under pathological conditions, including epilepsy.

5. Conclusion

Our findings demonstrate that physical activity leads to a notable increase in HDL and Vitamin D levels in the bloodstream following chronic seizure induction. These outcomes suggest that exercise might play a beneficial role in enhancing systemic health after seizure attacks, potentially aiding recovery and reducing long-term complications.[26]

Although different signaling pathways are involved in the protective effects of exercise, high levels of HDL are known as an anti-inflammatory and antioxidant factor, which might contribute to stabilizing neuronal membranes and improving cellular resilience.

The most important pathways include the glutamate and gamma-aminobutyric acid (GABA) signaling pathways, which are the primary excitatory and inhibitory pathways involved in the pathogenesis of epilepsy. The regulatory effects of exercise on glutamate and GABA synthesis have been reported.[56,57]

To clarify other potential pathways involved in the neuroprotective effects of exercise, we suggest that elevated levels of Vitamin D are associated with enhanced neuroprotection through modulating immune function, reducing oxidative stress, and maintaining calcium balance within neurons. Furthermore, our findings show an increase in the average number of dark cells in both the hippocampus and somatosensory cortex of epileptic rats, consistent with previous studies indicating that these brain regions are highly susceptible to seizure-induced excitotoxicity. However, exercise significantly reduces the number of dark cells, suggesting a protective effect. This neuroprotective influence might be linked to elevated serum levels of both HDL and vitamin D.

Our results demonstrate that regular physical exercise significantly modulates HDL and Vitamin D levels under epileptic conditions and contributes to the preservation of hippocampal neurons after seizure activity. These findings indicate that exercise might serve as a non-pharmacological strategy to mitigate seizure-induced neuronal damage. The increase in HDL and Vitamin D levels suggests that these factors might play important roles in mediating the neuroprotective effects of physical activity. Specifically, the regulation of lipid metabolism and Vitamin D status may be critical components of the brain's adaptive response to epileptic injury. Further studies are warranted to explore the molecular pathways through which HDL and Vitamin D exert their protective effects and to determine whether similar benefits can be replicated in human subjects with epilepsy.

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Conflict of interest

The authors declare that they have no competing interests.

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Ethics approval and consent to participate

All animal procedures were conducted in accordance with the ethical guidelines approved by the Research Ethics Committee of Iran University of Medical Sciences, Tehran, Iran (IR.IUMS.REC.1400.863).

Consent for publication

Not applicable.

Availability of data

The original contributions presented in the study are included in the article. Further inquiries can be directed to the corresponding authors.

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ACCSCIENCE PUBLISHING

Advanced Neurology

ORIGINAL RESEARCH ARTICLE

In vitro suppression of glioblastoma cell functions by TG100-115, a transient receptor potential melastatin 7 kinase inhibitor

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Abstract

Glioblastomas (GBMs) are highly aggressive and lethal primary brain tumors, known for their rapid proliferation, diffuse infiltration, and resistance to conventional therapies. Recent studies have highlighted the involvement of transient receptor potential melastatin 7 (TRPM7) in regulating GBM progression through its dual function as an ion channel and a serine/threonine protein kinase. TG100-115, initially characterized as a phosphoinositide 3-kinase γ/δ inhibitor, has recently been identified as a novel inhibitor of TRPM7 kinase. However, its potential pharmacological effects in GBM cells have not been fully elucidated. In this study, we investigated the anti-GBM effects of TG100-115 in U251 glioma cells. Cell viability and proliferation were assessed using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay, whereas cell motility and invasiveness were determined through wound healing and transwell assays, respectively. Western blotting was used to detect the expression of key proteins involved in the apoptotic and molecular signaling pathways. Our findings revealed that TG100-115 significantly diminished the viability of U251 cells by promoting apoptosis while concurrently inhibiting the migratory and invasive activities of GBM cells. Mechanistically, TG100-115 enhanced apoptotic signaling by modulating B-cell lymphoma 2 (Bcl-2), Bcl-2-associated X protein, and cleaved caspase-3 levels. It also altered the phosphorylation status of protein kinase B and cofilin – both critical for cell survival and cytoskeletal dynamics. In conclusion, these findings suggest that TG100-115, by targeting TRPM7 kinase, exhibits promising therapeutic potential for GBM treatment and provides novel insights into targeting TRPM7-associated pathways in aggressive brain tumors.

Keywords: Transient receptor potential melastatin 7 kinase; TG100-115; Glioblastoma; Proliferation; Migration; Invasion

1. Introduction

Glioblastomas (GBMs) are characterized by aggressive growth and invasiveness, significantly affecting patient survival, neurological function, psychological well-being, and overall quality of life.[1] Despite advancements in therapeutic strategies combining surgery, temozolomide chemotherapy, and radiotherapy, GBMs are associated with a poor prognosis, with a median survival of 14.6 months after diagnosis.[2] The ongoing challenges pertaining to drug resistance, the blood–brain barrier, and tumor heterogeneity highlight the need for new therapeutic approaches. Genomic analyses of gliomas have revealed a wide range of deregulated genes with somatic mutations, including *TERT*, *TP53*, *IDH1*, *ATRX*, and *TTN*, among others, providing important insights into glioma pathogenesis.[3-5] Building on these findings, recent studies have advanced the development of therapies targeting critical signaling pathways, including the epidermal growth factor receptor, phosphoinositide 3-kinase (PI3K)/protein kinase B (Akt)/mammalian target of rapamycin (mTOR) axis, and vascular endothelial growth factor, along with the exploration of immune checkpoint inhibitors.[6] However, the lack of effective treatment options underscores the urgent need for deeper exploration of the molecular mechanisms of GBM to discover novel therapeutic targets.

Transient receptor potential melastatin 7 (TRPM7) is a non-selective divalent cation channel that facilitates the influx of ions such as calcium, magnesium, and zinc. TRPM7 contains an unusual α-kinase domain within its C-terminal region, enabling its dual function as both an ion channel and a kinase.[7] TRPM7 is widely expressed in various tissues and has been implicated in the progression of multiple malignancies, including pancreatic,[8] breast,[9] gastric,[10] and nasopharyngeal cancers,[11] as well as GBM.[12] Accumulating evidence indicates that TRPM7 plays a pivotal role in the progression of GBM. Inhibition of TRPM7 using compounds, such as carvacrol and waixenicin A, has been shown to suppress GBM cell proliferation, migration, and invasion both *in vitro* and *in vivo* while inducing apoptosis in GBM cells.[12,13] Conversely, pharmacological activation of TRPM7 using naltriben enhances the migratory and invasive capabilities of GBM cells, highlighting its pro-tumorigenic function.[14] Mechanistically, TRPM7 regulates key oncogenic pathways, including the PI3K/Akt and mitogen-activated protein kinase (MAPK) kinase (MEK)/extracellular signal-regulated kinase (ERK)[12,13,15,16] pathways, and modulates the expression of matrix metalloproteinase 2, contributing to tumor cell invasion.[17] Furthermore, TRPM7 has been implicated in the maintenance of glioma stem-like cells through signal transducer and activator of transcription 3 and Notch signaling, and is activated by prostaglandin E2 via the prostaglandin E receptor 3/protein kinase A pathway to promote GBM cell proliferation and motility.[18] These findings collectively underscore TRPM7 as a critical regulator of GBM malignancy and a potential therapeutic target. However, the relative contribution of the kinase domain to GBM regulation, as compared to that of the ion channel, remains insufficiently defined.

The kinase domain of TRPM7 retains functional distinctiveness, with unique implications in human cancer pathogenesis, and mediates tumorigenic processes through the phosphorylation of specific substrates.[19] In contrast to the ion channel domain, which primarily mediates cell proliferation, the kinase domain is specifically required for cytoskeletal regulation and metastatic behavior. Notably, deletion or inactivation of this domain significantly reduces migratory and invasive capacities without affecting proliferation.[20,21] Therefore, selectively inhibiting the TRPM7 kinase domain may offer a therapeutic strategy to suppress cancer metastasis while minimizing effects on normal cell growth.

TG100-115, identified for its potent inhibition of TRPM7 kinase in 2017, demonstrates a potency surpassing the previously known TRPM7 kinase inhibitor, rottlerin, by over 70-fold.[22] In addition, TG100-115 significantly inhibits the migratory and invasive abilities of breast cancer cells.[22] TG100-115 was initially characterized as a potent inhibitor of PI3K, exhibiting selective affinity for the PI3K-γ and PI3K-δ isoforms. In rigorous animal models of myocardial infarction, TG100-115 demonstrated significant cardioprotective effects, reducing infarct size and preserving myocardial function.[23] At present, TG100-115 is the only potent inhibitor known to selectively target the TRPM7 kinase domain, offering valuable insights into the kinase-specific pathophysiological role of TRPM7 in GBM. Such inhibitors serve as important pharmacological tools for investigating the biological functions mediated by TRPM7 kinase activity.

In this study, we investigated the impact of TG100-115 on the proliferation, migration, and invasion of the GBM cell line U251. Furthermore, we explored its effects on apoptosis and potential molecular mechanisms, aiming to provide insights into novel therapeutic strategies for GBM treatment.

2. Materials and methods

2.1. Cell culture

Human GBM cell line U251 was obtained from the American Type Culture Collection (United States of America [USA]). Cell culture procedures followed

protocols previously described in earlier studies.[13,17] Briefly, U251 cells were cultured in 60 mm dishes with Dulbecco's Modified Eagle Medium (Gibco, USA), supplemented with 10% fetal bovine serum (Gibco, USA) and antibiotics (100 U/mL penicillin and streptomycin; Gibco, USA), under standard conditions of 5% carbon dioxide and 95% humidity at 37°C.

2.2. Cell viability and proliferation assay

U251 cells were plated onto 96-well plates at a density of 3,000 cells per well, with 100 μL of culture medium. After 24 h of attachment, the cells were subjected to another 24-h treatment with either dimethyl sulfoxide (DMSO; 0.1%, vehicle control) or TG100-115 at various concentrations (30, 60, 120, 180, and 240 μM). Subsequently, 10 μL of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) reagent (5 mg/mL) was added to each well, and the plates were incubated for 3 h at 37°C with 5% carbon dioxide. Throughout the incubation period, mitochondrial enzymes converted the yellow MTT into insoluble purple formazan. Following this, the medium was aspirated, and the formazan was dissolved in 100 μL of DMSO. Cell viability was assessed using a microplate reader (Synergy H1, Biotek, USA) by measuring absorbance at 490 nm, which corresponds to the formazan content. The cell viability was then calculated as a percentage relative to the control.

2.3. Calculation of half-maximal inhibitory concentration (IC_{50}) values

The half-maximal IC_{50} values of TG100-115 following 24-h incubation in U251 cells were calculated using non-linear regression analysis in GraphPad Prism 10. Log-transformed concentrations of TG100-115 and the corresponding cell viability data were fitted to a sigmoidal dose-response curve (with variable slope) to determine the IC_{50} value.[24]

2.4. Cell migration assay

Cell migration was measured using a wound healing assay, following established protocols.[12,13,17] Briefly, U251 cells were cultured in 24-well plates at a density of 1×10^5/mL per well. A monolayer of cells was scratched with a sterile 200 μL pipette tip to create a wound gap. After rinsing the wells with phosphate-buffered saline to remove detached cells, cultures were treated with either DMSO (0.1%) or TG100-115 at various time points (24, 48, and 72 h) or various concentrations. For each well, images from four different fields were captured using a digital camera connected to an Olympus phase-contrast microscope (×10 objective; CKX41, Olympus, Japan). The wound gap was quantified using the wound healing tool in NIH ImageJ (USA), and wound closure was determined as outlined in prior protocols.[12,13,17]

2.5. Matrigel invasion assay

The transwell assay was performed following the manufacturer's protocol. To evaluate the invasive capacity of U251 cells, BioCoat Matrigel invasion chambers equipped with 8-μm polycarbonate nucleopore filters (Cat. 354480, BD BioSciences, USA) were utilized. After being treated with DMSO (0.1%) or TG100-115 (150 μM) for 24 h, 100 μL of cells (2.5×10^4 cells/mL) in serum-free Dulbecco's Modified Eagle Medium were added to the upper compartment of the chamber. As a chemoattractant, 600 μL of complete medium was added to the lower chamber. Cells that invaded through the Matrigel-coated membrane to the underside of the insert were subsequently fixed with absolute ethanol and stained using 1% toluidine blue. Representative fields were imaged under a phase-contrast microscope (CKX41, Olympus, Japan), and the number of invading cells was quantified using NIH ImageJ (USA) via the cell counting module.

2.6. Western blotting

U251 cells were cultured in 35 mm dishes and grown until reaching approximately 60% confluency. Cells were then treated with either 0.1% DMSO or 150 μM TG100-115 in fresh culture medium and incubated for 24 h. Following treatment, cells were rinsed twice with ice-cold phosphate-buffered saline to remove the residual serum. Western blotting analysis was performed as previously described.[12,13,17] After blocking, the membranes were incubated overnight at 4°C with primary antibodies: Anti-cleaved caspase-3 (1:1,000; 9661S, CST, USA), anti-B-cell lymphoma 2 (Bcl-2; 1:1,000; 3498S, CST, USA), anti-B-cell lymphoma 2-associated X protein (Bax; 1:1,000; 2772S, CST, USA), anti-phosphorylated-Akt (p-Akt; 1:1,000; 9271S, CST, USA), anti-Akt (t-Akt; 1:1,000; 2920S, CST, USA), anti-phosphorylated-ERK1/2 (p-ERK1/2; 1:1,000; 5726S, CST, USA), anti-ERK1/2 (t-ERK1/2; 1:1,000, 4695S, CST, USA), anti-glyceraldehyde-3-phosphate dehydrogenase (1:5,000; 2118S, CST, USA), anti-phosphorylated-cofilin (p-cofilin; 1:1,000; ab12866, Abcam, USA), anti-cofilin (t-cofilin;, 1:1,000; ab134963, Abcam, USA), and anti-TRPM7 (1:1,000; ab85016, Abcam, USA). On the following day, mouse (1:5,000; 7076S, CST, USA) or rabbit (1:10,000; 7074S, CST, USA) secondary antibodies were applied, respectively. Protein bands were detected using a chemiluminescence reagent system (iBright Imaging System FL1500, Thermo Fisher Scientific, USA). Band intensities were quantified using NIH ImageJ (USA) for densitometric analysis.

2.7. Statistical analysis

All data are presented as means ± standard error of the mean. Student's *t*-test was employed to compare the control and treatment groups. One-way analysis of variance, followed by the Newman–Keuls *post hoc* test, was utilized to ascertain statistical significance for multiple comparisons. $p<0.05$ was considered to indicate a statistically significant difference.

3. Results

3.1. TG100-115 reduced U251 cell viability and inhibited cell proliferation

We assessed the impact of TG100-115 on U251 cell viability and proliferation through an MTT assay. As shown in Figure 1A and B, our findings show that the 24-h treatment with TG100-115 notably decreased U251 cell viability in a dose-dependent manner ($p<0.001$, $n = 4$), with an IC_{50} of 155.2 μM. The cell proliferation over time is illustrated in Figure 1C. In the control group, cell proliferation increased progressively over the culture period, reaching 108.0 ± 4.4%, 255.9 ± 5.7%, and 383.8 ± 5.4% at 24, 48, and 72 h, respectively. In contrast, treatment with TG100-115 (30 – 240 μM) significantly inhibited U251 cell proliferation at 24, 48, and 72 h, compared to the control group ($p<0.001$, $n = 3$). In Figure S1, numerous U251 cell colonies were observed in the control group 7 days after seeding in six-well plates, as indicated by the crystal violet staining. Treatment with TG100-115 (50 μM) resulted in a significant reduction in U251 cell colony formation to 47.0 ± 4.3%, compared to that observed in controls, 100.0 ± 5.1% ($p<0.01$, $n = 3$). These consistent findings across multiple assays and time points provided strong evidence that TG100-115 effectively inhibited U251 cell viability and proliferation.

3.2. TG100-115 induced apoptosis in U251 cells

We further investigated whether TG100-115 reduces the viability of U251 cells by promoting apoptosis. Cell images were captured 24 h after treatment with TG100-115 (30 –

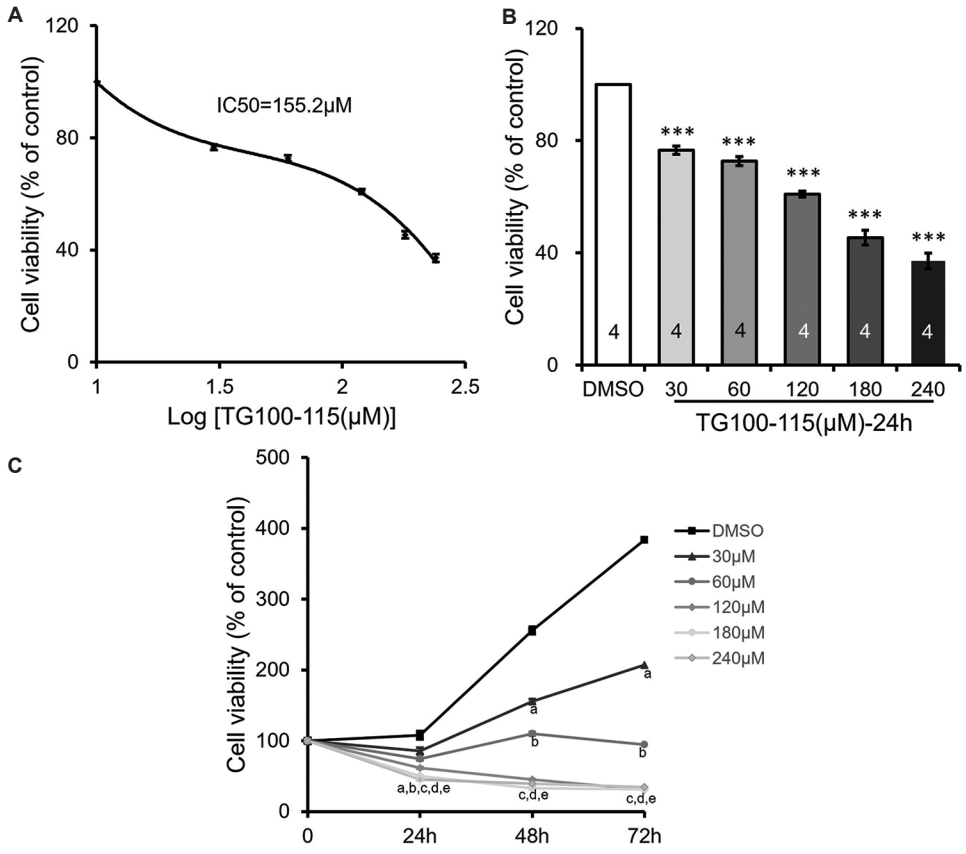

Figure 1. TG100-115 reduced the cell viability and proliferation of U251 cells. (A and B) U251 cells were treated with TG100-115 from 30 μM to 240 μM for 24 h. An MTT assay was used to evaluate the cell viability, and IC_{50} was calculated ($n = 4$). TG100-115 (30 – 240 μM) significantly inhibited U251 cell viability after 24 h (***$p<0.001$ versus DMSO; one-way analysis of variance with subsequent Newman–Keuls test, $n = 4$). (C) TG100-115 inhibited the proliferation of U251 cells. U251 cells were treated with TG100-115 from 30 μM to 240 μM for 24, 48, and 72 h, then an MTT assay was used to measure the proliferation (a, b, c, d, and e represent 30, 60, 120, 180, and 240 μM TG100-115 versus DMSO, respectively, $p<0.001$, $n = 3$).
Abbreviations: DMSO: Dimethyl sulfoxide; MTT: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide.

240 μM), revealing significant damage and the presence of visualized vacuoles when compared to the control group (Figure 2A, $n = 4$). This suggests that TG100-115 treatment resulted in morphological changes in the U251 cells.

To assess apoptosis, we conducted western blot analysis of cleaved caspase-3, Bcl-2, and Bax. The representative images are shown in Figure 2B, the raw blot images are provided in Figures S5–8. Cleaved caspase-3, an active form of the apoptosis-related cysteine peptidase,[25] showed a significantly higher ratio in TG100-115 (150 μM)-treated cells (34.5 ± 2.6%) compared to the control group (6.4 ± 0.7%, $p<0.01$, $n = 4$), as demonstrated in Figure 2C. Bcl-2 and Bax, pivotal regulatory proteins in apoptosis,[26] were also analyzed. Bcl-2, an anti-apoptotic protein, exhibited a significantly lower ratio in TG100-115 (150 μM)-treated cells (14.2 ± 5.3%) compared to the control group (48.5 ± 3.8%, $p<0.05$, $n = 4$), as shown in Figure 2D. Conversely, Figure 2E illustrates a significant increase in Bax level (97.4 ± 13.3%) after treatment with TG100-115 (150 μM) for 24 h, compared to the control group (45.7 ± 9.3%, $p<0.05$, $n = 4$). These findings collectively suggest that TG100-115 induces apoptosis in U251 cells.

3.3. TG100-115 suppressed U251 cell migration

To evaluate the impact of TG100-115 on the migratory behavior of U251 cells, a wound healing assay was performed. In Figure 3, cell images were captured at 0, 4, 8, 16, and 24 h following treatment with either DMSO (0.1%) or TG100-115 (150 μM), and the wound closure rate was analyzed. At 4, 8, 16, and 24 h, the control group exhibited wound closure rates of 14.8 ± 1.4%, 37.4 ± 1.4%, 48.3 ± 1.8%, and 66.1 ± 2.6%, respectively, all of which were notably faster than the TG100-115-treated group at the corresponding time points: 8.6 ± 1.2%, 20.2 ± 2.3%, 28.3 ± 3.4%, and 41.5 ± 2.2% ($p<0.01$, $p<0.001$, $n \geq 6$). This result suggests a significant and time-dependent reduction in the migratory capability of U251 cells treated with TG100-115 when compared to the control group. In Figure 4, we further investigated the migration of U251 cells with various doses of TG100-115 (60, 120, 180 μM) for 24 h, using DMSO (0.1%) as the control. The wound closure rate in the control group was 66.1 ± 2.6%, while in the treatment groups, it was significantly reduced to 48.8 ± 3.7%, 43.2 ± 3.7%, and 37.4 ± 1.4%, respectively ($p<0.01$, $p<0.001$, $n \geq 6$). This result indicates a significant reduction in the migration of U251 cells treated with TG100-115 compared to the control across different doses.

3.4. TG100-115 inhibited U251 cell invasion

The Matrigel transwell assay was utilized to assess U251 cell invasion. As depicted in Figure 5, U251 cell invasion was significantly reduced after 24-h exposure to TG100-115 at 150 μM. The relative invasiveness decreased from 100.0 ± 8.8% to 53.8 ± 3.8% with TG100-115 treatment ($p<0.01$, $n = 6$). This result indicates that, *in vitro*, TG100-115 diminishes the invasion of U251 cells.

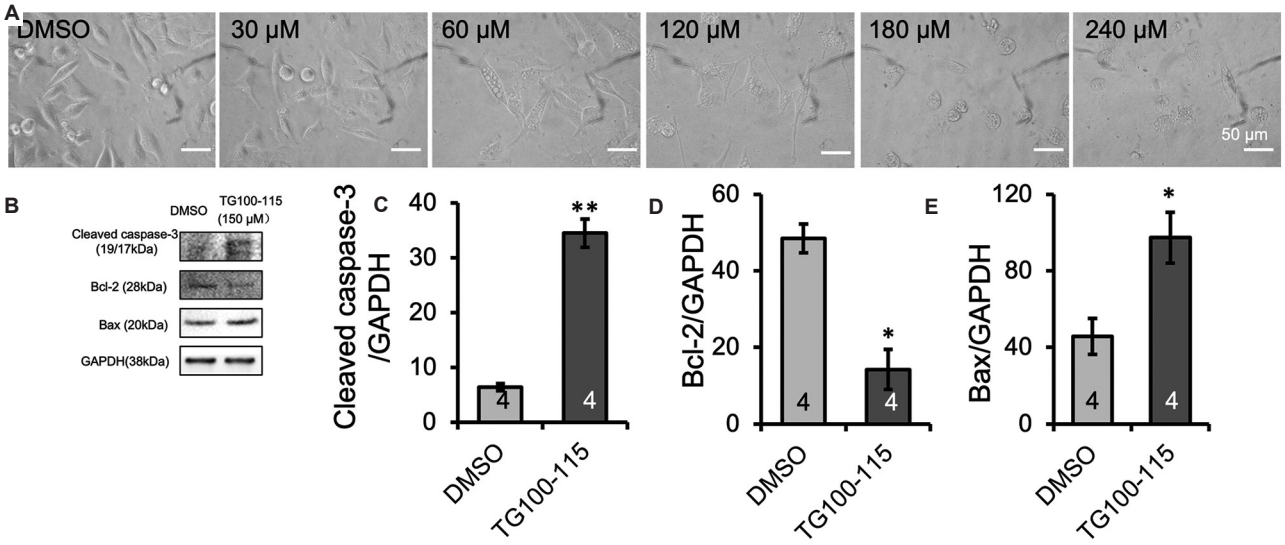

Figure 2. TG100-115 induced apoptosis in U251 cells. (A) Representative images of U251 cells with or without TG100-115 (30 – 240 μM) treatment for 24 h. Scale bar: 50 μm, magnification ×20. U251 cells treated with TG100-115 significantly showed cell damage and the presence of vacuoles when compared to the control group. (B) Representative images of western blotting results. (C) TG100-115 significantly increased the ratio of cleaved caspase-3/GAPDH. (D) TG100-115 significantly reduced the ratio of Bcl-2/GAPDH. (E) TG100-115 significantly increased the ratio of Bax/GAPDH.
Note: Statistical significance determined at *$p<0.05$ and **$p<0.01$ using Student's *t*-test, $n = 4$.
Abbreviations: Bax: B-cell lymphoma 2-associated X protein; Bcl-2: B-cell lymphoma 2; DMSO: Dimethyl sulfoxide; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase.

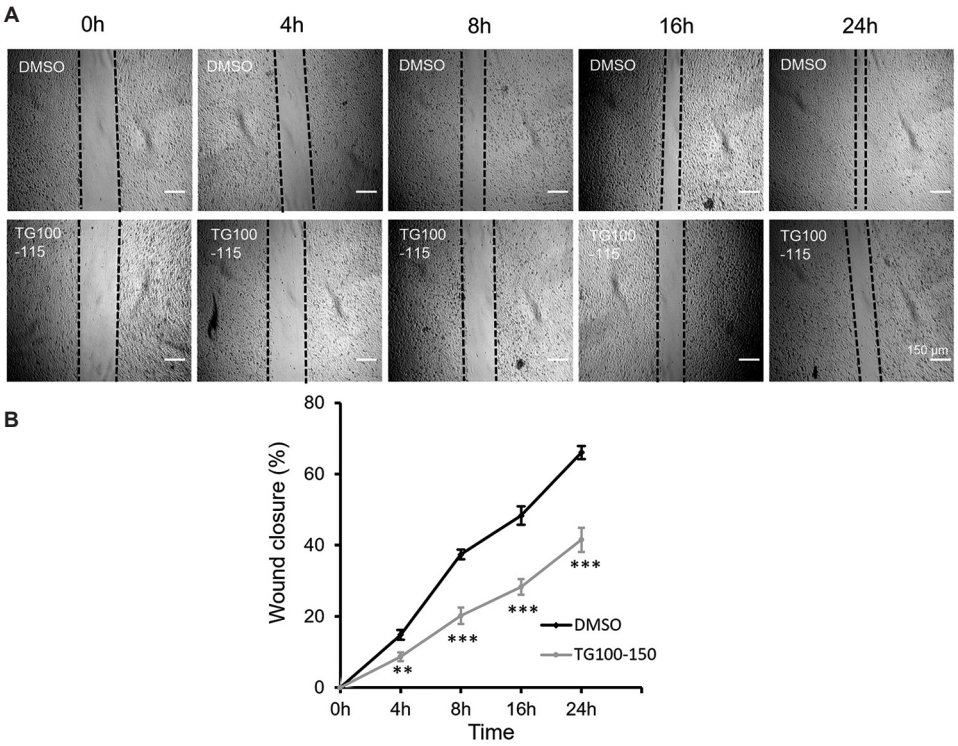

Figure 3. TG100-115 inhibited the migration of U251 in a time-dependent manner. (A) Representative images of wound healing. After being scratched with a 200 μL pipette tip, U251 cells were treated with TG100-115 (150 μM) or 0.1% DMSO. Then, the images were captured at 0, 4, 8, 16, and 24 h, and the gap closure was analyzed. Scale bar: 150 μm, magnification: ×10. (B) The wound closure of the TG100-115 treatment groups was significantly decreased compared to DMSO at the corresponding time points.
Note: Statistical significance determined at $^{**}p<0.01$ and $^{***}p<0.001$ versus DMSO using one-way analysis of variance with subsequent Newman–Keuls test, $n \geq 6$.
Abbreviation: DMSO: Dimethyl sulfoxide.

3.5. TG100-115 downregulated PI3K/Akt signaling

The PI3K/Akt and MAPK/ERK signaling pathways play key roles in regulating the proliferation, migration, and invasion of GBM cells.[16,27,28] Consequently, we explored the phosphorylation status of Akt and ERK1/2. In Figure 6A, TG100-115 (150 μM) markedly reduced p-Akt protein levels in U251 cells while leaving t-Akt protein levels unchanged, the raw blot images are provided in Figures S9-10. In Figure 6B, the p-Akt/t-Akt ratio was significantly reduced in TG100-115-treated cells (4.1 ± 1.1%) compared to the control group (21.1 ± 1.8%, $p<0.001$, $n = 4$). This indicates that TG100-115 treatment led to a notable decrease in the p-Akt/Akt ratio, suggesting a potential impact on the activity of the PI3K/Akt signaling pathway.

Subsequently, the protein levels of p-ERK1/2 and t-ERK1/2 were assessed. In cells treated with TG100-115 (150 μM), there was no significant alteration observed in the protein levels of both p-ERK1/2 and t-ERK1/2 compared to the TG100-115 group. Quantification of the p-ERK1/2/t-ERK1/2 ratio revealed no significant difference in TG100-115-treated cells (120.7 ± 6.7%) when compared to the control group (138.2 ± 10.1%, $p>0.05$, $n = 4$), as illustrated in Figure 6D, the raw blot images are provided in Figures S11-12. These findings suggest that TG100-115 did not affect the MAPK/ERK signaling pathway.

3.6. TG100-115 upregulated phosphorylation of cofilin

Cofilin, an actin-interacting protein capable of breaking down actin filaments, undergoes regulatory control through phosphorylation, leading to its inactivation. This dynamic regulation is essential for the proper functioning of cellular processes involving cytoskeletal restructuring and cellular motility.[29] Here, we investigated the effect of TG100-115 on cofilin regulation. In Figure 6A, the result revealed that TG100-115 treatment (150 μM) for 24 h increased the phosphorylation level of cofilin, with no change in total cofilin (t-cofilin), the raw blot images are provided in Figures S13-14. In Figure 6D, the ratio of p-cofilin/t-cofilin was significantly higher in TG100-115-treated cells (241.5 ± 22.8%) compared to the control group (34.7 ± 18.0%, $p<0.01$, $n = 4$). This indicates that TG100-115 influences the phosphorylation status of cofilin, shedding

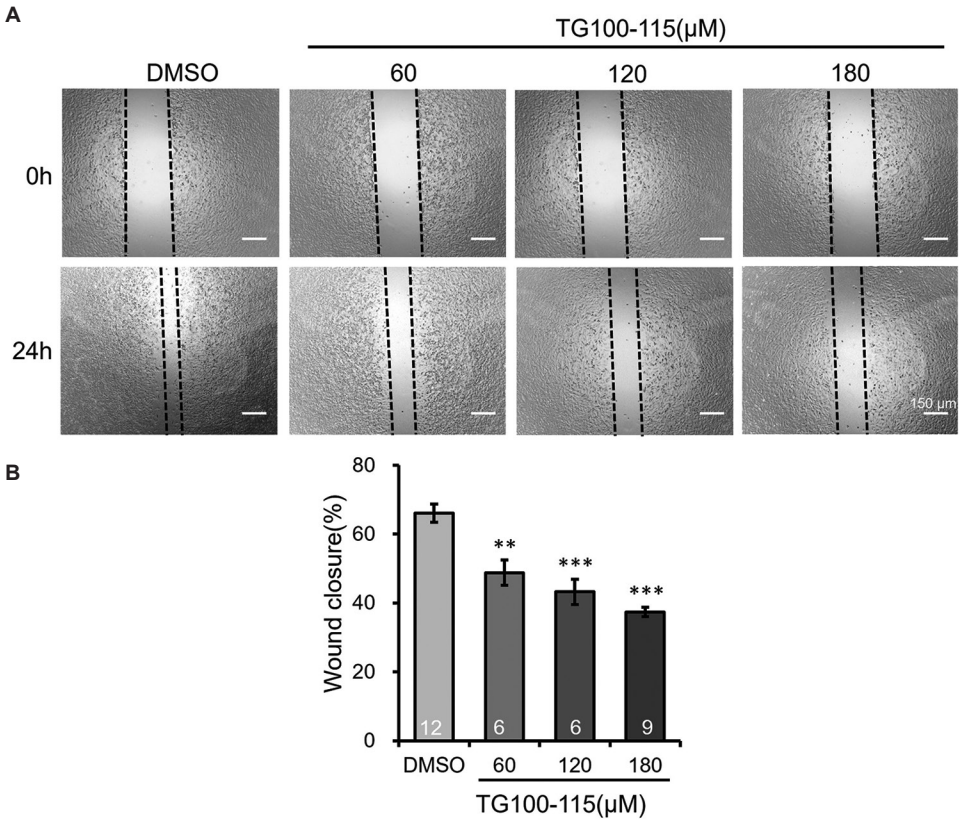

Figure 4. TG100-115 inhibited the migration of U251 cells at different doses for 24 h. (A) Representative images of wound healing. After being scratched with a 200 μL pipette tip, U251 cells were treated with TG100-115 at doses of 60, 120, 160 μM or 0.1% DMSO. Then, the images were captured at 24 h, and the gap closure was analyzed. Scale bar: 150 μm, magnification: 10×. (B) The wound closure of TG100-115 treatment at different doses was significantly decreased compared with DMSO.
Note: Statistical significance determined at $^{**}p<0.01$ and $^{***}p<0.001$ using one-way analysis of variance with subsequent Newman–Keuls test, $n \geq 6$.
Abbreviation: DMSO: Dimethyl sulfoxide.

light on its potential role in cellular processes associated with migration and invasion.

3.7. TG100-115 did not affect the protein expression of TRPM7

Finally, we investigated the impact of TG100-115 treatment on the protein expression of TRPM7 in U251 cells. Following a 24-h exposure to either DMSO (0.1%) or TG100-115 (150 μM), we assessed TRPM7 expression through western immunoblotting. As shown in Figure S2, the raw blot images are provided in Figure S16, TG100-115 exhibited no significant influence on the protein expression of TRPM7 when compared to the control group (19.1 ± 3.8% vs. 20.3 ± 4.6%, respectively, $p>0.05$, $n = 4$). This suggests that TG100-115 did not markedly influence the protein expression of TRPM7 under the conditions examined in U251 cells.

4. Discussion

This study demonstrates the role of TG100-115 in GBM cell biological functions. Our results showed that TG100-115 significantly reduced the viability and proliferation of U251 cells in a dose-dependent manner, with an IC_{50} of 155.2 μM. In addition, TG100-115 exhibited substantial inhibitory effects on U251 cell migration and invasion. Furthermore, we found that the anti-GBM activities of TG100-115 were involved in PI3K/Akt and cofilin-dependent signaling. In this study, experiments were initially performed using both U87 and U251 glioma cell lines, with preliminary data obtained from both models. However, due to variability in experimental conditions and inconsistencies in U87 cell status, only the U251 cell data have been reported here. Nevertheless, the relevant results from U87 cells are included in Figure S3, raw blot images are provided in Figure S17. This data demonstrated that TG100-115 significantly reduced U87 cell viability, migration, colony formation, and Akt phosphorylation, consistent with the findings observed in U251 cells. Thus, the results from both cell lines support the conclusion that TG100-115 exerts anti-GBM effects via similar mechanisms.

Our study showed that TG100-115 attenuated PI3K/Akt signaling. Akt, the central molecule of PI3K/Akt signaling, undergoes activation by phosphorylation, promoting tumor cell survival, proliferation, migration, invasion, and treatment resistance.[30] Recently, Akt was identified as a direct substrate of TG100-115,[31] and kinase-deficient murine neutrophils confirmed the essential role of TRPM7 kinase in neutrophil function via Akt/mTOR signaling.[32] Our findings exhibited that TG100-115 markedly reduced Akt phosphorylation. It should be noted that TG100-115 was previously recognized as a potent PI3K inhibitor, particularly targeting the PI3K-γ and δ isoforms.[22,23,32] We performed MTT and wound-healing assays using PIK-294, a selective blocker of PI3K p110δ. A PI3K p110δ inhibitor was specifically used because GBMs express a low level of PI3K p110γ.[33] In Figure S4A, our data revealed that PIK-294 significantly reduced the viability of U251 cells at higher doses (120 and 180 μM), but not at lower doses (10, 30, and 60 μM), with 24-h incubation. In contrast, TG100-115 significantly reduced cell viability even at a lower dose of 30 μM after 24 h of incubation. This data suggests that the PI3K inhibitory activity of TG100-115 could partially affect the reduction of cell viability.

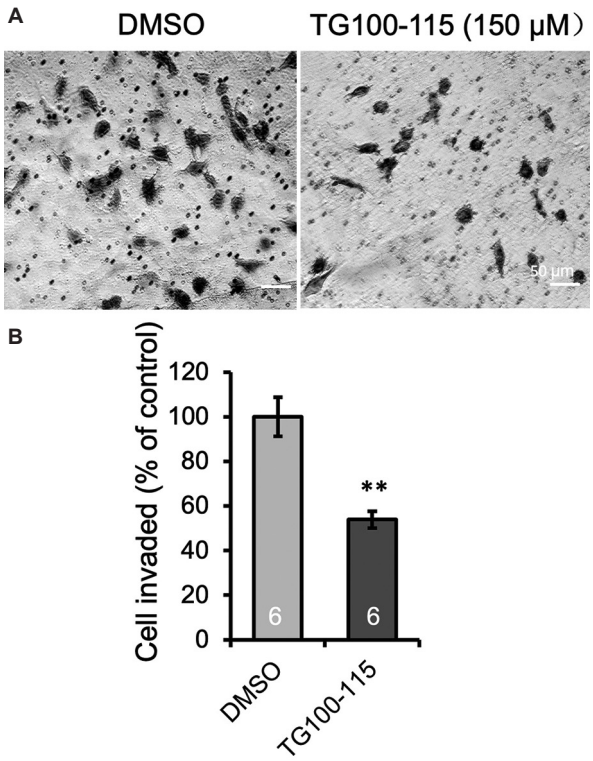

Figure 5. TG100-115 inhibited U251 cell invasion. (A) The representative images are from transwell experiments to detect *in vitro* cell invasion. Scale bar: 50 μm, magnification: ×10. (B) The invasion of TG100-115-treated cells (150 μM) was significantly decreased compared with DMSO (**p<0.01, Student's t-test, n = 6).
Abbreviations: DMSO: Dimethyl sulfoxide.

Previous studies have indicated that PI3K p110-δ is essential for glioma cell migration and invasion. CAL-101, a PI3K p110δ-specific inhibitor, has been shown to moderately reduce GBM cell proliferation and migration without significantly affecting tumor growth in GBM xenograft mouse models.[34] In our experiment, another PI3K p110δ-specific inhibitor, PIK-294, did not significantly influence the migration of U251 cells at concentrations of 60 and 120 μM (Figure S4B), likely due to the lower doses and different cell lines used. In contrast, TG100-115 significantly reduced migration at the same doses (Figure 2). This suggests that the anti-migration effect of TG100-115 on U251 cells may be mediated through its

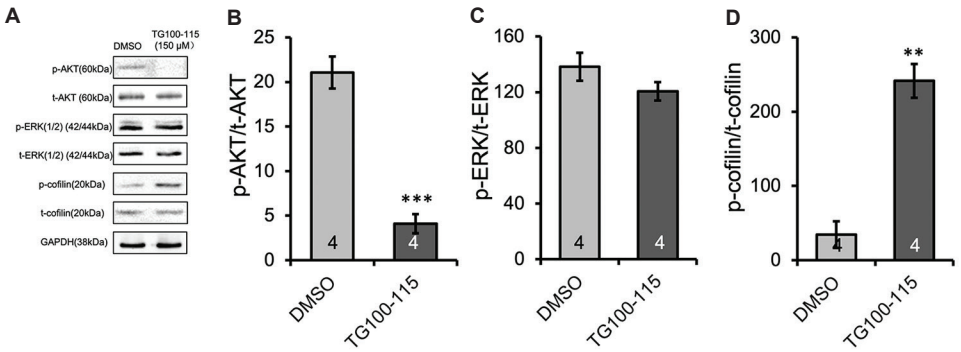

Figure 6. The underlying molecular mechanisms are mediated by TG100-115 in U251 cells. U251 cells were treated with TG100-115 (150 μm) for 24 h, and the protein expression was detected by western blotting. (A) Representative images of western blotting results. (B) TG100-115 significantly reduced the ratio of p-Akt/t-Akt. (C) TG100-115 did not change the ratio of p-ERK/t-ERK (p>0.05). (D) TG100-115 significantly increased the ratio of p-cofilin/t-cofilin.
Note: Statistical significance determined at **p<0.01 and ***p<0.001 using Student's t-test, n = 4.
Abbreviations: Akt: Protein kinase B; DMSO: Dimethyl sulfoxide; ERK: Extracellular signal-regulated kinase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase; p: Phosphorylated; t: Total.

inhibition of TRPM7 kinase, rather than PI3K blockade. Further studies using small interfering RNA targeting TRPM7 or kinase-mutated cell lines are needed to clarify the inhibitory activity of TG100-115 on TRPM7 kinase.

The MAPK/ERK pathway contributes to cancer progression and treatment resistance.[35] ERK1/2 is activated by MEK phosphorylation and phosphorylates transcription factors, influences gene expression, and promotes cancer, alongside GBM cell proliferation and invasion.[36] We previously reported that TRPM7 downregulation reduced the phosphorylation of ERK1/2.[12] However, we observed that TG100-115 showed no significant effect on ERK1/2, suggesting that TRPM7 regulates MAPK/ERK signaling potentially through its channel domain, but not its kinase domain. This is worthy of further investigation.

Bcl-2, an anti-apoptotic molecule, is overexpressed in GBM[37] and other types of cancer,[38] enhancing resistance to cytotoxic treatments.[39] Conversely, Bax, a pro-apoptotic Bcl-2 family member, when released from Bcl-2 inhibition through pharmacological means, promotes apoptosis in GBM cells both *in vitro* and *in vivo*.[26] Caspase-3 activation by cleavage promotes apoptosis.[40] Caspase-3 activity is modulated by the Bcl-2/Bax balance, where Bax leads to the release of cytochrome c and a subsequent cascade of caspase activations.[13,17] We found that TG100-115 diminished Bcl-2 expression and augmented Bax expression, while also increasing cleaved caspase-3 expression. Our results demonstrate that TG100-115 promotes GBM cell apoptosis through the Bcl-2/Bax/caspase-3 pathway, likely contributing to its inhibitory effects on GBM cell viability.

Cofilin is crucial for cell motility, particularly in tumor cell migration and invasion.[29,41] Its phosphorylated state suppresses activity, affecting actin filament dynamics and cellular movement, thus underscoring its importance in cancer metastasis.[29,42] Park *et al.*[43] have highlighted cofilin upregulation in human GBM tissues and its potential as a therapeutic target, with its inhibition reducing glioma cell motility.[43] Our laboratory has shown that TRPM7 inhibitors decreased cofilin activity, hindering GBM cell invasion and migration.[12,13] Consistently, this study also showed that TG100-115 increased cofilin phosphorylation. Intriguingly, recent research indicates that the TRPM7 kinase domain interacts with cofilin in neuroblastoma cells and mouse brains.[44,45] Cofilin phosphorylation can also be regulated by the Akt/mTOR pathway, influencing melanoma cell behaviors, which suggests cofilin as a potential downstream effector of Akt signaling.[46] Therefore, TRPM7 kinase may modulate cofilin activity through Akt-dependent signaling, affecting GBM migration and invasion.

TG100-115 was previously identified as a significantly more potent inhibitor of the TRPM7 kinase than other recognized inhibitors (exhibiting an IC_{50} of 1.07 ± 0.14 μM), as demonstrated by utilizing the cyclic adenosine monophosphate response element-binding protein peptide as an *in vitro* substrate and adopting an assay system for kinase inhibitory compounds.[22] Despite the demonstrated capability of TG100-115 to reversibly inhibit TRPM7-like currents, this effect required significantly higher concentrations, specifically 100 μM.[22] Interestingly, our study concluded that TG100-115 does not alter the protein expression of TRPM7, indicating that its inhibitory action is primarily linked to the kinase domain, rather than affecting the overall expression of the TRPM7 channel-kinase. Such findings highlight the inhibitory potency of TG100-115 in targeting the kinase-specific functions of TRPM7, making it a valuable tool for studying the functional implications of TRPM7 kinase in GBMs.

5. Conclusion

In summary, this study provides compelling *in vitro* evidence for the potential therapeutic efficacy of TG100-115 in GBMs. The potential molecular mechanism underlying the inhibitory effects of TG100-115 on cell viability, proliferation, migration, and invasion involves blocking TRPM7 kinase, thus altering multiple signaling pathways, including Bcl-2/Bax/caspase-3, Akt, and cofilin. These findings collectively position TG100-115 as a promising candidate for further exploration in preclinical and clinical studies.

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Conflict of interest

Hong-Shuo Sun is an Editorial Board Member, and Zhong-Ping Feng serves as an Associate Editor for this journal, but they were not in any way involved in the editorial and peer-review process conducted for this paper, directly or indirectly. Separately, other authors declared that they have no known competing financial interests or personal relationships that could have influenced the work reported in this paper.

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Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data

The data supporting this study can be accessed by submitting a request to the lead or corresponding author.

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ACCSCIENCE PUBLISHING

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SHORT COMMUNICATION

Alzheimer´s disease and related dementia as a component of a multiorgan senescence syndrome

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Abstract

Patients with Alzheimer's disease and related dementia (ADRD) have a higher risk for comorbidities than non-cognitively impaired age-matched individuals. Because ADRD is an age-related disorder, it was hypothesized that younger patients with ADRD have a lower prevalence of comorbidities than their older counterparts. To test this hypothesis, the following four patient cohorts were defined in the TriNetX Analytics Network database based on the presence or absence of International Classification of Diseases, Tenth Revision (ICD-10) codes G30 (Alzheimer's disease), and/or F01 (dementia in other diseases classified elsewhere) in their health care records who had health care visits from 2021 to 2023: individuals aged 65 – 80 years with and without ADRD diagnosis and individuals aged ≥90 years with and without ADRD diagnosis. Patients with ADRD in both age groups had a higher prevalence of comorbidities in almost all ICD-10 chapters than age-matched non-ADRD individuals. The younger ADRD cohort showed a comorbidity pattern that was significantly different than that of their age-matched cohort ($p < 0.0001$); however, it was not statistically different than the comorbidity pattern of the older ADRD cohort ($p = 0.80$). Similarly, the younger non-ADRD cohort showed a comorbidity pattern that was not statistically different than that of the older non-ADRD cohort ($p = 0.28$). These results indicated that ADRD diagnosis is associated with coincident multiorgan dysfunction in a pattern that is almost identical between the two different age groups. These data also suggested that cognitive impairment associated with ADRD is only a single component of a multiorgan senescence syndrome. Overall, this study revealed that optimizing the health care management of non-cognitive organ dysfunction in patients with ADRD may improve their overall health and, thereby, delay the progression of cognitive impairment.

Keywords: Alzheimer's disease; Dementia; Comorbidities; Multiorgan; Senescence; Syndrome

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1. Introduction

Alzheimer's disease and related dementia (ADRD) is a progressive neurodegenerative condition that can lead to a significant decline in cognitive function. Over the past few decades, extensive research has focused on potential therapies aimed at delaying or stopping the progression of cognitive decline associated with ADRD. Although treatments

that can significantly reduce beta-amyloid levels in the brain are available, clinically significant prevention or delaying of cognitive decline in patients with ADRD remains elusive.

Furthermore, ADRD has been reported to be associated with multiple comorbidities[1,2] using a variety of data sources, such as hospital discharge records,[3] medical claims data,[4,5] population-based studies,[6,7] and registries.[8-10] Moreover, studies have reported on the number and type of comorbidities that significantly impact the progression of cognitive decline, quality of life, and longevity of patients with AD.[8,11-14] However, as the prevalence of ADRD is age-related, it has generally been considered that ADRD-associated comorbidities are due to the aging process that differentially and independently affects multiple organ systems and not due to a common underlying pathophysiological process across organ systems.

The present study aimed to test the hypothesis that the comorbidity burden of older patients with ADRD (defined as 90+ years of age) is higher than that of younger patients with ADRD (defined as 65 – 80 years of age). Therefore, the relative prevalence of multiple medical disorders was evaluated in the following four specific cohorts using real-world data (RWD): individuals aged 90+ years with and without ADRD and individuals aged 65 – 80 years with and without ADRD.

2. Methods

2.1. Analysis using the TriNetX analytics network

The TriNetX Analytics Network contains RWD from anonymized electronic health records of more than 150 million Americans who have received care at over 80 health care organizations (HCOs) in the United States. This database contains information on signs, symptoms, diagnoses, demographics, laboratory data, procedures, and medications based on the International Statistical Classification of Diseases and Related Health Problems 10th Revision (i.e., International Classification of Diseases and Tenth Revision [ICD-10] codes). The healthcare payers include commercial, Medicaid, Medicare, and VA providers. TriNetX is compliant with the Health Insurance Portability and Accountability Act, the US federal law that protects the privacy and security of health care data, and any additional data privacy regulations applicable to the contributing HCO. Because this study used only deidentified patient records and did not involve the collection, use, or transmittal of individually identifiable data, it was exempted from the Institutional Review Board approval.

2.2. Inclusion and exclusion criteria

The TriNetX database allows for a targeted search of patients with specific characteristics. Four patient cohorts were defined within the TriNetX database using the following inclusion criteria as search terms:

1. United States patients only (150 million plus individuals are in the database)
2. Age 65 – 80 years (inclusive) OR ≥ 90 years
3. Health care visits occurred in 2021 – 2023
4. A diagnosis of AD (ICD-10 code G30) and/or unspecified dementia (ICD-10 code F03) was used to define the patients with ADRD, and the absence of a diagnosis of AD (ICD-10 code G30) or unspecified dementia (ICD-10 code F03) was used to define the non-ADRD patients.

2.3. Data compilation

Datasets were compiled for each of the four above mentioned cohorts. In particular, the prevalence of signs, symptoms, and diagnoses in each of the 22 ICD-10 chapters was extracted from a sampling of a maximum of 10,000 individuals from each of the 80+ HCOs. Scatter plots were generated for a visual comparison of the cohort datasets.

2.4. Statistical analysis

Risk ratios were calculated for cohort comparisons based on the prevalence of ICD-10 codes between cohort groups.

Pearson correlation coefficients were calculated between the data for each cohort comparison using the software available at https://www.socscistatistics.com/tests/pearson/default.aspx (accessed October 9, 2024).

The significance of the difference between two correlation coefficients was evaluated using the software available at https://www.analyticscalculators.com/calculator.aspx?id=104#google_vignette (accessed October 9, 2024).

3. Results

3.1. Demographics of patients aged 65 – and ≥90 years with and without ADRD with health care visits in 2021 – 2023

The TriNetX database contained data for 239,670 individuals aged 65 – 80 years with a diagnosis of AD (ICD-10 code G30) and/or unspecified dementia (ICD-10 code G30) who visited a health care provider in 2021 – 2023 as well as data for 13,071,730 patients without either of these diagnoses in the same age group.

The ≥90-year-old age cohort consisted of 184,060 individuals with a diagnosis of AD (ICD-10 code G30) and/or unspecified dementia (ICD-10 code G30) who visited a health care provider in 2021 – 2023 and 990,390 patients without either of these diagnoses during the same period.

As shown in Table 1, the 65 – 80-year-oldcohort with ADRD had a mean age of 74 years, and the same age range

Table 1. Demographics of individuals in the TriNetX database with and without diagnoses of Alzheimer's disease (ICD-10 code G30) and/or unspecified dementia (ICD-10 code F03) who had health care visits in 2021 – 2023, in the age groups of 65 – 80 and ≥90 years

Demographic characteristics	ADRD (G30 + F03) aged 65 – 80 years	No ADRD (G30 + F03) aged 65 – 80 years	ADRD (G30 + F03) aged 90+ years	No ADRD (G30 + F03) aged 90+ years
	(*n*=239,670)	(*n*=13,071,730)	(*n*=184,060)	(*n*=990, 390)
Mean age (years)	74	72	≥90	≥90
Sex (%)				
Female	54	53	67	60
Male	43	45	30	38
Unknown	2	2	3	2
Race (%)				
White	68	66	69	62
Black or African American	15	11	9	7
Asian	3	3	6	3
Other, mixed, unknown	14	20	16	28
Ethnicity (%)				
Non-Hispanic	74	65	74	63
Hispanic	6	4	4	3
Unknown	21	31	21	34

Note: Demographic data were generated from the TriNetX database using the abovementioned search terms. The database search included data on more than 150 individuals in the United States who visited a health care provider in 2021 – 2023.
Abbreviations: ADRD: Alzheimer's disease and related dementias; ICD-10: International classification of diseases, tenth revision.

cohort without an ADRD diagnosis had a mean age of 72 years. The mean age of the ≥90-year-old cohorts could not be calculated because specific age values >89 years are not provided in the TriNetX database to increase the anonymity of these patients.

Data on sex, race, and ethnicity are provided in Table 1; these data were generally consistent with previous epidemiological data for ADRD.

3.2. ICD-10 chapter code analysis of cohorts

Table 2 shows the prevalence of signs, symptoms, and disorders within each of the 22 major ICD-10 code chapters for each of the four study cohorts. To facilitate the comparisons of the chapter data, each decile of the prevalence data was coded by a different color and/or text bolding, as described in the table legend.

Prevalence rates in the ADRD cohorts were higher than those in the non-ADRD cohorts in 21 of the 22 ICD-10 chapters (there was a 0% prevalence of perinatal disorders in all four cohorts).

3.3. Risk ratios based on the prevalence of signs, symptoms, and disorders in the 22 ICD-10 chapters

Risk ratios were calculated using the prevalence data, as shown in Table 2, for each cohort comparison. As presented in Table 3 (column A), the 65 – 80-year-old individuals with ADRD had risk ratios of ≥2.0 in 6/22 chapters and ≥1.5 in 16/22 chapters than the 65 – 80-year-old individuals without ADRD. Similarly, the ≥90-year-old individuals with ADRD had risk ratios of ≥2.0 in 15/22 chapters and ≥1.5 in 17/22 chapters than the ≥90-year-old individuals without ADRD (Table 3, column B).

Furthermore, the pattern of risk ratios of individuals with and without ADRD in both the 65 – 80-year-old and ≥90-year-old cohorts was almost identical, as shown by scatterplots in Figure 1A and B.

The Pearson correlation coefficients for the data in Figure 1A were 0.88 ($p < 0.00001$) between 65 and 80-year-old individuals with and without ADRD and 0.86 ($p < 0.00001$) between ≥90-year-old individuals with and without ADRD.

Risk ratio comparisons were also performed between the younger and older cohorts with ADRD and without ADRD. As illustrated in Table 3 (column C), the ≥90-year-old ADRD cohort had risk ratios of ≥1.5 in 0/22 chapters compared with those of the 65 – 80-year-old ADRD cohort. Similarly, as presented in Table 3 (column D), the ≥90-year-old non-ADRD cohort had risk ratios of ≥1.5 in 0/22 chapters compared with those of the 65 – 80-year-old

Table 2. Prevalence of signs, symptoms, and diagnoses in ICD-10 chapters

ICD-10 codes	ICD-10 chapter categories	ADRD 65 – 80 visit in 2021 – 2023 (*n*=246,280)	NO ADRD 65–80 visit in 2021 – 2023 (*n*=710,490)	ADRD 90+ visit in 2021 – 2023 (*n*=183,250)	NO ADRD 90+ visit in 2021 – 2023 (*n*=536,110)
F01-F99	Mental, behavioral, and neurodevelopmental disorders	**98**	29	**98**	21
R00-R99	Symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified	**95**	69	**95**	**74**
G00-G99	Diseases of the nervous system	87	**38**	**79**	**35**
I00-I99	Diseases of the circulatory system	85	**57**	**93**	63
E00-E89	Endocrine, nutritional, and metabolic diseases	85	**56**	87	54
Z00-Z99	Factors influencing health status and contact with health services	85	**72**	82	64
M00-M99	Diseases of the musculoskeletal system and connective tissue	**73**	**54**	77	**50**
N00-N99	Diseases of the genitourinary system	68	40	**76**	44
K00-K95	Diseases of the digestive system	66	42	67	40
S00-T88	Injury, poisoning, and certain other consequences of external causes	60	**33**	68	**37**
J00-J99	Diseases of the respiratory system	**59**	**36**	62	**36**
D50-D89	Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism	**50**	22	**56**	29
A00-B99	Certain infectious and parasitic diseases	48	24	**50**	24
L00-L99	Diseases of the skin and subcutaneous tissue	43	27	45	27
V00-Y99	External causes of morbidity	41	**17**	49	23
C00-D49	Neoplasms	**37**	**32**	**35**	**30**
H00-H59	Diseases of the eye and adnexa	**34**	22	**38**	26
H60-H95	Diseases of the ear and mastoid process	25	**15**	**36**	20
U00-U85	Codes for special purposes	**15**	6	**14**	6
Q00-Q99	Congenital malformations, deformations, and chromosomal abnormalities	**10**	6	8	5
O00-O9A	Pregnancy, childbirth, and the puerperium	1	0	1	0
P00-P96	Certain conditions originating in the perinatal period	0	0	0	0

Note: The data provided represent the prevalence (i.e., the percentage of the cohort having at least one ICD-10 code in their medical record) within each of the 22 chapters of the ICD-10 code classification system. To facilitate the visual evaluation of the data, decile color coding was added for the prevalence values as follows:

0 – 9	**10 – 19**	20 – 29	**30 – 39**	40 – 49	**50 – 59**	60 – 69	**70 – 79**	80 – 89	**90 – 100**

Abbreviation: ADRD: Alzheimer's disease and related dementias; ICD-10: International classification of diseases, tenth revision.

non-ADRD cohort. Furthermore, the pattern of risk ratios of individuals in both the younger and older cohorts was almost identical, as depicted by the scatterplots in Figure 1C and D.

The Pearson correlation coefficients were 0.99 ($p < 0.00001$) for the data in Figure 1C between the younger and older ADRD cohorts and 0.98 ($p < 0.00001$) for the data in Figure 1D between the younger and older non-ADRD cohorts.

3.4 Statistical analyses of the comorbidity pattern of the prevalence of signs, symptoms, and disorders in the 22 ICD-10 chapters

The extent to which two correlation coefficients were significantly different from each other was evaluated by computing the z-score for the significance test and *p*-value.

In the comparison of correlation, the 65 – 80-year-old cohorts with and without ADRD (Figure 1A; r (20) = 0.88)

Table 3. Risk ratios of prevalence rates in ICD-10 chapters

Demographic characteristics			Risk ratios			
Color coding	ICD-10 codes	ICD-10 diagnosis categories	ADRD aged 65 – 80 versus No ADRD aged 65 – 80 (A)	ADRD aged 90+ versus No ADRD aged 90+ (B)	ADRD aged 90+ versus ADRD aged 65 – 80 (C)	No ADRD aged 90+ versus No ADRD aged 65 – 80 (D)
	A00-B99	Certain infectious and parasitic diseases	**2.0**	**2.1**	**1.0**	**1.0**
	C00-D49	Neoplasms	**1.2**	**1.2**	**0.9**	**0.9**
	D50-D89	Diseases of the blood and blood-forming organs and certain disorders involving the immune mechanism	**2.3**	**1.9**	**1.1**	**1.3**
	E00-E89	Endocrine, nutritional, and metabolic diseases	**1.5**	**1.6**	**1.0**	**1.0**
	F01-F99	Mental, behavioral, and neurodevelopmental disorders	**3.4**	**4.7**	**1.0**	**0.7**
	G00-G99	Diseases of the nervous system	**2.3**	**2.3**	**0.9**	**0.9**
	H00-H59	Diseases of the eye and adnexa	**1.5**	**1.5**	**1.1**	**1.2**
	H60-H95	Diseases of the ear and mastoid process	**1.7**	**1.8**	**1.4**	**1.3**
	I00-I99	Diseases of the circulatory system	**1.5**	**1.5**	**1.1**	**1.1**
	J00-J99	Diseases of the respiratory system	**1.6**	**1.7**	**1.1**	**1.0**
	K00-K95	Diseases of the digestive system	**1.6**	**1.7**	**1.0**	**1.0**
	L00-L99	Diseases of the skin and subcutaneous tissue	**1.6**	**1.7**	**1.0**	**1.0**
	M00-M99	Diseases of the musculoskeletal system and connective tissue	**1.4**	**1.5**	**1.1**	**0.9**
	N00-N99	Diseases of the genitourinary system	**1.7**	**1.7**	**1.1**	**1.1**
	O00-O9A	Pregnancy, childbirth, and the puerperium	**n/a**	**n/a**	**1.0**	**n/a**
	P00-P96	Certain conditions originating in the perinatal period	**n/a**	**n/a**	**n/a**	**n/a**
	Q00-Q99	Congenital malformations, deformations, and chromosomal abnormalities	**1.7**	**1.6**	**0.8**	**0.8**
	R00-R99	Symptoms, signs, and abnormal clinical and laboratory findings, not elsewhere classified	**1.4**	**1.3**	**1.0**	**1.1**
	S00-T88	Injury, poisoning, and certain other consequences of external causes	**1.8**	**1.8**	**1.1**	**1.1**
	U00-U85	Codes for special purposes	**2.5**	**2.3**	**0.9**	**1.0**
	V00-Y99	External causes of morbidity	**2.4**	**2.1**	**1.2**	**1.4**
	Z00-Z99	Factors influencing health status and contact with health services	**1.2**	**1.3**	**1.0**	**0.9**

Note: Risk ratios were calculated for cohort comparisons based on the prevalence of ICD-10 codes between two cohort groups.
Abbreviation: ADRD: Alzheimer's disease and related dementias; ICD-10: International classification of diseases, tenth revision.

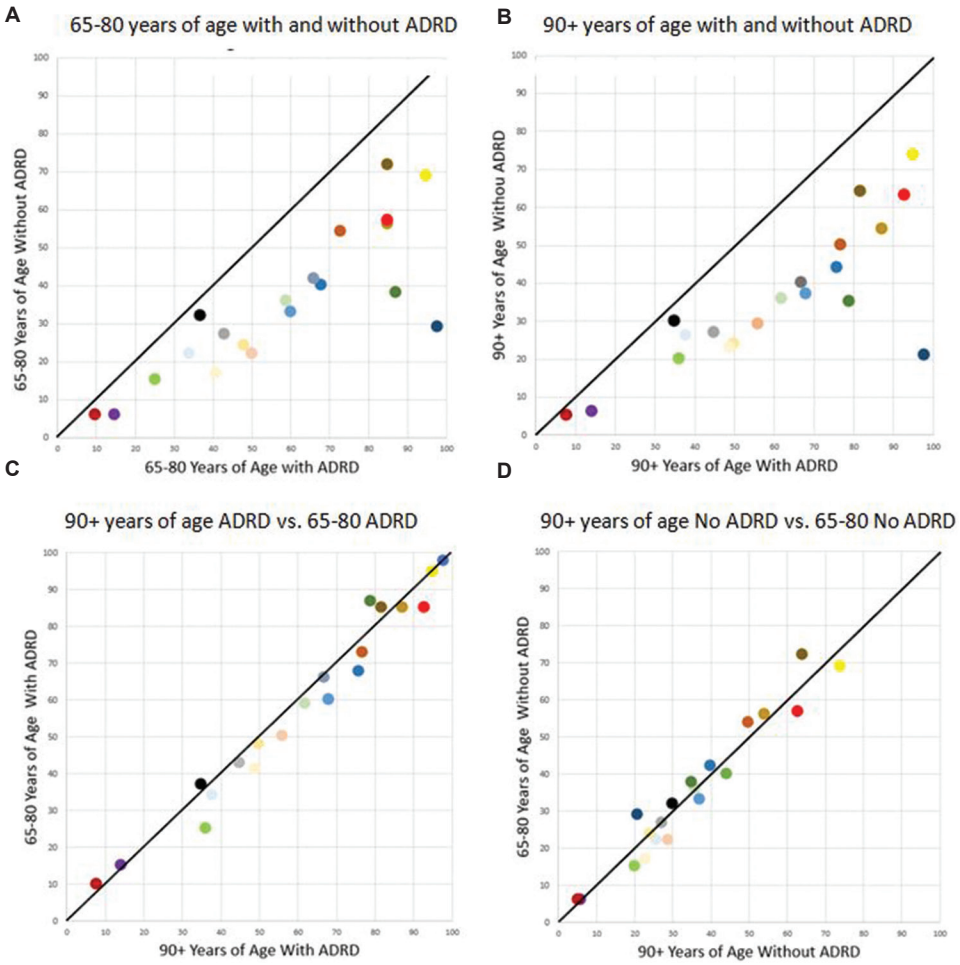

Figure 1. Scatterplots of ICD-10 code prevalence risk ratios of individuals aged 65–80 or ≥90 years with and without ADRD with health care visits in 2021–2023. (A) Comparative prevalence of ICD-10 chapter codes in 65–80 year olds with and without ADRD, (B) comparative incidence of ICD-10 chapter diagnoses in >90 year old individuals with and without ADRD, (C) comparative incidence of ICD-10 diagnoses in younger (65–80 years of age) versus older (90+ years of age) individuals with ADRD, and (D) comparative incidence of ICD-10 chapter diagnoses in younger (65–80 years of age) versus older (90+ years of age) individuals without ADRDs. Scatterplots were created using the ICD-10 code chapter prevalence data in Table 2 for various cohort comparisons. Data point color codes were assigned randomly to the ICD-10 code chapters to facilitate cohort comparisons (color codes are provided in Table 3). The data in all four scatter plots are positively correlated based on Pearson correlation coefficients: (A) r (20) = 0.88, $p < 0.00001$; (B) r (20) = 0.86, $p < 0.00001$; (C) r (20) = 0.99, $p < 0.00001$; (D) r (20) = 0.98, $p < 0.00001$.
Abbreviations: ADRD: Alzheimer's disease and related dementias; ICD-10: International classification of diseases, tenth revision.

and the ≥90-year-old cohorts with and without ADRD (Figure 1B; r (20) = 0.86), the z-score was 0.25 ($p = 0.80$), indicating no statistical difference between the pattern of comorbidity prevalence based on the presence or absence of ADRD in the two age groups.

In the comparison of correlation coefficients between the younger and older cohorts with ADRD (Figure 1C; r (20) = 0.99) and without ADRD (ADRD; r (20) = 0.98), the z-score was 1.1 ($p = 0.28$), indicating no statistical difference between the pattern of comorbidity prevalence based on age in patients with or without ADRD.

However, a comparison of the correlation coefficients between both the younger and older cohorts with ADRD and their aged-matched cohorts without ADRD revealed highly significant differences.

In the comparison of correlation coefficients between the 65 and 80-year-old cohorts with and without ADRD (Figure 1A; r (20) = 0.88) and the ≥90-year-oldcohort with ADRD (Figure 1C, r (20) = 0.99), the z-score was 3.9 ($p < 0.0001$). This result indicates that younger patients with ADRD have a comorbidity prevalence pattern that is significantly more similar to that of the older patients with ADRD than that of their age-matched non-ADRD cohorts.

Similarly, in the comparison of correlation coefficients between the ≥90-year-old cohorts with and without ADRD (Figure 1B; r (20) = 0.86) and the two age group of cohorts

without ADRD (Figure 1D, r (20) = 0.98), the z-score was 3.1 ($p < 0.002$). This result implies that younger individuals without ADRD have a comorbidity prevalence pattern that is significantly more similar to that of older individuals without ADRD than that of younger individuals with ADRD.

4. Discussion

The major finding of the present study was that the significant comorbidity burden associated with ADRD and the pattern of multiple organ involvement were statistically similar ($p = 0.80$) in younger and older ADRD cohorts. The hypothesis tested in this study was that the ADRD comorbidity burden in the 65 – 80-year-old cohorts is lower than that in the ≥90-year-old ADRD cohort due to the age-related prevalence of several clinical disorders. Hence, the hypothesis was not confirmed. Moreover, both the younger and older non-ADRD cohorts had a statistically similar ($p = 0.28$), but much lower, comorbidity burden. Considering that ADRD and several comorbid disorders are age-related, the results of this study were surprising because there were no statistical differences between the pattern of comorbidity prevalence based on age in patients with or without ADRD.

There is an extensive literature documenting multiple comorbidities associated with ADRD.[1-10] In general, the association between non-cognitive disorders and ADRD has been attributed to the fact that multiple medical conditions, such as ADRD, are age-related but unrelated in terms of a common pathophysiological basis.[2] Nevertheless, the present study showed that the risk ratios of age-related disorders in ADRD versus non-ADRD individuals were increased to a similar extent and pattern across multiple organ systems in both younger and older patients with ADRD. This observation suggests that ADRD and related comorbidities share a common pathophysiological mechanism. In particular, similar genetic and/or molecular factors related to the aging process may result in concurrent cellular and organ senescence. The concept of biological versus chronological age may provide a scientific basis for the data obtained in the present study.

Senescence is defined as the gradual deterioration of function in living organisms that is associated with biological aging. Senescence occurs at various rates in different organs but is, by definition, age-related. Concurrent organ senescence could explain the observed comorbid association of several seemingly unrelated clinical disorders with ADRD, implying that similar pathophysiological changes associated with biological aging at the cellular and organ level could explain the current dataset. As such, ADRD might be considered, at least in part, a component of a multiorgan senescence syndrome, rather than an independent disease associated almost exclusively with cognitive decline secondary to the accumulation of beta-amyloid in the brain.

The recognition of ADRD as a component of a multiorgan senescence syndrome has significant research and clinical management implications. At present, pharmaceutical research is overwhelmingly focused on the progressive cognitive impairment associated with ADRD. For instance, of the 3413 "Alzheimer Disease" research trials listed on the clinicaltrials.gov website (accessed on May 20, 2024), only 46 (1%) mentioned "comorbidities," despite the fact that approximately 1700 articles have been published on comorbidities of dementia. Routine evaluations of ADRD-related comorbidities in clinical research may allow for insights into efficacy benefits unrelated to cognition. However, patients with ADRD with significant medical comorbidities are generally excluded from most dementia research trials. Consequently, populations in research trials tend to be healthier, more educated, and significantly younger[15] than the general population of patients with ADRD.

At the clinical practice level, ADRD-related comorbidities have been recognized, and the need for their multimodal management has also been emphasized.[16-20] In fact, there are extensive data suggesting that optimized management of age-related comorbid disorders, which are not often associated with cognitive decline, could delay, or slow, the cognitive decline associated with ADRD. As a specific example, cataracts are considered an age-related, but pathophysiologically unrelated, comorbidity of ADRD.[21,22] As depicted in Table 3, a risk ratio of 1.5 was observed for diseases of the eye and adnexa (ICD-10 chapter H00-H59) in both aged-matched ADRD and non-ADRD cohorts. Although cataracts are unequivocally age-related, the prevalence of eye and adnexa disorders only slightly increased in the older versus younger cohorts with and without ADRD (Table 3). Furthermore, cataract surgery is associated with a decreased risk for ADRD compared with that in a nonsurgical group.[23,24] Unfortunately, despite this benefit, cataract surgery rates have been shown to decrease steeply in patients with ADRD within a year of the initial ADRD diagnosis, whereas a slow age-related increase in cataract surgery rates continued in the non-ADRD cohort.[25] Further studies are required to determine whether cataract removal in the early stages of ADRD can retard the anticipated progressive cognitive decline.

The field of geroscience has generated an extensive body of research on the roles of various factors in the aging process, such as telomere shortening, mitochondrial dysfunction, epigenetic changes, cellular senescence, and

stem cell depletion.[26] The concept that the central nervous system is unaffected by the underlying pathophysiological changes associated with age-related changes appears meaningless. The results of the present study suggest that increased efforts to evaluate and modulate the pathophysiological basis of systemic aging as well as current focus on beta-amyloid are effective approaches to improve ADRD management.

Therapeutic approaches for ADRD that target potentially common biological processes related to aging are limited. For instance, only a single small trial ($n = 5$) titled "Senolytic Therapy to Modulate Progression of Alzheimer's Disease" using dasatinib and quercetin has been completed.[16] A larger trial with 48 individuals is ongoing (NCT04685590). A senolytic approach to ADRD has the potential to significantly improve or delay the progression of multiple comorbidities associated with ADRD.

Furthermore, physical exercise, cognitive stimulation, maintenance of normal body weight, socialization, and avoidance of tobacco and drug abuse are all clinically relevant approaches for the optimal management of age-related disorders, including cognitive decline. In fact, exercise is an effective intervention in nearly all age-related disorders.[27-29] Exercise alone exerts a beneficial effect on all-cause mortality as well as multiple medical conditions, such as cardiovascular diseases, depression, obesity, osteoarthritis, osteoporosis, type 2 diabetes, and ADRD. The observation that a single intervention can be beneficial for multiple age-related disorders is also consistent with the hypothesis that similar pathophysiological components underlie several age-related disorders.

5. Conclusion

Similar pathophysiological components related to biological aging may underlie several age-related disorders. This study emphasizes the need for additional research on addressing the comorbidities of ADRD to improve the overall health status and quality of life of patients with ADRD. The current concept that ADRD-associated cognition is solely attributable to beta-amyloid pathophysiology, without optimizing the management of comorbid disorders, should be reconsidered. As part of a comprehensive and integrated care program, comorbidities should be evaluated and addressed at all stages of ADRD. Increasing evidence suggests that a comprehensive clinical management strategy could delay or slow cognitive decline and improve the overall health of patients with ADRD.[20,26,30,31] Therefore, future studies should determine the extent, if any, to which the optimized clinical management of ADRD-related comorbidities can retard the clinical progression of ADRD.

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Conflict of interest

The author declares that he has no competing interests.

Author contributions

This is a single-authored paper.

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Not applicable.

Consent for publication

Not applicable.

Availability of data

Data are available from the corresponding author on reasonable request.

Further disclosure

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CASE SERIES

Reduction in the number of subcortical white matter fibers in subcortical band heterotopia: A case series

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Abstract

Subcortical band heterotopia (SBH) is a developmental disorder in which neuroblasts migrating from the periventricular zone to the cortex cannot reach their final destination. Subcortical bands might interfere with axonal development. Therefore, this study examined white matter organization in 10 healthy controls and 2 patients with drug-resistant epilepsy (referred to as patients 1 and 2), in whom diagnostic magnetic resonance imaging revealed subcortical bands of gray matter. Diffusion tensor imaging, advanced tractography methods, and functional imaging were performed in patient 2. The number of subcortical fibers decreased significantly in both patients. The heterotopias interrupted the propagation of the tracking algorithm. Only a small number of tracts were generated from inside the heterotopias. According to the literature and our findings, SBHs appear to differ in their localization, extension, and structure. Some heterotopias might allow the propagation of tractography, thereby creating the impression that tracts are passing through, whereas others interrupt the propagation, as observed in our cases. In addition, a reduction in the number of subcortical white matter fibers was observed. These findings may have consequences in pre-surgical planning. The generalization of seizures might be facilitated by a non-reduction in SCWM fibers and tracts passing through the band, and these patients might benefit more from extensive resections of the epileptogenic zone than others.

Keywords: Subcortical white matter fibers; Subcortical band heterotopia; Tractography; Drug-resistant epilepsy

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1. Introduction

Subcortical band heterotopia (SBH) is a developmental disorder in which neuroblasts migrating peripherally from the periventricular zone cannot reach their final destination within the cerebral cortex.[1] Most SBH cases are linked to *de novo* mutations of the *DCX* gene; however, mutations in other genes such as *LIS1* and rarely *TUBB* have been detected.[2,3] In case of SBH with *DCX* mutation, affected hemizygous men tend to present

with a more severely affected phenotype than heterozygous women, wherein neurons expressing the non-mutated gene might migrate appropriately.

Clinically, patients with SBH exhibit a wide spectrum of features. Typical symptoms include mental retardation and high seizure rates, depending on the degree of manifestation of the heterotopia.[4,5] Invasive electroencephalogram (EEG), single photon emission computed tomography, and experimental data suggest that the heterotopia and overlying cortex contribute to the development of epilepsy.[6-9] Accordingly, no specific EEG patterns have been described in the literature.[10]

Early and recent magnetic resonance imaging (MRI) studies using tractography have shown a rather "isolated" band caused by interruptions of central or more peripheral white matter tracts at the inner respective outer border of the SBH.[11-13] Thus, epileptic discharges from the overlying cortex might be difficult to propagate to generalization. However, according to histology, this "isolation" does not appear to be real. "All subtypes of inhibitory GABAergic interneurons intermingled with pyramidal neurons were observed in the white matter. This network of differentiated neurons with its dendritic and axonal ramifications suggests the presence of functional neuronal circuitry, and there is strong observational evidence that the two cortices were anatomically and functionally interconnected."[14, p.1838]

Owing to this ostensible discrepancy between imaging and post-mortem findings, we examined the subcortical white matter (SCWM) between the malformation and overlying cortex and between the central white matter (CentWM) and longer tracts as well as explored the organization of the malformation using diffusion tensor imaging (DTI)–based tractography.

2. Case series

The retrospective study had been approved by the local ethics committee of CEDIMAT, and informed consent was obtained from each participant.

2.1. Controls and patients 1 and 2

Overall, 2 patients with drug-resistant epilepsy, in whom MRI revealed an SBH, and 10 healthy age-matched volunteers (control group), who had undergone MRI previously for other reasons, were enrolled.

Patient 1 was a 24-year-old woman who had been suffering from tonic and rarely from clonic seizures since the age of 13 years. Her seizure rate initially reached up to 50/day, which was reduced by a combination of carbamazepine, valproic acid, and lacosamide. Cognitively, she had mental retardation with spells of aggressive behavior and reduced mobility of her right extremities. Her EEG showed bifrontal spike-wave discharges. The scan had to be performed under general anesthesia.

Patient 2 was a 22-year-old woman who started experiencing tonic–clonic convulsions at the age of 12 years and presented with episodes of "loss of look and absentia." Her EEG revealed frequent interictal spike-wave discharges. Under lamotrigine and valproic acid treatment, her seizure rate could be reduced to 2 – 5 episodes/month. She had no neurologic or neurologic deficit and worked successfully as a psychologist.

2.2. MRI

All scans were performed using a 3T Philips Achieva scanner and included the following sequences:

- T1-weighted: 3D turbo field echo MPRAGE, repetition time/echo time (TR/TE) = 6.73/3.11 ms, number of sagittal slices = 180, reconstructed voxel size = 1 mm^3.
- DTI: Number of gradient directions = 32, b = 0 and 800 s/mm^2, measured voxel size = 2 × 2 × 2 mm, number of slices covering the whole head = 60, SENSE factor = 2.
- Functional MRI (patient 2 only): Echo planar imaging–gradient echo sequence: TR = 2000 ms, number of slices with 3-mm thickness = 34, gap = 0.75 mm, field of view = 192 mm, voxel size = 2.38 × 2.38 × 2 mm, and number of slices covering the whole head = 34.

2.3. Post-processing of data

Post-processing of DTI data was performed using the program "MRtrix3" (https://www.mrtrix.org), supported by "FSL" (FMRIB Software Library, https://fsl.fmrib.ox.ac.uk/fsl). MRtrix uses multishell multitissue constrained spherical deconvolution to improve fiber tracking in voxels containing crossing fibers and partial volume effect, anatomically constrained tractography to reject streamlines ending in biologically implausible tissues such as the cerebrospinal fluid (CSF), and spherical deconvolution informed filtering of tractograms, which corrects the overestimation of longer streamlines.

DTI data from images arranged in stacks were denoised and corrected for ringing artifacts and EPI distortion, and fiber distribution orientation was then estimated. The T1-weighted images were segmented into tissue types such as gray matter, CentWM, and CSF and coregistered to the DTI. For general tractography, streamlines were calculated from the coregistered gray matter–white matter border templates for the whole brain. Tracts within the SCWM were edited from these streamlines by excluding all tracts that entered into the CentWM. Vice versa, CentWM tracts

were calculated.[15] Unfortunately, this method included parts of the band heterotopias in both patients; however, to avoid rater-induced bias for comparison between patients and controls, using individually drawn region of interest (ROI) appeared more reliable.

The number of tracts was recorded, and DTI parameters such as fractional anisotropy (FA), mean diffusivity (MD), longitudinal diffusivity (LD), and radial diffusivity (RD) were taken from the abovementioned ROIs and compared with the parameters of the control group. Finally, individual ROIs were interactively placed into the gray matter bands of the patients, and DTI parameters were recorded from these ROIs.

Differences between patients and controls were examined by the Mann–Whitney U-test for significance ($P < 0.05$). This test is not parametric but uses ranks instead of means to estimate differences between groups and does not afford homogeneity of group variances. Thus, the number of group members does not affect the result.

Functional MRI of patient 2 was performed using a 30-s block design of motor, speech, and visual paradigms and evaluated via Statistical Parametric Mapping (SPM, https://www.fil.ion.ucl.ac.uk/spm). Activated ROIs were used as seeding areas for fiber tracking.

2.4. Result of T1-weighted images and FA maps of patients 1 and 2 and controls

Patient 1 exhibited a continuous band of the subcortical gray matter of varying diameters extending through both hemispheres from frontal to occipital (Figure 1A), whereas in patient 2, heterotopy was confined to the parieto-occipital areas and appeared band-like only in some regions, but was more voluminous in others (Figure 1B). In all areas, a thin belt of white matter was interposed between the heterotopia and the overlying cortex. In both cases, the cortex overlying the heterotopia was pachygyric with coarse folding. Coregistered FA maps revealed increased intensities within the heterotopic bands, and in patient 2, these intensities in some areas appeared as extensions of the CentWM crossing the heterotopic mass (Figure 1C and D).

2.5. General tractography and SCWM and CentWM tracts of patients 1 and 2 and controls

In both patients, the course of most streamlines generated via general tractography appeared to be "interrupted" by the heterotopias, and only a small number of tracts "passed" through the bands (Figure 2).

Long corticospinal tracts or radiation of the corpus callosum did not pass the band in patient 1 and had more lumpish peripheral extensions than those in a control case (Figure 3).

Editing subgroups of tracts by the introduction of no-go areas, such as the previously segmented cortex or CentWM, showed that the number of subcortical tracts significantly reduced ($P = 0.030$), and they appeared to be packed more loosely in patients than in controls (Table 1). Conversely, the reduction in the number of CentWM tracts in patients remained slightly below the level of significance ($P = 0.076$). DTI parameters obtained from SCWM and CentWM ROIs were not significantly different between the two groups.

2.6. Tracts and DTI parameters generated from heterotopias of patients 1 and 2

In both patients, seeding ROIs placed into and strictly confined to the heterotopias gave origin only to a small

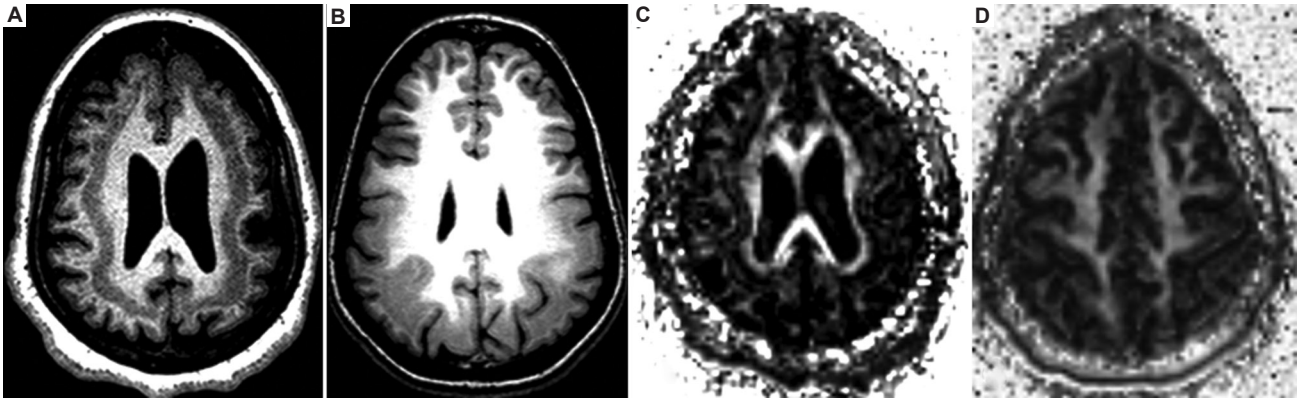

Figure 1. Magnetic resonance imaging. T1-weighted images (A and B) and FA maps (C and D). Patient 1 showed a band heterotopia extending throughout both hemispheres, whereas in patient 2, the heterotopia was confined to the parieto-occipital region. In both cases, FA maps showed increased signal within the bands, which appeared to be more structured in patient 2, particularly on the right (D). A subcortical area free from gray matter was clearly visible in patient 1, but it could be identified as a small strip of white matter in patient 2. The overlying cortex appeared pachygyric.
Abbreviation: FA: Fractional anisotropy.

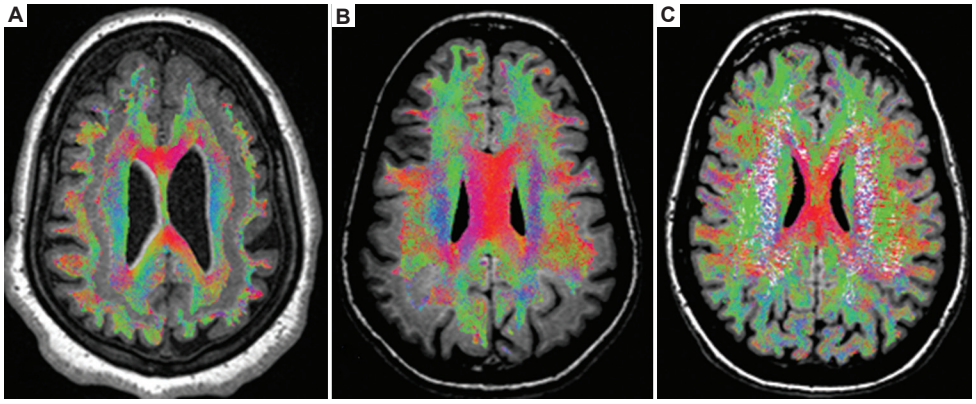

Figure 2. Results of general tractography. In both patients (A and B), the bands were clearly visible as structures free from tracts. The tracts in the subcortical space appeared less numerous and less densely packed (A) or even invisible (B) compared with the control scan, where fibers extended far into the periphery (C). Tracts passing through the bands were rarely observed, except for a right frontal tract in patient 1 and small occipital tracts in patient 2.

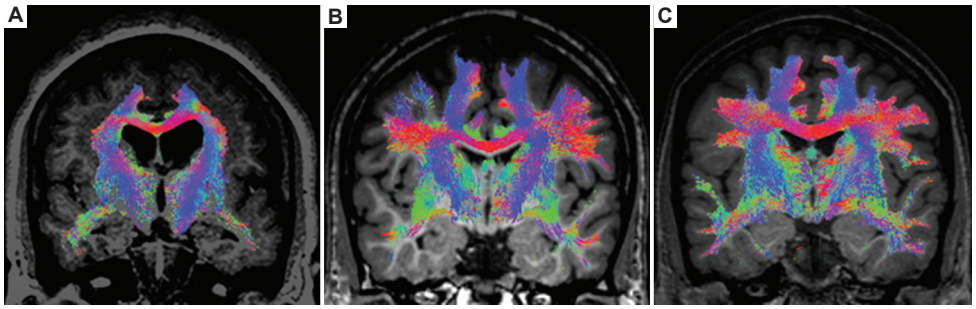

Figure 3. Corticospinal tracts generated from the regions of interest placed in the internal capsule. In patient 1 (A), tracts did not cross the bands, and in patient 2, the tracts (B) had more lumpish peripheral extensions than those in a healthy control (C).

number of tracts. Outside the heterotopia, these tracts propagated into the CentWM in both patients and into the right subcortical zone in patient 1 (Figure 4).

In both patients, most DTI parameters (MD, LD, and RD) derived from these ROIs were lower than those obtained from SCWM ROIs and even from CentWM ROIs; however, FA values were comparable (Table 1).

2.7. Tracts generated from activated areas in patients 1 and 2

In patient 2, activations were generated using functional MRI through hand movements and vision and speech in the pre- and post-central gyrus, occipital lobe, and left fronto-opercular areas as expected. From these ROIs, except for the left occipital ROI, CentWM and SCWM fibers could be generated (Figure 5).

3. Discussion

For the first time, a reduction in the number of white matter "fibers" as tracked by advanced DTI-based tractography could be demonstrated in two patients with SBH. Compared with the normal control group, a significant reduction was observed in the number of SCWM fibers,

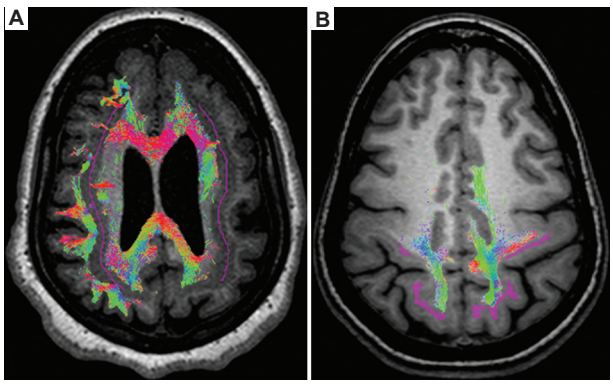

Figure 4. Tracts generated exclusively from the heterotopias. Only in a few circumscribed areas, tracts could be generated from the regions of interest placed exclusively inside the heterotopias and overlaid on the basic image in patients 1 (A) and 2 (B).

but not in the number of CentWM fibers. This reduction might be due to a generally affected development of white matter tracts in heterotopic disorders. In case of SCWM, the reduction may be caused by heterotopic cellular infiltration, as observed in SBH cases.[13,14] This reduction in the number of fibers, as observed in our cases, corresponds to the previously reported disorganization of U-fibers,

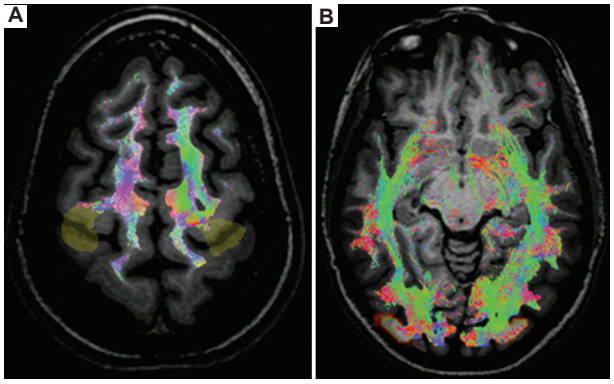

Figure 5. Tracts generated from activated areas in functional magnetic resonance imaging. In patient 2, activations induced by hand movements and vision were localized in typical areas, and their regions of interest gave rise to tractographies of the corticospinal tracts (A) and optic radiation (B).

which has been attributed to seizures originating from the heterotopic gray matter that might exhibit "altered white matter connectivity."[16] However, DTI parameters obtained from this region were not different from those of a healthy control group.

As previously mentioned, the band is usually described to interrupt the tracts present on its way between the CentWM and cortex (Table 2).

Conversely, Erickson *et al.*[17] revealed that "eigenvectors were found to pass through the band in an aligned fashion." This finding has been replicated using more advanced DTI techniques and is more in line with an early post-mortem case report, in which the band was histologically found to be interrupted by fiber bundles running from the CentWM to SCWM.[18] However, only a small number of "passing-through" tracts were noted in our two cases. This was also applicable to longer tracts generated from special ROIs as the corticospinal tracts and radiation of the corpus callosum in patient 1.

In patient 2, in whom the heterotopia was confined to the parieto-occipital region, long tracts extended into the periphery; however, these fine ramifications were not noted in the controls. A similar finding, tract reduction only in heterotopic areas, was reported in patients with SBH accompanied with an X-linked Alport syndrome.[19] Beyond these regions, tracts could be generated in patient 2, for example, from the cortical areas activated during functional MRI, where no underlying malformation was present.

It remains unclear whether this interruption of tracts at the borders of the heterotopy a real finding or is caused by propagation issues of the tracking algorithm. Neuropathological reports revealed a large zone of hypomyelinated brain parenchyma within the band.[4] The cellular arrangement within the band is unstructured[14] or might show a laminar appearance; however, it was less well-organized than in the overlying cortex.[20,21] Similarly,

Table 1. Number of subcortical and central white matter tracts and DTI parameters of these regions as well as those from the heterotopias

	Patient 1	Patient 2	Control (Mean)	Control (Standard deviation)
No. of SCWM tracts	502225*	476515*	3251372.2*	1329843.11
No. of CentWM tracts	471004	585255	734084.4	180669.19
FA SCWM	0.194	0.228	0.204	0.0259
FA CentWM	0.285	0.37	0.322	0.0232
MD SCWM (mm^2/s)	1.145	0.959	1.063	0.1666
MD CentWM (mm^2/s)	0.916	0.804	0.829	0.0970
LD SCWM (mm^2/s)	1.354	1.177	1.235	0.1414
LD CentWM (mm^2/s)	1.201	1.137	1.124	0.1235
RD SCWM (mm^2/s)	1.04	0.85	0.936	0.1013
RD CentWM (mm^2/s)	0.773	0.638	0.684	0.0869
FA band heterotopia	0.187	0.23		
MD band heterotopia (mm^2/s)	0.739	0.788		
LD band heterotopia (mm^2/s)	1.046	0.983		
RD band heterotopia (mm^2/s)	0.665	0.691		

Note: *Significant difference (P=0.030).
Abbreviations: SCWM: Subcortical white matter; CentWM: Central white matter; FA: Fractional anisotropy; MD: Mean diffusivity; LD: Longitudinal diffusivity; RD: Radial diffusivity.

Table 2. Reports of DTI-based tractography in subcortical band heterotopia

Authors	Main findings related to DTI tractography
Erikson *et al.*[17]	Tracts may pass or end within the bands, and fibers may originate from the bands
Lee *et al.*[11]	Tract disruption and decreased anisotropy within band heterotopia
Lu *et al.*[12]	Disruption of frontal connections in band heterotopia
Hoischen *et al.*[19]	Disruption of white matter tracts in band heterotopia
Iannetti *et al.*[16]	Structural disorganization of U-fibers in band heterotopia
Kini *et al.*[24]	Neurite organization within bands was more similar to white matter than to gray matter
Chiba *et al.*[5]	Corticospinal tract abnormalities depend on the severity of band heterotopia
Zhou *et al.*[13]	Disruption of white matter tracts in band heterotopia

Abbreviation: DTI: Diffusion tensor imaging.

apparent synaptic connections of glutamatergic and GABAergic neurons were noted in an ACTG1 variant with SBH and agenesis of the corpus callosum.[22]

FA maps, which usually are regarded as indicators of fiber quality,[23] revealed slightly increased values within the heterotopias and appeared to be structured in some areas in patient 2. Otherwise, the paucity of tracts that could be generated directly from the heterotopias may indicate that this structure was disorganized and did not represent real white matter extensions. The FA values of these heterotopias were similar to those of the SCWM of patients and controls but well below that of CentWM. Kini *et al*.[24] reported a similar finding, whereas FA values were reduced in the case reported by Lu *et al*.[12]

In the future, knowledge of whether tracts passing or not passing through the band heterotopy might help in planning the extent of neurosurgical resections in drug-resistant cases.[25] In animal experiments, a normotopic cortex was the major contributor to epilepsy, and interictal-like discharges secondarily propagate to the band heterotopia.[8] Thus, the generalization of seizures is facilitated by a non-reduction in SCWM fibers and tracts passing through the band, and these patients might benefit more from extensive resections of the epileptogenic zone than others.

4. Conclusion

SBH malformations differ by their localization, extension, and structure. Some heterotopias exhibit a more structured appearance and might allow the propagation of tractography, thereby creating an impression that tracts are passing through, whereas others do not, as observed in our cases. This might influence pre-surgical planning in patients with severe and drug-resistant seizures. Furthermore, it does not necessarily indicate a loss of functionality in cases where heterotopia is limited to some regions, as observed in patient 2, because changes in functional connectivity might compensate for this deficit, as described previously in the sensorimotor network.[26]

Acknowledgements

This report is dedicated to Dr. Diogenes Santos Viloria, who died during the evaluation of data. We will always remember him as a great contributor to the community of epileptologist, as a true friend and over all, a Dominican "grandseigneur."

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: All authors
Formal analysis: Peter Stoeter, Diogenes Santos-Perez
Investigation: Peter Stoeter, Diogenes Santos-Viloria, José Bido
Methodology: Peter Stoeter, Diogenes Santos-Viloria, José Bido
Writing–original draft: All authors
Writing–review & editing: Peter Stoeter

Ethic approval and consent to participate

This retrospective study was approved by the local ethic committee of CEDIMAT (code of approval: CEI 137). Consent was obtained from the patients prior to their participation.

Consent for publication

Patients gave informed written consent that their data, which were measured in a clinical context, may be published.

Availability of data

Data can be requested from the corresponding author.

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ACCSCIENCE PUBLISHING

Advanced Neurology

CASE REPORT

Cognitive and autonomic dysfunction associated with a novel *RYR2* variant: A case report

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Abstract

Variants in the ryanodine receptor 2 (*RYR2*) gene are primarily associated with catecholaminergic polymorphic ventricular tachycardia. However, recent studies have also identified potential links to neurological pathologies, including Alzheimer's disease, benign epilepsy of childhood, and neurodevelopmental disorders. Despite these findings, there is limited data on the association between *RYR2* variants and additional neurological symptoms, such as autonomic dysfunction. This case report describes the clinical progression of a father-son pair, who carry a novel pAsn2517Ser variant in the *RYR2* gene, identified through genetic studies. The report highlights distinct neurological manifestations in both individuals: the father exhibited Alzheimer's-like cognitive dysfunction, while the son presented with an autonomic disorder. This case aims to provide additional information on the role of RyR2 in the brain and the symptomatology associated with pathological variants. Given that both father and son share the same *RYR2* mutation, the observed neurological manifestations are likely attributable to this genetic alteration. These cases offer novel clinical insights into the role of cytoplasmic calcium regulators and their impact on the neurological system.

Keywords: Ryanodine receptors; RyR2; Autonomic dysfunction; Alzheimer's disease

[†]These authors contributed equally to this work.

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1. Background

Ryanodine receptors (RyRs) are calcium ion channels located on the sarcoplasmic reticulum in cardiac and skeletal muscle cells.[1] Intracellular calcium release, triggered by depolarization through L-type calcium channels, is mediated by RyRs, which facilitate the rapid release of Ca^{2+} from the sarcoplasmic reticulum into the cytosol.[2,3] These homotetrameric protein ion channels exist in three isoforms: RyR1, predominantly expressed in skeletal muscle;[4] RyR2, primarily found in cardiac muscle and also Purkinje cells of the cerebellum, hippocampus, and cerebral cortex; and RyR3, expressed in hippocampal neurons, the thalamus, Purkinje cells, and the corpus striatum.[5]

Mutations in both RyR1 and RyR2 have been associated with severe pathological conditions. RyR1 mutations are associated with central core disease, exertional rhabdomyolysis, and malignant hyperthermia. RyR2 mutations, meanwhile, are

implicated in catecholaminergic polymorphic ventricular tachycardia (CPVT) and right ventricular dysplasia Type 2 (AVRD2).[1]

The *RYR2* gene encodes a 105-exon, 565 kDa monomer that assembles into homotetrameric proteins, incorporating four 12 kDa FK-506 binding proteins.[6] The RyR2 protein contains a large cytosolic N-terminal domain (approximately 4,300 residues) and a smaller C-terminal transmembrane domain (approximately 500 residues) that spans the channel pore. Most regulatory components of the channel are located in the cytosolic domain, with mutations predominantly occurring in four conserved regions of the RyR2 protein: domain 1 (amino acids 77 – 466, domain 2 (amino acids 2246 – 2534), domain 3 (amino acids 3777 – 4201), and domain 4 (amino acids 4497 – 4959).[7]

While RyR2 mutations have been extensively studied in the context of cardiac physiology and disease, recent research has begun to investigate their associations with adverse neurological outcomes.[8] These neurological outcomes include sudden unexplained death in epilepsy, benign epilepsy of childhood, and Alzheimer's disease, as well as Alzheimer's-like signaling changes in the brain following COVID-19 infection.[9,10] Studies in murine models have revealed that RyR2 mutations affect glutamate release and reduce the spreading depolarization potential in the dorsal medulla, a critical control center for the respiratory rhythm.[11] These findings suggest a link between RyR2 mutations and extracardiac manifestations.

In particular, RYR2 mutant mice have demonstrated increased spreading depolarization across the neocortex and the dorsal medulla's autonomic microcircuits,[12] a phenomenon implicated in heightened seizure activity. Consequently, RYR2 mutations are now considered a potentially novel neurocardiac calcium channelopathy, with relevance to epilepsy, Alzheimer's disease, and autonomic dysfunction.[13,14]

2. Case presentation

2.1. Patient 1: The Father

A 54-year-old man was referred for evaluation of neurological symptoms, including headaches and cognitive difficulties. He reported experiencing headaches up to 3 times/week, accompanied by blurred vision, dizziness on standing, progressive weakness in the arms and legs, and depression. His hypertension was identified as a contributing factor to his headaches. His past medical history included obstructive sleep apnea with inconsistent adherence to continuous positive airway pressure treatment and post-concussive syndrome following a motor vehicle collision. Neuropsychological testing indicated cognitive performance within the average range, although he exhibited persistent deficits in executive functioning, particularly in phonemic fluency, verbal set-shifting, and visuomotor integration.

In reference to Figure 1, the patient's family history was notable for multiple neurologic and psychiatric disorders, including a brother with bipolar disorder, a mother with early-onset Alzheimer's disease, and a maternal grandmother with dementia. More distant relatives reportedly had behavioral problems, obsessive–compulsive disorder, and seizures.

On physical evaluation, the patient's blood pressure was elevated at 159/117 mmHg. Cognitive assessment revealed impairment, with a Montreal Cognitive Assessment score of 22/30. Cranial nerve function was intact, and motor strength was graded at 5/5 bilaterally in the arms and elsewhere. Reflexes were normal, and coordination was intact. However, pinprick sensation was decreased bilaterally above the elbows and on the left leg to the mid-calf. He also exhibited mild balance difficulties during tandem gait testing.

The patient was prescribed propranolol to manage his hypertension and migraines. Magnetic resonance imaging revealed mild atrophy of the temporal lobes at the Sylvian fissure bilaterally and an empty sella. Following the initiation of propranolol, the patient reported a resolution of headaches and blurred vision, but his cognitive difficulties persisted. Over the subsequent 6 months, his symptoms progressed to include worsening remote and recent memory deficits, impulsive behaviors, and mood swings. He was started on donepezil, which stabilized his cognitive scores, and additional mood-stabilizing medication was introduced, yielding positive effects.

Genetic testing, prompted by his family history of early-onset Alzheimer's disease, identified a novel *RYR2* variant (pAsn2517Ser). In addition, the patient was found to have intermediate enzymatic activity for catechol-O methyltransferase, methylenetetrahydrofolate reductase, and cytochrome P450 2D6.

2.2. Patient 2: The Son

The second patient in this case report is the 19-year-old son of Patient 1. He has a complex medical history that includes seizures, migraines, severe episodic dysautonomia, and post-traumatic stress disorder. His dysautonomia was characterized by orthostatic hypotension and postural tachycardia, with episodes accompanied by headaches, confusion, and increasing agitation.

Magnetic resonance imaging revealed no evidence of acute territorial infarct, intracranial hemorrhage,

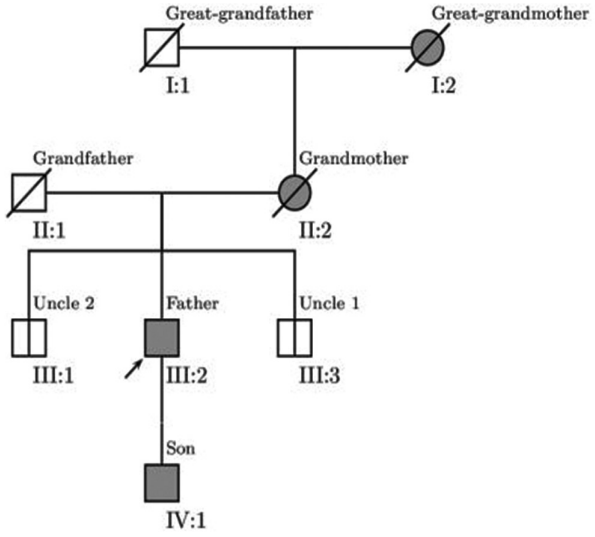

Figure 1. Family pedigree of Patient 1 (the father). Patient 1 and Patient 2 (Patient 1's 19-year-old son) share the *RYR2* pAsn2517Ser and *LRRK2* mutation pMet1646Thr mutations. Patient's 1 mother (deceased) had moderately severe Alzheimer's disease, diagnosed at the age of 64 – 65, and passed away at 67 due to gastrointestinal issues for which she refused treatment. She was also treated for hypertension. During her illness, she experienced sundowning at night and, toward the end of her life, developed social phobias. An autopsy revealed moderate to severe atrophy in cortical and limbic structures. Patient 1's Brother 1 (Uncle 1): No known medical problems; Patient 1's Brother 2 (Uncle) 2: Diagnosed with bipolar disorder. Patient 1's grandmother: Alzheimer's presented at 84. Image created with: http://pedigrees.varphi.com/cgi-bin/pedigree.cgi

intra-axial mass, or midline shift. A small focus of fluid-attenuated inversion recovery and T2 signal hyperintensity was observed at the level of the right corona radiata, in close proximity to the anterior limb of the right internal capsule, consistent with prior imaging findings. No significant changes were noted, and overall, the imaging impression was unremarkable.

On physical examination, the patient exhibited slowed processing speed and poor attention. Cranial nerve function was intact, and motor strength was 5/5 throughout. Reflexes were decreased at 1/4 across all tested regions. Pinprick sensation was decreased bilaterally in the mid-forearms and upper thighs. Gait was normal, though a mild postural tremor was observed.

Genetic testing was performed due to the early onset of symptoms and the father's diagnosed autonomic and cognitive issues. Next-generation sequencing technology was employed to analyze mitochondrial nuclear DNA from multiple sources, including peripheral blood, muscle, liver, saliva, and urine. The analysis targeted known variants in genes associated with autism, developmental delay, dysautonomia, fatigue, gastrointestinal dysmotility, hypotonia, migraine, seizures, ventricular tachycardia, brain abnormalities, familial dysautonomia, and mitochondrial dysfunction. The results revealed no large deletions or known deleterious mutations. However, genetic testing identified variants in both *RYR2* and leucine-rich repeat kinase 2 (*LRRK2*) genes. The patient was confirmed to carry the same *RYR2* (OMIM 18090) variant (pAsn2517Ser) as his father. The genetic testing documentation concluded that the *RYR2* variant is the most likely cause of the disease progression observed in the patient, given the absence of other significant genetic abnormalities.

3. Discussion

This case report highlights the association between a novel *RYR2* variant to a unique phenotype characterized by Alzheimer's-like cognitive dysfunction and global autonomic dysregulation. The identified missense mutation, *RYR2* pAsn2517Ser, results in an asparagine-to-serine substitution at amino acid 2517 in the cytosolic domain of the RyR2 protein. This mutation, localized to the BSolB region of RyR2, has not been previously characterized. Although unconfirmed, current theories suggest that mutations in this region may enhance sensitivity to sarcoplasmic reticulum luminal calcium or destabilize the closed conformation of the RyR2 channel.[15] Both mechanisms could disrupt calcium homeostasis, contributing to the observed neurological and autonomic manifestations.

Calcium homeostasis plays a critical role in autonomic stability. While this case does not involve calcium channel antibodies, dysautonomia – particularly orthostatic hypotension – has been documented in cases linked to calcium dysfunction.[16] The destabilization of the RyR2 function in this specific domain could provide a plausible mechanism for the son's pathology.

Although mutations in RyR2 account for up to 50% of CPVT cases, fewer studies have investigated their impact on extracardiac processes.[17] The clinical presentations in this case report suggest that the *RYR2* mutation may extend beyond cardiac phenotypes, influencing cognitive and autonomic functions. Temporal lobe atrophy observed on imaging, combined with a family history of dementia, further supports the hypothesis of a connection between this mutation and these clinical manifestations.

Emerging research suggests that RyR2 function is essential for activity- and experience-dependent dendritic spine formation in the hippocampus, processes crucial for memory acquisition.[18] Patients with Alzheimer's disease exhibit downregulated *RYR2* expression, which may contribute to reduced dendritic spine density and impaired memory.[19] Alternatively, RyR2-mediated endoplasmic

reticulum Ca^{2+} leaks may activate Ca^{2+}-dependent signaling pathways involved in Alzheimer's disease pathogenesis.[20] Although the father has not been diagnosed with Alzheimer's disease, his memory dysfunction may reflect these underlying mechanisms. In addition, *RYR2* mutations have been implicated in neurocardiac channelopathies, which may explain the autonomic dysfunction observed in this case. This case report underscores the need for further research to clarify this suggested association.

4. Conclusion

RYR2 variants have been extensively studied in the context of CPVT and AVRD2. Emerging evidence suggests that these variants also impact other organ systems, including the brain. In this case report, both the father and son presented with neurological symptoms linked to an *RYR2* mutation (p.Asn2517Ser). The father exhibited cognitive dysfunction, consistent with previous studies implicating *RYR2* variants in the development of Alzheimer's-like disease. In contrast, the son displayed symptoms of autonomic dysfunction that could not be attributed to known causes, such as mitochondrial disease, vitamin deficiencies, or structural abnormalities. Given the shared *RYR2* variant and the established role of RyR2 in the brain, this case highlights the potential for *RYR2* mutations to manifest in diverse neurological phenotypes. Future studies are needed to identify and characterize additional patients with this *RYR2* mutation to further elucidate its phenotypic spectrum and underlying mechanisms.

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Conflict of interest

The authors declare that they have no competing interests.

Author contributions

Conceptualization: Anna Hohler, Okeanis Vaou
Formal analysis: Liam Power, Justin Ho, Charly Edmiston, Leilani Miranda
Investigation: Liam Power, Justin Ho, Charly Edmiston
Writing-original draft: Liam Power, Justin Ho, Charly Edmiston, Leilani Miranda
Writing-review & editing: Liam Power, Justin Ho, Charly Edmiston, Leilani Miranda, Anna Hohler

Ethics approval and consent to participate

This retrospective case study was conducted with ethics approval waived by the Institutional Review Board of St. Elizabeth's Medical Center. Written informed consent to participate in the study was obtained from the patients and their guardians.

Consent for publication

Written informed consent for publication of their details was obtained from the patients and their guardians.

Availability of data

All data generated or analyzed during the study are included in the article. Further enquiries can be directed to the corresponding author.

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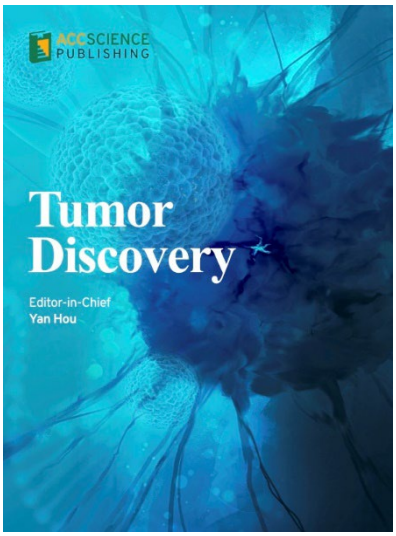

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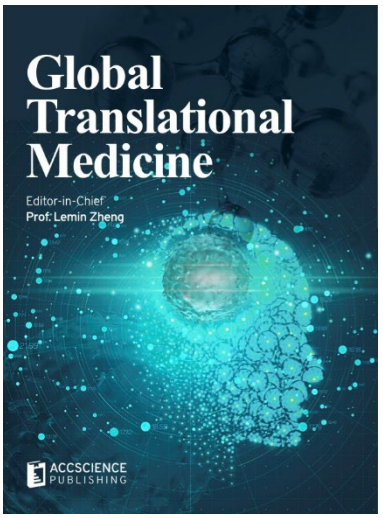

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