Neurodegenerative Diseases and Potential Early Detection Methods

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ABSTRACT

Neurodegenerative diseases create a significant financial and emotional toll on society. Prion diseases are a type of progressive neurodegenerative diseases that are irreversible and fatal. They are characterized by long incubation periods and defective protein folding that make them infectious. Other neurological diseases such as Alzheimer's and Parkinson's also have similar characteristics and are hence called prion-like diseases. Historically, these diseases were unable to be clearly diagnosed until significant progression had taken place. In fact, many diagnoses were not made until post mortem examination was performed. Early detection of these diseases is key to finding a cure. Other bodily structures could possibly reveal clues related to the presence of neurodegenerative disease. In this review, we introduce and explain the nature of the diseases, current detection methods and the future of early detection methods.

Introduction

Neurodegenerative diseases are a class of progressive, irreversible, age-related diseases characterized by destruction of neurons in the central nervous system. They predominantly cause either cognitive problems such as in the case of Alzheimer's disease (AD) or motor problems such as in the case of Parkinson's disease (PD) or Amyotrophic Lateral Sclerosis (ALS). [1] A group of rare conditions called prion diseases can also cause neurological problems which are usually fatal. They are caused by a misfolded version of a normal host prion protein and are characterized by long incubation periods and rapid progression and are always fatal.

Neurodegenerative diseases take a significant toll on people. More than 6 million Americans are living with AD. This number is expected to grow to 13 million by 2050. Deaths due to AD and other forms of dementia increased by 16% during the COVID-19 pandemic. More than 10 million people worldwide have PD. In the U.S alone, about 60,000 people are diagnosed with PD every year. While case counts for prion diseases are not as large, between 1 and 1.5 cases occur every year. On an average about 300 people die every year in the US alone.

These diseases cost the U.S. economy billions of dollars each year in direct health care costs and lost opportunities; it is estimated that $100 billion per year is spent on Alzheimer disease (AD) alone. In addition to the financial costs, there is an immense emotional burden on patients and their caregivers. As the number of elderly citizens increases, these costs to society also will increase.

Prion and Prion-like Neurodegenerative Diseases

There are many neurodegenerative diseases that wreak havoc on the brain that are typically diagnosed later in their progression (Figure 1) and this paper will focus on prion and prion-like diseases.
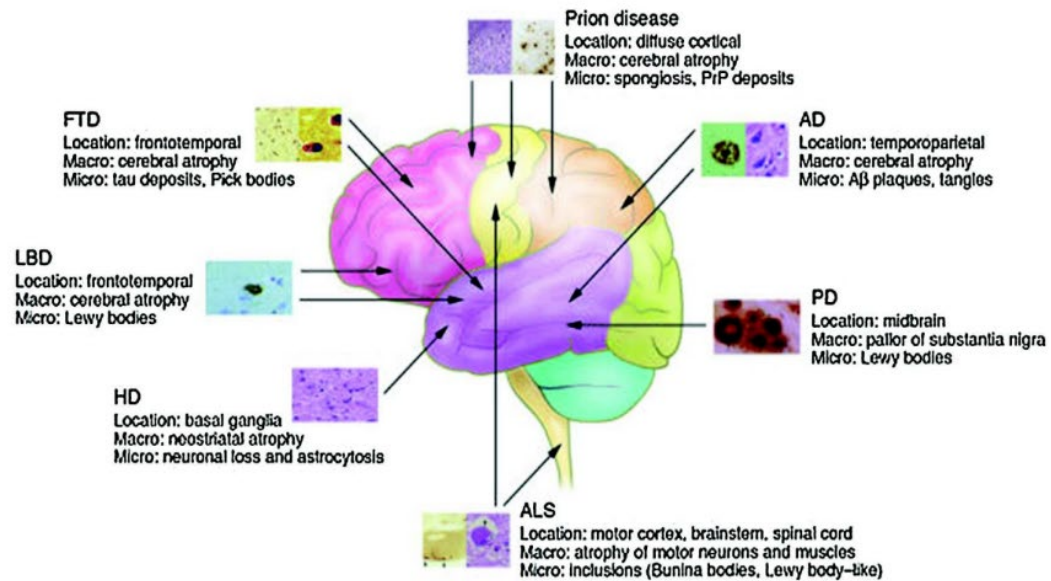
Figure 1. Neurodegenerative diseases and the primary regions of the brain that they affect. FTD: Frontotemporal dementia. LBD: Lewy body dementia. HD: Huntington's disease. ALS: Amyotrophic Lateral Sclerosis. PD: Parkinson's disease. AD: Alzheimer's disease. [26]

Prion Diseases

Transmissible Spongiform encephalopathies (TSE) are a class of transmissible neurodegenerative diseases that are known to occur in both humans and animals [6]. TSEs are called spongiform because the affected brain looks like a sponge with holes. In 1982, Nobel prize winner, Stanley Prusiner, coined the term prion ("proteinaceous infectious disease") to describe the self-propagating protein that is responsible for TSE.

The term prion was introduced to distinguish the novel class of infection from viruses and viroids as well as to emphasize the role of proteins in the infection [12]. Prion proteins occur in both non-infectious and infectious forms. The only difference of the infectious form is that it takes on a different folded shape - from alpha-helix to beta- sheet.

The most common disease involving human transmissible spongiform encephalopathies is Creutzfeldt-Jakob disease (CJD) [3, 8, 9]. The cerebral cortex is most commonly affected by CJD and it can occur in three ways: sporadically, inherited, and through transmission from infected individuals. Sporadic CJD is the most common form of prion disease in humans. The person's normal prions spontaneously change into the infectious form and then alter the prions in other cells in a chain reaction. The inherited form is caused by mutation in the prion protein gene that causes the prions to be shaped in an abnormal way. This genetic change can be transmitted to the offspring. Infectious prions can be transmitted through contact with infected tissue, bodily fluids or contaminated medical instruments. Over the last 40 years, the death rate has increased by about 1% on average. Normal sterilization procedures such as boiling water and irradiation do not prevent transmission.

Other forms of prion diseases are extremely rare and include the following: Variant CJD - also known as "mad cow disease", occurs when humans eat meat from cows that have the prion disease. According to the FDA, in 2019, 232 people worldwide have become sick with vCJD. Most lived in the UK at some point in their life. The brainstem is commonly affected by vCJD.

Gerstmann-Sträussler-Scheinker disease (GSS): -this disease is caused by mutation in the PRNP gene. PRNP gene provides instructions for making the prion protein. About 1 to 10 in 100 million cases of GSS occur worldwide every year.

Fatal familial insomnia (FFI) - this affects the thalamus, the part of the brain that controls the sleep wake cycle [5]. The most common symptom is sleep disturbance. This disease occurs when a variant in the PRNP gene is inherited in an autosomal dominant pattern. FFI is found in about 50 families worldwide [7].

Alzheimer's Disease

AD is a common fatal neurodegenerative disease. Post mortem brain reports of AD patients have been found to contain plaques composed of beta amyloid [10, 11]. Beta amyloid varies in size from 39 to 43. Similar to the prion proteins, beta amyloid forms fibers with different molecular structures and are toxic to neurons. Similar to prions, beta amyloid accumulates in the extracellular space and is found to be infectious in animal studies. Besides the Beta amyloid, AD patients also have accumulation of Tau proteins. Tau is a microtubule associated protein that promotes intracellular transport. In AD patients, tau is found to accumulate in a step wise orderly manner throughout the brain similar to the prion disease. AD starts by affecting the entorhinal cortex and the hippocampus. The Entorhinal cortex is mainly responsible for memory, navigation and perception of time. Hippocampus is used for learning and memory. As AD progresses, the disease affects other parts of the brain such as the cerebral cortex. Memory loss, wandering, taking longer to complete tasks, increased aggression and mood changes are common symptoms.

Parkinson's Disease

PD is caused by selective destruction of dopaminergic neurons in the substantia nigra through the accumulation of proteins into lumps called Lewy body [5, 12]. Alpha synuclein proteins are involved in the accumulation process. These proteins have been observed to propagate similar to prion proteins. PD affects the basal ganglia, the part of the brain that controls movement. PD patients also have low levels of dopamine, a chemical that is involved in movement. As a result, people with PD have balance and coordination problems along with tremor and slowness of movement, also called bradykinesia.

These diseases are typically not able to be diagnosed until late in their pathological progression and oftentimes not confirmed until post mortem study is completed. However, recent technological developments have allowed us to start diagnosing some of these diseases earlier. Since these disorders have similar pathophysiology could these new diagnostic techniques be used to detect all of them at an earlier stage of progression?

Early Detection Methods for Neurodegenerative Diseases

There are a number of different diagnostic tests that are starting to be used to diagnose neurodegenerative diseases at an early stage (summarized in Table 1). Below we provide more detail of how some of these are being used.

Table 1. Diagnostic Methods Currently used to Detect Neurodegenerative Diseases

Method	Clinical applications

Brain Diffusion Magnetic Resonance Imaging (MRI)	Early identification of Ischemic stroke, differentiation of cysts, necrotic tumors, encephalitis, assessment of cortical lesions in **CJD**, extent of axonal injury, grading of gliomas, and prostate lesions
Blood tests	**Prion Disease, AD, PD**
Genetic testing	**AD**, Cancers, **PD**
Cornea scans	Identification of infective keratitis, Diabetic neuropathy, **PD, Prion Disease**
Gastrological dysfunction	**PD**
Optical Coherence Tomography (OCT)	Central serous retinopathy, Diabetic retinopathy Glaucoma, Macular degeneration Macular holes, **AD, Prion Disease, PD**

(PD=Parkinson's Disease, AD= Alzheimer's Disease, CJD=Creutzfeldt-Jakob disease)

Brain Diffusion Magnetic Resonance Imaging (MRI)

The diffusion magnetic resonance imaging (dMRI) technique uses the properties of Brownian motion of water molecules within a voxel of tissue [14, 15]. Tissue structures such as cell membranes impede the diffusive motion of water molecules, meaning tissue with dense cellularity can be distinguishable from tissue with less densely packed cells, or pure fluid. A gradient magnetic field is applied on the tissue and the loss in signal is determined. The loss of signal will be proportional to the rate of motion of the water molecules so densely packed cells, such as a clot, tumor or lesion would show up as a bright spot in the MRI.

Event based modeling of the MRI data can further enhance the understanding of the disease progression. For example, event-based modeling could be used to identify the epicenter and lesion propagation of prion disease. One of the most common subtypes of sporadic CJD has been found to originate in the neocortex and propagate to subcortical regions. Another subtype has an epicenter in the precuneus region of the brain. Event based modeling has been used in identifying disease initiation and propagation in other prion like diseases such as AD.

Blood Tests

The presence of prions could be identified in the blood using a technique called protein misfolding cyclic amplification (PMCA) [16, 17]. PMCA relies on the nature of proteins to clump abnormally and change into prions. In the PMCA technique, a small amount of abnormal protein along with an excess amount of normal protein are incubated. The misfolded protein is blasted with ultrasound, breaking them into smaller protein chains thereby increasing the number of misfolded proteins. By repeating this process, the mass of normal proteins is converted to misfolded proteins. This technique has been shown to identify infectious prions in as little as a single oligomer. Alzheimer's disease can also be detected by checking for mis-foldings in the beta oligomers using PMCA.

Extracellular vesicles are emerging as an early detection method of AD and prion diseases [21]. For example, exosomes, a subtype of extracellular vesicles, are found to flow through the blood-brain barrier.

Exosomes contain proteins and biomarkers associated with pathogenesis and progression of AD.

Genetic Testing

Genetic testing is a technique used to look for changes in a person's genes or changes in the amount, function, or structure of key proteins coded by specific genes [18]. There are 3 different types of genetic testing - chromosomal studies, DNA studies and biochemical studies. In chromosomal studies, the number and structure of chromosomes are analyzed from samples collected from blood or tissue. In DNA studies, errors in DNA due to duplication, deletion, repetition and mutation are identified. In biochemical studies, abnormalities in the proteins that regulate the chemical reactions in the body are analyzed.

In the case of AD, genetic testing is used to find the mutant genes that cause early onset of the disease. These genes include amyloid protein precursor (APP), presenilin-1(PSEN1) and presenilin-2 (PSEN2). Multiple genetic factors have been identified for Parkinson's diseases. Mutations in the SNCA gene occur in the early onset of PD. The SNCA gene encodes alpha-synuclein which is known to form clumps in PD. LRRK2 has been shown to cause late onset PD. PARK1 encodes the protein parkin that helps cells break down and recycle proteins. PARK7 encodes the protein DJ-1, which protects against mitochondrial stress. The PINK1 protein can protect against mitochondrial stress.

Corneal Imaging

Corneal Imaging is used to assess the structure and function of the cornea and the anterior segment to diagnose and treat several ocular diseases [19]. Three different techniques are used to characterize the corneal surface. In the first method, a concentric ring pattern from the Placido disc is reflected and this reflection reveals the shape of the cornea. In the second method, which is called the scanning slit technique, a slit of light is scanned and captured using a camera to create a map of the anterior and posterior corneal surfaces. This technique is also used in corneal confocal microscopy. In the third technique, known as Scheimpflug imaging, a rotating camera is used to photograph corneal cross-sections that are illuminated by slit beams at different angles.

Corneal imaging has been shown to be able to detect prion diseases. The agent that causes the variant version of CJD is found to commonly deposit in lymphoid tissue such as spleen and tonsils. The cornea has lymphoid tissue in the form of corneal dendritic cells. Vision problems related to cornea that appear early in the vCJD disease onset can be a way to detect one subtype of CJD. In a recent study, corneal confocal microscopy was used to detect small fiber neurodegeneration in Parkinson's disease

Optical Coherence Tomography (OCT)

OCT is a way for ophthalmologists to image the back of the eye including the macula, optic nerve, retina and choroid [20]. The scans produce a resolution of 10 microns which is much better than MRI. It works by shining a beam of light through a transparent window such as cornea and measuring the light reflected by tissues at different depths.

Retinal scans using OCT can detect key changes in blood vessels and nerve fibers that may provide biomarkers for early detection of AD. OCT scans have revealed that the retinal nerve fiber layer was also thinned in cases of sporadic CJD as well as PD.

Gastrological Dysfunction

Constipation and other gastrointestinal problems occur years before clinical diseases of Parkinson's disease. The gastrointestinal tract is affected and analysis of this could possibly serve as an early detection method.

Methods

Existing literature was initially chosen based on finding the fundamental papers in the field. These were selected through the utilization of key search words including "prion disease", "neurodegenerative disease", "early detection methods". Building upon this, additional papers were obtained utilizing more complex search terminology and searching specific early detection methods such as "extracellular vesicles" and "blood testing" for more in-depth research. Careful consideration was used to identify what papers demonstrated how the methods can be used to detect neurodegenerative disease and why they work. All in all, websites were scoured for various types of papers that covered methods thought to be the most crucial to identifying ways to quickly and accurately detect neurodegenerative disease. The major websites accessed included PubMed, CDC.gov, and NCBI.gov.

Future Directions

MRI is the most common method currently used to detect prion and prion like diseases such as AD and PD. Other imaging tests that are also commonly used are computed tomography and positron emission tomography. Computed tomography creates a cross sectional slice of the body using a computer. The computer reads the signals generated by a rotating, narrow beam of x-ray that is aimed at a portion of the body.

Positron emission tomography uses a radioactive compound called florbetapir F18 to measure and display changes in the metabolic processes or the physiological activities such as blood flow, chemical compositions and absorption. For example, during the test for AD, the radioactive compound is injected into the body [22]. The compound attaches to the plaques, if present. Then pictures are taken of the brain. If plaques are present then these are highlighted in the pictures. The presence of plaques does not necessarily mean that AD will develop in the future with any certainty. However, the plaques can be used as an early indicator of AD.

A special type of single photon emission computerized tomography called the dopamine transporter scan, is used to detect PD [22]. In this test a radioactive tracer compound called Ioflupane is injected in the bloodstream. The compound attaches itself to a molecule found on dopamine neurons called the dopamine transporter. When scanned, people with PD will have smaller signals compared to normal scans, in a part of the brain called the striatum where the ends of dopamine neurons are meant to be. Other common types of testing include Cerebrospinal fluid (CSF) testing and Electroencephalography (EEG) to detect prion diseases, memory testing to diagnose AD and other routine screening tests.

Early detection testing does not have to be a direct examination of the brain. Techniques such as Corneal Imaging could show potential for future detection methods as it examines another part of the body to identify neurodegenerative disease, as well as checking for gastrointestinal dysfunction [2]. Interestingly, skin disorders such as seborrheic dermatitis and hyperhidrosis are common and often overlooked symptoms of PD [25]. A skin biopsy can be performed by first giving a local pain-relieving injection. Then the skin cells are shaved or a section of the skin is removed. Skin biopsies are taken from three locations: the back, lower thigh or from the leg above the ankle. The pathologist then processes the tissue sample and examines it under a microscope to determine if there is any disease present. This could make skin biopsies a unique option used to identify early biomarkers of PD.

Additionally, optical coherence tomography and blood testing also show promise as they can be used to identify more than one neurodegenerative disease (AD, Parkinson's, prion disease) [24]. Continued innova-

tion in the development of novel early detection methods as well as reimagining current methods could go a long way in helping to allow any treatments to be more effective for those suffering with incurable neuro-degenerative diseases.

Conclusions

Neurodegenerative diseases like Alzheimer's disease, Parkinson's disease, and prion disease are irreversible, fatal, and pose a huge burden to society, both emotionally and economically, depleting millions of dollars for medical expenses to slow down and treat symptoms although no cures or precise early detection methods have been developed. Methods such as examining other bodily structures to identify the presence of disease, or novel techniques like gene and blood tests show promise in being able to detect these diseases earlier as they diverge from the usual methods that often do not detect disease until late in their progression and a number of symptoms are already present. By increasing the scope of techniques used to identify neurodegenerative disease, we may be able to detect them earlier and slow down their progression. Finding parallels between the different diseases and using methods that work for multiple diseases like OCT scanning may reduce the financial burden of managing them, as well as the emotional burden of getting diagnosed with neuro-degenerative disease when it becomes too late.

Acknowledgments

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