Chapter

Protein Detection in Clinical Diagnosis and Management of Prevalent Neurodegenerative Diseases and Metabolic Disorders

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Abstract

An accurate diagnosis gives leeway to cost-effective treatments. However, many diseases continue to evolve; hence, their etiology is sometimes missed due to the procedures used during diagnosis. Protein-related diseases include proteopathies (proteinopathies) such as neurodegenerative diseases and metabolic disorders like protein-energy malnutrition and some hormonopathies. Hormonopathies are associated with the change in the production of hormones. Diabetes mellitus, a type of hormonopathy, is reviewed in this work alongside neurodegenerative diseases and protein-energy malnutrition. This chapter aims to elucidate more on the diagnosis of these diseases considering the structure and function of their proteins viz-a-viz their deficiencies and hyper-production in man. Their pathogenesis and the principles underlying their diagnosis are further discussed to optimize the management of these diseases among patients.

Keywords: prion, proteinopathy, hormonopathy, marasmus, kwashiorkor, alzheimer, parkinson, huntington, diabetes, neurodegenerative disease

1. Introduction

Medical laboratory diagnosis has given hope to detect and efficiently treat or manage diseases, and protein-related diseases are not an exception. The diagnosis ranges from the least like urinalysis to the highly sophisticated methodologies involving molecular techniques such as polymerase chain reaction. This chapter discusses the neurodegenerative disease and the pathologic conditions associated with protein-derived hormones and protein-energy malnutrition, focusing on their diagnosis and management.

Proteinopathy (proteopathy) is a disease mainly characterized by the production of aberrant proteins. Here, there could be a defect in the structure or function of the proteins produced, which could also reflect in the over-secretion or under-secretion

of these proteins. Neurodegeneration involves a gradual loss of neuronal structure or function, invariably leading to cellular death [1]. Neurodegenerative proteinopathies (proteopathies) are neurodegenerative diseases that have abnormally produced proteins that could result from the changes in the structure and function of these proteins. Their pathological examination reveals their link with aberrant proteins. Their prevalence in the United States of America is shown in **Table 1**. Popular

Disease	Age-adjusted deaths per 100,000
Prion disease	0.1319
Alzheimer's disease	233.8
Parkinson's disease	65.3
Frontotemporal dementia	66.7
Amyotrophic lateral sclerosis	5

Table 1.
Prevalence of some common neurodegenerative diseases in the United States [1–5].

Disease	Host	Route of exposure
Kuru	Fore people of New Guinea	Ritualistic cannibalism
CJD:		
Iatrogenic	Human	Invasive hospital equipment, implants, transplant organs, and tissues
Familial	Human	Germline mutations in the *PrP* gene
Sporadic	Human	Somatic mutation or spontaneous conversion of PrP^{c} into PrP^{sc}
Variant CJD		Ingestion of bovine prions
Gerstmann–Straussler–Scheinker syndrome	Humans	Germline mutations in *PrP* gene
Fatal familial insomnia	Humans	Germline mutation in the *PrP* gene (D178N, M129)
Sporadic fatal insomnia	Humans	Somatic mutation or spontaneous conversion of PrP^{c} into PrP^{sc}
Scrapie	Sheep	Genetic mutation in sheeps
Bovine spongiform encephalopathy	Cattle	Infection by prion contaminated meat and bone meals
Transmissible mink encephalopathy	Mink	Infection with prions from sheep or cattle
Chronic wasting disease	Mule, deer, elk	Unknown
Feline spongiform encephalopathy	Cats	Infection with beef contaminated with prions
Exotic ungulate encephalopathy	Greater Kudu, Nyala, Oryx	Infection with prion contaminated bone meal and meat

PrP, prion protein; PrP^{c}, cellular prion protein; PrP^{sc}, scrapie isoform of the prion protein; CJD, Creutzfeldt–Jacob disease.

Table 2.
Pathogenic feature of the types of prion disease [1, 2].

Disease	Protein	Histopathological identification
Prion disease [1, 2]	PrP^{Sc}	PrP^{sc} amyloid plaques
Alzheimer's disease [6, 7]	Aβ	Aβ amyloid plaques
	Tau	Paired helical filaments in neurofibrillary tangles
Parkinson's disease [8–10]	ά-Synuclein	Lewy bodies
Frontotemporal dementia [1, 2]	Tau	Straight filaments and paired helical filaments
Pick's disease [1, 2]	Tau	Pick bodies
Amyotrophic lateral sclerosis [1, 2]	Neurofilament	Neuronal aggregates
Huntington's disease [1, 2, 11, 12]	Huntington	Nuclear inclusions of CAG repeats
Spinocerebellar ataxia [1, 2]		
Type 1	Ataxin 1	Nuclear inclusions
Type 2	Ataxin 2	Cytoplasmic inclusions
Machado–Joseph disease	Ataxin 3	Nuclear inclusions

Table 3.
Protein detection in prevalent neurodegenerative diseases.

examples of neurodegenerative diseases are amyotrophic lateral sclerosis, Parkinson's disease, Alzheimer's disease, and Huntington's disease, which may be grouped as non-infectious neurodegenerative proteinopathies, and prion diseases considered infectious neurodegenerative proteinopathies [1, 2]. The pathogenic feature of the variety of prion disease, an infectious neurodegenerative proteinopathy, is shown in **Table 2**, while protein deposition seen in most common neurodegenerative diseases is shown in **Table 3**.

2. Infectious neurodegenerative proteinopathies

2.1 Prion diseases

Infectious neurodegenerative proteinopathies are proteinopathies that are communicable diseases, and their characteristic symptom is neurodegeneration in humans. Prion disease is an excellent example of such a disease. Some types of prion disease, also known as transmissible spongiform encephalopathies (TSEs), such as Creutzfeldt–Jakob disease (CJD), Kuru, Gerstmann–Straussler–Scheinker syndrome (GSS), and fatal familial insomnia, are a group of fatal neurodegenerative diseases. They are incurable but manageable; they can affect humans and animals and are sometimes transmitted to humans by infected meat products. The clinical progression is over weeks, progressing to akinetic mutism with a median disease duration of 20 weeks. Prodromal features, present in around 30% of cases, include depression, fatigue, weight loss, headaches, insomnia, general malaise, and ill-defined pain sensations. Additionally, we see myoclonus and mental deterioration, while neurological features include pyramidal signs, extrapyramidal signs, cerebellar ataxia, and cortical blindness [13–15]. The pathogenic features of the prion disease are shown in **Table 2**. Risk factors for prion disease are positive family history, eating meat infected by "mad cow disease," infection from receiving contaminated organs or tissues including corneal tissue, or contaminated medical equipment [16].

The central feature of the pathogenesis seen in most types of prion disease is the post-translational conversion of host-encoded, normal, healthy, cellular prion protein (PrP^{C}) to an abnormal infectious isoform, termed scrapie isoform of the prion protein (PrP^{Sc}) or (PrP^{res}), which is an alternatively folded variant of the cellular prion protein, PrP^{C} [15–17]. This misfolding of PrP^{Sc} is possible due to the more important content of the β-sheet structure, which aggregates to form medium and large-size polymers [18]. Studies have proposed different functions of PrP^{C}, such as the roles in apoptosis, neuroprotection, oxidative stress, transmembrane signaling, cell adhesion, myelination, and trafficking of metal ions. The critical event in the stages of prion disease is the structural and conformational change of PrP^{C} to the disease-associated misfolded form, PrP^{Sc} [18]. This conversion changes PrP^{C} from a protein characterized by alpha-helices to a partially protease-resistant misfolded protein filled with beta-sheets (β-sheets). Proteinase K (PK) partially digests PrP^{Sc} and is often used to determine the presence of misfolded PrP^{Sc}. PrP^{Sc} accumulates in different brain regions as distinct types of deposits depending on the animal species and strains of the infectious agent. The incapacitation of the critical biological function of PrP^{C} is one possible mechanism by which PrP^{Sc} formation might lead to degeneration of neurons. Another possible mechanism by which PrP^{Sc} formation might be linked to the disease is by direct toxicity of the misfolded protein [15–18]. **Table 2** shows the list of prion diseases and their routes of infection. Prion disease affecting animals has been included for academic purposes; however, our discussion is focused on those affecting humans.

2.2 Etiology and clinical manifestation

Human prion diseases can be grouped etiologically into sporadic, inherited, and acquired forms [13]. The following paragraphs shall consider the different etiological classifications of prion diseases, stating the respective examples.

2.2.1 Sporadic cases

More than 80% of the occurrence is sporadic cases of human prion disease, which presents as Creutzfeldt–Jakob disease (sporadic CJD). The cause of sporadic CJD is unknown, although it is hypothesized to include somatic mutation of the prion protein gene (*PRNP*) or the spontaneous conversion to PrP^{Sc} form of PrP^{C}. A polymorphism that occurs at residue 129 of human PrP (encoding either methionine (M) or valine (V)) strongly influences the susceptibility to human prion diseases. About a third of Europeans are homozygous for the more frequent methionine allele, half are heterozygous, and a tenth is homozygous for valine. Homozygosity at *PRNP* codon 129 is a causal factor to the development of sporadic and acquired CJD. Polymorphic homozygosity favors the occurrence of most sporadic CJD. This susceptibility factor is also vital in the inherited forms of CJD, most especially in vCJD. All hospitalized cases studied so far have been homozygous for codon 129 methionine of *PRNP*. Additionally, a haplotype for *PRNP* susceptibility has been identified, indicating additional genetic susceptibility to sporadic CJD at or near the *PRNP* locus [15–18].

Classical sporadic CJD presents with a rapidly progressive multifocal dementia predominantly with myoclonus. The onset is usually in the 45–75 years age group, with the median age at death of 68 years. The clinical progression expends over weeks, progressing to akinetic mutism with a median disease duration of 5 months. Prodromal features, present in about 30% of cases, include fatigue, insomnia,

headaches, weight loss, depression, malaise, and non-specific pain sensations. In addition to mental deterioration and myoclonus with cerebellar ataxia, frequent additional neurological features include extrapyramidal signs, pyramidal signs, and cortical blindness [13, 18].

Atypical forms of sporadic CJD are seen in about 10% of cases of CJD, and they have a longer duration of a clinical course spanning over 2 years. Here, cerebellar ataxia is seen instead of cognitive impairment. Hence, it is termed ataxic CJD [19]. Heidenhain's variant of CJD refers to conditions in which cortical blindness is marked with severe involvement of the occipital lobes. The panencephalopathic type of CJD is more common in Japan; it presents with extensive degeneration of the cerebral white matter and spongiform vacuolation of the gray matter [20].

2.2.2 Inherited prion disease

About a fifth of human prion diseases is associated with autosomal dominant pathogenic mutations in *PRNP* [21–23]. The mechanism by which pathogenic mutations in *PRNP* cause prion disease is yet to be elucidated; however, it is believed that in most cases, it involves a mutation that leads to an increased tendency of PrP^{C} to form PrP^{Sc}. Even though pieces of evidence abound in congruence with this, this may partly be related to the decreased thermodynamic stability of mutated PrP^{C} [24, 25].

Traditionally, inherited prion diseases have been classified by the presenting clinical syndrome, falling into three main sub-divisions: GSS, CJD, or FFI. GSS is seen in people in their 40s; it classically presents as chronic cerebellar ataxia with pyramidal features with dementia seen much later in a clinical course that is usually longer than in classical CJD [13, 23]. Fatal familial insomnia (FFI) has its pathognomonic feature as progressive chronic insomnia, dementia and dysautonomia, selective thalamic degeneration, and is mainly associated with a missense mutation at codon 178 of *PRNP* (3); its sporadic form with no causative mutation in *PRNP* have been reported [13, 23, 24]. Another form of inherited prion disease, though extremely rare, is variably protease-sensitive prionopathy (VPSPr). VPSPr is similar to CJD; however, the protein is less sensitive to digestion. It is more likely to affect people in their seventh decade of life with a family history of dementia. The existence of phenotypic overlap between individuals with different mutations and even in family members with the same *PRNP* mutation indicates that accurate classification of inherited human prion diseases should be based upon mutation alone [24–26]. Due to the extensive phenotypic variability associated with inherited prion disease and its ability to mimic other neurodegenerative conditions, notably Alzheimer's disease, *PRNP* analysis should be considered in all patients with undiagnosed dementing ataxic disorders [13, 23, 26].

2.2.3 Acquired prion disease

Human prion diseases are transmissible diseases; their acquired forms have, however, until recently, been confined to rare and unusual situations. They include the iatrogenic CJD, Kuru, and variant CJD.

The two most prevalent causes of iatrogenic CJD occurring through the medical procedure are the implantation of grafts of dura mater and treatment with growth hormone derived from the pituitary glands of human cadavers [13]. Less frequent causes of human prion disease have been associated with the iatrogenic transmission of CJD during corneal transplantation, infected electroencephalographic (EEG) electrode implantation, and surgical operations using contaminated instruments or

apparatus [13, 27, 28]. The clinical presentation in iatrogenic forms of human prion disease appears to be related to their etiology and, in particular, the route of exposure to human prions [13]. Peripheral routes of infection are commonly associated with more extended incubation periods and usually present with a Kuru-like syndrome, in which ataxia is common, while dementia is rare at the onset. Conversely, patients with dura mater graft-related exposure to human prions, in which infectivity is placed proximal to the brain, usually have a clinical presentation that looks like sporadic CJD, although exceptions with unusual clinical features have been reported [13, 27–29].

Kuru is a disease that used to be predominant among cannibals in the Fore tribe of the Eastern Highlands in Papua New Guinea, but it is now rare due to consistent enlightenment and rules that abolished such culture [29, 30]. It is caused by eating prion ladened human brain tissue. The central clinical feature of Kuru is progressive cerebellar ataxia, and in sharp contrast to sporadic CJD, dementia is late and may be absent. A prodrome and three clinical stages consisting of an ambulatory stage, a sedentary stage, and a tertiary stage have been described [13, 23, 29]. Remarkably, Kuru demonstrates that incubation periods of infection with human prions can exceed 50 years [29]. The *PRNP* codon 129 genotype has been identified to have a pronounced effect on Kuru in terms of the incubation periods and susceptibility, and most elderly survivors of the kuru epidemic are heterozygotes [26, 30, 31]. The glaring survival advantage for codon 129 heterozygotes gives a cue for a robust basis for selection pressure in the Fore clan [13, 23, 26]. However, analyzing the global haplotype diversity and frequency of the alleles responsible for coding and noncoding polymorphisms of *PRNP*, an older and widely spread balancing selection at this locus has more unusual variation because of heterozygote advantage is suggestive [23, 29, 31]. Only a few human genes present evidence for balancing selection. With the biochemical and physical evidence of cannibalism on five continents, one explanation is that cannibalism resulted in prion disease epidemics in human prehistory, thus imposing balancing selection on *PRNP* [23, 29, 31].

The variant CJD is an infectious type of disease that is related to "mad cow disease." Eating meat that has been inflicted with bovine spongiform encephalopathy (BSE) may cause the disease in humans, as seen in the United Kingdom years back [13, 27]. The meat may cause abnormal development of normal human prion protein. The disease is associated with iatrogenic conditions. This disease usually affects younger people and is rare in most developed nations [13, 27, 32].

2.3 Diagnosis of prion

Prion disease can be provisionally diagnosed using the clinical signs and symptoms presented alongside the taking of history. Neurologic and visual examinations could be done to ascertain nerve damage and vision loss. Prion diseases such as CJD can be diagnosed via MRI, PET, and CT scans of the brain and body; and spinal tapped cerebrospinal fluid (CSF). Electroencephalogram, which analyses brain waves, could also be used; this painless test requires placing electrodes on the scalp. At the same time, some centers choose to do blood and urine tests, which involves immunologically based analysis. Raised cerebrospinal fluid 14-3-3 protein, S-100, and neuronal-specific enolase (NSE), although unspecific for CJD, may be helpful diagnostically in the appropriate clinical context [13].

Prions lack DNA or RNA, so PCR or other nucleic acid-based tests cannot identify them. Hence, the strategy is to mix the test material with the proteinase K (PK)

enzyme, which digests the regular portion of prion protein but cannot digest any of the portions, which appears abnormal. Some other techniques aim at detecting the residual protein (PrP^{Sc}) after digestion. Methods relying on PK digestion are less sensitive than those that do not rely on it because the former reduces the small amount of original PrP^{Sc} captured [33].

The most sensitive, crucial, precise, but uncommon immunoassay method of confirmatory diagnosis is by identifying the disease-causing PrP isoform (PrP^{Sc}) using the conformation-dependent immunoassay (CDI) laboratory method [15, 34, 35]. The CDI is the only immunoassay that measures both the protease-resistant and protease-sensitive forms of PrP^{Sc} [14]. The CDI was developed to quantify PrP^{Sc} in tissue samples from mammals producing prions. Sandwich CDI represents a rapid, robust, powerful tool to study prions in bodily fluids of CJD/vCJD patients, with a turnaround time of 12–24 hours [15, 34]. Safar et al., in their experiment, showed the superior performance of the CDI in diagnosing prion disease compared to the routine neuropathologic examination and immunohistochemistry (IHC). Hence, they proposed using CDI in place of these earlier mentioned methods [14, 33].

2.4 Managing prion diseases

Prion diseases rarely have a cure; hence they are managed using certain medications, which could slow their progress. This management focuses on keeping people with these diseases as safe and comfortable as possible despite progressive and debilitating symptoms.

Effective anti-prion agents may have broader implications due to the adverse effects associated with them. Several therapeutic approaches include polyanionic, polycyclic drugs such as pentosan polysulfate (PPS), which prevent the conversion of PrP^{c} to PrP^{res} and might also sequester and down-regulate the protease-resistant prion protein (PrP^{res}). Polyanionic compounds might also help to clear PrP^{res}. Treatments aimed at the laminin receptor, an essential accessory molecule in converting PrP^{c} to PrP^{res}—neuroprotection, immunotherapy, siRNA, and antisense approaches, have provided some experimental cues [28].

In drug development, the PrP^{C}, PrP^{Sc} (PrP^{res}), or the process of its conversion are the targets. Pentosan polysulphate (PPS) is presumed to act as a coreceptor for PrP on the cell surface in competition with endogenous heparin sulfate proteoglycans and shows the ability to inhibit the formation of new PrP^{Sc} in neuroblastoma cells. Quinacrine is thought to prevent PrP^{Sc} polymerization by stabilizing PrP^{C} and reducing its conversion to PrP^{Sc}. Doxycycline reverses the protease resistance of PrP^{Sc} extracted from CJD brains and prolongs the survival of animals experimentally infected with prions, even when given at the onset of clinical signs [33, 36–38]. Active and passive immunization are two significant aspects of immunotherapy. Resveratrol is an essential compound with antioxidant, anti-allergy, anti-aging, and neuroprotective activities, and it has been reported to eliminate prion replication *in vitro* and prion infection *in vivo*. The ubiquitin (Ub)-proteasome system (UPS) is the first line of defense in degrading soluble misfolded proteins. Conversion from PrP^{C} into PrP^{Sc} may involve chaperones and Ub ligases for UPS-dependent protein quality control. Enhanced UPS aims to stimulate the degradation of PrP^{Sc}. The autophagy-lysosome system is another quality control system to remove the misfolded proteins [36–38]. Studies have alluded that rapamycin can activate autophagy *in vitro* and delay disease onset in rodents with prion disease [39]. Autophagy could also lead to PrP^{Sc} clearance in cell models and prolong the lifespan of prion-infected mice [36].

3. Non-infectious neurodegenerative proteinopathies

3.1 Alzheimer disease

Alzheimer's disease (AD), a progressive neurodegenerative disorder, is the leading cause of dementia among geriatrics [6, 40]. It affects over 27 million persons worldwide, and prediction shows that over 86 million people would be affected by 2050 [7]. It is characterized by difficulty solving problems, memory loss, disorientation in time and space, among others [41]. This disease was first described in 1906 at a conference in Tubingen, Germany, by Alois Alzheimer [41]. Aging seen in the absence or presence of dementia of the Alzheimer type (DAT) is associated with loss of weight; hence, accelerated weight loss of idiopathic origin may herald the onset of DAT, aiding its clinical diagnosis [42]. The significant risk factors of this multifactorial disease include apolipoprotein E 4, hypercholesterolemia, genotype, traumatic brain injury, family history, age, obesity, hypertension, diabetes, and low level of education [6].

A complex array of molecular events has been implicated in the pathogenesis of AD. The major pathological characteristics of AD brains are senile, neurofibrillary tangles, plaques, and neuronal loss [6, 7, 40, 41]. The pathogenic mechanism implicated here seems elusive; however, oxidative stress has been identified as a leading factor in the initiation and progression of the ailment [43]. The excessive reactive oxygen species may be generated from mitochondria dysfunction and aberrant accumulation of transition metals, while the abnormal accumulation of amyloid-beta (Aβ) and tau proteins appears to promote the redox imbalance leading to neurotoxicity [41–43]. Additionally, oxidative stress may augment the production and aggregation of Aβ and facilitate the phosphorylation and polymerization of tau, leading to a vicious cycle that promotes the initiation and progression of AD [43]. Researches are gradually drifting from the simple assumption of the original amyloid hypothesis to new theories of pathogenesis, which include gamma oscillations, cerebral vasoconstriction, prion transmission, growth hormone secretagogue receptor 1α (GHSR1α)-mediated mechanism, and infection [44].

3.1.1 Diagnosis

The disease morphologically features an overall loss of synapses and neurons and an overall reduction in brain volume. The neuropathologic examination has been identified as the gold standard for diagnosing Alzheimer's disease (AD). However, popular opinion has it that histologic examination is the best indicator of AD diagnosis. Thus, an autopsy may gradually become the gold standard for determining clinical diagnostic accuracy rates [7, 45, 46]. A routine examination is better done with magnetic resonance (MR) or computed tomographic (CT) imaging. In the early onset of the disease, coronal MR images have been helpful to document or quantify the atrophy of both the hippocampus and entorhinal cortex. At the same time, subtraction and volumetric MR techniques can be used to quantify and monitor rates of regional atrophy and dementia progression. Positron emission tomography (PET) coupled with single-photon emission CT is helpful in the differential diagnosis of AD from other dementias associated with the cortical and subcortical dementias and may also be of prognostic value. Values from the MR are also used to monitor treatment effects in clinical trials of antidementia agents and cognitive enhancers [7, 40, 46].

Additionally, PET studies have shown that subtle abnormalities may occur at the prodromal stages of AD and in subjects bearing susceptibility genes. PET ligands

may be of value in identifying amyloid plaques. Functional MR-based memory challenge tests are also beneficial [7, 40, 46].

Peripheral biomarkers are also beginning to gain ground in the diagnosis of Alzheimer's disease. This gives room for presymptomatic detection of disease, which could be valuable for monitoring the efficacy of disease interventions during clinical trials. CSF has long remained the sample of choice for biomarkers for many scientists until some Australian scientists developed theirs using blood. A biomarker panel that was about 85% sensitive and 93% specific was developed. The plasma markers in this biomarker panel that was significantly increased were cortisol, pancreatic polypeptide, β_2 microglobulin, insulin-like growth factor binding protein 2, and vascular cell adhesion molecule 1. There was also CD40, carcinoembryonic antigen, matrix metalloprotein 2, macrophage inflammatory protein 1α, superoxide dismutase, and homocysteine. In AD, these markers were decreased (apolipoprotein E, epidermal growth factor receptor, hemoglobin, calcium, zinc, interleukin 17, and albumin). This panel of plasma biomarkers was proven to be efficient, as it distinguished individuals with AD from cognitively healthy control subjects with high precision [7, 46].

Nevertheless, the prominent causal factors for AD development are genetic mutation involving genes encoding for proteins such as presenilin 1 (PSEN1), presenilin 2 (PSEN2), and amyloid precursor protein (APP). Usually, at an early age between the third and fifth decade of life, about half of the carriers of such mutations develop AD-type dementia. The hallmark of AD includes the accumulation of Aβ as senile plaques and aggregating hyperphosphorylated tau-mediated neurofibrillary tangles, NFTs for short [6, 7].

3.1.2 Management

Previous researches implicated an instability in the homeostasis of neuronal Ca^{2+} in age-related cognitive impairment associated with Alzheimer's disease (AD). This is seen when increased oxidative stress and impaired energy metabolism associated with senescent neurons lead to malfunctioning proteins that control membrane excitability and subcellular Ca^{2+} dynamics. Toxic forms of amyloid β-peptide (Aβ) may trigger Ca^{2+} influx into neurons by inducing membrane-associated oxidative stress or forming an oligomeric pore in the membrane, thus, exposing neurons to excitotoxicity and apoptosis. During AD, mutations in the β-amyloid precursor protein and presenilins may compromise the normal proteins in the plasma membrane and endoplasmic reticulum. With time, knowledge of the actions of Ca^{2+} upstream and downstream of Aβ gave a cue to developing some prophylactic or curative interventions for AD [47].

Alzheimer's disease is managed, not cured; the only medications approved for managing the disease are used for mild to moderate AD. These drugs are the cholinesterase inhibitors (ChEI): tacrine, rivastigmine, donepezil, and galantamine. While for moderate to severe AD is memantine, a noncompetitive *N*-methyl-D-aspartate (NMDA) receptor inhibitor, which blocks the excess release of glutamate assumed to be related to cholinergic damage [6, 48, 49].

Research has shown no additional benefit of combination therapy involving vitamin E (2000 IU/day) and selegiline (10 mg/day). However, due to low cost and relative safety, vitamin E was recommended in addition to ChEIs to slow AD progression. Some other treatments, such as Ginkgo biloba, anti-inflammatory drugs, and hormone replacement therapy, have been suggested as possible treatments, although with insufficient evidence [6, 48, 49].

Another treatment option is a combination of memantine and donepezil, and the combination therapy (Namzaric®) was recommended in 2014 to treat individuals with moderate to severe AD who are stabilized on donepezil and memantine therapy. The multi-target-directed ligands (MTDLs) approach currently focuses on designing hybrid molecules that simultaneously regulate multiple biological targets. Moquin is a drug, which has been developed as a potential anti-AD candidate because of its MTDL design capacity. However, combination therapy (CT), including ChEIs and memantine, currently constitutes the best and effective treatment for individuals displaying moderate-to-severe AD. Additionally, CT exhibited better clinical efficacy than monotherapy, along with similar tolerability and safety [6].

3.2 Parkinson disease

Parkinson's disease (PD) is a neurodegenerative clinical syndrome characterized by at least two of four cardinal features: bradykinesia, rigidity, resting tremor, and impairment of postural balance leading to disturbance of gait and falling. James Parkinson, an English physician, was the first to describe this disease, when he called it the "shaking palsy" in 1817 and also coined the term, *paralysis agitans* meaning the shaking palsy, since then, there is still a lack of understanding of the causes of PD [8, 50]. Parkinson's disease may be mistaken with the regular essential tremor; however, the difference in both is that the tremor in Parkinsonism occurs predominantly at rest, while that of essential tremor is seen during actions. Also, tremor in Parkinsonism is unilaterally seen in the arms or legs, while essential tremor bilaterally affects both upper limbs. Bradykinesia is usually the most troublesome symptom. Patients report slowness in performing their daily activities. Falls and swallowing problems are classic signs of late Parkinson's disease; however, if they occur early and are accompanied by unresponsiveness to treatment, they may indicate multiple system atrophy or progressive supranuclear palsy. Early dementia and other features could indicate Lewy body dementia, vascular Parkinsonism, or corticobasal degeneration. Young patients with Parkinsonism (aged <40 years) should always be evaluated for changes in the values of their serum copper and ceruloplasmin levels, with a 24-hour urine collection for copper excretion and slit-lamp examination for Kayser–Fleischer rings in consideration of Wilson's disease [51].

The pathognomonic feature of PD is a loss of the pigmented, dopaminergic neurons of the substantia nigra pars compacta in the brain, with the appearance of intracellular inclusions known as Lewy bodies. During the 1960s, researchers identified a fundamental defect that is a hallmark of the disease: the loss of brain cells that produce an essential chemical, dopamine, which helps direct muscle activity. Gradual loss of dopamine-containing neurons is a feature of normal aging; however, most people do not lose 70–80% of the dopaminergic neurons that cause symptomatic PD. In the absence of treatment, PD gradually deteriorates into a rigid, akinetic state where patients cannot care for themselves within 5–10 years. Death may result from complications of immobility, such as aspiration pneumonia and pulmonary embolism [50, 51].

3.2.1 Diagnosis

This aims to identify ubiquitous Lewy bodies in microscopic postmortem studies, a feature of cell death associated with the disease. However, the clinical diagnosis in PD includes cardinal motor symptoms such as akinesia, rigidity, and tremor [8].

Diagnosis of Parkinsonism involves structured clinical examinations or autopsies. The central pathology in PD is the degeneration of pigmented neurons in the brainstem. Through a microscope, intracellular Lewy bodies are easily identified. The neurons located in the substantia nigra pars compacta are the most affected, resulting in dopamine depletion to its major projection area, the striatum. The depletion culminates into an overactive subthalamic nucleus, which increases the activity of the major inhibitory output nuclei such as the globus pallidus and substantia nigra pars reticulata, resulting in increased inhibition of thalamic activity and problems with motor output. Lewy bodies in the cortex and deeper structures are the main features that distinguish Parkinson's disease (PD) from dementia with Lewy bodies (DLB), a type of neurodegeneration sharing similarities with Alzheimer's and Parkinson's [9].

Due to the presence of Lewy-type α-synucleinopathy in the submandibular glands of PD patients, some scientists considered the feasibility of submandibular gland biopsy for diagnosing PD. Hence, immunohistochemical staining was considered for Lewy-type α-synucleinopathy [10]. Some studies also considered performing needle core biopsies of the submandibular gland in living patients with PD to assess Lewy-type α-synucleinopathy (LTS). Although it was a small-scale study, this tissue biopsy method may be valuable for confirming PD in patients being considered for invasive medical interventions and research studies of other PD biomarkers [52].

Autopsy remains the main definitive diagnostic tool. Some studies provided evidence that unilateral onset of symptoms with features that include tremor and at least one of bradykinesia and rigidity with an efficient initial response to L-dopa have been the best predictors of the pathological diagnosis. In a fifth of the cases, a different neurological disorder was diagnosed at autopsy from that diagnosed during life. Neurological imaging studies with computed tomography or MRI do not reveal any specific changes related to Parkinson's disease. However, most neurologists perform brain imaging tests to rule out rare conditions requiring a different treatment regimen and management strategies, such as normal pressure hydrocephalus or focal lesions. Functional imaging of brain regions affected by Parkinsonism with either positron emission tomography (PET) or single-photon emission tomography SPECT has been proposed [51].

3.2.2 Management

For the efficient management of medical conditions, the risk–benefit ratio is considered; the aim here is to make the patient experience wellness as close to normal function as possible without having side effects from therapy. Hence, the appropriate multi-disciplinary approach must be utilized.

Some factors are considered to determine the optimal choice for the individual patient at different phases. These include the following:

1. Level of patient disability in terms of performing a daily routine which includes work. Here L-dopa may be indicated. Dopamine agonists may be efficient for patients with mild to moderate disabilities. For very mild symptoms, anticholinergic drugs or amantadine may be considered [51].

2. Prevention of response fluctuations. The initial use of dopamine agonists may attenuate the risk of developing dyskinesias, "wearing off" and "on–off fluctuations."

3. Age of the patient. Those that occur at younger ages (aged <65 years) are more tolerable to medications and may bear a lower risk of side effects. Geriatrics often have more challenges with cognitive and psychiatric side effects, and physicians should be cautious while administering anticholinergics and amantadine. Dopamine agonists may also present more side effects in elderly patients [51].

4. Side-effect profile of the drug under review. If a patient is worried about potential drowsiness or may not tolerate a change in mental status or already has cognitive impairment, then a dopamine agonist may not be a good choice. Ankle edema may be exacerbated by amantadine or dopamine agonists [51].

5. Cost for patients without health care coverage. Generic L-dopa/carbidopa and bromocriptine may be the most affordable [51].

Pharmacological attempts to restore dopaminergic activity with levodopa and dopamine agonists have successfully alleviated many of the clinical features of PD. A complementary approach has been to resuscitate the normal balance of cholinergic and dopaminergic influences on the basal ganglia with anticholinergic drugs. The availability of effective pharmacological treatment has drastically altered the prognosis of PD; in many cases, good functional mobility can be achieved for many years, and the life expectancy of well-managed patients is increased substantially [50]. It is important to emphasize that PD therapy must be individualized and tailored to the specific needs of each patient using a basic algorithm [53, 54].

Treatment of early PD with mild symptoms benefit from nonpharmacological therapy such as exercise and relaxation techniques. However, monoamine (MAO) inhibitors such as selegiline, rasagiline, and safinamide; dopamine agonists; or anticholinergic medications are added to ameliorate conditions [50, 53]. Levodopa or dopamine agonist could be added to ease the challenges associated with the motor neurons [50, 53]. Decades of clinical observation have validated levodopa as the most effective primary medicinal agent [50]. Entacapone (Comtan) and rasagiline (Agilect) could help hold brief pending when PD has progressed and the medications seem inefficient in relieving symptoms [50]. Surgical and experimental therapeutics should be considered as the disease progresses and motor complications (including motor fluctuations and dyskinesias) develop [53]. Inosine, which increases urates, and Isradipine, a calcium channel blocker, when added, are treatments designed to prevent the accumulation of toxic α-synuclein. Monoclonal antibodies directed at aggregated α-synuclein in some patients with Parkinson's disease also provided evidence of strong target engagement and CNS penetration [53].

Optimizing the pharmacologic treatment for both motor and non-motor symptoms is critical; however, nutritional interventions cum counseling could also be planned to manage weight gain or loss of weight efficiently. The optimization of levodopa pharmacokinetics and avoidance of interaction with proteins; improvement in gastrointestinal dysfunction such as dysphagia and constipation; prevention and treatment of nutritional deficiencies either the micronutrients or vitamins could systematically be employed [55]. However, other therapeutic interventions such as continuous pump therapies with apomorphine or parenteral levodopa or the implantation of electrodes for deep brain stimulation could also be considered [8]. **Table 3** shows the type of protein detected in some popular neurodegenerative diseases, including those not discussed in this work.

3.3 Huntington disease

The disease got its name from the physician George Huntington, who first described it in late 1872. Huntington's disease is a hereditary, autosomal dominant, progressive neurodegenerative disease associated with a single abnormal gene on chromosome 4. The pathogenesis is initiated by a CAG (glutamine) trinucleotide expansion in exon 1 of the Huntingtin (*HTT*) gene, which is found at the short arm of chromosome 4p16.9. The normal function of the Huntington gene *HTT* is not known, but it may be involved in sustaining the cyclic adenosine monophosphate response element-binding protein, intracellular signaling, and obviating toxicity of neurons. Earlier studies suggest that the conjugation of the striatum-protein-rich Ras homolog with mutant HTT (mHTT) could lead to cellular toxicity. Although, why this protein causes cellular toxicity is poorly understood. Some evidence suggests that the interaction of the mHTT protein and the group 1 metabotropic glutamate receptors may be at the root of the delayed onset [11, 12, 56].

Huntington's disease (HD) is clinically characterized by cognitive dysfunction, abnormal involuntary movements, behavioral disturbance, and psychiatric disease. In abnormal involuntary movements, symptoms may include chorea, dystonia, rigidity, akathisia, bruxism, swallowing disorders, myoclonus, impaired manual dexterity, impaired global motor capacities, and gait and balance disorders. Cognitive dysfunction may present with impaired executive functions, bradyphrenia, language and communication disorders, and social cognition impairments. Behavioral disorders could be associated with memory disorders, disorientation, and visuospatial and visual perceptual disorders. Psychoanalysis of the patient could reveal depression, suicidal ideation or attempts, irritability, apathy, anxiety, obsessions, impulsivity, sexual disorders, hallucinations, sleep disorders, urinary incontinence, pain, dental pain, excessive perspiration, weight loss, hypersalivation, reduced lung function, and respiratory muscle strength [11, 12, 57].

The disease typically lasts 15–20 years, with dementia, mutism, dystonia, and bradykinesia becoming the classic symptoms in advanced forms of the disease. The mean age at onset is between the third and fifth decade of life, with a range of 2–85 years. Juvenile Huntington's disease (JHD) is when the first symptoms and signs appear before the second decade of life. The symptoms of young and old patients vary, as the younger patient presents with an overwhelming rigidity (Westphal variant), while the geriatric becomes bed-bound with rigidity and flexion contractures in the limbs [11, 58].

Pathologically, diffuse neurodegeneration is seen in the cortex and the striatum. The medium spiny neurons are the primary neurons affected, marked with the conspicuous presence of γ-aminobutyric acid and enkephalin. These neurons typically project into the lateral globus pallidus. With time, this degenerative process progresses to the rest of the basal ganglia with subsequent dissemination, reaching the cortex and substantia nigra. Aggregates of mHTT are seen within the nucleus and cytoplasm during microscopy. Inclusion bodies containing a complex of Huntingtin and other soluble mHTT are seen in the neurons. The cause of the cell death seen in this disease is yet to be delineated between the accumulation of the mHTT conglomerate or the soluble form of the protein when toxic. Glutamate, dopamine, and γ-aminobutyric acid are considered the most affected neurotransmitters in HD; hence, they are the focus of current pharmacological interventions [11, 12, 58].

The prevalence among the European is at 4–8 cases per 100,000, While America has not had a general epidemiological study since 1993. It is rare in Japan and Finland but common in Scotland and Venezuela. At the same time, there are inadequate data from Africans, Black Americans, and those in Eastern Asia [58].

3.3.1 Diagnosis

After accessing the clinical signs and symptoms presented, a genetic test called predictive test is requested. A DNA test showing abnormal CAG expansion (or repeats) in the *HTT* gene can be used to confirm the diagnosis in symptomatic individuals. The CAG (cytosine (C), adenine (A), and guanine (G)) repeats seen in the juvenile HD is over 55 in most cases. While for the elderly, it is about 36–40. The longer the repeats, the younger the age of the patients [11]. Biomarkers could also be exploited. With biomarkers, identification of mHTT in CSF could be a positive indication [12].

3.3.2 Management

Due to the myriad of disorders involved in this disease, it benefits more from symptomatic management rather than a definitive cure. This symptomatic management which is multi-disciplinary in approach, includes physical therapy, gastrostomy device, and medications such as antidepressants and antipsychotics—the chorea benefits from atypical antipsychotic drugs, which include olanzapine and tetrabenazine. Irritability benefits from an atypical antipsychotic drug in severe cases, but in mild cases, the use of selective serotonin reuptake inhibitor (SSRI), an antidepressant, may suffice. For obsessive–compulsive thoughts and actions, experts recommend SSRIs. For dystonia, physical therapy and injection of botulinum toxin are advocated [11, 58].

Since this disease involves the production of aberrant proteins, targeting the DNA or RNA may form a basis for drug discovery [12].

4. Metabolic disorders

Most metabolic disorders are nutrition-based disorders that are a result of the diet and lifestyle of the patients. Among these nutrition-based diseases, some ailment result from undernutrition due to food scarcity, leading to insufficient energy. At the same time, some result from overnutrition due to the insufficient capacity of the hormones regulating these nutrients. In this section, the focus is made on protein-energy malnutrition and diabetes mellitus, a type of hormonopathy.

4.1 Protein-energy malnutrition

The World Health Organization considers malnutrition in the context of both undernutrition and overnutrition. It could be described as the cellular imbalance between the supply of nutrients and energy and the body's demand to ensure healthy development, maintenance, and specific functions [59]. The term protein-energy malnutrition (PEM) includes kwashiorkor, marasmus, and intermediate states of marasmic-kwashiorkor. Those below 5 years may present a mixed picture of marasmus and kwashiorkor or milder forms of malnutrition. Malnutrition among children leads to waned immunity and increases susceptibility to diseases. Inadequate access to nutritious foods due to rising food prices is a common cause of malnutrition [60]. In the former times, rising food prices or food scarcity was induced by war; however, in recent times, terrorism and climate change could be implicated. Areas close to the desert may be experiencing an acute food shortage due to severe drought and other effects of climate change.

Studies have shown that those between 6 and 12 months are most affected, with over half of this population studied presenting with PEM and a third of those 13–24 months having PEM. Among these studies, marasmus is the most prevalent form of PEM, affecting a third of the population studied. Diarrhea and malaria are the associated co-morbidities popular with this disease, with over 60% of these populations coming from the lower socioeconomic status. The case fatality rate was 40.1%, with the males having more prevalence at 50.9%. Mortality among the marasmic-kwashiorkor and the unclassified group was 53.3 and 54.5%, respectively [61, 62].

The World Health Organization estimates that about two-thirds of all deaths occurring among pediatrics in developing countries could be attributed to malnutrition. Therefore, improving nutrition is crucial for reducing high infant and under-five mortality rates, the proportionate physical growth, the social and mental well-being of children, and academic achievement [61, 62]. Sub-Saharan Africa suffers the most from PEM around the world.

4.1.1 Diagnosis

The assay of total protein and albumin helps diagnose PEM, as early detection helps obviate the challenges associated with severe forms of PEM. In the final stage of wasting, reduced plasma albumin concentration ensues due to the adaptation of the human system to a protein-deficient diet. The development of marasmus reveals energy deficiencies in the diet, which leads to the change of the regular pattern of proteins. It is also observed that a decrease in serum albumin and total protein in PEM was due to reduced synthesis of protein resulting from inadequate intake of dietary protein. PEM in children is associated with a more significant deficiency of total protein, which may be as low as 50% of the child's total protein in severe cases. These reductions of total serum protein and albumin are prominent in kwashiorkor and marasmus [63].

In the absence of a diagnostic facility, the nutritional status of children is determined by clinical examination, history, and anthropometric measurements, which include height-for-age, weight-for-age, weight-for-height, head circumference, mid-upper arm circumference, and skinfold thickness which could be compared to the reference charts of the World Health Organization [60].

4.1.2 Management

A healthy balanced meal is advocated to fortify the immunity of infants and children under the age of 2. However, due to the inability to decipher the best description of a healthy balanced meal for children of such ages in most rural areas, exclusive breastfeeding is strongly advised for the first 6 months after delivery. While locally available meals and fruits rich in vitamin C, coupled with proteins such as eggs, are augmented with breastfeeding between the first 6 and 24 months of delivery. This daily intake of an egg and vitamin C-rich foods (or tablets) for at least one month is based on the need to boost the immunity and replenish the worn-out tissues of these pediatrics [64, 65]. The prevention of PEM cannot be overemphasized as it is associated with a high mortality rate among children under the ages of five in Sub Sahara, Africa [61, 62].

4.2 Hormonopathy

Hormonopathy is a term used in describing a disease associated with a change in the production of hormones. In these conditions, there can be over-secretion or

under-secretion of hormones or even the production of aberrant proteins. Endocrine proteinopathies, which are grouped under hormonopathy, are diseases associated with peptide- or protein-derived hormones. They are characterized by the hyper- or hypo-secretion of these proteins or an aberration in their structure and function. Protein-derived hormones include insulin, prolactin, ACTH, gastrin, parathyroid hormone, oxytocin, leptin, ADH and growth hormone. This section focuses on the most prevalent endocrine proteinopathy related to insulin, a disease called diabetes mellitus.

4.2.1 Diabetes mellitus

Diabetes causes severe life-threatening complications, such as hyperglycemic coma, hypoglycemic coma, severe impairment of renal function, blurred vision, memory loss, insulin allergy, and acute neuropathy. Managing it requires dietary control, physical exercise, and insulin administration. Demographic data is based on the patient's age, sex, location, and income. Clinical data is divided into physical signs and laboratory results. Physical signs are those obtained via physical examination of the patient, like BMI (body-mass index), pulse rate, and blood pressure, while the laboratory results are based on the blood sugar levels [66, 67].

An expert system determined by a set of rules used to make decisions is known as a rule-based expert system. Developing this expert system requires a knowledge of the engineering process in which the rules used by human experts are collated and translated into an appropriate form for computer processing [66, 67]. The rule-based expert system is utilized in this section.

4.2.2 Diagnosis

Laboratory results are associated with laboratory tests, like blood and urine tests [66–70]. Using the rule-based expert system, diagnosis is classified into:

I. Test urine for glucose and ketones.

II. Measure random or fasting blood glucose:

- Fasting plasma glucose ≥7.0 mmol/L
- Random plasma glucose ≥11.0 mmol/L

III. Oral glucose tolerance test:

- Fasting plasma glucose 6.1–6.9 mmol/L
- Random plasma glucose 7.0–11.0 mmol/L

4.2.3 Management

The management of diabetes can be classified into three categories.

I. Type-I (juvenile) Diabetes- only Insulin is used.

II. Gestational Diabetes- only Insulin is used, and

III. Type-II Diabetes- the oral hypoglycemic agent, low-carbohydrate diet, and sometimes Insulin is used. In this category, there are unique treatments:

- The drugs for obese and lean patients.
- The specific drugs for patients with challenges with their organs, such as renal diseases, lactic acidosis, and liver disease [69, 70].

5. Conclusion

Early and accurate diagnosis of protein-related diseases saves cost and prevents rapid deterioration of these disease conditions. It also prevents the waste of time and resources used in managing a misdiagnosed condition. The exposition in this chapter should be an eye-opener to the public and stakeholders in the public health domain to harp on the need for early diagnosis, treatment, or management of these diseases for improved health for all.

Health professionals and researchers are encouraged to give further research attention towards discovering a cure and treatment of these protein-related diseases. This can be done by utilizing the knowledge garnered from protein synthesis and the post-translational modification of proteins.

This work which is a summary of the prevalent neurodegenerative diseases and some metabolic disorders has taken the first step to elucidate on how proteins formed the basis of some modalities involved in the diagnosis and treatment of a few of these diseases; the onus lies on researchers in this field to consolidate on it and bring succor to the ailing population.

Acknowledgements

Special thanks to all those who diagnose and manage protein-related diseases in Nigeria; to those children of Northern Nigeria who became the first experience in PEM; to the geriatrics in Mgbidi, Oru west, whose health challenges continue to be an inspiration.

Conflict of interest

The authors declare no competing interests.

Notes/thanks/other declarations

Ohanube A.K. Goodluck conceptualized the work and wrote the section on neurodegenerative diseases and protein-energy malnutrition; Obeta M. Uchejeso wrote the section on Hormonopathy and Introduction; Ikeagwulonu Richard wrote the abstract and conclusion. All authors reviewed the work and approved the final manuscript.

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