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Peripheral Biomarkers for Early Detection of Alzheimer's and Parkinson's Diseases

Thein Than Htike[1] · Sachin Mishra[1] · Sundramurthy Kumar[1] · Parasuraman Padmanabhan[1] · Balázs Gulyás[1]

Abstract
Neurological disorders are found to be influencing the peripheral tissues outside CNS. Recent developments in biomarkers for CNS have emerged with various diagnostic and therapeutic shortcomings. The role of central biomarkers including CSF-based and molecular imaging-based probes are still unclear for early diagnosis of major neurological diseases. Current trends show that early detection of neurodegenerative diseases with non-invasive methods is a major focus of researchers, and the development of biomarkers aiming peripheral tissues is in demand. Alzheimer's and Parkinson's diseases are known for the progressive loss in neural structures or functions, including the neural death. Various dysfunctions of metabolic and biochemical pathways are associated with early occurrence of neuro-disorders in peripheral tissues including skin, blood cells, and eyes. This article reviews the peripheral biomarkers explored for early detection of Alzheimer's and Parkinson's diseases including blood cells, skin fibroblast, proteomics, saliva, olfactory, stomach and colon, heart and peripheral nervous system, and others.

Keywords Peripheral biomarkers · Alzheimer's disease · Parkinson's diseases · Early detection · Neurodegenerative diseases

Introduction

Despite various advancements in neurology in the last few decades, there are many mysteries in understanding the pathophysiology of several neurological diseases especially with the approach and possibilities of diagnosing and treating these abnormalities. Identifying novel biomarker for diagnosis, prognosis, and treatment is very important for neurodegenerative diseases (NDs). There are various key hurdles for identifying proximal biomarkers in neurological disorders including limited tissue availability from pathological site, requirement of lumbar puncture and cerebrospinal fluid (CSF) exploration, difficult to know biomarker sources in CNS disorders, low-quality clinical diagnosis, the complexity of brain tissue and functions, and absence of validation models. Peripheral biomarkers play an important role in solving these limitations and providing non-invasive alternate solutions for disease diagnosis. The exploration of biomarker models for early diagnosis is indeed an important goal for a better management of the neurological diseases. An ideal early diagnosis biomarker is supposed to be sensitive and specific to the initial neurological changes that occurs and should be able to differentiate among the diseased condition and normal aging conditions. Peripheral tissues are found to be indicators of cognitive and biological changes of brain and are supposed to differentiate the changes occurring during diseased conditions with normal conditions. This review article discuss about various peripheral biomarkers being discovered by researchers for early detection of Alzheimer's disease (AD) and Parkinson's disease (PD).

Alzheimer's Disease

AD is considered the most common cause of dementia, and its medication costs around $172 billion per year in the USA alone. Twenty-four million people are supposed to be suffering from this disease presently, and the predication of it being four times is till year 2050 [1, 2]. There are three forms of AD. Mild cognitive impairment (MCI) is an early phase of AD and 40 to 60% is progressing toward AD. When AD occurs in young people of 30 years old and below, it is called as early onset or familial AD. It is associated with genes like amyloid

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β-protein precursor (AβPP), presenilin-1 (PS1), and presenilin-2 (PS2). The most common form is the last-onset AD and mostly affects elderly people of 65 years old and above [3, 4]. It is counted as incurable and fatal NDs. The clinical symptoms include progressively losing of memory, declining of learning and executive function. Histopathologically, AD is diagnosed by death of neurons in the hippocampus, cerebral cortex, and other areas of the brain, finding deposit of amyloid β (Aβ) plaques in extracellular and neurofibrillary tau tangles (NFT) in intracellular regions [5, 6]. Figure 1 provides an overview of the early symptoms and peripheral biomarkers for early detection of AD.

Blood Cells as Biomarkers

There are many reports finding abnormalities or early changes of red blood cells (RBCs), white blood cells (WBCs), and platelets in AD. Protein kinase C (PKC) plays an important role in synapse formation and is involve in the function of memory. Decreased PKC levels, its activity, and cellular localization have been found in the studies of AD patient brains [7]. PKC signaling pathways disrupts in AD patients and also in animal models [8].
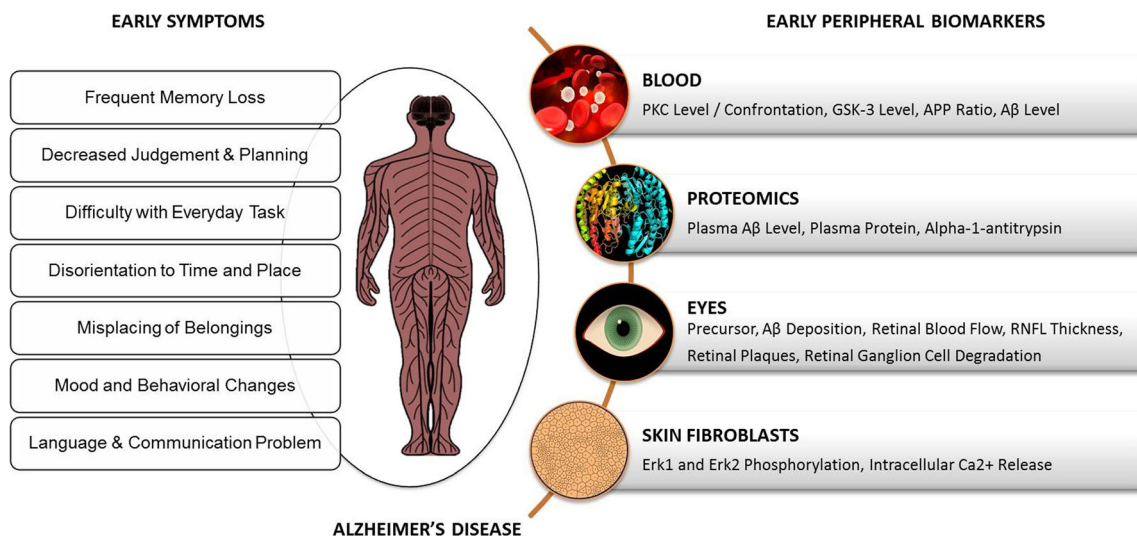
In RBCs, alteration of PKC conformations is observed in both AD patients and healthy controls. Janoshazi et al. developed a method in 2006 which used fluorescence probe and spectrum to measure conformational changes of PKC. These conformational changes were not found in RBCs of PD patients who have no dementia [9]. Hye et al. in 2005 measured isoforms of glycogen synthase kinase-3 (GSK-3) protein and its activity in WBC of three groups which are divided as AD, MCI, and elderly normal control subjects (Fig. 2). In AD and MCI groups, GSK-3 activity was decreased but total GSK-3 protein was increased [10]. In the mitochondria of primary neurons, in transgenic mice which were expressing familial

AD-linked forms of human APP, blocking GSK-3 activity by tau ablation can inhibit Aβ-induced transport deficits in anterograde axonal transport [11]. Soluble amyloid precursor protein alpha (sAPPalpha) also inhibits GSK-3 activity by blocking beta-site APP-converting enzyme 1 (BACE1). It can cause a reduction of tau and Aβ [12]. Investigation of GSK-3 activities might be a useful peripheral biomarker to diagnose early phase of AD.
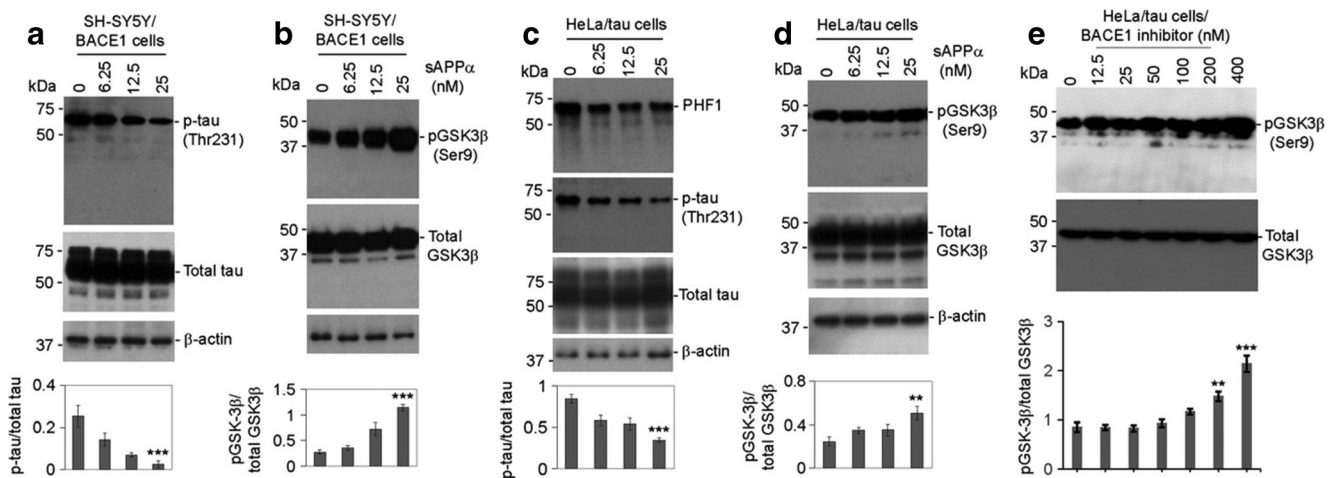
Abnormalities on AD platelets are shown in Fig. 3 [13]. Tang et al. studied blood platelet levels targeting 22-kDa fragment in 10 age-matched healthy patients and 31 AD patients. In this study, both Aβ level and activation of BACE1 were increased but decreased APP ratios and decreased activation of ADAM10 were also observed [14]. Baskin group did the similar research and found a decreased APP ratio in AD patients who were normal in PD and hemorrhagic stroke (HS) patients [15]. APP isoform ratio of 120 to 110 kDa was significantly lower in carriers who have mutation for autosomal-dominant AD compared with non-carriers. In the caudate nucleus and precuneus, APP is inversely correlated with amyloid imaging [16]. Lower ratio of APP provides a potential peripheral biomarker for AD's diagnosis, but further explorations are needed. Table 1 enlists the blood cells for peripheral biomarkers of AD.

MicroRNAs in the Plasma

MicroRNAs (miRNAs) are attractive molecules and considered one of the candidates for blood-based biomarkers in neurodegenerative diseases such as AD. MiRNAs are ~22 nt small non-coding RNAs that bind to the 3′-untranslated region of their target mRNAs to direct post-transcriptional repression of the target genes by forming the RNA-induced silencing complex, which leads to mRNA destabilization or translational inhibition [17, 18]. Some miRNAs are encapsulated in microvesicles, such as exosomes, and present as a relatively

EARLY SYMPTOMS

- Frequent Memory Loss
- Decreased Judgement & Planning
- Difficulty with Everyday Task
- Disorientation to Time and Place
- Misplacing of Belongings
- Mood and Behavioral Changes
- Language & Communication Problem

ALZHEIMER'S DISEASE

EARLY PERIPHERAL BIOMARKERS

BLOOD
PKC Level / Confrontation, GSK-3 Level, APP Ratio, Aβ Level

PROTEOMICS
Plasma Aβ Level, Plasma Protein, Alpha-1-antitrypsin

EYES
Precursor, Aβ Deposition, Retinal Blood Flow, RNFL Thickness, Retinal Plaques, Retinal Ganglion Cell Degradation

SKIN FIBROBLASTS
Erk1 and Erk2 Phosphorylation, Intracellular Ca2+ Release

Fig. 1 Early symptoms and peripheral biomarkers for early detection of AD

Fig. 2 Treatment with sAPPa decreases tau phosphorylation and increases GSK-3β (Ser9) phosphorylation in SH-SY5Y/BACE1 cells and HeLa/tau cells. SH-SY5Y/BACE1 cells (**a**, **b**) and HeLa/tau cells (**c**, **d**) were treated with sAPPa at indicated concentrations for 12 h. Cell lysates were prepared for WB analysis of both total and phosphorylated levels of tau and GSK-3β. **a**, **c** Phosphorylation status of tau was detected by anti-phospho-tau (p-tau (Thr231)) and PHF1 antibodies. Total tau (phosphorylated and non-phosphorylated) was detected by tau-46. **b**, **d** Phosphorylation status of GSK-3β (pGSK-3β (Ser9)) was detected by anti-phospho-GSK-3β (Ser9) antibody. **e** HeLa/tau cells were treated with BACE1 inhibitor LY2886721 at indicated concentrations (0, 12.5, 25, 50, 100, 200, and 400 nM) for 12 h and then cell lysates were prepared for WB analysis of both total and phosphorylated GSK-3β. These results indicated that the GSK-3β activity was dependent on BACE1 inhibition. In addition, BACE1 inhibitor dose-dependently induced inhibitory pGSK-3β (Ser9) phosphorylation in SH-SY5Y/BACE1 cells (date not

shown). Densitometry analysis shows the band density ratio of p-tau (Thr231) to total tau and pGSK-3β (Ser9) to total GSK-3β as shown below each figure panel. WB results are representative of two independent experiments for pGSK-3β (Ser9) and total GSK-3β, and three experiments respectively for PHF1, p-tau (Thr231), and total tau. A *t*-test revealed a significant difference in pGSK-3β (Ser9) to total GSK-3β and p-tau to total tau ratios for both SH-SY5Y/BACE1 cells and human tau stable transfected Hela cells treated with either 6.25, 12.5, or 25 nM sAPPa compared with control (0 nM). In addition, a significant difference in pGSK-3β (Ser9) to total GSK-3β ratio was observed for HeLa/tau cells treated with either 200 or 400 nM BACE1 inhibitor compared with control (0 nM) (**P < 0.05; ***P < 0.001). Aβ40, 42 peptides were undetectable by Aβ ELISA of the conditioned media from either cell line with or without sAPPa treatment (data not shown). Republished figure with permission from [12]

stable form in biofluid, including serum or plasma [19]. Downregulation of exosome miR-193b and upregulation of both exosomes miR-135a and miR-384 were observed in the serum of AD patients compared with normal patients. Among three miRNAs, miR-384 was the best candidate biomarker [20]. A significant upregulation of miR-455-3p expression in the serum, in the brains of AD patients, in the cerebral cortex of amyloid precursor protein transgenic mice, in the human and mouse neuroblastoma cells treated with the amyloid-β (1-42) peptide, revealed biomarker characteristics [21]. MiR-501-3p levels were downregulated in the serum of AD patients but remarkably upregulated in the brains of same donors and overexpression in the cultured cells (a human neuroblastoma cell line of SH-SY5Y cells). Serum-miR-501-3p is indicator that reflects pathological events occurring in AD patients' brains [22]. Both miR-125b and miR-181c were downregulated in the serum of AD patients compared with that of normal controls [23]. MiR-125b is also decreased in the serum of AD patients as compared with non-inflammatory normal disease controls (NINDCs) (accuracy of 82%) [24]. Six candidate miRNAs (miR-9, miR-29a, miR-29b, miR-34a, miR-125b, and miR-146a) in plasma of AD patients and normal subjects were measured. Two plasma miR-34a and miR-146a levels in AD patients were significantly lower than in control

subjects [25]. These miRNAs maybe useful for biomarker for AD diagnosis. Table 2 enlists the microRNAs as potential peripheral biomarker of AD.

Proteomics Approach for Biomarker

Amyloid plaques play a major role in the pathogenesis of AD. It is primarily composed of Aβ peptides which were derived from APP. In present time, diagnosis of AD is mainly based on clinical symptoms and neuroimaging. There is no gold standard central or peripheral biomarker to diagnose AD, and there is urgent need to develop promising biomarkers especially for the early-stage diagnosis. To know early stages of AD, measuring Aβ in plasma is an inexpensive as well as non-invasive method. But previous cross-section studies in humans show that plasma Aβ of AD patients is not much different from normal controls. More inconsistent plasma Aβ results were found in different mouse models. Longitudinal studies appear to be more promising than cross-section studies in both human and mouse models [26].

Before and during early stages of AD, plasma Aβ 40 and Aβ 42 were elevated in some patients but decline on the late stage of disease. Aβ 40 and Aβ 42 are strongly correlated and
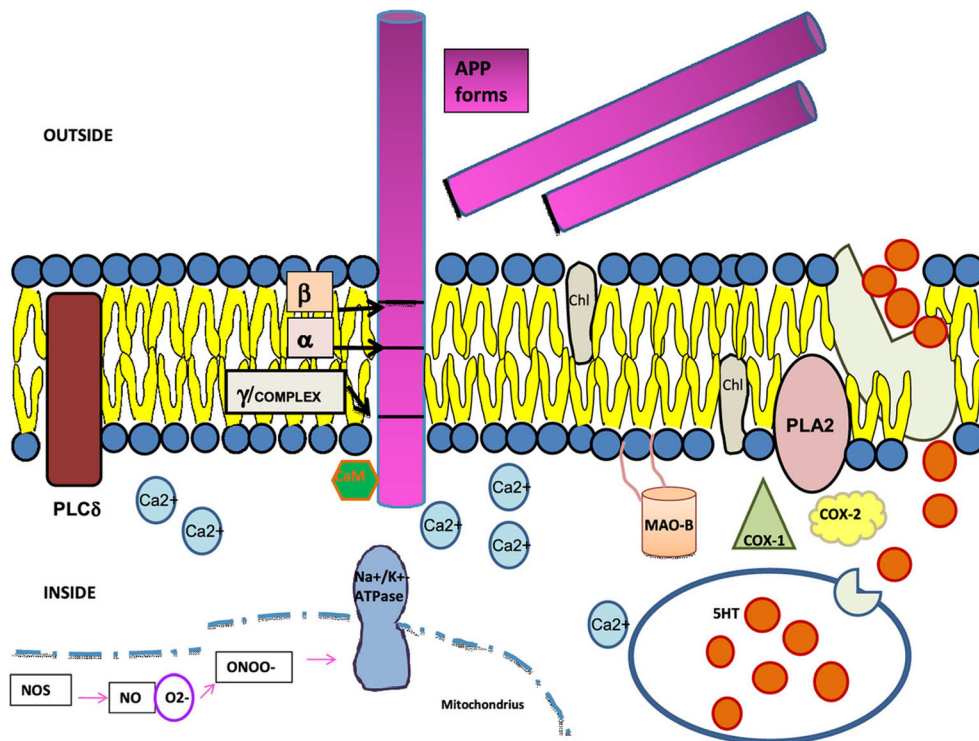
Fig. 3 Abnormalities on AD platelets. Some membrane (secretases, phospholipases), cytosolic (monoamine oxidase, cyclooxygenases), and mitochondrial activities (nitric oxide synthase, sodium potassium ATPase pump) are compromised in AD platelets. Alterations are evident in the APP processing itself, membrane fluidity, and cholesterol levels; in serotonin levels/uptake and intracellular Ca^{2+} levels; and in nitric oxide and peroxynitrite production. Abbreviations: PLCδ, phospholipase C δ; β, β-secretase; α, α-secretase; γ-secretase complex; APP forms, amyloid precursor protein forms; CaM, calmodulin; chl, cholesterol; MAO-B, monoamino-oxidase B; PLA2, phosholipase A2; COX-1, ciclooxigenase-1, COX-2, ciclooxigenase-2; 5HT, 5-hydroxytryptamine; NOS, nitric oxide synthase; NO nitric oxide; $O2^-$ superoxide anion; $ONOO^-$ peroxynitrite; Na+/K+-ATPase, sodium potassium ATPase pump; Ca2+, calcium. Adapted with permission from ref. [13]

increasing with age. Increased level of plasma Aβ 42 is directly related with mortality in AD patients [27]. Other studies showed that both an increase and no change can occur in plasma Aβ 42 level in normal-aging human without dementia [28–31]. Decreased Aβ 42 level in CSF is related with the AD

and disease progression, but variation in human plasma Aβ is inconsistent [32], especially in sporadic AD.

Ray and group did a study to find significant inflammatory biomarkers in AD patients and non-demented control subjects which found 18 significant inflammatory biomarkers with nearly 90% accuracy in examining 120 cell signaling plasma

Table 1 Blood cells for peripheral biomarkers of AD

Blood cells	Patients group	Remark	References
Red blood cells	Patient groups: AD ($n = 33$); control ($n = 25$)	PKC conformation in RBC is altered in AD patients as compared with RBC from healthy control	[9]
White blood cells	Patient groups: AD ($n = 60$); MCI ($n = 33$); control ($n = 20$)	In both AD and MCI patients group, isoforms of GSK-3 protein was increased but its activity is decreased	[10]
Platelets	Patient groups: AD ($n = 31$); control ($n = 10$)	β secretase activity↑, Aβ↑, α-secretase activity↓, Aβ PP isoform ratios (120–130 to 110 kDa)↓	[14]
	Patient groups: PD ($n = 8$), HS ($n = 6$), AD ($N = 10$), controls ($n = 11$)	APP ratios were normal in the patients with PD and HS but declined in AD patients	[15]
	Patient groups: ADAD mutation carrier ($n = 15$); non-carrier ($n = 12$)	Decreased APP ratio in mutation carrier. APP is inversely correlated with brain amyloid	[16]

ADAD, autosomal dominant Alzheimer's disease; AβPP, amyloid β-protein precursor; GSK-3, glycogen synthase kinase 3; HS, hemorrhagic stroke

Table 2 MicroRNAs as potential peripheral biomarker of AD

MiRNA	Study group	Comment	Reference
MiR-501-3p	Survey group: AD ($n = 27$), NC ($n = 18$). Validation group: AD ($n = 36$), NC ($n = 22$)	MiR-501-3p, let-7f-5p, and miR-26b-5p were significantly deregulated in AD patients: a) Downregulated in the serum of AD patients b) Upregulated in the brains of same AD donors and in the human neuroblastoma cell line	[22]
MiR-125b and MiR-181c	AD ($n = 105$); NC ($n = 150$)	Downregulated in AD patients upregulated in normal controls	[23]
MiR-125b	AD ($n = 22$); NINDCs ($n = 18$); INDCs ($n = 8$); FD ($n = 10$)	Decreased in the serum of AD patients	[24]

AD, Alzheimer's disease; NC, normal control; NINDCs, non-inflammatory normal disease control; INDCs, inflammatory normal disease control; FTD, frontotemporal dementia

protein. It can also differentiate between AD patients and non-demented control subjects. The baseline amounts of these proteins can also be distinguished MCI patients and other different dementia pathologies by 81% accuracy. There could be modified links which cause significant changing of cell signaling proteins. These links should be explored more in future [33]. Two research groups had shown a diagnostic accuracy of 60–70% [34, 35], and it could be a potential peripheral biomarker.

Hye et al. in 2006 did a case-control study with an idea to identify different proteins in the plasma of two groups, 50 AD patients and 50 elderly control subjects by using 2D gel electrophoresis and mass spectrometry. A group of 511 people including AD, other NDs, and elderly normal control subjects were used for validation. Image analysis showed disease cases with 80% specificity and 56% sensitivity while analysis of mass spectrometry showed proteins involved in disease pathology. Elevated complement factor H (CFH) precursor and alpha-2-macroglobulin (alpha-2M) are specific for AD and severity. To improve sensitivity and specificity, other assays would be necessary but CFH, alpha-2M, and other proteins are potential peripheral biomarkers for diagnosis of AD [36].

Liao's group, using 2-DE, found that spot densities of six plasma proteins in AD group and non-demented group were different. Plasma level of alpha-1-antitrypsin and apolipoprotein J were tested again because of their involvement in amyloid plaque formation. Although plasma α1 antitrypsin was more elevated in the AD group (77% sensitivity and 88% specificity) than non-demented group, no difference in total

Table 3 Proteomic studies of peripheral biomarkers for the diagnosis of AD

Protein	Study group	Remark	References
Aβ40 and Aβ42	530 individuals	Before and during the early stages of AD, increased Aβ40 and Aβ42 but decline in late stage of AD	[27]
↑plasma protein: CCL18, CXCL8, Ang-2, ICAM-1, IGFBP-6, IL-11, and Trail-R4. ↓plasma protein: CCL5, CCL7, CCL15, TNF-α, PDGF-BB, M-CSF, G-CSF, EGF, GDNF, IL-1α, and IL-3	Alzheimer's disease patients ($n = 85$), non-demented controls ($n = 79$), MCI ($n = 47$), other dementia ($n = 11$), other neuro disease ($n = 21$), and rheumatoid arthritis ($n = 16$)	Differentiation of 18 inflammatory biomarkers. Accuracy is nearly 90% between AD and NDC	[33]
↑α1 antitrypsin, ↑DBP, ↓IHRP, ↓PKAC1, and ↓Apo J precursor	2D-PAGE, LC-MS/MS, Alzheimer's disease patients ($n = 10$), and non-demented controls ($n = 10$)	↑α1 antitrypsin in AD group, no difference in total apolipoprotein J concentration; sensitivity = 77%; specificity = 88%	[37]

CCL, chemokine containing a C–C motif; CXCL, chemokine containing a C–X–C motif; Ang-2, angioprotein-2; ELISA, enzyme-linked immunosorbent assay; EGF, epidermal growth factor; G-CSF, granulocyte-colony stimulating factor; GDNF, glial derived neurotrophic factor; ICAM-1, intercellular adhesion molecule-1; IGFBP, insulin-like growth factor-binding protein; IL, interleukin; M-CSF, macrophage-colony-stimulating factor; MCI, mild cognitive impairment; PDGF-BB, platelet-derived growth factor BB; TRAIL-R4, TNF-related apoptosis-inducing ligand receptor-4; TNF-α, tumor necrosis factor-α

apolipoprotein J concentration was found [37]. Plasma proteomic analyses identified oxidized form of alpha-1-antitrypsin in AD patients [38, 39].

In a study, inflammatory biomarkers, plasma IL-6 was increased and, plasma tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) was decreased in AD group ($n = 40$) compared with normal control group ($n = 40$). These two biomarkers were significantly correlated with cerebrospinal fluid levels [40]. Table 3 enlists various proteomic studies of peripheral biomarkers for the diagnosis of AD.

Eyes as Peripheral Biomarkers

Lens

In the lens of rats and monkeys, Frederikse and colleagues found Alzheimer's precursor protein (beta APP) and A-beta [41]. In 2002, they also found that Aβ deposition, defect in fiber cell membrane which is as similar as found in human cataract in mouse models of Down syndrome-carrying human AbetaPP (*hAbetaPP*) gene [42]. More lens cataract with enhanced Aβ immune-reactivity is detected in transgenic mice with AD beta-amyloid rather than wild-type mice [43]. Goldstein and colleagues in 2003 first discovered Aβ1–40 and Aβ1–42 in lenses of both AD patients and control patients [44]. The concentration of Aβ in lens can compare with amount of concentration found in the brain. Also, concentration of Aβ1–40 in primary aqueous humor is comparable with CSF.

In AD patients, increased deposits of electron-dense Aβ immunoreactive aggregates within lens fiber-cell cytoplasm at supranuclear sub region of lens and supranuclear cataracts were found but not in the control subjects [44]. Juliet Moncaster and co-workers analyzed lenses obtained patients from Down syndrome (DS), AD and normal controls. Same result such as Aβ aggregates within fiber cell cytoplasmic and

opacification with increased Aβ deposition at supranuclear region were reported. These findings are identical to the lens pathology in AD [45]. In AD and DS, accumulation of Aβ shows linking between lens and brain pathology [44, 45]. These findings revealed Aβ accumulation as a key pathogenic determinant linking lens and brain pathology in both DS and AD [44, 45]. In 2013, Kerbage et al. did the study to detect Aβ deposit in the lens. They used specific fluorescent signature of ligand which bound Aβ in the supranucleus region of lens. And then it was detected by a laser scanning device. Figure 4 shows fluorescence decay rates for bound (red) and unbound (green) compound to Aβ peptide. They found two important results. In both AD and control groups, the highest measurement of ligand bound fluorescence signal was obtained from deeper regions of the supranucleus (SN). A twofold differentiation factor between control groups and AD patients was also noted [46].

Surprisingly, three reports showed opposite result. Michael and colleagues reported that no beta-amyloid in lenses was found in cortical cataracts of both control and donors with AD [47]. Ho et al. reported that β-amyloid, phospho-tau, and α-synuclein are present in different forms at lower levels or do not deposit in the eye [48]. Although participants with AD biomarkers positive (positron emission tomography-Pittsburgh compound B (PET-PiB) and CSF levels of Aβ42) have cataracts and cortical light scattering more advanced than biomarker-negative participants, no one showed significant statistical data progressing to AD [49].

Retinal Nerve Fiber Layer

Bearish et al. studied peripapillary retinal nerve fiber layer (RNFL) in nine AD patients and eight age-matched control subjects by using optical coherence tomography (OCT). They found reduced retinal blood flow with narrow veins and significant thinning of RNFL in the superior quadrant. But RNFL

Fig. 4 Fluorescence decay rates for bound (red) and unbound (green) compound No. 11 to Aβ peptide. Increase in fluorescence intensity ($\delta I \sim 2\times$) and decay rates of bound ($\tau = 2.4$ ns) compound No. 11 vs. unbound ($\tau = 1.4$ ns) to Aβ. Fluorescence lifetime image of aggregated Aβ peptide in color-coded representation. Figure as originally published in ref. [46]
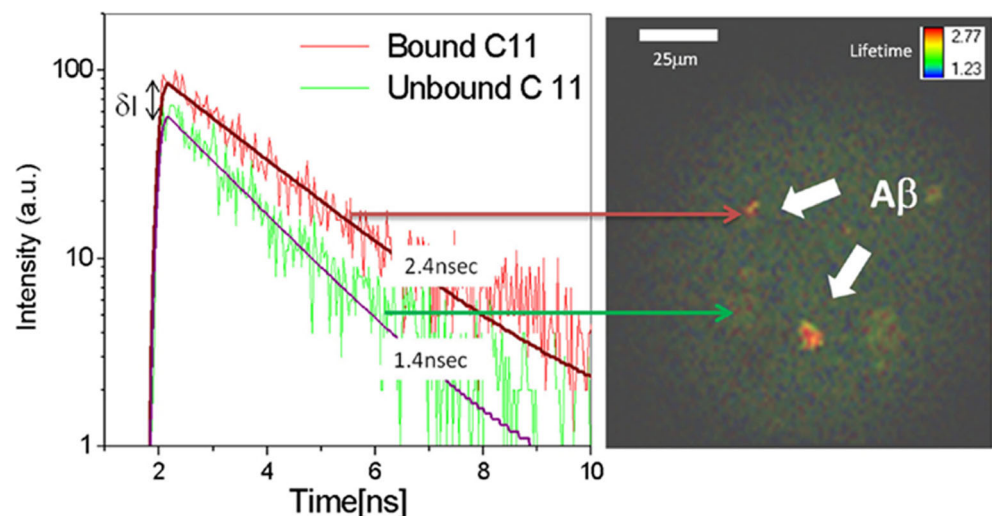
Fig. 5 Spectral domain optical coherence tomography of the retinal nerve fiber layer (RNFL) in a patient with Alzheimer's disease. The RNFL of the superior quadrant is reduced to the 0–5 percentiles for age (red), but the nasal, temporal, and inferior quadrants are normal (green). Adapted with permission from ref. [51]
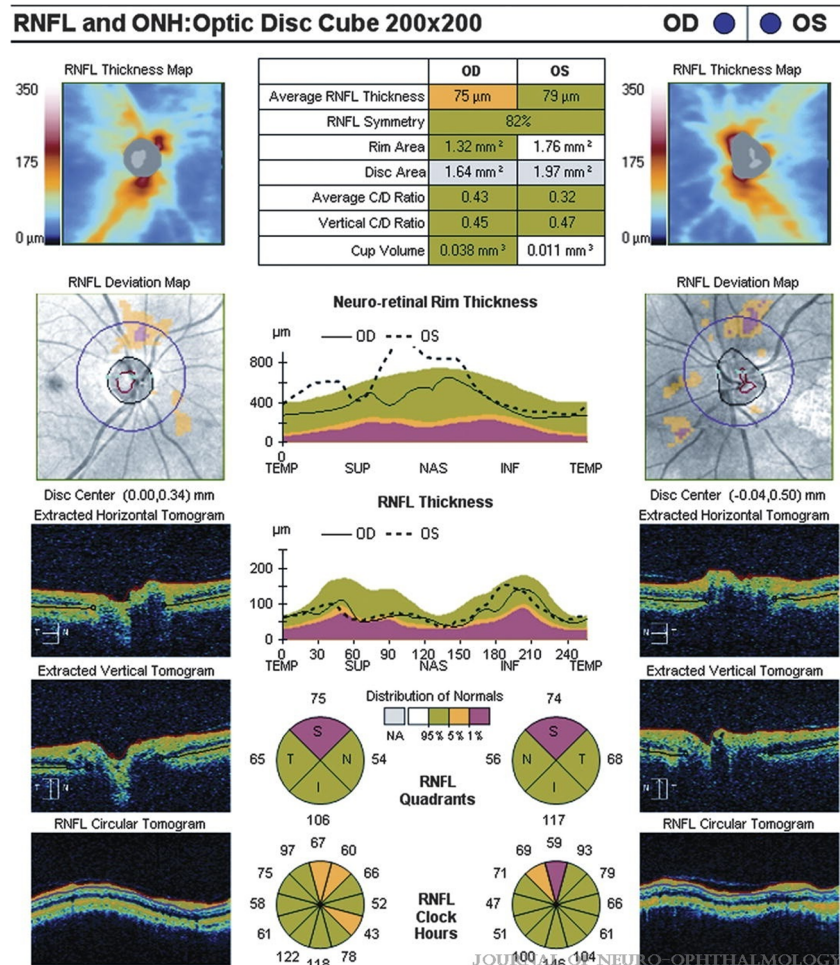
thickness in inferior, temporal, or nasal parts showed no significant differences [50].

Three research [51–53] also reported RNFL thinning in the superior and inferior quadrant. Figure 5 shows spectral domain OCT of the RNFL in a patient with AD. Gao et al. revealed that RNFL thinning in the superior quadrant as well as RNFL thickness at 12:00 position in the superior quadrant [54]. There is no relationship between RNFL thickness and mini-mental state examination score, but decreased thickness of RNFL in all quadrants is found in the AD group compared with the control group. These findings suggest that analysis for RNFL thickness may be useful in diagnosis of early stage of AD [55].

Retinal Vessels

For retinal vascular abnormalities in AD, a study reported thinning of blood column diameter with decreased blood flow in major superior temporal venule. Laser Doppler device was used to measure these parameters [50]. Studying of the retina from AD transgenic mice revealed neuro-inflammation, elevated microvascular Aβ deposition, Aβ plaques, and hyperphosphorylated tau. Immunotherapy for Aβ

showed retina plaques are clear but it worsens in retinal amyloid angiopathy. So, investigations such as non-invasive retinal imaging are good technique to know early diagnosis and severity of AD [56, 57]. Thirteen significant retinal vascular parameters were found in AD ($n = 25$) and healthy controls ($n = 123$). The method was that 19 vascular calibers were calculated by retinal photography in largest six arterioles and six venules (see more in [58]). Retinal photography together with vascular analysis could be a potential biomarker to detect early AD.

Retinal Ganglion Cells

Blanks et al. in 1989 showed degeneration of retinal ganglion cells (RGCs) in AD patients. The degeneration is unique due to finding of frothy and vacuolated appearance in the cytoplasm of RGCs, but no neurofibrillary tangles were found. In all examined AD patients, amyloid angiopathy is found but none of neuritic plaques is found in optic nerves or retinas [59]. In 1996, they found a decrease of 25% in total neurons in the ganglion cell layer of the central retina of AD patients ($n = 9$) compared with

age-matched control subjects ($n = 11$) [60]. Retinal ganglion cell loss is also found in the retinas of the AD patients [61].

Williams et al. found that the dendritic integrity of RGCs was significantly decreased without RGC loss in Tg2576 mice in comparison with age-matched wild-type controls. RGC dendrites are confined to the inner plexiform layer of the retina, imaging techniques that focus on this layer, rather than the loss of RGCs, may provide a sensitive biomarker for monitoring neural damage in AD [62]. Another study by Lu et al. found that RGCs of the APP/PS1 double transgenic mice with AD group was comparatively much less than the control group [63]. Targeting RGCs combined with imaging techniques need to be explored more in future to diagnose early phase of AD [61–63].

Biomarkers for Skin Fibroblasts

Extracellular-Signal-Regulated Kinases (Erk1/2)

Zhao's group in 2002 did a research study and found that abnormal phosphorylation of Erk1 and Erk2 was induced by bradykinin (BK) in cultured skin fibroblasts of AD patients compared with normal control subjects [64]. In 2004, Veeranna found that in the brain of AD patients, there is an association between phosphorylated Erk1, Erk2, and activated calpains. They found that in AD patients' brain, intensities of phosphorylated Erk1 and Erk2 band is relatively increased but not in the brains of control subjects [65].

A research by Khan and Alkon in 2006 revealed that internally controlled AD-specific different phosphorylation ratio of Erk1 and Erk2 caused by bradykinin, a small nanopeptide which is PKC activator and natural inflammatory agonist [66]. It induced PKC-mediated Erk1 and Erk2 phosphorylation in two different types of human skin fibroblasts, fresh-taken skin fibroblasts, and banked skin fibroblasts. Skin fibroblasts samples were obtained by punch biopsy from total 264 cases including AD group, non-AD group, and age-matched control group. AD-Biomarker Index (AD-Index) was used to calculate quantitative imaging of the phosphorylated Erk1 and Erk2 bands. AD-Index positive was considered having AD and zero or negative as non-AD dementia. In 42 autopsy-confirmed cases out of total 264 cases, clinical diagnosis for AD had 78% sensitivity and 20% specificity while AD-biomarker diagnosis had 97% sensitivity and 100% specificity (overall accuracy was 98%). For validation, AD-biomarker diagnosis accuracy was 100% for dementia cases with duration no longer than 4 years while clinical diagnosis was only 52% accurate. The pathophysiologic relevance of AD-biomarker measurement was also tested by examining $A\beta 1$–42-induced abnormal AD-biomarker phenotype [67]. Therefore, Erk1/2 phosphorylation of skin fibroblasts could have important clinical utility such as for increasing diagnostic certainty in early phase of the AD.

Calcium

Altered Ca2+ homeostasis is found in brain of the AD patients and in the peripheral tissue. The first report by Peterson et al. in 1985 demonstrated that decreased Ca2+ uptake is found in human skin fibroblasts of AD patients compared with age-matched controls [68].

In cultured skin fibroblasts from aging and AD patients, 70 and 81% decline of cytosolic-free calcium had been reported when compared with fibroblasts from age-matched controls and young adult donors [69]. In the fibroblast cells of aging patients and AD donors, various drugs treatment (serum, 3,4-diaminopyridine, N-formyl-methionyl-leucyl-phenylalanine, and bradykinin) can transiently elevate cytosolic-free calcium, but it is slower and lesser in young patients. Alteration of both dynamic and resting calcium homeostasis has been found in cultured skin fibroblasts from aging patients and AD donor [70]. There was more alteration of calcium homeostasis and mitochondrial functions in AD than normal aging [71]. In mitochondria of skin fibroblasts from histologically confirmed AD patients, calcium uptake is less than in mitochondria of fibroblasts from age matched controls. In mitochondria of AD patient, more calcium uptake than controls was seen after exposing with free radial.

Table 4 Skin fibroblasts for peripheral biomarkers of AD

Biomarker	Tissue and study group	Comment	Reference
Erk1 and Erk2 phosphorylation	-Cultured skin fibroblasts -AD patients group and control group	Abnormalities in Erk1 and Erk2 phosphorylation	[64]
Quantitative imaging of the phosphorylated Erk1 and Erk2 bands	-AD-biomarker measurements in 42 autopsy-confirmed cases out of 64 autopsy examinations	AD-Biomarker Index AD-Index (positive), considered having AD diagnosis AD-Index (negative or 0), considered to have a non-AD dementia	[66]
Intracellular Ca2+ release by IP3 generation	-Fibroblasts of AD patients	Ca2+ response is measured by standard Ca2+ fluorescence imaging techniques	[75, 77]

PKCε, protein kinase c epsilon; s-APPα,d soluble amyloid precursor protein

Treating with U-74500A, the antioxidant drug or deferox-amine, iron chelator, prevents free radial, inducing increased calcium uptake of control mitochondria and it only protects partially in Alzheimer's mitochondria. It showed that in the mitochondria of Alzheimer's fibroblast cells, calcium transport processes is impaired and sensitivity for oxygenic free radicals is increased [72]. But one research group in 1992 reported that ionic calcium levels in cytoplasm of Alzheimer's fibroblast cell have no pathological or diagnostic value [73]. Because of this result, several research groups focuses on different methods for Ca2+ to be used as a peripheral biomarker. Etcheberrigaray et al. in 1994 reported that K+ channel blocker TEA can increase intracellular Ca2+ in normal skin fibroblasts but response to TEA stimulation is low in cells of few familial AD cases and sporadic AD cases [74].

Low-dose Bradykinin can induce intracellular Ca2+ release by IP3 generation, activating phospholipase C and evokes enhanced Ca2+ signaling in AD fibroblasts [75, 76]. Because of these findings, response of Ca2+ in skin fibroblasts after stimulation with TEA or bradykinin was measured by using standard Ca2+ fluorescence imaging techniques. The biochemical response was reported as the ratio of percent response after TEA stimulation and brady-kinin stimulation [75, 77]. Targeting Ca+ homeostasis and Ca+ signaling in the skin fibroblast could be a potential peripheral biomarker for AD. Table 4 enlists the skin fibroblasts for peripheral biomarkers of AD.
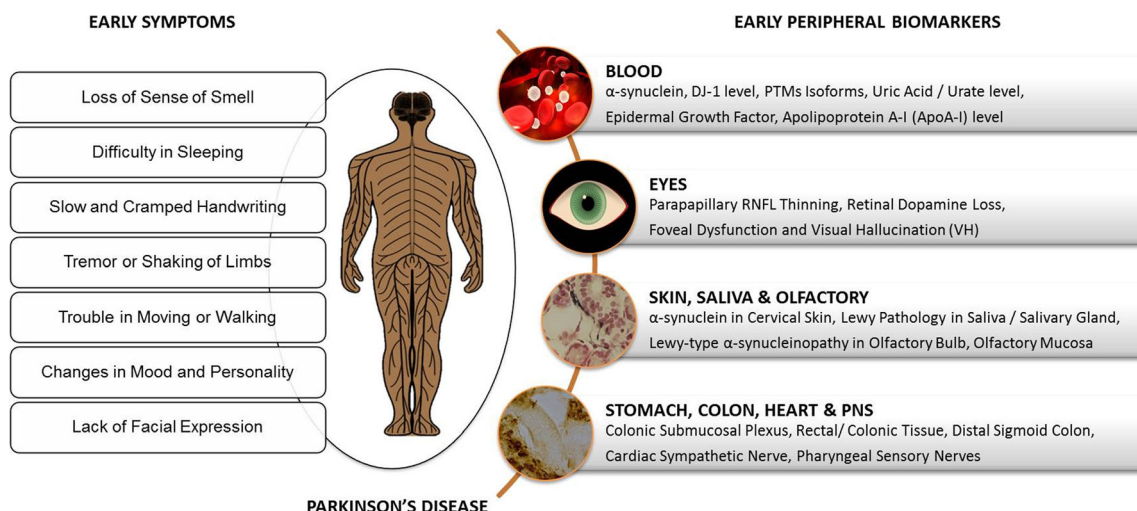
Parkinson's Disease

PD is a chronic progressive and second most common ND. It is a chronic and progressive movement disorder and mostly occurs in elderly people of 60 years and above. It is characterized by a significant reduction of dopaminergic neurons in the substantia nigra together with physical signs (rigidity of muscle on passive movement, instability in posture, resting tremor and akinesia). Currently, there are no definitive diagnosis criteria or biomarkers [78, 79]. Clinical signs and symptoms and post mortem examination are the criteria to diagnose PD. Specific, sensitive, and economical central or peripheral biomarkers are needed to diagnose early stage of disease, severity, differential diagnosis, prognosis, and treatment of PD. In the following sections. peripheral biomarkers explored for early diagnosis of PD are discussed. Figure 6 provides an overview of the early symptoms and peripheral biomarkers for early detection of PD.

α-Synuclein in Blood

Histologically, PD is characterized by the presence of inclusions known as Lewy bodies, which is a composition of α-synuclein, in the intracytoplasmic, degeneration, and reduction of dopaminergic neurons in the substantia nigra of brain. It is also found in other parts of the brain such as the thalamus, hippocampus, neocortex, and cerebellum [80, 81]. It is an unfolded protein, it is soluble, and polymerization of α-synuclein becomes fibrils. Accumulation of fibrils found inclusions known as glial cytoplasmic inclusions, Lewy neuritis, and bodies. It is a highly acidic, heat-stable amino acid compound (140 amino acid; 14 kDa) [82–84]. Its filaments are found in many NDs. The role of α-synuclein in blood and peripheral tissue in PD is discussed.

Initially, α-synuclein was considered an intracellular protein, but identification in both CSF and plasma is the evidence that it is secreted from cells. An effort for α-synuclein in CSF as a diagnostic biomarker have been underway and found that total α-synuclein in CSF is lower in PD patients than healthy control subjects [85, 86]. Figure 7 shows CSF α-synuclein concentrations in study groups [85]. α-Synuclein protein (alphaS) aggregates from a monomer to assemblies such as oligomers, protofibrils, and mature fibrils. While the alphaS

Fig. 6 Early symptoms and peripheral biomarkers for early detection of PD
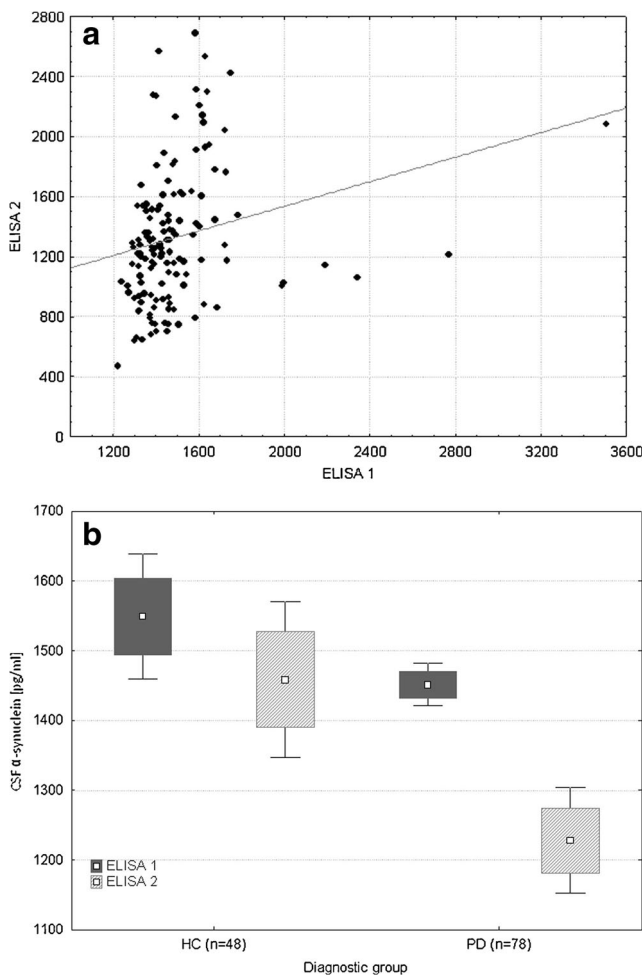
Fig. 7 CSF α-synuclein concentrations in study groups. **a** Scatter plot for CSF α-synuclein values from patients with early Parkinson's disease and healthy subjects, as measured by two different and independently operated ELISA systems (ELISA 1 and 2). Concentrations measured by ELISA 1 and 2 correlated positively by Pearson's analysis (0.229; $p = 0.01$). **b** Graphs in whisker-box plot format summarize results for the total CSF α-synuclein concentration (pg/ml) shown in (**a**). HC, healthy controls; PD, Parkinson's disease. Adapted with permission from ref. [85]

concentration in CSF is significantly decreased in patients with PD, alphaS oligomer concentration was elevated in CSF and in the blood of PD patients. It showed that alphaS in the CSF and in the blood could be a peripheral biomarker for PD [87].

Three research groups tried to measure total α-synuclein [88], phosphorylated α-synuclein [89], and oligomeric forms of α-synuclein [90]. Total α-synuclein was measured by Tinsley and colleagues. They developed a new ELISA specifically for quantifying α-synuclein in human plasma using a novel α-synuclein-specific antibody that has very high sensitivity and signal. Although the study was done on small cohort of patients and control subjects, it is promising and has potential as peripheral biomarker [88].

Alpha synuclein can be phosphorylated at serine129 (P-S129) by kinase(s). The presence of highly phosphorylated

α-synuclein in Lewy bodies suggests that it has an important pathological role. In healthy brain, only 4% phosphorylated alpha-synuclein is found while approximately 90% has been observed in the brains of PD patients. Phosphorylated alpha-synuclein is toxic to dopaminergic cells. Significant elevation of P-S129 α-synuclein leads to accumulation and increased cell death [91–93].

One research group developed a novel ELISA method that detects only oligomeric α-synuclein soluble aggregates. They found significantly elevated plasma levels of α-synuclein oligomeric forms ($P = 0.002$) which were obtained from 34 PD patients compared with 27 control subjects (specificity of 0.852 and sensitivity of 0.529) [90]. The mean plasma level of phospho-α-synuclein was higher than the controls ($P = 0.053$). It is not different for oligo-α-synuclein, total α-synuclein, or oligo-phospho-α-synuclein from 38 PD patients and 30 healthy control participants [89]. Studying for various forms of α-synuclein is still underway, although the results are promising and have potential to be the peripheral biomarkers.

α-Synuclein in Peripheral Tissue

Skin Samples

Michell et al. in 2005 reported that a low rate of α-synuclein (19%) was detected in skin biopsy samples of 16 PD patients and concluded that diagnosis of PD and its severity is not related with α-synuclein level. The results showed that skin α-synuclein would not be an appropriate diagnostic biomarker for PD [94]. But phosphorylated α-synuclein was found in the cervical skin site of all patients with Idiopathic PD (IPD) but not in the controls. This result significantly distinguishes IPD from other forms of parkinsonism. It may be useful as a biomarker especially for differential diagnosis [95].

Saliva and Salivary Gland

A research group tried to find biomarker for PD in saliva. The result showed significantly low α-synuclein level and a specific protein in the saliva of PD patients. The measurement of α-synuclein in the saliva have more advantage than other biological fluids such as plasma and blood because it can be obtained easily and with less contamination than blood [96].

Studying tissue sections of submandibular glands from patients with PD ($n = 9$), Lewy body disease ($n = 3$), multiple system atrophy ($n = 2$), and control subjects ($n = 19$) using α-synuclein immunohistochemistry revealed that Lewy body pathology in all PD patients but not in patients with multiple system atrophy and controls [97].

In another study, they used the same salivary gland with a different method. From submandibular gland of living
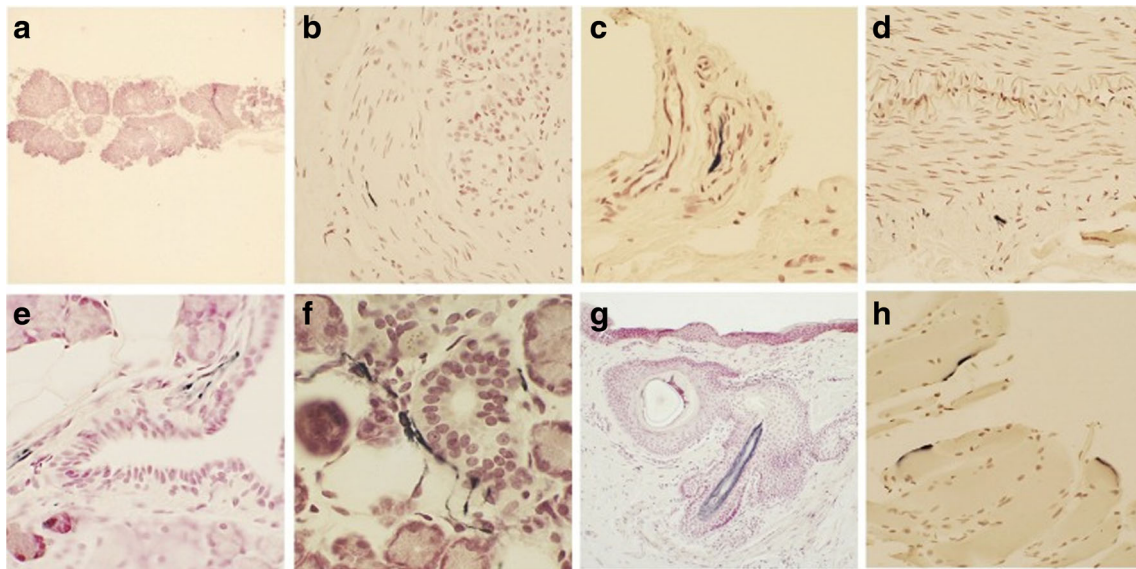
Fig. 8 Photomicrographs of needle core tissue from submandibular gland biopsies of subjects with Parkinson's disease. Sections were stained with an immunohistochemical method for phosphorylated α-synuclein (see "Methods"). Only structures immunoreactive for phosphorylated α-synuclein (black) and morphologically consistent with nervous tissue were considered to represent a positive biopsy finding. Sections were counterstained with neutral red. **a** Typical needle core tissue sample. **b** Single immunoreactive nerve fiber within a nerve fascicle running in connective tissue stroma adjacent to glandular parenchyma. **c** Single immunoreactive nerve fiber within a stromal nerve fascicle. **d** Immunoreactive puncta within the adventitia of a small artery in the stroma. **e** Several immunoreactive nerve fibers running beneath duct epithelium. **f** Immunoreactive nerve fibers interweaving among parenchymal serous gland cells. **g** Non-specific staining (black) of hair follicle within a fragment of skin. **h** Non-specific staining (black) of the edges of muscle fibers. Adapted with permission from ref. [98]

patients, needle core biopsies are obtained and found a Lewy-type α-synucleinopathy in 75% of cases [98] (Fig. 8). α-Nuclein was detected in the labial salivary glands of both in PD cases (60%) and in control cases (30%) [99]. Saliva and salivary gland alone is not a diagnosis biomarker but in combination with other tests can be a potential biomarker.

Olfactory

In neurological disorders including PD, a common feature is the olfactory dysfunction. Duda et al. [100] used antibodies to detect α- and β-synucleins. They found abnormal dystrophic neurites in the olfactory epithelium of both, PD patients and controls. In the olfactory mucosa, the most abundant synuclein is the α-synuclein. It is most significantly found in olfactory receptor neurons [100]. Lewy-type α-synucleinopathy (LTS) involvement in the olfactory bulb occurs in an early stage of PD. Beach and colleagues in 2009 reported that in PD, sensitivity is 95% and specificity is 91% compared with elderly control [101]. But one report in the same year found that in the olfactory epithelium of both PD patients and healthy control subjects, the expression or distribution of α-synuclein showed no histochemical differences [102].

Stomach and Colon

Lebouvier's group, in 2008 paper reported that 80% of ascending colon biopsies from submucosa of PD patients showed positive staining for phosphorylated α-synuclein-immunoreactive neurites [103].

In 2010, they found Lewy pathology in the colonic biopsies of PD patients (72%) which are obtained from submucosal plexus of ascending and descending colon but not in the control subjects [104]. Pouclet and co-workers in 2012 demonstrated that 55% of patients with PD have Lewy pathology in the mucosa and submucosa but not in the control subjects [105]. Lewy neurites were found by same group in the colonic biopsies from submucosa of rectal, ascending and descending colon in PD patients (23 to 65%) but not in controls [106].

Biopsies taken from distal sigmoid colon by flexible sigmoidoscopy from patients with early stage of PD (n = 7), control subjects (n = 23), and subjects with inflammatory bowel disease (n = 23). All PD patients (100%) showed α-synuclein staining in nerve fibers in colonic submucosa but not in the control samples as shown in Fig. 9 [107]. α-Synuclein inclusions were also detected in the gastric mucosa [108]. In vivo evidence of α-synuclein in peripheral tissue with underlying PD may be useful and it can be a safe tool providing development of peripheral biomarkers.

Fig. 9 αSYN immunohistochemistry of colon at × 40 magnification (**a**, **c**, **e**) and × 120 magnification (**b**, **d**, **f**). Nerve fiber staining was seen in PD subjects (**a**, **b**). Some subjects with inflammatory bowel disease showed a pattern of immunostaining in round cells of unknown origin (**c**, **d**). Controls and most subjects with inflammatory bowel disease showed no α-synuclein immunostaining (**e**, **f**). Scale bar in (**e**) and (**f**) represents 50 and 20 μm and applies to (**a**, **c**, **e**) and (**b**, **d**, **f**), respectively. Adapted with permission from ref. [107]
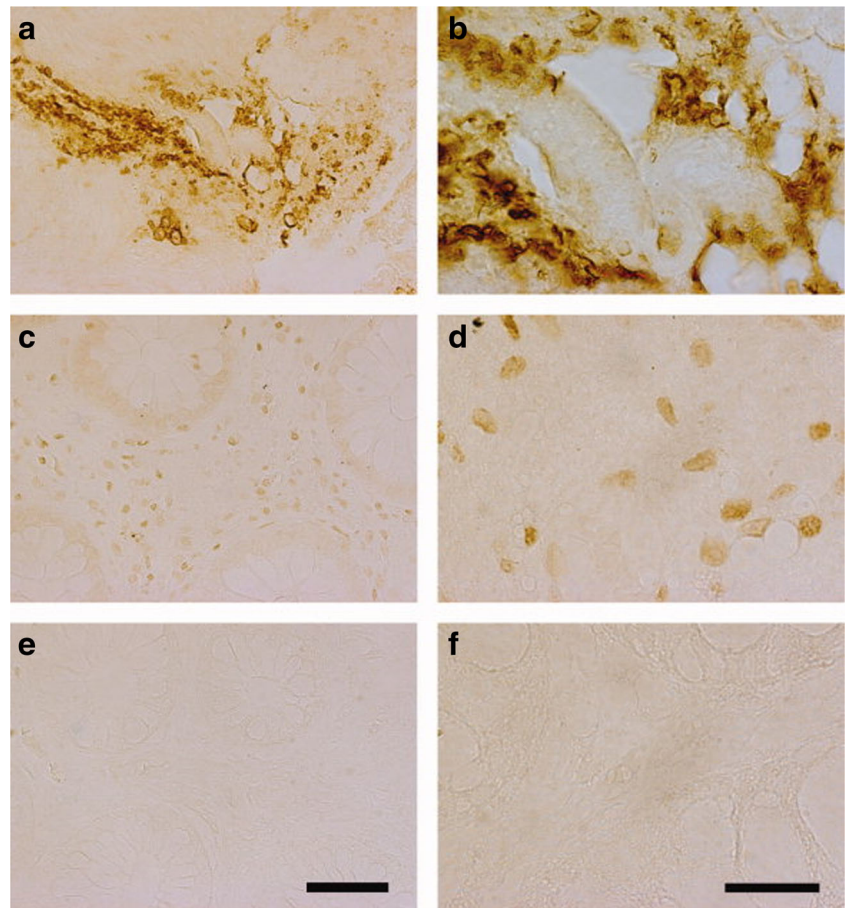
Heart and Peripheral Nervous System

Both sympathetic nerve and heart tissue from 11 PD patients and 7 incidental Lewy body disease patients (iLBD) were examined. Results showed that Lewy bodies and α-synuclein were found in the neurites in PD patients (9 out of 11) and 7 iLBD patients (100%) [109].

Phosphorylated α-synuclein aggregates were found in the cardiac sympathetic nervous system in 90% of iLBD cases and 60% of PD cases, but no phosphorylated α-synuclein is found in control subjects [110]. α-Synuclein positive neurites were also found in epicardium in PD cases ($n = 14$) and iLBD cases ($n = 11$), but not in normal controls ($n = 4$) [111].

Biopsies from gastric myenteric and submucosal plexuses were taken from five autopsy cases. The brains of the autopsy cases also staged for Parkinson-associated synucleinopath and immunocytochemistry was done. In neurons of the submucosal Meissner plexus, α-synuclein immunoreactive inclusions were observed [112].

Biopsies from pharyngeal sensory nerves from autopsy bodies of PD ($n = 10$) and controls ($n = 4$) was taken by Mu et al. to detect Lewy pathology and immunohistochemistry for phosphorylated α-synuclein. Axonal α-synuclein aggregates were found 100% in PD cases, but no α-synuclein aggregates were found in control cases [113]. Table 5 enlists α-synuclein in peripheral tissue.

Other Biomarkers from Blood

DJ-1

DJ-1 mutations are associated with PD, both familial and sporadic types. DJ-1 ELISA kit was used to detect DJ-1 in serum of both PD patients and control subjects. Two research groups in 2003 and 2008 reported that secreted DJ-1 level is not only different in two groups but also there is no association with age and severity of PD. Moreover, there is no correlation with level of oxidative stress [114, 115].

In 2010, Hong's group did a research in PD patients ($n = 117$), AD patients ($n = 50$), and healthy controls ($n = 50$) by using highly sensitive Luminex technology. They found that contamination of blood in CSF also influenced the level of DJ-1. When several major confounders were controlled to control blood contamination, the result showed that DJ-1 is decreased in PD vs. controls or AD. There was no correlation between severity of PD and level of DJ-1 [116].

Table 5 α-Synuclein in peripheral tissue

Type of tissue	Study group	Method	Comment	Reference
Skin biopsy	P ($n = 20$), C ($n = 30$)	IHC	Phosphorylated α-synuclein positive in all IPD patients but not in the controls	[95]
Submandibular salivary gland	PD ($n = 15$)	IHC	Lewy-type alpha-synucleinopathy (LTS) positive in 75% of cases	[98]
Submandibular salivary gland	PD ($n = 9$), iLBD ($n = 3$), MSA ($n = 2$), C ($n = 19$)	IHC	Lewy pathology (LP) in all PD patients but not in MSA and controls	[97]
Labial salivary glands	PD ($n = 3$), C ($n = 3$)	IHC	Alpha-nuclein in about 60% of PD cases and about 30 % of control cases	[99]
Olfactory mucosa	PD ($n = 5$), C ($n = 11$)	IHC	In all PD patients and all controls, abnormal dystrophic neurites were identified in the olfactory epithelium. The most abundant synuclein in the olfactory mucosa is α-synuclein.	[100]
Olfactory bulb	PD ($n = 58$), C ($n = 69$)	IHC	Sensitivities (95%) and specificities (91%) for PD vs. elderly control	[101]
Biopsies from colonic submucosal plexus	PD ($n = 29$), C ($n = 10$)	IHC	Lewy pathology in 72% of patients with PD but not in the controls	[104]
Biopsies from colonic submucosa, rectal/colonic tissue	PD ($n = 9$), C ($n = 10$) PD ($n = 26$), C ($n = 9$)	IHC	55% of PD patients showed Lewy pathology but no Lewy pathology in controls 23 to 65% of PD patients showed Lewy neurites but no Lewy neurites in controls	[105, 106]
Distal sigmoid colon	PD ($n = 7$), C ($n = 23$), and subjects with inflammatory bowel disease ($n = 23$)	IHC	α-Synuclein in nerve fibers of colonic submucosa in all PD patients but not in the control samples	[107]
Cardiac sympathetic nerve	PD ($n = 14$), ILB ($n = 11$), C ($n = 4$)	IHC	α-Synuclein-positive neurites in iLBD and PD (100%), control subjects (0%)	[111]
Pharyngeal sensory nerves	PD ($n = 10$), C ($n = 4$)	IHC	Axonal α-synuclein in all PD patients (100%)	[113]

C, control; IHC, immunohistochemistry; iLBD, incidental Lewy body disease; PD, Parkinson's disease; MSA, multiple-system atrophy

In the same year, one research group reported the similar result. There was no correlation between severity of PD and levels of DJ-1. DJ-1 level was decreased (more than 95% in RBCs, 1 to 4% in platelets and 1% or less than 1% in WBCs and plasma). It showed that platelet contamination and hemolysis could be significant factors affecting DJ-1 level in serum or plasma and blood. The results showed that DJ-1 in blood is not specific biomarker to diagnose, to know progression and severity of PD [117].

After this setback, researchers are trying to focus on the total DJ-1 and its isoforms. In a study of a group of 119 people including control subjects, AD patients and patients with different PD stages revealed 7 DJ-1 isoforms, 4 major post-translationally modified (PTMs), and no significance in the level of total DJ-1 in blood samples of control subjects and PD patients. Four major PTMs are methionine oxidation, cysteine oxidation, phosphorylation, and 4-hydroxy-2-nonenal (HNE) adduction. Among four PTMs, only HNE-modified DJ-1 isoforms is decreased in control subjects, AD patients, and early-stage PD patients while it is increased in late-stage PD patients. A validation study for 114 people was done, and the result was confirmed. Because PTM isoforms are candidate biomarkers only for late-stage PD [118], more studies are needed to identify PTM isoforms to diagnose early-stage PD.

Uric Acid

Serum urate is found to reduce the risk of PD. It is derived from purine and has potent antioxidant action. Church and Ward's group in 1994 reported that in the brain of PD patients, at substantia nigra region, uric acid (54%) and dopamine (85%) levels were significantly reduced [119]. Davis and colleagues did the hypothesis among men. From 1965 to 1968, they measured serum uric acid level in 7968 men. From that group of study, 92 men suffered IPD. Forty percent reduction in IPD incidence was found in men whose uric acid concentration was above the median at the time of measurement [120]. Two groups found that serum uric acid level is inversely related with PD [121, 122] and gout is inversely related with risk of PD [123]. In men, a higher dietary urate index and high plasma urate concentration were correlated with lower risk of PD [124]. But one report revealed that in women, urate is not strongly associated with lower risk of PD [125]. Urate has neuro-protectant, antioxidant, metal-complexing properties, and higher urate level is related with lower risk of PD. Urate/uric acid can be identified as a promising biomarker of the PD [126, 127].

Epidermal Growth Factor

In most of the PD patients, cognitive impairment is developed together with PD. From a group of 70 PD patients, baseline
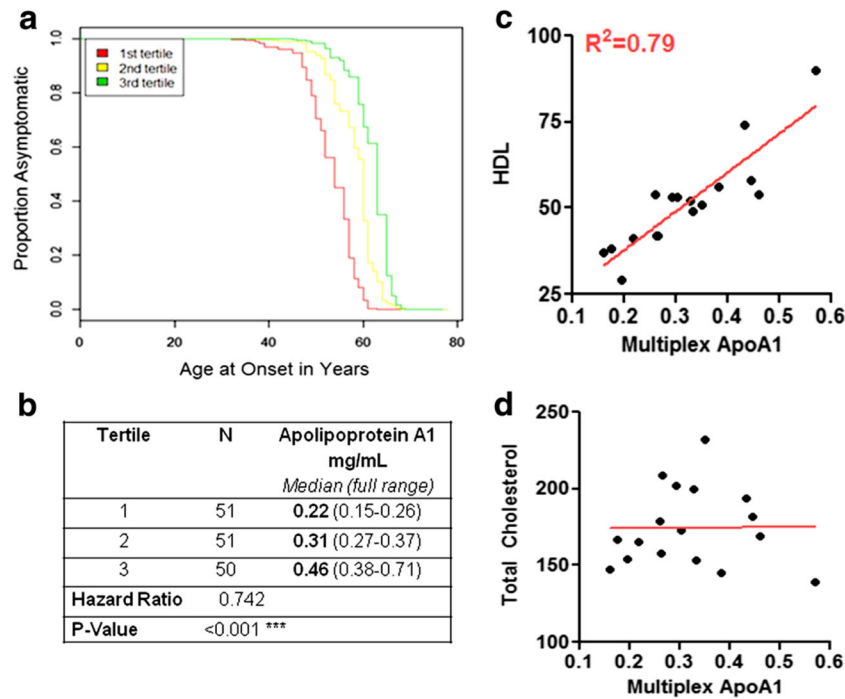
Fig. 10 Apolipoprotein A1 (ApoA1) as a biomarker for age at Parkinson's disease (PD) onset. **a** Low ApoA1 levels are predictive of early age at PD onset in the discovery cohort. Survival curves of time to PD onset are shown here for the lowest tertile of ApoA1 plasma levels (*red line*), middle tertile (*yellow line*), and highest tertile (*green line*), as measured by multiplex immunoassay. Outcomes for age at PD onset between the different tertiles were significantly different ($p < 0.001$, hazard ratio = 0.742, 95% confidence interval = 0.606–0.909), after adjustment for age at plasma draw and gender. **b** Number of individuals (*N*) and ApoA1 plasma values (median and full range in mg/ml) for each tertile are listed. ***p value < 0.001. **c** High-density lipoprotein (HDL) levels (mg/dl) are shown on the *y*-axis; ApoA1 levels (mg/ml) are shown on the *x*-axis. HDL levels correlate well with immunoassay ApoA1 values, as reflected by an R^2 of 0.79. **d** In contrast with HDL levels, total cholesterol levels do not correlate with ApoA1 levels. Total cholesterol levels (mg/dl) are shown on the *y*-axis; ApoA1 levels (mg/ml) are shown on the *x*-axis. Adapted with permission from ref. [130]

levels of different proteins ($n = 102$) in the plasma determined by using a bead-based immunoassay. Cognitive status at baseline and cognitive status at annual follow-up were determined by Mattis Dementia Rating Scale-2 (DRS). Among 11 proteins identified, epidermal growth factor (EGF) was the best candidate. If baseline cognitive test scores are poor, it is associated with low levels of EGF. For people with intact baseline cognition, cognitive decline to DRS is eightfold greater risk at follow-up. Separate study of 113 PD patients was also done using another technical platform to know relation between levels of the top biomarker and cognitive performance. They found that a weaker result than previous 70 PD patients study group, but there was significant correlation between cognitive performance and EGF plasma level [128].

Same study to assess cognitive functions and EGF in serum was done in a group of 65 drug-naive PD patients. Baseline and 2-year follow-up study showed that cognitive functions of the frontal and temporal portion of PD patients are correlated with serum EGF levels and performance on posterior and frontal cognitive function. Although EGF is a candidate biomarker for early cognitive impairment in PD, further studies are needed to be a diagnosis marker for early stage of PD [129].

Apolipoprotein A-I

The role of biomarkers may improve diagnosis for NDs, but none of the biomarker is a definitive biomarker to diagnose early stage of PD. Many studies have shown decreased levels of apolipoprotein A-I (ApoA-I) in CSF and serum in PD patients compared with healthy controls [130–133].

To identify proteins which were associated with PD, 96 proteins were measured by multiplex immunoassay in a group of 152 PD patients. Second study was to identify top candidate biomarkers and its plasma levels in 187 PD patients by ELISA method. Third study is to confirm the association between top biomarker and PD in the group of 134 people who are at risk but asymptomatic. To evaluate the association of dopaminergic system integrity and plasma protein levels, they used dopamine transporter (DAT) imaging, and the best candidate protein biomarker is apolipoprotein A1. Early onset of PD is correlated with low levels of ApoA1 [130, 131]. DAT deficit in putamen is also related with lower plasma levels of ApoA1 [130]. Figure 10 describes ApoA1 as a biomarker for age at PD onset. A higher ApoA1 level has relative protection against risk of PD, and drug such as statin can modify Apo-A1 level.

Table 6 MicroRNAs as potential peripheral biomarker for PD

miRNAs	Study group	Comment	References
miR-19b, miR-195, and miR-24	PD ($n = 109$), NC ($n = 40$)	a) Downregulation of miR-19b b) Upregulation of miR-195 and miR-24	[136]
miR-29c, miR-146a, miR-214, and miR-221	PD ($n = 138$), NC ($n = 112$)	Significantly downregulated in PD patients. MiR-221 is the most promising biomarker	[137]
a) miR-1, miR-22, and miR-29 b) miR-16-2, miR-26a2, and miR30a	PD ($n = 8$), NC ($n = 8$)	Aix differentially expressed miRNAs: a) Can distinguish non-treated PD from healthy subjects b) Can differentiate treated PD patients from untreated patients	[139]
miR-181c, miR-331-5p, miR-193a-3p, miR-196b, miR-454, miR-125a-3p, and miR-137	PD ($n = 31$), NC ($n = 25$)	7 overexpressed miRNAs in PD patients	[140]

miR, microRNA; NC, normal control; PD, Parkinson's disease
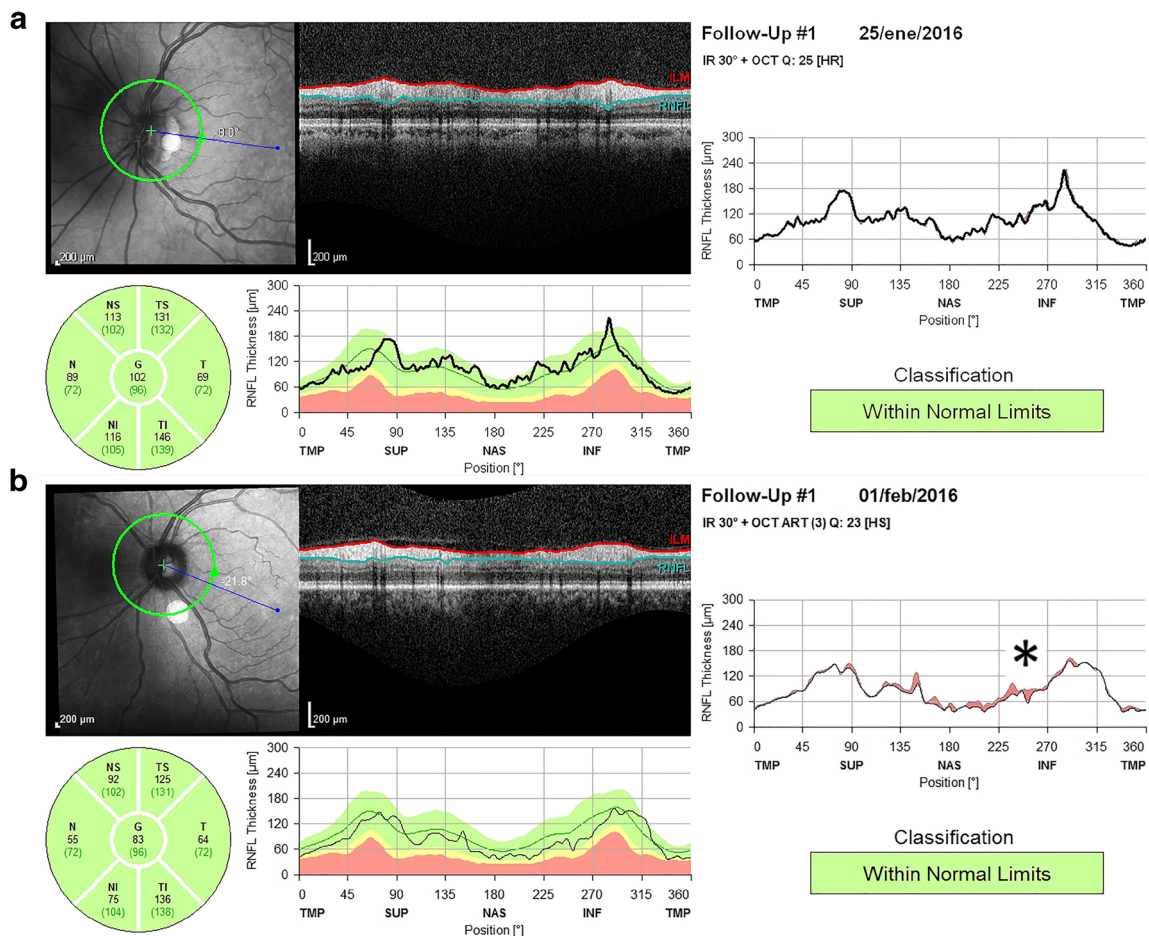
Fig. 11 Retinal nerve fiber layer analysis by OCT in a patient with PD and a healthy control. **a** Healthy control. **b** Parkinson's disease patient. The OCT report compares current analysis with the baseline evaluation (5 years prior). The graphic on the right side of each report shows marked changes (thinning) in the RNFL. Compared with the healthy control, the patient shows progressive RNFL loss in the superior, nasal, and inferior sectors of the optic nerve, marked with an asterisk. Republished figure with permission from [142]

Continuous use of statin is associated with decreased PD risk [134] while stopping using of statin is associated with PD [135].

MicroRNAs

MicroRNAs (miRNAs) are non-protein coding transcripts that post-transcriptionally regulate gene expression during development. RNAs encapsulated in exosome-like microvesicles in serum were extracted analyzed by quantitative reverse transcription polymerase chain reaction (qRT-PCR). Results showed a downregulation of miR-19b and upregulation of miR-195 and miR-24 in PD patients when compared with normal patients [136]. Sixteen serum miRNAs were measured in PD patients and control subjects. Four miRNAs (miR-29c, miR-146a, miR-214, and miR-221) were significantly downregulated in PD patients, and miR-221 is the most promising biomarker [137]. Other study showed that four serum miRNAs (miR-141, miR-214, miR-146b-5p, and miR-193a-3p) were significantly decreased [138]. Analysis of peripheral blood from PD patients and control subjects identified six differentially expressed miRNAs. MiR-1, miR-22, and miR-29 expression levels allowed distinguishing non-treated PD from healthy subjects while miR-16-2, miR-26a2, and miR30a differentiated treated PD patients from untreated patients [139]. Studying of plasma by another research group identified seven overexpressed miRNAs (miR-181c, miR-331-5p, miR-193a-3p, miR-196b, miR-454, miR-125a-3p, and miR-137) [140]. These microRNAs may represent novel biomarkers for the early detection and diagnosis of PD. Table 6 enlists the MicroRNAs as potential peripheral biomarker for PD,

Eyes as Potential Biomarker

As unmyelinated axons from retina are directly synapsing into the central nervous system, retina is considered a prominent environment to study the neurological diseases. Pathologic changes are identified in the RNFL of the eye and associated with neurological conditions. Because of it, eyes are the unique environment to find a surrogate biomarker for early stages of neurodegeneration.

OCT is a non-invasive tool used for measuring tissue at micro meter resolution [141]. The parapapillary RNFL represents axons of the ganglion cells and a thinning of this nerve in PD was first reported by Inzelberg's group in 2004 (Fig. 11) [143]. Later several other research groups found the same result [144–147].

Both eyes from 42 untreated newly diagnosed PD patients and 40 healthy controls were examined by using spectral-domain OCT. They found that the mean RNFL thickness is less in PD patients when compared with healthy controls. Selective thinning of the RNFL was observed in temporal region of both PD patients and controls [148]. Jimenez's group in 2014 did the same research in larger group. They found that peripapillary RNFL thickness gradually decreased while evolution and severity of PD progress [149, 150]. Retinal dopamine loss, foveal dysfunction, and visual hallucination (VH) are noted in PD patients [150]. Although one paper in 2011 reported that there was no difference of RNFL thickness in PD patients ($n = 51$) and healthy control subjects ($n = 25$) [151], other research groups demonstrated the gradual diminishing of RNFL thickness over time together with validating OCT as potential research to find a diagnosis biomarker.

Discussion

During the early research, it was believed that neurological diseases are associated with brain only, but further, through the discovery of various biomarkers, it is proved to be also affecting the peripheral tissues. For AD, amyloid beta, the main pathological hallmark, is found in the brain as well as peripheral tissues. Decreased PKC level, alteration of PKC conformation in RBCs [7, 8], increased GSK-3 protein in WBCs [10], and reduced APP ratios in platelets [14] were found in the AD patients. Finding plasma Aβ in cross-sectional studies of human and mouse showed no significance, but longitudinal studies were more promising [26]. Other studies showed both increasing and decreasing of Aβ in AD patients [29–31]. Moreover, changes in human plasma Aβ is inconsistent, especially in sporadic AD [32].

CFH precursor and alpha-2-macroglobulin (alpha-2M) elevation are specific for AD and correlated with disease severity. But alternative assays would be necessary to improve sensitivity and specificity [36]. Alpha-1-antitrypsin and apolipoprotein J were involved in AD amyloid plaque formation. By validation with either ELISA or Western blot, plasma concentration of Alpha-1-antitrypsin showed a difference between AD patients and controls but no difference for apolipoprotein J [38, 39]. Other plasma proteins were also investigated as potential biomarkers (see more in [152]), but targeting plasma proteins as peripheral biomarkers are not promising at the current moment.

Aβ deposition is found in supranuclear region of the lens together with Aβ aggregates in cytoplasm of the lens fiber cell [45, 46]. But other researchers reported that no Aβ and only lower levels in different forms are present in the lens of AD patients and in controls with cortical cataract [47, 48]. So different groups reported different results, and more research are needed to use lens as a peripheral biomarker.

Narrow retinal veins, thinning of the major superior temporal venule blood column diameter, and thinning of RNFL

at superior quadrant are found in AD patients [50–54]. Results for RNFL, retina artioles, and veins are promising, and further investigations are needed. Degeneration of RGCs [59], reduced number of the RGCs, and reduced dendritic integrity of RGCs were found in both AD patients and transgenic mice [61–63]. Decreased numbers of neurons in the ganglion cell layer (GCL) of the central retina (fovea/foveola/parafoveal retina) in the eyes of AD patients were also observed [153]. Targeting RGCs combined with imaging techniques could be a potential peripheral biomarker for AD in the future [61–63, 153].

Erk1/2 phosphorylation in skin fibroblasts has higher sensitivity and specificity for AD than clinical diagnosis [64–67]. Dynamic and resting calcium homeostasis is altered in skin fibroblasts of aged and AD donors [70]. Alzheimer's fibroblast mitochondria are found to be impairing calcium transport process and increasing the sensitivity to oxygenic free radicals [72]. Targeting Ca+ homeostasis and Ca+ signaling in the skin fibroblast showed promising results but still need more tests to be used as a peripheral biomarker.

AlphaS are found in both CSF and blood with different forms such as α-synuclein, phosphorylated α-synuclein, and oligomeric forms of α-synuclein. Reports showed different results. Studying for various forms of alpha-synuclein is still underway, but the results are promising [87–90]. α-Synuclein results in skin samples are not promising. One research group [94] reported that α-synuclein in skin samples has no diagnosis value while another research group [95] reported that it can distinguish between IPD and other forms of parkinsonism.

Low α-synuclein level and a specific protein was detected in saliva of Parkinson's patients with an extinction coefficient of 27.25 M cm^{-1} [96]. Targeting salivary gland alone is not a diagnosis biomarker but in combination with other tests can be a potential biomarker [97–99]. Although α-synuclein is most abundant synuclein in olfactory mucosa, reports for α-synuclein in olfactory mucosa are controversial, showing positive and negative results [100–102].

α-Synuclein is found in submucosa of descending, ascending, rectal, and sigmoid colon [104–108]. Lewy bodies and α-synuclein are found in heart tissue, epicardium, cardiac sympathetic nervous system, submucosal Meissner plexus, and pharyngeal sensory nerves. The results are promising, but all tests are invasive and stressful for patients [109–113].

Total DJ-1 or α-synuclein in plasma alone is not useful as biomarkers for PD diagnosis or progression [117], but only one PTM isoform of DJ-1 is a candidate biomarker to know late stage of PD [118].

UA is decreased in nigrostriatal dopamine neurons in parkinsonian patients [119] and high serum levels of uric acid were linked with significant decrease in risk of PD. Urate/uric acid can be identified as a promising biomarker of the PD [121, 122, 125, 126]. Significant relationship between EGF and cognitive impairment in PD was observed [128,

129]. Low-plasma Apo-A1 is associated with earlier PD onset and greater putaminal DAT deficit [130, 131]. The thickness of the RNFL in PD, as evaluated by OCT, gradually diminishes if the evolution and severity of PD progress [149, 150], but one research showed no difference [151]. The availability of different studies with the same biomarkers shows sometime conflicting results, and the cause of these discrepancies can be related to limitations in standardization of these biomarkers, variation in laboratory conditions, interpretation of experimental data, procedure for acquisition and analysis of samples, varying severities of abnormalities conferring different prognoses, the selection of study group, period of observation or prediction, and many other factors [154]. With the advancements in researches of peripheral biomarkers for early detection of NDs, although the developments are not perfectly reliable and sensitive, further studies will explore the potentials.

Conclusion

The present review article comprehensively highlighted the significant research models being explored for early detection of Alzheimer's and Parkinson's diseases as peripheral biomarkers. It can be conferred that there are very few definitive peripheral biomarker present till the current research scenario. As reliable diagnosis of neurodegenerative diseases are still based on clinical symptoms, post-mortem result, and immature molecular imaging due to limited capabilities of central biomarkers, the discovery of early, sensitive, specific, and economically effective peripheral biomarker for definite diagnosis, prognosis, and treatment of Alzheimer's and Parkinson's diseases is the requirement of the near future.

Compliance with Ethical Standards

Research Involving Human Participants and/or Animals Not applicable

Informed Consent Not applicable

Conflict of Interest The authors declare that they have no conflict of interest.

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