Review Article

Use of ocular biomarkers as a potential tool for early diagnosis of Alzheimer's disease

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Alzheimer's disease (AD) is the most common neurodegenerative disease worldwide which unfortunately has no known effective cure to date. Despite many clinical trials indicating the effectiveness of preclinical treatment, a sensitive tool for screening of AD is yet to be developed. Due to multiple similarities between ocular and the brain tissue, the eye is being explored by researchers for this purpose, with utmost attention focused on the retinal tissue. Besides visual functional impairment, neuronal degeneration and apoptosis, retinal nerve fiber degeneration, increase in the cup-to-disc ratio, and retinal vascular thinning and tortuosity are the changes observed in the retinal tissue which are related to AD. Studies have shown that targeting these changes in the retina is an effective way of reducing the degeneration of retinal neuronal tissue. Similar mechanisms of neurodegeneration have been demonstrated in the brain and the eyes of AD patients. Multiple studies are underway to investigate the potential of diagnosing AD and detection of amyloid-β (Aβ) levels in the retinal tissue. Since the tissues in the anterior segment of the eye are more accessible for *in vivo* imaging and examination, they have more potential as screening biomarkers. This article provides a concise review of available literature on the ocular biomarkers in anterior and posterior segments of the eye including the cornea, aqueous humour (AH), crystalline lens, and retina in AD. This review will also highlight the newer technological tools available for the detection of potential biomarkers in the eye for early diagnosis of AD.

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Alzheimer's disease (AD) is a degenerative disorder of the nervous system, which affects over 10% of people older than 65 years.[1] AD is the most common cause of dementia worldwide.[2-5] Dementia affects approximately 47 million people worldwide, and with the rapid increase in the ageing population, this number is expected to increase to more than 131 million by 2050.[6]

Pathological characteristics of AD are deposition of extracellular amyloid-β (Aβ) plaques and intracellular hyperphosphorylated tau aggregates known as neurofibrillary tangles (NFTs) [Fig. 1].[7,8] Other pathological features include Hirano bodies which are eosinophilic rod-like bodies and granulovacuolar degeneration.[9] These lesions are associated with synaptic network dysfunction leading to progressive brain atrophy, which in turn results in longitudinal cognitive decline.[10]

Current diagnostic tools mainly include clinical evaluation, subjective methods, or invasive tests, which have limited accuracy. Definite diagnosis of AD is only confirmed by postmortem detection of extracellular Aβ plaques and intraneuronal NFTs.[11-16] Clinically, it is also important to exclude other conditions that may result in similar cognitive impairment, for example, Parkinson's disease (PD), depression, hypothyroidism, vitamin deficiencies, and drug interactions.[17] The existing clinical methods have very limited sensitivity and specificity, and clinical diagnoses have been considered inaccurate in at least 10–15% of cases.[18]

There has been significant progress in the diagnosis of AD with the development of advanced technological tools such as magnetic resonance imaging (MRI), positron emission tomography (PET) imaging, blood-based biomarkers (Aβ), and cerebrospinal fluid (CSF) biomarkers (Aβ and tau).[19-24] In genome-wide association studies, researchers have identified more than 20 genes, which increased the risk of AD. However, the exact mechanism of associations of AD with these genes yet remains unknown.[25-29] Studies on amyloid precursor protein (APP), presenilin-1 (PS1), and presenilin-2 (PS2) mutations have been promising.[30] However, these tools are invasive and expensive, therefore for screening and diagnostic purposes, their applicability remains limited. Newer noninvasive and less expensive methods of an early and definitive diagnosis of AD are needed to better serve the population at risk.

For the development of a novel noninvasive screening and diagnostic tool, the ocular examination sector appears promising.[31] Embryologically, the origin of the eyes and brain are similar. The anterior neural tube forms the eyes and later gives rise to the forebrain. PAX6, which plays a pivotal role in neurogenesis, is

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also key to the development of the visual field.[32-34] Neurons in the retina are similar to the neurons in the cerebral cortex in many ways.[35] Retinal neurons also form complex neural networks, similar to those in the cerebral cortex.[36,37] AD not only causes neurodegenerative changes in the brain but also produces structural and functional alterations in the retinal neurons and vasculature.[1] The similarity between ocular and cerebral tissue suggests that ocular manifestations may be used as early biomarkers of AD.[7]

In AD patients, changes in the neural and non-neural ocular tissues including accumulation of Aβ and degeneration of retinal axonal and neural tissue have been demonstrated in various studies.[38-41] Optical retinal imaging platforms are also being developed, which can detect Aβ plaques in the retina of AD patients.[42-47] Researchers have also considered the assessment of pupillary responses, and retinal vasculature and blood flow as biomarkers of AD.[48-50]

A sensitive screening biomarker of AD measurable at an early stage of the disease would pave the way for newer potential therapies, help to identify the population at risk and improve the management of AD patients. This review article examines the evidence for potential ocular biomarkers of AD.

Relationship between Pathophysiology of AD and Ocular Manifestations

Relationship between AD and their possible ocular manifestation has been emphasized in numerous studies. Published literature suggests that biomarkers specific to AD, also play an important role in the degeneration of retinal tissue and the impairment of visual function.[51,52] Multiple studies have shown the relation between AD and glaucoma, suggesting both to be considered age-related neurodegenerative diseases, having some common pathophysiological features.[53-58] It has also been shown that, at the molecular genetic level, AD and age-related macular degeneration (AMD) share a common disease mechanism and similar pathological signalling defects.[59,60] There is growing evidence that drusen, which are considered the hallmark of AMD, have a vital component- Aβ, which is considered the hallmark of AD.[61,62] Besides deposition of Aβ, AD and AMD share many other similarities including neuroinflammation, microvascular abnormalities, and metabolic and oxidative stress.[61-63]

Pathological changes in ocular structures

- Cornea-[64]
  - Keratoepithelin (Ker) and PS1/PS2 expression
  - Decreased corneal sensation
  - Increased dendritic cell density.
- Aqueous-[44]
  - Presence of Aβ
  - Presence of APP.
- Pupil-[65-67]
  - Atypical pupillary response to cholinergic antagonists
  - Reduced latency and amplitude of pupillary light reflex (PLR)
  - Increased pupillary size.
- Lens-[68]
  - Aβ deposition in the crystalline lens
  - Cataractous changes in the crystalline lens.
- Retina-[15,69-73]
  - Aβ deposition in the retina
  - Reduced retinal blood flow
  - Thinning of retinal nerve fiber layer (RNFL)
  - Degeneration of retinal ganglion cell (RGC)
  - Reduced number of RGC axons.
- Choroid-[74]
  - Reduced choroidal thickness.
- Optic Nerve-[75]
  - Increased cup-to-disc ratio
  - Increased disc pallor.

Clinical visual manifestations of AD

The visual pathway is affected at various levels in AD [Table 1].

Visual acuity

Although in many studies, no significant difference was found between the VA of AD patients and that of control population, it has been observed that AD patients have decreased VA under low luminance.[76,77] In addition, the ability to recognize pictures in low spatial frequency is hampered in AD patients.[78] Another important factor, which affects the VA is the increased risk of cataract in AD patients.[79]

Contrast sensitivity

As compared to the control population, AD patients have markedly reduced CS and the difference is detectable even in the early stages of AD.[80,81] The quality of life of AD patients is adversely affected by a deficit in CS.[82] Furthermore, AD patients have been shown to have improved CS with anticholinesterase inhibitor, donepezil.[83] It has also been shown that in AD patients, the reading latency is increased and reading speed is decreased.[84,85]

Colour vision (CV)

Some studies have indicated that the majority of AD patients are tritanomalous (blue axis deficient) but this finding has been contradicted by some other studies.[86,87] However, colour discrimination error in patients of AD is invariably demonstrated.[87]

Visual field (VF)

VF changes in AD are caused by synaptic dysfunction and neurodegenerative changes resulting from the accumulation of Aβ.[65] Assessment of VF by automated perimetry using frequency doubling technology (FDT) has shown that the loss of sensitivity, particularly in the inferior VF, occurs in AD patients, and furthermore, the correlation between the degree of VF changes and the degree of dementia has also been observed.[88,89]

Motion perception

AD patients have higher thresholds for motion detection as compared to control population. A study had also found a correlation of increased threshold with the severity of dementia.[90] Furthermore, relatively higher thresholds for motion detection across all spatial and temporal frequencies are observed in AD patients.[91]

Table 1: Effects on the visual pathway in AD

Tissue	Area	Manifestations
Primary visual cortex		Altered VA
		Altered CV
		Reduced Visual attention
	Medial temporal visual cortex	Depth and motion perception
Retina, RGC, RNFL		Altered CS

Depth perception and stereopsis

AD patients have reduced depth perception and stereopsis as compared to control population. Also, the correlation has been found between stereopsis performance and cognitive assessment scores.[66]

Ocular motor functions

AD patients have poor visual attention and dysfunctions of ocular motility. AD patients also have slower reaction time, increased latency, and difficulty in suppressing reflexive saccades. These factors often make target fixation difficult for AD patients.[92] AD patients have decreased activity in the oculomotor areas of the cerebral cortex, and exhibit significantly more anti-saccade errors.[93] In AD patients, the convergence angle has also been observed to be smaller.[94]

Ophthalmic signs and ocular biomarkers of AD

Cornea

In AD patients, a higher prevalence of corneal lattice dystrophies has been demonstrated, owing to deposition of Ker, pathological amyloid, in the corneal stroma.[95] PS1/PS2 are also expressed in the corneal epithelium of AD patients.[96] Investigators have observed that AD has profound direct and indirect degenerative effects on the corneal sub-basal nerve plexus (SNP).[97] There are corneal nerve fiber pathology and decreased corneal sensation in patients with neurodegenerative diseases.[64,98,99] Further studies have demonstrated overall decreased corneal nerve fiber number and density, and increased density of dendritic cells in AD patients.[97,98]

Aqueous humour (AH)

There is a multitude of similarities between AH and CSF. Blood-aqueous and blood-CSF barriers also share various common features.[99] Researchers have identified Aβ in the AH of non-AD patients undergoing cataract surgery.[69] It has also been suggested that Aβ and APP derivatives may be produced in the retina, and thereafter transported into the AH via the vitreous humour.[99] Presence of Aβ has also been demonstrated in AH of patients with pseudoexfoliation syndrome (PEX) and glaucoma; linking these pathological conditions with AD.[53] Considering these studies, it would be reasonable to examine the AH for the presence of APP and Aβ, as a biomarker for AD.

Pupil

It has been well established that AD patients are deficient in acetylcholine (ACh), resulting in changes in the pupillary system and altered PLR.[68] The root cause of ACh deficiency is a degenerative pathological change in the Edinger–Westphal nucleus and nucleus basalis of Meynert. In AD patients, repetitive stimulation of the PLR is less pronounced, with decreased amplitude and latency.[64] A significant correlation has also been found between increased pupillary size and Aβ and tau levels in CSF.[70] This may be a potential biomarker for AD patients.

Lens

AD patients show a progressive loss in the transparency and cataractous changes (particularly supranuclear) of the otherwise transparent crystalline human lens. In AD patients, an increase in aggregation and accumulation of misfolded abnormal protein in the crystalline lens has also been demonstrated.[68] Studies have demonstrated a certain degree of similarity in the fundamental mechanism of cataract formation and that of neurodegenerative conditions of the brain.[69] Animal studies have demonstrated APP and Aβ expression in cultured crystalline lenses, indicating that AD-associated pathologic mechanisms may be associated with the development of age-related cataract.[68] It has also been demonstrated that PS1/PS2 are expressed in the mammalian crystalline lens.[30] Supranuclear cataracts and lenticular deposition of Aβ have also been observed in Down's syndrome, which is associated with early-onset AD. Thiamine (vitamin B1) deficiency and the resulting oxidative impairment, has also been linked to AD pathology.[17]

Retina

Multiple studies suggest that in AD patients, brain and retina share many hallmark pathological features. Therefore, the early detection of the retinal manifestations of AD may prove to be a promising tool for screening, diagnosing, and monitoring the progression of the disease [Fig. 2].[36,40-44]

(a) Retinal Vasculature

There are multiple structural and functional similarities between cerebral and retinal vasculature, and various studies have observed the associations between AD and parameters of retinal vascular parameters, owing to these similarities.[38,41-44] Studies have demonstrated that retinal vessels of AD patients have reduced the caliber of retinal veins, reduced arteriolar and venular fractal dimensions, smaller, more tortuous and sparse retinal vessels, and reduced flow of blood.[48,49,69-73] These findings suggest that AD progression may be monitored using retinal blood flow as a tool. Studies also suggest that changes in retinal blood flow parameters appear earlier than the neuronal loss.[7] Researchers, studying ocular metabolism by retinal oximetry, found that in AD patients, the oxygen saturation in retinal arterioles and venules was higher as compared to the non-AD population.[73] In AD patients, Aβ accumulates in retinal and cerebral vascular walls, and studies suggest that cerebrovascular pathology may also be associated with AD.[48]

(b) Retinal Thickness

In AD, RGCs are supposed to be the primary targets of cell loss.[7] Diffuse degeneration of axons in the optic nerves of the majority of AD patients has been revealed in an early postmortem study.[69] Histological studies have confirmed the extensive loss of RGCs and increase in astrocytes in the RGC layer of AD patients.[15] *In vivo* evidence of RGC degeneration comes from the measurement of RNFL by optical coherence tomography (OCT).[70] In AD patients, various patterns of RNFL loss have been observed, including sectoral loss of RNFL in the superior, nasal, and inferior retina.[15,69] Research also suggests that with the progression of AD, RNFL thickness decreases and a significant correlation between the reduction of macular volume and degree of cognitive impairment is observed.[78] Recent studies have also demonstrated a correlation between a decrease in the inner plexiform layer (IPL) thickness and a decrease in volume of the temporal and occipital cerebral cortex.[79]

Detection of apoptosing retinal cells (DARC) is a novel technique of *in vivo* imaging using confocal laser scanning ophthalmoscopy (CLSO). DARC has provided additional evidence of degeneration of RGC in patients with AD.[15]

(c) Retinal Fluorescence

Correlation of retinal changes with clinical features of AD has been suggested by studies using fluorescence lifetime imaging ophthalmoscopy (FLIO). Drusen, in the peripheral

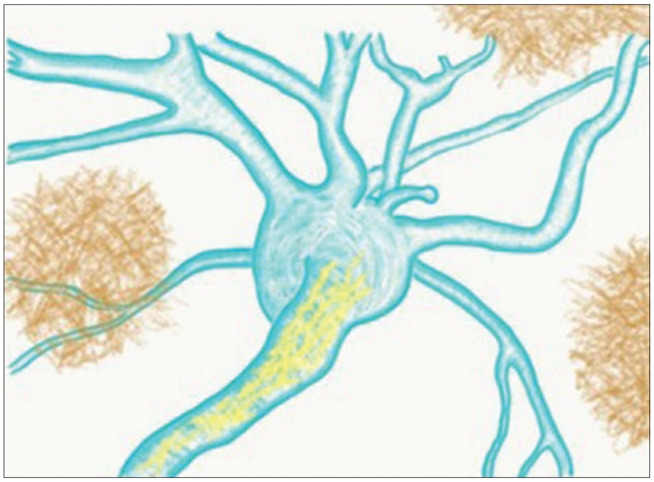

Figure 1: (Reproduced from Reference No. 52) Histopathological features of AD. Extracellular Aβ plaques *(brown)* and intracellular hyperphosphorylated tau aggregates (NFTs) *(yellow)* in a neuron

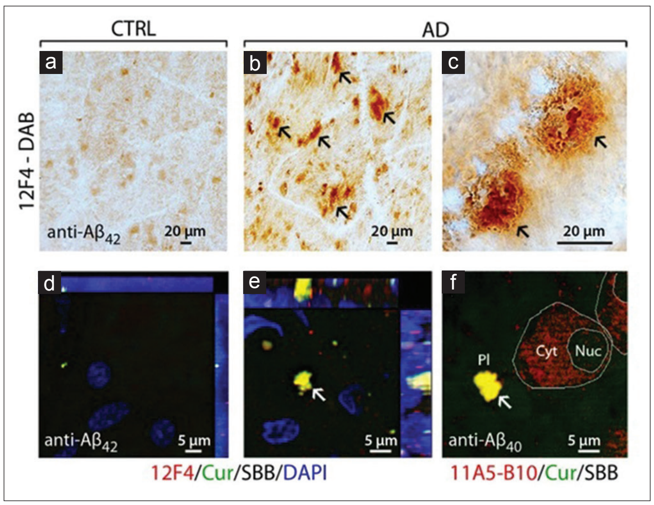

Figure 3: (Reproduced from Reference No. 44) flat-mount retinas from control subjects (ctrl) compared with AD patients stained with anti-Aβ_{42} C-terminal-specific antibodies (12F4) and visualized with peroxidase-based labelling (DAB). (a) Control. (b and c) Aβ plaques along a retinal blood vessel. (d and e) Aβ_{42} detected by fluorescence labelling (*yellow*), using curcumin (cur) staining (*green*), 12F4 staining (*red*), and 4′,6-diamidino-2-phenylindole (DAPI) nuclear staining (*blue*). Sudan Black B (SBB) suppresses nonspecific autofluorescence. (f) Extracellular Aβ and cytosolic Aβ_{40} using Cur and anti-Aβ_{40} C-terminal-specific antibodies (11A5-B10) staining. *Arrows* indicate Aβ plaques

retina of AD patients, has been identified using ultra-wide-field fluorescence imaging.[71] These may prove as potential biomarkers for early detection of AD.

(d) Retinal Aβ accumulation

The pathological hallmark of AD is retinal Aβ deposits, resulting in neuronal cell death and synaptic dysfunction.[65] Numerous studies on animal models have identified the accumulation of Aβ and NFTs in various retinal layers, *viz.* RNFL, RGC, IPL, and outer retina.[70-72] While RGC degeneration and cell loss have been repeatedly demonstrated in postmortem samples of retinal tissue from AD patients, other

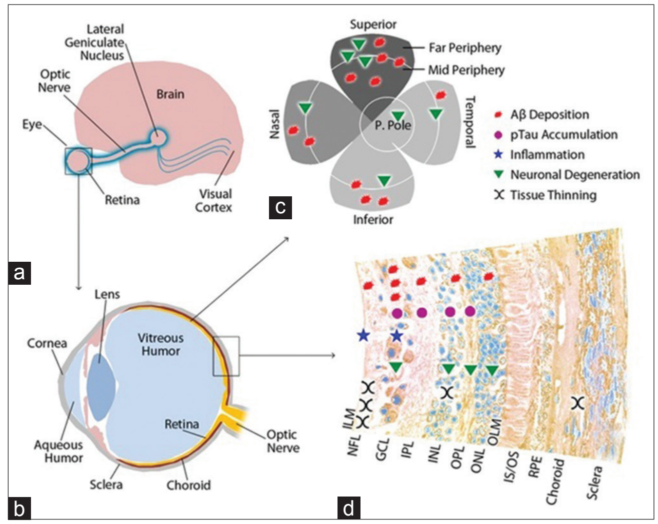

Figure 2: (Reproduced from Reference No. 44) Retinal manifestations of AD. (a) Visual pathway. (b) Sagittal section of the eye. (c) Retinal flat-mount showing the geometric distribution of pathology. Darker shading indicates NFL thinning. (d) Tissue layers of retinal and outer coats of the eye showing the distribution of disease pathology

studies have been contradictory, and have found either no or limited evidence of Aβ accumulation.[72,73]

Studies using several labelling techniques have confirmed the presence of extracellular Aβ plaques, containing Aβ_{40} and Aβ_{42}, and intracellular Aβ_{40} in retinal tissue of AD patients [Fig. 3].[44]

In a study, deposition of Aβ aggregates were observed around and within melanopsin-staining subtype of RGCs (mRGCs) in retinal tissue of AD patients.[92] Studies have also demonstrated Aβ in retinal drusen, a hallmark of AMD. Drusen containing Aβ are found to be associated with photoreceptor apoptosis and RPE degeneration.[47,69] Peripheral retinal drusen have a clinically significant association with AD.[47] These findings suggest that retinal Aβ imaging may be used as a potential biomarker for AD.

Choroid

Some animal studies have observed the reduced thickness of choroidal tissue in AD subjects.[41,74] In a recent imaging study of the choroid, decreased choroidal thickness was demonstrated in AD patients.[76] Age-dependent deposition of Aβ aggregates in the choroidal blood vessels has also been reposted in an animal study.[90] However, more studies and research are needed to establish choroidal changes as a biomarker for AD.

Optic nerve

RGC apoptosis and optic nerve head changes have been observed in postmortem AD retinas. These changes include cellular shrinking and cellular swelling with vacuolization.[36,38,41] Optic nerves of AD patients show degeneration of almost half of the RGC axons, particularly of the larger "M" cell type RGCs. Multiple studies, using OCT and CLSO, have demonstrated increased pallor of the optic disc and increased the cup-to-disc ratio.[75,97,98]

Conclusions and Future Implications

In an era of revolutionary technology and emerging therapies, the quest for potential reliable biomarkers of AD is still ongoing.

Researchers are still puzzling with the question of diagnosing AD before the onset of symptoms. Currently, diagnosis of AD relies on the clinical signs and symptoms of the disease, neuroimaging of the brain, and CSF markers. Modern imaging techniques, such as PET and fMRI, are useful tools providing opportunities for a more accurate diagnosis. However, these investigative techniques are very expensive and have limited availability. These factors render the population-wide screening of AD as a challenging question. Furthermore, cerebral changes appear as a sign often in the later stages of the disease, after symptoms of brain dysfunction and cognitive decline already have set in.

In multiple ways, the eyes and the brain share neural and vascular similarities, leading the investigators towards the pursuit and discovery of potential ocular biomarkers. These biomarkers may prove to significantly influence the methodology of diagnosis of AD and prompt intervention with emerging treatment options, in a hope to save the patients from the disabling clinical course and complications of the disease.

The cornea and AH are still under early phases of research related to AD; hence, more studies are needed to establish their possible role as biomarker tissues. AD, being a neurodegenerative condition, may also have direct or indirect neurotoxicity on the corneal nerve plexus. This possible correlation is also worth exploring. Multiple studies suggest the correlation between changes in the crystalline lens and pathologic changes in the brain in AD, making the crystalline lens a promising tissue as a biomarker for diagnosis of AD. However, further validation of these conclusions, by more imaging studies demonstrating Aβ deposition in the crystalline lens, is needed to conclusively rely on this biomarker.

Presence of Aβ in the retina of AD animal models and humans has been observed in multiple studies. Furthermore, studies have also noted that the appearance of Aβ plaques in retina precedes with that in the brain. These data have opened the possibility of diagnosing AD at an early stage, and of monitoring the effect of emerging novel therapeutic agents. Changes in RNFL thickness and decreased RGC density and function have been noted in AD in many studies. Similarly, retinal vasculature is another promising tissue, which can be used as biomarkers of AD, subject to further research supporting this hypothesis.

While multiple studies are currently underway to evaluate novel ocular biomarkers, and the results look promising, at present these biomarkers are not yet conclusively validated for clinical use for diagnosis of AD, and their implementation to the population for screening purpose remains a challenge. Consequently, there is still a need for developing novel biomarkers, which are more economical, more specific, and could diagnose AD before the cognitive decline sets in.

In summary, while recent studies strongly indicate the potential of ocular biomarkers as screening tools for early diagnosis of AD, further research and more evidence are needed. If the quest for the novel ocular biomarker is successful, it may pave the way to a new dawn for both patients and physicians dealing with AD.

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Conflicts of interest

There are no conflicts of interest.

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