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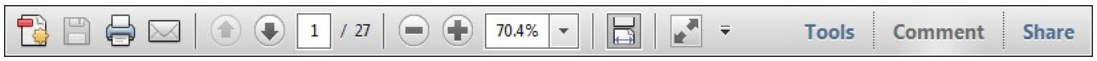

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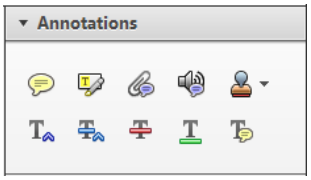

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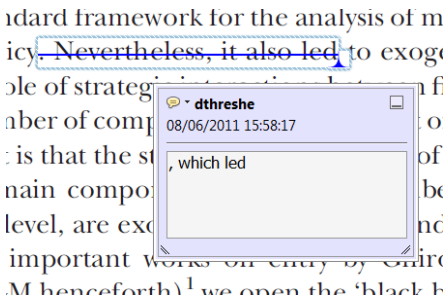

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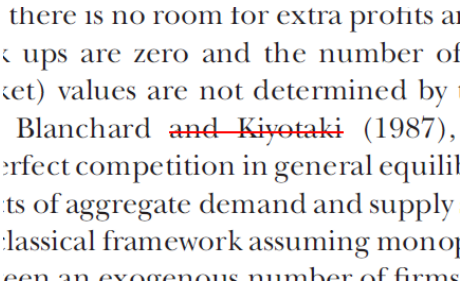

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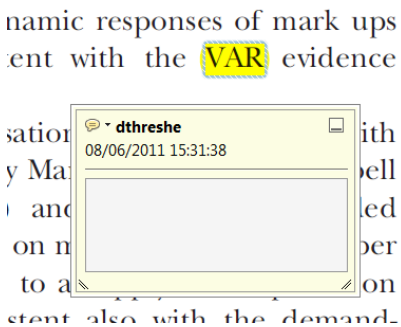

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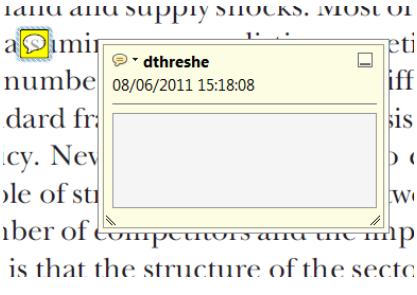

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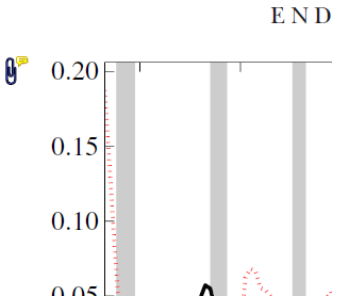

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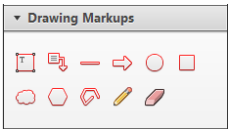

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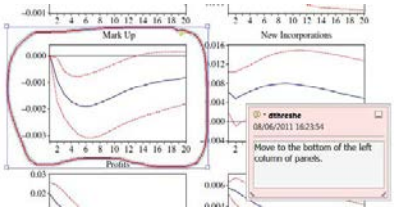

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Review Article

Can the retina be used to diagnose and plot the progression of Alzheimer's disease?

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ABSTRACT.

Alzheimer's disease is a neurodegenerative disease and the most common cause of senile dementia. It impairs the quality of life of a person and their family, posing a serious economic and social threat in developed countries. The fact that the diagnosis can only be definitively made post-mortem, or when the disease is fairly advanced, presents a serious problem if novel therapeutic interventions are to be devised and used early in the course of the disease. There is therefore a pressing need for more sensitive and specific diagnostic tests with which we can detect Alzheimer's disease in the preclinical stage. The tau proteins and beta-amyloid proteins start to accumulate 20 years before the symptoms begin to manifest. Detecting them in the preclinical stage would be a potential breakthrough in the management of Alzheimer's disease. A high degree of clinical suspicion is needed to correlate problems in cognition with the changes in the eye, particularly the retina, pupil and ocular movements, so that the disease can be detected early and managed in the prodromal phase. In this systematic review, we ask the question whether the retina can be used to make a specific and early diagnosis of Alzheimer's disease.

Key words: Alzheimer's disease – diagnosis – preclinical – progression – retina

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Introduction

About 46.8 million people in the world are living with dementia in the year 2015. This number is predicted to double every 20 years reaching 131.5 million in 2050. Alzheimer's disease is the most common cause of senile dementia worldwide and accounts for 60–80% of all the cases of dementia (Prince et al. 2015). Alzheimer's disease is an age-related neurodegenerative disorder, characterized by cortical and hippocampal atrophy. The clinical symptoms are loss of short-term memory, impaired judgement and learning, poor visuospatial perception and reduced executive functions progressing to a stage when the patient may not recall previously well-remembered information (Hardy 2006). The initial symptoms are mistaken for ageing or stress (Waldemar et al. 2007). The disease progresses through three stages – mild, moderate and severe. The early stage is marked by episodes of forgetfulness and confusion in unfamiliar situations. In the middle stage, there is difficulty in remembering recently learned information, deep confusion in some situations and problems with sleep. In the late stage, there is poor ability to think, difficulty in speaking, and the patient becomes dependent on the caregivers, which poses a serious social, economic and psychological problem to the society.

The cause for most Alzheimer's cases is still not known. Only in 1–5% of cases, genetic differences have been identified (Reitz & Mayeux 2014). The basic pathology is intracellular hyperphosphorylated tau protein in the form of tangles and deposits of beta-amyloid protein. Beta-amyloid protein is also the main constituent of senile plaques (Ferreira et al. 2007; Chafekar et al. 2008; Sakano & Zako 2010). There is amyloid deposition in the cortex and hippocampus. These pathological changes lead to dysfunction of synapses and loss of neurons. The uncoupling of tau proteins from the microtubules results in microtubule disassembly.

Most of the drug treatments are based on the cholinergic hypothesis, which states that the cause of AD is reduced synthesis of the neurotransmitter acetylcholine (Francis et al. 1999). The medications currently used to treat the cognitive problems of AD are tacrine, rivastigmine, galantamine, donepezil (which are acetylcholinesterase inhibitors) and memantine, which is a NMDA receptor antagonist. There is evidence for the use of these drugs in mild-to-moderate Alzheimer's disease (Birks 2006) and some evidence for their use in the advanced stage. The drug used for the treatment of advanced AD dementia is donepezil, but the benefit from its use is small (Birks & Harvey 2006). Overall, these drugs do not help to stop or reverse the progression of the disease.

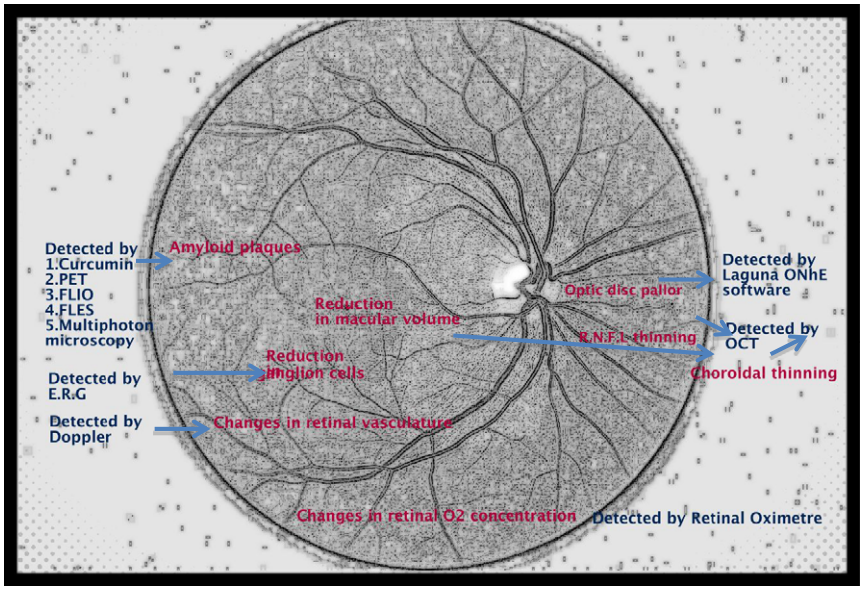
The recognition of the pathophysiological mechanisms in AD has led to identification of targets for the development of specific drugs. More than 200 compounds are undergoing phase 2 and phase 3 trials (Hampel 2012). These drugs have been divided into anti-amyloid agents and drugs that target other pathological pathways. Immunotherapeutic agents like bapineuzumab, solanezumab, gantenerumab, ponezumab and crenezumab have a high affinity to antigenic
6 determinant epitopes of beta-amyloid. They are then recognized by the B and T cells and cleared from the brain (Burstein et al. 2013). A vaccine CAD106 undergoing phase 1 trial may be able to reduce beta-amyloid accumulation by binding to the amyloid aggregates and blocking cellular toxicity. Other drugs directed against tau protein are methylene-blue (Rember), NAP (AL-108) and lithium. The former two favour the stabilization of microtubules, while lithium salts prevent tau hyperphosphorylation (Takashima et al. 2013). A phase III clinical
7 trial 'Anti-Amyloid Treatment in Asymptomatic Alzheimer's Disease' is recruiting cognitively normal adults
8 whose brain scans show evidence of amyloid build-up, which places them at risk for memory loss and cognitive
9 decline, to determine whether an anti-amyloid investigational drug, solanezumab (a monoclonal antibody), can slow memory and cognitive decline and affect the build of amyloid plaques which is shown by brain imaging and other biomarkers in these people. Further trials are needed to target disease modification in patients with very mild clinical symptoms (Dubois et al. 2010).

The ocular changes in Alzheimer's disease include decreased vision, abnormal pupillary reaction, decreased contrast sensitivity, visual field changes, loss of retinal ganglion cells and retinal nerve fibre layer, peripapillary atrophy, increased cup–disc ratio, retinal thinning, tortuosity of blood vessels and deposition of beta-amyloid in the retina. The changes in the brain are at molecular level and disease progression is difficult to detect, requiring methods that are expensive and invasive. When the disease has progressed considerably, with significant damage to the brain, a diagnosis of 'probable AD' is made (McKhann et al. 1984). Changes in the brain are a sign of late stage AD and the symptoms of cognitive decline have already set in. Recent studies suggest that the changes in the retina occur early, thereby suggesting the possibility for earlier diagnosis and subsequent treatment of AD (Koronyo et al. 2011). The development of sensitive biomarkers to detect these changes in a timely fashion is therefore needed. Advanced modern techniques have made it possible to visualize the changes in the retina at a very fine level. By detecting neurodegenerative changes in the eye, the hope is that we may be able to diagnose, manage and plot the progression of Alzheimer's disease at a very early stage (Heaton et al. 2015). The fact that the retina develops from the diencephalic invagination of the pluripotent cells allows the retina to share many morphological similarities with the brain (Ran et al. 2009) and the eye is easily accessible because it is not enclosed by the skull or spine. Therefore, the retina can be used to study CNS disease and its pathology.

Biomarkers are a subclass of endogenously occurring indicators, which signify the pathological state (Rachakonda et al. 2004). An ideal biomarker is specific, sensitive, non-invasive, inexpensive, reproducible, suitable for large-scale population screening and readily available. This challenging set of criteria makes the use of current biomarkers limited (Cedazo & Winblad 2010; Humpel 2011). There are various changes in the retina in Alzheimer's disease, which can be detected using non-invasive retinal imaging techniques (Fig. 1). They are described subsequently in this review with their advantages and disadvantages. The National Institute of Aging (NIA) has set certain guidelines to make an accurate diagnosis of AD focusing on the following stages of Alzheimer's disease:

(1) Dementia
(2) Mild cognitive
(3) Preclinical
(4) Changes observed in an autopsy

According to these guidelines, the following categories of biomarkers have been defined (Clifford et al. 2011):
(1) Category one describes the biomarker of amyloid accumulation and tau protein, which can be detected in brain by amyloid imaging using PET scan, and levels of tau protein in the CSF (Engler et al. 2006; Schoonenboom et al. 2008; Mulder et al. 2010; Nordberg 2010).
(2) The second category describes the marker of neuronal degeneration and injury-tau protein in the cortex measured by C.S.F. analysis. The brain volume changes are measured by M.R.I (Dierks et al. 2000; Sunderland et al. 2003; Maheswaran et al. 2009; Luckhaus et al. 2010).
(3) The third category describes changes at physiological level cell



42 **Fig. 1.** Retinal changes in AD and methods of detecting them using non-invasive retinal imaging techniques.

LOW RESOLUTION COLOR FIG

death, synaptic damage, oxidative stress and inflammation. How useful these measures are is still being determined (Kiddle et al. 2014). The fact that some of these markers are invasive led the NIA to conclude that extensive work was needed before these guidelines were adopted for clinical purposes.

Methods

Systematic review

We searched the MeSH database for all human and animal reviews, clinical and comparative studies, published in any language in the past 5 years from March 2011. Initial search terms were 'retina' and 'Alzheimer Disease/diagnosis'. Additional publications were identified from references in the preliminary literature and subsequent updates. Devices selected for analysis do not constitute a comprehensive list of worldwide research but were chosen for significant progress addressing the question if the retina can be used to diagnose and plot the progression of Alzheimer's disease, comparing the strength of evidence in humans with experimental animals and establishing whether they are specific and sensitive.

Data extraction

We initially screened all studies identified in the systematic search of the online databases by abstract and title in the last 5 years. Irrelevant or duplicate studies were removed, and the remaining articles were assessed for eligibility by full-text review. Data extracted from these studies included: title, authors, publication year, aim of study, study type, disease focus, results and suggestions made by authors.

Results

Using the MeSH database, 102 papers were identified, of which 51 were included as they were in the last 5 years. These 51 were screened by abstract and title only. Twelve seemed ineligible at this stage, which included two reviews. These two reviews were excluded because they were not specific to the question addressed in this systematic review. Three were excluded, as they were an abstract-only conference presentation with no specific content. The total number of papers that were included was 36. Of 36 papers included, seven were review articles.

Results of review of systematic evidence

Optical coherence tomography. The changes in the retina in Alzheimer's disease generally comprise thinning of the nerve fibre layer and loss of the retinal ganglion cells, which can be detected utilizing optical coherence tomography (OCT). The current resolution of commercial OCT is 15 μm. The advantages are that it is fast, non-invasive, readily available and inexpensive (Cost et al. 2006). Spectralis OCT resolves the retina to 5 μm or even less, acquires images quickly and distinguishes the retinal layers. Faster acquisition improves the reliability of the technique. The disadvantage of OCT is that it cannot be used to distinguish between the different types of dementia because of low sensitivity. Moreno–Ramos have characterized retinal changes across different types of dementia including dementia with AD, dementia with Lewy bodies and Parkinson's-associated dementia (Moreno Romos et al. 2013). No statistically significant differences in RFNL thickness between different types of dementia have been observed.

Studies have shown that the cognitive impairment in Alzheimer's disease as determined by mini-mental status examination has a positive correlation with the reduction in macular volume (Parnell et al. 2012). In the patients with mild cognitive impairment, which is an early stage of Alzheimer's disease, significant reduction in the retinal nerve fibre layer has been shown in studies (Iseri et al. 2006). It has been documented in studies, that the first affected area is the macular area in Alzheimer's disease, which is followed by a significant decline in the peripapillary thickness of the retinal nerve fibre layer. The most sensitive area is the superior inner macula (Garcia-Martin et al. 2014). In patients with Alzheimer's disease, there is a typical inferior field loss, which corresponds to the superior loss of the retinal nerve fibre layer (Trick et al. 1995). The most sensitive protocol for detecting this subclinical atrophy is the Nsite Axonal Analytics application of Spectralis OCT.

In a meta-analysis consisting of 11 studies with 380 patients with Alzheimer's disease, 68 of who had mild cognitive impairment, and 293 healthy controls, retinal nerve fibre layer thickness was measured by means of OCT (Li et al. 2009). The mean retinal thickness was reduced in mild cognitive impairment (p value 0.031) and in AD (p value 0.0001). The study found significant decrease in thickness in each quadrant, reflecting the fact that the degenerative process affects the entire retina (Li et al. 2009). This study supported the fact that analysis of the retinal nerve fibre layer with OCT could be used to monitor the progression in Alzheimer's disease patients and also the effectiveness of future treatment in patients with AD.

With the development of spectral domain OCT and shift towards the longer wavelengths of light, choroidal thickness and area can be measured (Margolis & Spaide 2009; Ikuno et al. 2010; Manjunath et al. 2010). Gharbiya et al. (2014) have reported significant thinning of the choroid. The main disadvantage is that inability of choroidal OCT to distinguish AD from other diseases like glaucoma (Alonso Canerio et al. 2013; Park et al. 2014; Zhang et al. 2014). Whether the patients with mild cognitive impairment and retinal thinning have a higher incidence of conversion to AD is not still determined and studies are needed to address this question (Winblad et al. 2004). Spectralis has a good sensitivity of 94.3% and specificity 95%. In a study by Martin et al. (2016), 150 patients with AD and 75 age-matched healthy controls were analysed using macular scans and association between retinal layer thickness, disease duration and AD severity was evaluated. Patients with AD had reduced thickness in the retinal nerve fibre layer, ganglion cell, inner plexiform and outer nuclear layers ($p < 0.05$). The inner layers of the retina were more affected in patients with long-duration disease and the ganglion cell and retinal nerve fibre layer thickness were inversely corelated with AD duration and severity, indicating that ganglion cell reduction was associated with increased axonal damage and may predict greater disease severity.

Bambo et al. (2014) found out significant thinning in the superior and inferior retinal nerve fibre layer in Cirrus OCT and significant thinning in the inferior and infero-temporal retinal nerve fibre layer in Spectralis

OCT. They also found that colour vision assessment is better corelated with disease severity (Tas et al. 2015). Risacher et al. (2013) found that disturbances in contrast sensitivity are present even in the early stages of AD. They also demonstrated a correlation between contrast sensitivity and the mini-mental status score. They also found a significantly positive correlation between colour confusion index (to assess the severity of dyschromatopsia) and Lanthony 15D test with disease duration (0.973, $p = 0.027$). Thus, colour vision impairment could be a good biomarker for diagnosis and follow-up of AD (Bamboo et al. 2015). Colour vision assessment is better correlated than RNFL thickness with disease activity in other neurodegenerative diseases, such as multiple sclerosis (Gundogan et al. 2007).

The results of 10 studies, showing the comparative analysis with statistical significance of the retinal findings, choroidal findings and changes in the optic nerve volume as measured by OCT in patients with AD, patients with mild cognitive impairment and controls are summarized in Table 1.

Doppler and retinal photography to detect changes in retinal vasculature. In amyloid angiopathy, beta-amyloid deposits in the walls of arteries and arterioles result in a narrowed lumina and eventually occlusion (Ellis et al. 1996; Vinters et al. 1996; Jellinger 2002; Tian et al. 2006). The retinal vascular changes in AD include narrow veins, decreased retinal blood flow in veins, reduced complexity of the branching pattern, reduced optimality of the branching geometry and less tortuous venules (Heaton et al. 2015).

The first study that reported the retinal vascular changes in AD was a small participant study by Berisha et al. (2007). Further studies carried out by Frost et al. and Chang et al. suggest that retinal venular narrowing is associated with AD and the reason for this is the increased collagen deposition in the cerebral veins (Cheung et al. 2014). With the help of advances in retinal imaging, measurements of the minute abnormalities in the retinal microcircuitry enable accurate results (Witt et al. 2006). In a study of 52 subjects (10 AD, 21 mild cognitive impairment and 21 normal controls), blood column diameter, blood speed and blood flow were measured in a major temporal retinal vein using retinal laser Doppler flowmetry. This study reported that blood flow abnormalities might precede the neurodegenerative changes in the brain in Alzheimer's disease (Feke et al. 2015; Fig. 2).

A comparative analysis of 11 studies comparing the ocular and haemodynamic parameters, with statistical significance in patients with AD and healthy controls, have been described in Table 2.

The Australian Imaging, Biomarkers and Lifestyle Flagship study of Aging is the first study that correlated the changes in the retinal blood vessels to the plaque burden in the brain. Venular branching asymmetry factor and arteriolar length to diameter ratio are higher in healthy individuals with high plaque burden (Frost et al. 2013). Retinal photography using 500 canon CR Dgi after dilating the pupil with 1% tropicamide was used in the patients with the help of a semi-automated computer-assisted program – Singapore1 Vessel Assessment Software. This software uses the optic disc, placing a grid with the centre of the optic disc, identifying the vessel type and calculating the retinal vessel parameters. A study using this software found out that the patients having the lower value of venular fraction or lower arterial tortuosity were more likely to develop AD after the adjustment of age, smoking, hypertension, diabetes, cardiovascular, cerebrovascular and drug use. However, this study could not demonstrate association between vascular parameters and mini-mental status examination.

In a study by Carol et al. (2014), 136 dementia patients with AD and 290 age-matched controls were studied, retinal photographs were taken, and retinal microvascular parameters were measured. They included 136 dementia patients with AD and 290 controls. Persons with narrower venular calibre, decreased arteriolar and venular fractal dimension, increased arteriolar and venular tortuosity were more likely to have Alzheimer's disease. These changes in the retinal microvasculature may indicate similar pathophysiological changes in the brains of patients of AD.

Atherosclerosis Risk in Communities (Knopman et al. 2016) developed software in which the vessel calibre was measured within a zone that was 0.5–1 disc diameter away from the optic disc margin. Central retinal artery equivalent, vein equivalent and arteriovenous ratio are the parameters which measure changes in vascular calibre. Changes in the retinal vascular width and branching angle occur early in the course of AD during the point when there is asymptomatic plaque deposition. The following haemodynamic parameters and retinal zones were defined:

1 Fractal dimension: Property of self-similarity in 2-dimensional spaces.
2 Tortuosity: Degree of straightness or waviness of a vessel.
3 Bifurcation angle: First angle subtended between the two daughter vessels at each bifurcation.
4 Branching coefficient: Branching vessel width/trunk vessel width.

Table 1. The results of 10 studies, showing the comparative analysis with statistical significance of the retinal findings, choroidal findings and changes in the optic nerve volume as measured by OCT in patients with AD, patients with mild cognitive impairment and controls.

	AD	MCI	Control	P value	Author
1. Average	84.7 ± 10.6	85.8 ± 10	94.3 ± 11	0.05	Kesler et al. (2011)
RFNL	64.75 ± 15.18	86.03 ± 7.26	103.57 ± 8.94	0.001	Ascaso et al. (2014)
thickness	91.83 ± 6.23		97.49 ± 6.09	0.001	Bayhan et al. (2015)
(in μm)	97.4		99.2	0.106	Polo et al. (2015)
	95		89	0.001	Bamboo et al. (2015)
	89.4		100.9	0.002	Bamboo et al. (2015)
	65 ± 6.5		72 ± 3.8	0.001	Kerbas et al. (2012)
2. Choroidal	221.48 ± 40.33		251.86 ± 48.03	0.01	Bayhan et al. (2015)
Thinning	200 ± 70.7		266 ± 93.5	0.001	Gharbiya et al. (2014)
3. Optic nerve Volume	3.68 ± 0.62		4.19 ± 0.60	0.001	Kusbeci et al. (2012)

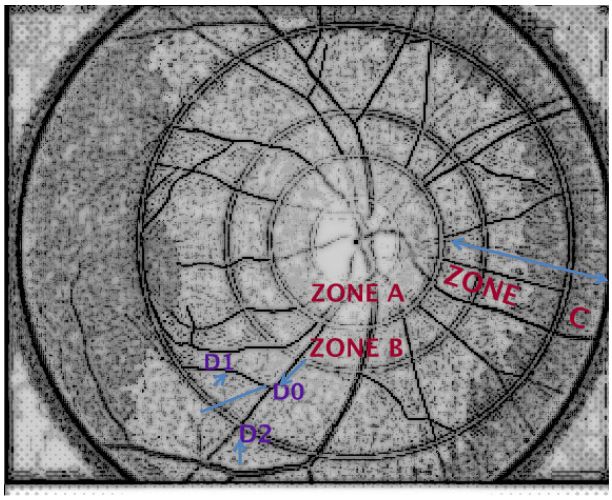


Fig. 2. Retinal zones utilized for retinal vascular analysis: Zone A: The region from 0 to 0.5 disc diameters away from the disc margin. Zone B: The region from 0.5 to 1.0 disc diameters away from the disc margin. Zone C: The region from 0.5 to 2.0 disc diameters away from the disc margin. D0 is the main branching trunk. D1 and D2 are the branches, and the branching angle is the angle between the perpendicular and D1 and D2.

Table 2. A comparative analysis of 11 studies comparing the ocular and haemodynamic parameters, with statistical significance in patients with AD and healthy controls.

	AD (Mean)	Control (Mean)	p Value	Study
Visual acuity	74.3 ± 3.3	74.3 ± 5.8	0.97	Berisha et al. (2007)
Intra-ocular pressure	14.4 ± 4.4	13.3 ± 3.3	0.53	Berisha et al. (2007)
Superior quadrant RNFL (in μm)	92.2 ± 22.6	113 ± 10.7	0.02	Berisha et al. (2007)
Central retinal arterial equivalent	112.8	110.8	0.029	Berisha et al. (2007)
Central retinal venous equivalent	159	158.9	0.951	Berisha et al. (2007)
Arterial tortuosity	0.876	0.921	0.03	Williams et al. (2015)
Venous tortuosity	1.016	1.035	0.458	Williams et al. (2015)
Arteriolar branching angle	78.3	79.17	0.523	Williams et al. (2015)
Venular branching angle	80.38	79.86	0.599	Williams et al. (2015)
Retinal arteriolar 02 concentration	94.2 ± 5.4%	90.5 ± 3.1%	0.028	Cheung et al. (2014)
Retinal venular 02 concentration	51.9 ± 6%	49.7 ± 7%	0.02	Cheung et al. (2014)

Frost et al. (2013) have reported a sensitivity of 81.2% and specificity of 75.7% using 13 retinal vascular parameters in patients with AD (Frost et al. 2013). A large number of population-based studies have shown the association of retinal changes with cognitive impairment. Retinal arteriolar narrowing, venular widening and suboptimal retinal bifurcation are associated with impaired cognitive performance (Parisi 2003; Witt et al. 2006; Reitz et al. 2011; Frost et al. 2013; Al Fiadh et al. 2014). Sparse retinal microvascular network is also associated with AD. The strength of association for these parameters is weak (Williams et al. 2015). Future work will be needed to evaluate whether the changes alone can serve as an independent biomarker in AD.

Image analysis software. The fact that there is homology between the cerebral and retinal circulations helps us to use the retinal microvascular changes as a biomarker in dementia through non-invasive and easily accessible retinal imaging techniques. Cheung et al. (2014) studied retinal photographs in patients with AD. They used a laser Doppler device to assess retinal blood flow in patients with AD. They found out thinning in the retinal microvasculature of patients with AD as compared to the controls. Retinal vascular imaging can give a clue to the vascular mechanisms contributing to the development of AD. The Laguna ONhE software, which is colorimetric analysis software, can be used to assess the colour of the optic disc. The optic disc in patients with AD is pale due to perfusion alteration and axonal loss in the early stages. This can be used an early biomarker in AD (Bambo et al. 2015). The mean haemoglobin percentage and haemoglobin content in the outer ring, which corresponds to the neuroretinal rim, is significantly lower in patients with AD. In glaucoma patients, this program evaluating haemoglobin in the vertical intermediate sectors and centre/periphery haemoglobin has 89.1% sensitivity and 95.1% specificity, which is similar to the other tests.

Johnson et al. (2002) were the first to report beta-amyloid protein in the subvesicular component of a drusen in eyes with age-related macular degeneration. Further studies by Csincsik et al. (2016) have found that in patients with AD, there is significantly higher prevalence of hard drusen in the peripheral retina ($p < 0.05$) which increase significantly in the 2-year follow-up period ($p < 0.05$) suggesting that imaging the drusen could be used to plot the progression of AD. Ultra wide-field and OCT images were acquired by Optos 200TX and Optos OCT SLO. Seventy-two healthy controls and 46 patients with AD (mini-mental score examination <20) were analysed for presence, absence and progression of drusen at baseline and at 2-year follow-up. The development of AD is associated with thinning of the nerve fibre layer and accumulation of drusens, which reflect atrophy and plaque deposits in the brain. Ultra wide-field angle imaging might become a useful tool in monitoring the progression of AD.

Heather Whitson et al. conducted a study where 15 patients with mild cognitive impairment (MCI)/prodromal AD, 15 patients with moderate AD, 17 cognitively normal adults were enrolled. They underwent examination, spectral domain optical coherence tomography, wide-field fundus, colour and autofluorescence photography and stereo disc photography. Duke Optical Coherence Tomography Retinal

Analysis Program (DOCTRAP) software was used to measure nerve fibre layer thickness. Retinal specialists graded drusens and the proportion of participants with evident drusen were compared by chi-square test. After one-year follow-up, they have reported that peripheral drusen are visible in 66.7% of patients with AD as compared to 35.7% patients with mild cognitive impairment or 35.5% controls patients (p value is 0.06), and this finding was bilateral in 16 of 17 subjects (Whitson et al. 2016).

A non-invasive retinal imaging device has been developed by Cedars–Sinai Medical Centre that can detect changes that occur 15–20 years before the clinical diagnosis. According to Frost (Frost et al. 2013), in the initial results in 40 patients, the test could
28 distinguish between AD and non-AD patients with 100% sensitivity and 84% specificity. In the data from 40 patients, amyloid levels detected in the retina were correlated with brain amyloid levels as shown by PET imaging. An average increase in 3.5% retinal amyloid during a 3.5-month period suggested that the technique could be used for monitoring the response to therapy as well.

Retinal oximetry. Jong et al. (2011) examined vascular patterns in dementia and reported that wider retinal venules are associated with an increased risk of vascular dementia. The use of spectrophotometric retinal oximetry measures oxygen saturation in blood vessels. Another study has confirmed that the retinal oxygen saturation in retinal arterioles and venules is significantly elevated (Einarsdottir et al. 2015). Oxygen delivery is the product of the blood flow and the arteriovenous difference in oxygen concentration. As reported by Berisha et al. (2007) if the blood flow is reduced, there is a decrease in oxygen delivery, which explains the elevated oxygen saturation in these patients. Retinal oximetry is a non-invasive test with a potential to detect not only ocular but systemic vascular health. However, the reliable
29 evaluation and monitoring of retinal architectural changes have been limited by the subjective nature by which these parameters are assessed.

Electroretinography. An early complaint of patients with AD is visual disturbance. Visual field, colour vision, contrast sensitivity and visual perception are affected in patients of Alzheimer's disease. Instead of recording the electroencephalogram, the electroretinogram can be used as an alternative to detect pathology in the early stages. There is a significant delay in N35, P50 and N95 components of the pattern electroretinogram. Reduction in b-wave amplitude occurs and can be explained due to the reduced number of ganglion cells in the retina of patients with AD. Visually evoked potential shows changes in patients with AD, which are detected when the pathology is advanced. There is latency of the flash P2 component of the cortical VEP. There is impairment of synaptic organization in AD. This occurs before the neuronal loss, and therefore, it is a better indicator of the cognitive decline (De Kosky & Scheff 1990; Jindal 2015). In patients with AD, ERG had a specificity of 93% and a sensitivity of 70% and the results were similar to the figures published by Coben et al. (1990). As the defects are at many steps from the retinal ganglion cells to the cortex, these tests could be used as screening tools for early AD detection (Dehabadi et al. 2014).

PET imaging. Detecting amyloid plaques in the retina of patients is a diagnostic tool, and it holds great promise in the future. PET scan is a nuclear medicine, functional imaging technique used to measure metabolic processes in the body. The PET scan detects a pair of gamma rays emitted indirectly by a tracer, which is introduced in the body on a biological active molecule. PET scan imaging uses (C-11 or F18) Pittsburgh compound-B (PiB; Mulder et al. 2010), which is biologically active. With this technology, the plaques can be detected at the same time as when the symptoms arise and therefore the disease can be picked up and managed early and efficiently (Schoonenboom et al. 2008). When the disease progresses and symptoms become manifest, there is increase retention of Pittsburgh compound in the plaques and therefore the progression can be monitored as well (Sunderland et al. 2003). The disadvantage of this promising technique is that in one-third of the patients with cognitive impairment not due to AD also have retention of Pittsburgh compound-B PiB in the plaques. This technique is also expensive and has limited availability. Microglial activation has a mitochondrial marker, which is expressed, in low levels in resting microglial cells but when there is inflammation cognitive, this marker increases, and in the advanced stages of the disease, specific radio ligands have been used with positron emission tomography (PET) imaging. Amyvid™(florbetapirF18),Vizamyl™(flutemetamol) and Neuraceq™ (florbetaben F18) are radioactive tracers which have been recently approved by the U.S. Food and Drug Administration for positron emission tomography (PET) imaging of the brain to estimate the beta-amyloid plaque density. This technique carries the risk of potentially toxicity and is also invasive. Various positron emission tomography approaches show a similar sensitivity (range 80.0–100%) and specificity (range 62.0–90%) as compared to CSF fluid tau sensitivity (range 73.3–100%) and specificity (range 70.0–92.4%; Gaugler et al. 2013).

Research by Synder et al. (2016) described a non-invasive approach to find Alzheimer's early PET and OCT scans to observe microscopic details. Sixty-three patients who were high risk for Alzheimer's disease based on emerging symptoms and family history were included. The researchers first conducted PET scans to establish the extent of the participants' beta-amyloid accumulation. Then, they performed OCT scans and compared the results. They used a technique called blue laser autofluorescence in conjunction with the OCT. The OCT scan cannot directly detect beta-amyloid proteins, but it revealed shadow-like inclusion bodies that correlated well with the level of beta-amyloid close to the retina revealed by the PET scan. The surface area of the inclusion bodies increased as a function of cortical amyloid burden with a greater increase in the inner plexiform layer which is rich in cholinergic activity.

If the OCT reveals evidence of beta-amyloid, a PET scan could be performed for a more detailed assessment. This is the first study to demonstrate the results in a preclinical AD population.

Curcumin. Yang et al. (2004) have reported that curcumin, which is a naturally occurring phytochemical extract from the rhizome of *Curcuma longa* L. (Reinke & Gestwicki 2007) binds to the beta-plated structure of amyloid can be used as a staining agent for the amyloid plaques and it also

decreases their density (Ho et al. 2012). When injected i.v or administered orally, it has been seen in mouse models that it crosses the blood brain barrier and stains the amyloid plaques in the brain (Yang et al. 2005; Garcia Alloza et al. 2007) and retina (Dhillon et al. 2008). This technique is very specific and has been used in live transgenic mice with high resolution and specificity (Krantic & Torriglia 2014). A significant advantage of curcumin is that it is safe even at higher doses (12 g per day) and even when it is used over longer periods (Reinke & Gestwicki 2007). The disadvantages are limited bioavailability due to poor absorption, rapid metabolism and quick elimination from the body (Aggarwal et al. 2003; Ringman et al. 2005; Begum et al. 2008). Methods have been developed recently to increase the stability and increase the availability *in vivo* (Anand et al. 2007). Curcumin derivatives can be used as a marker in PET scanning as suggested by Ryu et al. (2006). Researchers at the Commonwealth Scientific and Industrial Research Organization in Australia used curcumin fluorescence imaging to highlight beta-amyloid in the retina and correlated them with the PET findings in the brain. The ability of curcumin to cross the blood brain barrier, bind to the beta-amyloid plaques and dissolve them with less toxicity makes it a promising tool to diagnose and treat AD. According to the researchers, the retinal amyloid test was able to differentiate between Alzheimer's disease and non-Alzheimer's disease with 100% sensitivity and 80.6% specificity. The retinal amyloid test is a potential initial screen that can complement the currently used tests and could potentially be delivered as part of regular eye checks.

Fluorescent ligand eye scanning system (FLES). To distinguish between probable patients with AD and healthy individuals, a new technique called the Fluorescent Ligand Eye Scanning (FLES) System is being developed. This technique has a sensitivity of 85% and a specificity of 95%, ($p < 0.001$). It uses a laser eye scanning device and a fluorescent marker to mark the plaques (Sadowsky et al. 2014). Currently, this technique is in the phase 2 of clinical trials. A high concordance between amyloid PET and FLES has been found. Pilot studies have suggested the safety, feasibility and utility of distinguishing patients with AD from healthy controls. Further studies on a large-scale population with multiple comparisons and autopsy validation are needed to confirm the prognostic utility of the test (Sadowsky et al. 2014). The researchers found that the patients with Alzheimer's disease and healthy volunteers were differentiated with a sensitivity of 85% and specificity of 95%. In addition, FLES showed a strong correlation with amyloid PET imaging. This appears to give us a way to predict Alzheimer's changes in the brain before they occur, and it may give us a simple and cost-effective way to gauge disease progression and treatment benefit.

Fluorescence lifetime imaging ophthalmoscopy. Another new technique called fluorescence lifetime imaging ophthalmoscopy (FLIO) has been developed which demonstrates changes in the retina of patients of AD. It uses a scanning laser ophthalmoscope coupled to an excitation pulse laser BLD 440 and detects fluorescence lifetime (Jentsch et al. 2015). It measures fluorescence lifetime by measuring time correlated single photon counting in two spectral channels. In patients with AD, the fluorescent component of the second channel correlates with the mini-mental status examination and tau protein concentration in the C.S.F. In a study by Jentsch et al. 16 patients with AD, the FLIO parameters of the second fluorescent component significantly corelated with mini-mental score examination as well as tau protein concentration in the CSF. The limitation of the study was low number of subjects, but the strong correlation with Alzheimer's disease was interesting and a study with large number of subjects should be planned in the future to confirm the initial results.

Diffuse tensor imaging is another technique, which can demonstrate that the damage in patients with AD extends down the visual pathway. In patients with AD, diffuse tensor imaging shows increase in total diffusivity, radial diffusivity and decreased fractional isotropy in the optic nerves (Nishioka et al. 2015). This technique is sensitive and specific and has been used to demonstrate beta-amyloid plaques *ex vivo* in mouse and human retina and *in vitro* screening of chemical compounds for amyloidogenesis.

The sensitivity and specificity of fluorescence in the detection of amyloid-β in human retinas of patients with Alzheimer's disease (AD) have been studied. It is an accepted marker of disease, used for diagnosis post-mortem, so its presence in the retina presents an opportunity for a non-invasive diagnostic. *Ex vivo* retinas were stained with thioflavin S and flat mounted from eyes of those with a diagnosis of AD ($n = 19$) and those with no history of AD ($n = 18$) and excluded those with a diagnosis of glaucoma. Retinas were examined using fluorescence in both transmission and confocal scanning microscopy for amyloid-β deposits. In diseased retinas (defined by an AD diagnosis and positive fluorescence), the number of fluorescing deposits also had polarization contrast. Thioflavin S staining in the retina shows 84.2% sensitivity and 72.2% specificity in detecting AD. The use of crossed circular polarization showed a high specificity to amyloid-β deposits but produced some false negatives. This method shows promise for the imaging of amyloid-β in the retina of the living eye as a marker of AD (Emptage et al. 2014).

Studies in animal models

Some promising techniques for the future have been used and tested in animal models. Multiphoton microscopy is a technique using fluorescent dyes AO1-987 and CRANAD-2, which emit light in the near-infrared spectrum. It has been used in animal models. The advantage of this technique is that it is specific and can be used in PET scan by radiolabelling (Ran et al. 2009). The disadvantage is that the procedure is highly invasive requiring a cranial window.

Direct visualization of the beta-amyloid plaque in the retina could diagnose AD preclinically. The neuropathological hallmarks have been confirmed in the retina using transgenic mouse models of AD. Whole mount retinae of transgenic mice have demonstrated beta-amyloid structures in the retina (Ning et al. 2008; Liu et al. 2009; Peterz et al. 2009). Histological examination of the retinal cross sections reveals elevated amyloid plaques in the RGCs, RNFL and superficial retinal layers (Ning et al. 2008; Peterz et al. 2009).

Using retinal imaging techniques, plaque formation and clearance

following immunotherapy, in which altered myelin antigen is loaded on dendritic cells, can be used *in vivo* and can be used to demonstrate the progress of the disease. The effects of the therapy can be visualized by plaque reduction, and this can be very promising for therapy assessment in humans. Future studies are needed in this direction so that the disease progression can be monitored in humans (Yosef et al. 2012). Altered excitability in the neurons has been reported in the hippocampus in a transgenic mice model of Alzheimer disease. Future studies detecting altered neuronal excitability in the retina are needed to confirm its use as an early diagnostic marker (Krantic & Torriglia 2014). Schon et al. observed the progression of tau protein pathology *in vivo*, using laser-scanning ophthalmoscope. They could also demonstrate hyperphosphorylated tau protein in the human retina. They suggested the use of fluorescent probes targeting the tau proteins as a biomarker with great potential in the future.

Combining clinical and retinal imaging biomarkers

Pupillometry is a sensitive technique for the detection of early cholinergic deficits. It has been proposed that pupillometry can be used as a diagnostic tool in early stages of AD (Lim et al. 2013). Reductions in pupillary responses have been reported, but the effect is highly variable and it is difficult to distinguish between patients with AD and patients with Parkinsonism or vascular dementia. Ability to fixate, saccades and pursuit movements are affected in patients with AD (Sadun et al. 1987; Fletcher & Sharpe 1988; Zaccara et al. 1992). These when combined with a high degree of clinical suspicion and retinal findings could be very sensitive in the diagnosis of Alzheimer's disease.

Conclusion

As there is little evidence for the use of currently available drugs in AD, there is an ongoing and increasing need to identify more AD-specific and sensitive biomarkers in the retina, to detect the disease in the preclinical stage allowing neuroprotective interventions to slow the progression of the disease.

Whether the retina can be used to make a specific diagnosis of Alzheimer's disease and monitor progression is still open to question. Retinal examination combined with other ocular tests is a highly promising clinical biomarker for AD. As the earliest complains in AD are visual disturbances, visual function tests especially colour vision can facilitate the detection of AD. The decline in retinal structure and function as documented by OCT and ERG suggests the potential of using retinal examinations, which are easy to perform with other ocular tests. With improvements in retinal imaging techniques like Doppler, FLIO and FLES, which are non-invasive and inexpensive, the early diagnosis of AD is possible with a detailed and early assessment of the degenerative changes in the retinal neurons and axons. They also enable to detect the build-up of beta-amyloid in the eye, early in the disease progression.

Recent advances like dynamic vessel analysis, retinal oximetry (Harazny et al. 2011) and adaptive optic retinal imaging (Moreno Romos et al. 2013), provide a detailed analysis of the retina including the retinal oxygen concentration, blood flow, foveal capillary network and choroidal vasculature enabling retinal vasculature screening to be used as a method for population screening of AD (Moreno Romos et al. 2013). The retinal amyloid test is a potential screening test that can complement the currently used tests and could potentially be delivered as part of regular eye checks. Curcumin and peripheral drusens are very promising techniques to monitor the progression of the disease.

A greater familiarization with these techniques is required. The awareness of the available imaging and analysis methods could improve the ability to understand the clinical relevance of the retinal vascular changes that can be used as very useful clinical biomarkers.

The definitive diagnosis currently requires a combination of many biomarkers with a high degree of clinical suspicion to correlate problems in cognition with the changes in the eye, particularly the retina, pupil and ocular movements, so that the disease can be detected early and managed in the prodromal phase. Careful characterization of cognitive changes and exclusion of normal tension glaucoma is critical.

More future work focusing on the diagnostic, prognostic and therapeutic value of using the retina, as a biomarker to detect and monitor progress in Alzheimer's disease, will still be needed. With the increasing evidence suggesting the use of retina as a non-invasive biomarker, it is very likely that one day the retinal biomarkers will be used as an early method of detecting groups at increased risk of developing Alzheimer's disease.

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