Title: Retinal Ganglion Cell Complex in Alzheimer’s Disease: Comparison of Ganglion Cell Complex and Central Macular Thickness in Alzheimer’s Disease and Healthy Subjects Using Spectral Domain-Optical Coherence Tomography (SD-OCT)

Running title: Ganglion Cell Thickness in Alzheimer’s disease

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Abstract

Introduction: Alzheimer’s disease (AD) is the commonest form of dementia worldwide. The modalities to diagnose AD are generally expensive and limited. Both CNS and Retina are derived from cranial neural crest, so the changes in retinal layers may reflect the changes in CNS tissue. Optical Coherence Tomography (OCT) machine can show the delicate retinal layers and is widely used in retinal disorders. The purpose of this study is to find a new biomarker to help clinicians diagnose AD by the means of retinal OCT examination.

Methods: After considering the inclusion and exclusion criteria, 25 patients with mild and moderate AD and 25 normal matched subjects were enrolled in the study. OCT was done for all eyes. Central Macular thickness (CMT) and Ganglion Cell Complex (GCC) thickness were calculated. Groups were compared using SPSS 22 software.

Results: Both GCC thickness and CMT were significantly decreased in patients with AD, compared to healthy age and sex matched subjects.

Conclusion: Retinal changes, specifically CMT and GCC thickness, may reflect the AD process in brain. OCT may be considered as a non-invasive and inexpensive means to help diagnose the AD.

Keywords: Alzheimer’s disease, optical coherence tomography, ganglion cell complex, central macular thickness
**Plain language summary:**

In our study, we provide a new, safe and relatively inexpensive tool, called optical coherence tomography (OCT), for detection of Alzheimer`s disease. OCT is widely used for evaluation of ocular diseases, specially the Retinal disorders. Because both retina and central nervous system (CNS) have the same embryologic origin, changes in the ocular structure may show the changes in the CNS tissue.

**Highlights:**

This study shows that ocular examination is a good tool for detection of Alzheimer`s disease.
1. Introduction

Alzheimer’s disease (AD) is the commonest form of dementia, characterized by the gradual and progressive worsening of cognitive function (Scheltens P et al., 2016). The incidence increases with age, and with the increase in aging population worldwide, dementia becomes a major public health problem. The main pathologic change is the accumulation of amyloid β (Aβ) in CNS, which is derived from an abnormal processing of amyloid precursor protein (APP). Intracellular neurofibrillary tangles (NFTs) of tau protein are also observed. Some biomarkers are currently used for the diagnosis of AD. There is a biomarker classification system for the diagnosis of AD, called A/T/N system (Jack et al., 2018). Word A states for aggregation of Aβ in CNS, by CSF evaluation or PET-Scan. T refers to aggregated tau protein, which was also shown by CSF analysis or PET-Scan; and N states for neuronal injury, detected by PET-scan, structural MRI, and CSF total tau protein. These procedures are generally costly and invasive; so many of them are not used widely in clinical practice [Sanchez et al., 2018- Lad EM et al., 2018- Grossman et al., 2010- Thal Li et al., 2006]. So, it seems interesting to find a relatively low cost and non-invasive biomarker to help diagnose and follow-up the patients with AD.

Retina is an embryological extension of the brain; both are derived from cranial neural tube. A study showed that, in addition to CNS, Alzheimer-related neurodegenerative changes also occur in the retina and optic nerve (Hinton DR, Sadun AA, Blanks JC, Miller CA., 1986). Beta-amyloid plaques and neurofibrillary tangles are shown in retina (Dentchev et al., 2006). So any change in the retinal structure may somehow reflect the pathologic process in brain. Axons of retinal ganglion cells form the retinal nerve fiber layer (RNFL). The optic nerve formed by RNFL and then passes to the brain. Some histopathologic studies report the reduction in both RNFL and ganglion cell layer (Hinton et al., 1986- Srinivasan S, Efron N., 2019).

Optical coherence tomography (OCT) is a non invasive, easy to use and relatively low cost method which can provide a detailed examination of the retinal cells. OCT can distinguish different retinal layers and measure each layer thickness. A routine OCT exam measures both RNFL, which provide quantity of neuronal axons, and ganglion cell complex, which shows the quantity of dendrites and cell bodies of retinal ganglion cells. Ganglion cell complex (GCC) is defined as the sum of the thickness of RNFL, GCL (Ganglion Cell Layer) and IPL (Inner Plexiform Layer) [Figure 1].
Figure 1: Optical Coherence Tomography (OCT) result of a normal retina. Retinal layers are shown individually. Ganglion Cell Complex (GCC) is composed of Nerve Fiber Layer, Ganglion Cell Layer and Inner Plexiform layer.

These quantitative measures can show the health status of the neuronal structure. Paquet et al. (2007), Danesh-Meyer HV, Birch H, Ku JY et al. (2006), Parisi V (2001), Berisha F, Feke GT, Trempe CL, McMeel JW, Schepens CL (2007), Moschos et al. (2012), Kromer R (2014), Larrosa JM et al. (2014), Polo V et al. (2014), Lu Y et al. (2010), Marziani E et al. (2013), Garcia-Martin ES et al. (2014), Salobrar-Garcia E et al. (2014) measure the peripapillary RNFL (PRNFL) in patients with AD. Nearly all of these studies report PRNFL thinning. Although some recent studies show no significant decrease in PRNFL thickness in patients with AD compared with normal population (Domingo Sánchez et al, 2018). Evaluation of GCC thickness in AD was the subject of some other studies. For example, it was shown that Inner Plexiform Layer (IPL) thickness decreased earlier than the other layers (Srinivasan S, Efron N...
One study suggested that evaluating macular GC-IPL (Ganglion cell-Inner Plexiform Layer) thickness may be more sensitive than RNFL layer in the OCT exam of patients with AD (Gupta VK et al. (2016)). Rui Tao et al. (2019) found that GCC thickness is significantly decreased in AD patients and also in patients with mild cognitive impairment, compared with healthy subjects. Decreased GC-IPL thickness is associated with decreased performance and more severe disease (Cunha JP et al., 2016).

Macula is the region of the retina, which is by the definition, has more than two layers of GCL. Choi SH, Park SJ, Kim NR (2016), Gao LY, Liu Y, Li X, Bai Q, Liu P (2015) and Cheung CY et al. (2015) shown that macular thickness and macular volume decrease in patients with AD.

In this study, we aimed to evaluate the GCC layer and central macular thickness (CMT) in the patients with AD and compare it with the healthy age and sex matched subject. Although a lot of studies exist about the RNFL thickness in patients with AD, there are relatively few studies about the GCC thickness; also there is no previous similar study on Iranian patients with AD.

2. Materials and methods

This case-control study was conducted in Shahid Sadoughi general hospital (Yazd province, Iran). The study was approved by the medical research ethics committee of the hospital and adhered to the principles of declaration of Helsinki. Patients with diagnosis of mild to moderate AD was referred by one experienced neurologist were referred for ophthalmic examination. The diagnosis of dementia was made clinically through minimal mental state examination (MMSE), in which the patient is assessed and given a score according to his/her level of education. Patients with MMSE score of 24-30 was considered as mild, and patients with score of 18-23 as moderate stage of disease. Neuroimaging and clinical investigation was done to rule out secondary causes of dementia. Any patient with psychiatric disorders, like depression, anxiety and psychosis has been excluded from the study. The patients with severe AD were also excluded because of possible poor cooperation. Written informed consent was obtained from all of study subjects and/or their legal representatives. The control group was selected, after obtaining written informed consent, from the ophthalmology clinic. The control group was age and sex matched
with the case group. Full ophthalmic examination was done for all subjects, including BCVA (Best Corrected Visual Acuity), IOP (intraocular pressure) by Goldman tonometer, anterior segment examination with Slit Lamp (SLE), and ophthalmoscopy after pupil dilation with mydriatic drop (1% tropicamide). The subjects with following conditions were excluded: BCVA less than 0.3 (as determined by Snellen chart), refraction with spherical equivalent more than +5/-5 diopter, IOP more than 20 mm Hg, significant corneal opacity or dense cataract (because both conditions alter the OCT measurement signal), or any abnormal acquired or congenital finding in optic nerve and retinal exam. Because presence of glaucoma can alter the results of OCT exam, any patient with history of glaucoma or positive family history was also excluded. Also any subject with history of alcohol abuse, previous ocular surgery and previous blunt or perforating globe trauma were excluded.

After considering the inclusion and exclusion criteria, 25 patients with AD and 25 normal matched subjects were enrolled in the study. Both eyes of all of the subjects were enrolled, so 50 eyes were examined in both groups.
Spectralis Domain (SD)-OCT was done using Spectralis OCT machine (Heidelberg Engineering, Heidelberg, Germany) for all eyes. Images with significant artifacts or low quality score were excluded.
The measurements were done using standard 9-subfields defined by ETDRS (Early Treatment of Diabetic Retinopathy Study) (ETDRS research group investigators, 1991). This standard protocol includes 3 concentric circles centered on the fovea, with the diameter of 1, 3, and 6 mm. The 1-3 mm and 3-6 mm rings were divided to superior, inferior, nasal and temporal quadrants. So, nine areas were studied at each exam: superior 3mm (S3), inferior 3mm (I3), nasal 3mm (N3), temporal 3mm (T3), superior 6mm (S6), inferior 6mm (I6), nasal 6mm (N6), temporal 6mm (T6) and center of macula (C) [Figure 2].
Figure 2: ETDRS standard grid used for retinal thickness report. 1: central 1mm, 2: Superior 3mm (S3), 3: Nasal 3mm (N3), 4: Inferior 3mm (I3), 5: Temporal 3mm (T3), 6: Superior 6mm (S6), 7: Nasal 6mm (N6), 8: Inferior 6mm (I6), 9: Temporal 6mm (T6).
Ganglion Cell Complex (GCC) thickness was defined as the sum of RNFL, ganglion cell layer (GCL) and inner plexiform layer (IPL). These layers were automatically segmented and thickness was measured. Because the GCC is naturally thin at the fovea, GCC thickness measurements were not done in the central 1 mm circle; in this area, the Central Macular Thickness (CMT) was measured instead.

Statistical analysis using software SPSS 22 (SPSS Inc, Chicago, IL, USA) was performed. Categorical data were presented as frequencies by percentage and continuous ones were presented as standard deviation and mean value. The student t-test was used to compare the results between two eyes, and p-value of less than 0.05 was considered as significant.

3. Results

Patient demographics and clinical characteristics

A total of 25 patients with AD (50 eyes) were included in the study. 25 healthy subjects (50 eyes) were also enrolled. In the case group, 8 patients (32%) were male and 17 patients (68%) were female. The female to male ratio in the control group was the same, with a p-value=1. The mean age of patients with AD was 80.44 years (SD=8.66). The mean age of control group was 79.64 years (SD=9.3). The p-value was 0.757, showing that there is no significant difference between two groups [Table 1]. In the case group, 2 patients (8%) had mild AD and 23 patients (92%) had moderate AD.

SD-OCT (Optical Coherence Tomography) findings

The mean CMT was 265.15 μ in the healthy group (SD= 16.30), compared with the mean CMT of 233.60 μ in the case group (SD= 13.55) [Table2]. The p-value was 0.0001, showing significant decrease of CMT in patients with AD.

The mean GCC thickness in N3 area was 111.43 μ (SD=10.67) in healthy controls and 92.10 μ (SD=11.29) in AD patients. Mean GCC thickness in T3 area was 104.94 μ (SD=7.56) in control
group and 89.42 μ (SD=8.16) in AD group. The mean thickness of GCC in S3 area was 113.78 μ (SD=9.17) in controls and 93.03 μ (SD=12.83) in patients. The mean GCC thickness was 109.35 μ (SD=13.14) in I3 area in healthy subjects and 95.70 μ (SD=12.81) in AD group. P-value for areas N3, T3, and S3 was 0.0001, and for area I3 was 0.001. Mean GCC thickness in control group in N6 area was 106.63 μ (SD=11.89) and 90.03 μ (SD=12.70) in AD patients. The mean thickness of GCC in T6 area was 86 μ (SD=10.54) in healthy group and 74.45 μ (SD=9.12) in patients. The mean GCC thickness in S6 area was 93.37 μ (SD=11.52) in control group and 82.72 μ (SD=12.90) in case group. Mean thickness of GCC in I6 area was 94.72 μ (SD=16.28) in control group and 82.50 μ (SD=10.60) in AD patients. P-value was 0.0001 for N6 area, 0.009 for T6 area and 0.003 for S6 and I6 area [Table 2]. As the results show, the difference of GCC thickness between healthy group and case group reached statistical significance in all areas.

4. Discussion

It is proved that in Alzheimer’s disease, the degenerative changes occur in all parts of visual system. Deposition of amyloid β throughout the retina, retinal vasculature, and choroid was shown in histopathological studies. The amyloid β deposits mainly in the inner retina and optic disc (London A, Benhar I, Schwartz M (2013), Dentchev et al. (2006)). This deposition is found to be toxic for the retinal ganglion cell neurons.

The modalities of diagnosis in AD are expensive and limited by low sensivity and specificity. Up to now, there is no proven biomarker for the diagnosis of AD (Kwon JY et al. (2017)- Gharbiya M et al. (2014), van de Kreeke JA et al. (2019)). It seems that evaluation of retina can provide a useful biomarker for detection of neuronal changes in the CNS. Optical Coherence Tomography is a non-invasive device, which can provide detailed evaluation of retinal layers. OCT is widely used in the diagnosis and following up the patients with retinal disorders. This modality enables the physician to evaluate and quantitatively assess each individual retinal layer. Optic nerve is actually made of RNFL, the axons of ganglion cells and then passes to the brain to connect to lateral geniculate body. There is a theory that the retrograde degeneration of axons from the brain can alter the structure of retinal ganglion cells (Domingo Sánchez, et al., 2018). So, OCT seems a potential good device to detect pathologic processes in CNS, such as Alzheimer disease.
OCT can measure RNFL, GCC and central macular thickness, providing objective measurement of ganglion cell bodies and their dendrites and axons. As mentioned in the Introduction section, peripapillary RNFL thickness was previously reported to be decreased in AD. Macular thickness and macular volume were also shown to be decreased in patients with AD. Cheung et al. (2015) and Lad EM et al. (2018) reported that there is a significant reduction of GCC thickness in patients with AD, compared to healthy ones. Some studies showed that IPL thickness decreased earlier than the other layers (Paquet C et al. (2007), Moschos et al. (2017), He XF et al. (2012), Koronyo-Hamaoui M et al. (2011), Gupta VK et al. (2016)). Macular GC-IPL thickness may be more sensitive than the RNFL layer in the evaluation of AD patients (Cunha JP et al., 2016). Van der Kreeke et al. (2019) did not find any significant change in GC-IPL thickness between amyloid β positive and negative individuals. Rui Tao et al. (2019) found that GCC thickness is significantly decreased in AD patients and also in patients with mild cognitive impairment. They also found a remarkable association between brain volume and retinal changes. Marziani et al. (2013) reported significant GCC reduction in patients with AD, compared with healthy subjects. Cheung et al. (2015) measured sum of GCL and IPL and reported significant decrease in thickness of these layers in AD patients. Choi et al. (2016) also measured the macular ganglion cell- IPL layer thickness and found that decrease of this layer’s thickness is associated with clinical disease progression. In fact, ganglion cell loss begins in the macula during the degenerative process, possibly because of high RGC density in this region (Curcio CA, Allen KA., 1990). This observation was proved by histopathological studies (Blanks JC, Torigoe Y, Hinton DR, Blanks RH, 1996). In a study on a mouse model of AD by Williams et al. (2013), changes in dendritic of RGCs occurred before cell loss, suggesting use of inner retinal layers for detection of neurodegeneration. Our results were consistent with the results of the previous studies.

Central macular thickness (CMT) shows the health status of retinal neurons. In our study, CMT was significantly decreased in patients with AD compared to healthy subjects. This is consistent with many other studies (Choi SH et al. (2016), Gao LY et al. (2015) and Cheung CY et al. (2015)).

Up to our knowledge, this is the first study evaluating GCC thickness by OCT in Iranian patients with AD; the use of OCT is not popular between Iranian neurologists for the detection or following up the neurologic disorders. So this study may help neurologists to use a non-invasive,
easy to use and relatively low-cost device to diagnose Alzheimer’s disease, in conjunction with ophthalmology consult.

Our study has some limitations; first of all, the sample size was relatively small to detect the difference between patients and healthy group. Second, although we exclude any patient with signs of glaucomatous optic neuropathy or intraocular pressure more than 20 mm Hg, this cannot completely exclude glaucoma patients, because the patients did not take visual field test. Third, the control group did not undergo extensive neurologic testing to definitely rule out the early or preclinical AD. Fourth, most of our patients had moderate AD, limiting the study to detect possible early changes in patients with mild AD. Actually, in the moderate stages of AD the diagnosis is made with little challenges, and more challenges were encountered in the diagnosis of mild cognitive impairment; so future studies will be needed to target this group of patients. Finally, we did not assess the relation between other variables, such as Functional Assessment Staging (FAST) score, with GCC thickness, which can be considered in future studies.

Use of OCT markers in AD patients may also limited because of poor patient compliance due to cognitive impairment. The common ocular conditions occurring in the older age, for example age related macular degeneration (ARMD), glaucoma, dense cataract or any retinal complications of other systemic diseases such as diabetic retinopathy can interfere with the OCT results interpretation. The use of OCT itself to detect changes due to AD maybe limited because of a severely dense cataract, dense corneal opacity, vitreous hemorrhage and any other ocular condition which can interfere with OCT signal. Presence of any congenital retinal abnormality can also limit the use of this technology.

In conclusion, in the present study we evaluate and compare the thickness of GCC and CMT by means of OCT, in patients with AD and healthy age and sex matched controls. Up to best of our knowledge, this is the first OCT study about AD effects on retina in Iran. Our results show significant thinning of GCC and CMT in AD patients compared with control group. Larger sample size will be needed for better evaluation of OCT parameters in AD. It is better to evaluate patients with mild degree of AD, because this may help to detect possible early AD-related changes in the retina. Follow up studies are also needed to evaluate the changes in retinal OCT over the time.
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Methodology: all authors
Investigation: all authors
Writing – Original Draft: Zahra Farzinvash
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