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Oral Citicoline and Diabetic Retinopathy: Effects on Macular Ganglion Cell Layer Thickness

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Abstract: Diabetic retinopathy (DR) is the main cause of blindness at a productive age, especially in developing countries including Indonesia. Damage to the retinal neural unit occurs to precede vascular disorders and inflammation. Citicoline has been used widely as a neuroprotector and proven in vitro and in vivo to maintain the function of retinal ganglion cells (RGC) in glaucoma. The objective of the study is to measure the macular ganglion cell layer (GCL) thickness after administration of citicoline in patients with DR. All subjects with DR receive 500 mg citicoline administered orally once daily for 2 weeks. Before and after citicoline administration, all subjects undergo examinations of visual acuity, funduscopy, intraocular pressure (IOP), fundus photos, and measurements of GCL thickness of the macula by *ocular computed tomography* (OCT). There are 13 subjects with DR, most of whom are female (69.2%), aged between 40-60 years (53.6%), and have bilateral DR (65%). Non-proliferative diabetic retinopathy (NPDR) is found in 30.8% of subjects and macular edema in 53.8% of subjects. The average macular GCL thickness is 59.23 $\pm$ 31.83 um. After citicoline administration, there is a GCL thickness increase significantly in subjects with proliferative diabetic retinopathy (PDR). The thinning of GCL is shown in subjects with diabetic macular edema (DME). Citicoline may play a role in maintaining the neural unit of retina, especially the ganglion cell layer. Further studies are needed to explore the effect on each stage of diabetic retinopathy.
Keywords: oral citicoline, ganglion cell layer, macula, diabetic retinopathy

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Introduction

Diabetes mellitus is known currently as a disease that has become a global epidemic.(Wong & Sabanayagam, 2020) Diabetic retinopathy is a microvascular complication as the main cause for blindness at productive age in almost all countries in the world including Indonesia.(Adriono, 2011) As many as 30% of people with diabetes mellitus are predicted to experience diabetic retinopathy and 10% of them are at risk of experiencing severe low vision even blindness.(Ogurtsova et al., 2017) The latest prevalence study of DR in Mataram city proves a similar result that is as many as 28.36% of patients with diabetes mellitus experience DR.(Monalisa, 2021)

The current pathogenesis of DR includes the occurrence of neurodegeneration, inflammation, and retinal microvascular damage.(Joseph D. Boss et al., 2017) Understanding the pathophysiology of diabetic retinopathy is necessary for the search for biomarkers that can indicate the presence or absence of worsening of DR even though it's still in the beginning phase of the

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disease. It includes inflammatory biomarkers, neurotrophins, and retinal layer thickness through OCT examination.(Garcia-Ramirez et al., 2009)

Citicoline is known as a neuroprotective drug in cases of disorders of the brain such as stroke and Alzheimer's. Currently, citicoline has also been proven in vitro and in vivo to maintain the function of retinal ganglion cells in glaucoma cases through inhibition of glutamate toxicity cascade, prevention of oxidative stress, and increasing neurotrophins.(Iulia et al., 2017) Citicoline has identical chemistry with endogenous cytidine-5'-phosphocholine (CDP-choline) formed from choline and cytidine. It is an essential precursor in the formation of phosphatidylcholine as a component of cell membranes.(Faiq et al., 2019) The structure of citicoline allows citicoline to enter past the oral route to then be hydrolyzed in the liver and reformed in the body tissues, especially neuron cells.

Citicoline works through the preservation of cardiolipin, sphingomyelin, phosphatidylcholine, and phosphatidylethanolamine; ensures the availability of phosphatidylcholine, stimulates glutathione synthesis, lowers the level of the membrane thus preventing excitotoxicity due to glutamate, guards the function of mitochondria thus preventing damage due to oxidative stress as well as delays the onset of nerve apoptosis, synthetizes myelin which is important for nerve membrane integrity, increases acetylcholine synthesis, and prevents endothelium dysfunction.(Faiq et al., 2019)

Cell membranes require a high supply of phosphatidylcholine to run its function. Phosphatidylcholine together with phosphatidylethanolamine guards intracellular and extracellular membrane integrity through replacement and neurotransmission thus playing a role in maintaining homeostasis and neuron function. Citicoline stimulates sphingomyelin synthesis, a key metabolite to stabilize the membrane of retinal ganglion cell axons so that they can survive the redox imbalance, repair their ability to clean themselves, and defend against inflammatory mediators.

Its use for neurodegenerative cases is well-known and extensive in cases of stroke, Alzheimer's, and Parkinsons. The use of it then inspired the study of its role in neurodegenerative cases of the eye, such as glaucoma, optic neuropathy, and diabetic retinopathy.(Oddone et al., 2021) Retinal ganglion cells are neurons with long myelinated axons to provide rationalization use of citicoline. Study on diabetic retinopathy shows that oral citicolines have the potential to improve N95 amplitude in electroretinogram examination, although this has not been statistically proven.(Utami et al., 2019) Oral administration has been shown to have a protective effect on retinal CGL cells in cases of glaucoma. Topical citicoline administration is also done through stage research for use in RD.(Parravano et al., 2020)

Management for diabetic retinopathy is still aimed at curative treatment for moderate and severe DR, such as retinal photocoagulation laser, anti-VEGF treatment, and vitrectomy.(Flaxel et al., 2020) Approximately 30% of patients do not respond to laser photocoagulation and anti-VEGF therapy.(Busch et al., 2019) Can citicoline give protection to the retina of patients with diabetic retinopathy? There is evidence of the effect of citicoline on retinal cells, GCL thickness, and macular RNFL so that it can act as an alternative adjuvant therapy for diabetic retinopathy. Not only acts as therapy but also as prevention for the progression of diabetic retinopathy. This study aims to analyse the effect of oral citicoline on macular GCL thickness as part of neural unit of retina in patients with diabetic retinopathy.

The retina consists of 10 layers, a combination of neurosensory cells consisting of ganglion cells, glial cells, bipolar cells, and amacrine cells, photoreceptors, and in the outermost section there are retinal pigment epithelial (RPE) cells.(Lechner et al., 2022) Examination to assess cell retinal thickness is currently being performed through ocular computed tomography (OCT). This examination can measure each layer in detail when the refractive media is in clear conditions. This examination is noninvasive and takes less than 5 minutes to get results.

Materials and Methods

This is a prospective uncontrolled study in patients with diabetic retinopathy, where patients who meet the criteria of research subjects will undergo a series of examinations before and after oral citicoline administration. This study is conducted at the NTB Provincial Hospital from September to November 2023.

All subjects undergo ophthalmology examinations such as best-corrected visual acuity (BCVA), intra-ocular pressure (IOP), fundus photo, and macular GCL thickness through OCT examination. Data are generated from direct measurements of study subjects by using lens correction, indirect/ direct fundoscopy, OCT machine, fundus photo, and non-contact tonometry. Data are taken at the polyclinic NTB Provincial Hospital eyes by trained officers and ophthalmologists on duty.

The subject of the study is a population with the following criteria: eye polyclinic patients who have a diagnosis of diabetic retinopathy both old and newly diagnosed at NTB Provincial Hospital, aged over 18 years old, are willing to participate in this study, and have undergone treatment to lower blood sugar. Exclusion criteria are undergone intravitreal anti-VEGF in the last 3 months, history of vitrectomy, have or have

had eye diseases such as uveitis, glaucoma, other N II disorders, having a refractive media disorder that interferes with vision inspection fundus photos and OCT, undergoing topical/systemic steroid therapy, blunt trauma, penetrating trauma eyes, and history of using oral citicoline in the last 3 months. All subjects matched with the criterias above will be included in the study consecutively during the study period.

The study has been approved by the Ethics Committee of Medical Faculty University of Mataram as mentioned in the letter of approval no. 217/UN18.F8/ETIK/2023.

Diabetic retinopathy is diagnosed according to Diabetic Retinopathy Disease Severity Scale and International Clinical Diabetic Retinopathy Disease Severity Scale.("Grading diabetic retinopathy from stereoscopic color fundus photographs--an extension of the modified Airlie House classification. ETDRS report number 10. Early Treatment Diabetic Retinopathy Study Research Group," 1991; Wilkinson et al., 2003) As for macular GCL thickness, the OCT machine records GCL thickness in at least 4 segments of the macular area as listed in the OCT results in micrometer. The results will be written in numeric data.

Patients with diabetic retinopathy in the Eye Polyclinic of the NTB Provincial Hospital who meet the criteria research criteria and are willing to participate in the study filled the informed consent form. All subjects are undergoing ophthalmic examinations: BCVA, IOP, direct/ indirect funduscopy, fundus photo, and macular OCT. Citicoline is given for 2 weeks with daily dose is 500 mg tablet taken after meal. The same examinations will be conducted at the same place in the next 2 weeks.

Result and Discussion

A total of 26 patients at the NTB Provincial Hospital's eye clinic with diabetic retinopathy during the study period fulfilled the research inclusion criteria, and 13 subjects matched the requirements to become research subjects. Those thirteen (13) unselected patients were excluded because they were diagnosed as having cataracts, so the refractive media are cloudy, and OCT cannot be done, had undergone anti-VEGF injections within 3 months, and did not come at the time of data retrieval.

Subjects who succeeded in joining the research have characteristics that are mostly women, with age range between 40-60 years. (table 1)

Table 1. Characteristics of subjects.

Variable	N= 13 (100%)	Mean
Age		58.77 ± 6.19
<40 years	-	
40-60 years	7 (53.6%)	
>60 years	6 (46.2%)	
Gender		
Men	4 (30.8%)	
Women	9 (69.2%)	

Based on ophthalmology examinations, most subjects (53.8%) had good BCVA, based on criteria for normal vision, which is equal to or better than 6/18, 5 subjects with low vision, and 1 patient with blindness. Diabetic macular edema (DME) was found in most subjects, followed by NPDR and PDR. (table 2)

Table 2. Clinical characteristics of study subjects.

Variable	N= 13 (100%)	Mean
BCVA		
≥6/18	7 (53.8%)	
< 6/18- 3/60	5 (38.5%)	
< 3/60	1 (7.7%)	
Diagnosis of RD		
NPDR	4 (30.8%)	
PDR	2 (15.4%)	
DME	7 (53.8%)	
Macular GCL Thickness		59.23 ± 31.83

Macular GCL thickness before citicoline administration in the NPDR stage was greater than in the PDR and DME stages. Likewise in the examination after administration of citicoline. A statistically significant difference was found in the thickness of the macular GCL in PDR subjects before and after citicoline administration. (table 3)

Table 3. Macular GCL thickness before and after oral citicoline administration

Variable	Frequency N=13 (100%)	Before (mean+ SD)	After (mean+ SD)	p
GCL Thick-ness		59.23 ± 31.83	59.54 ± 27.39	0.287
NPDR	4 (30.8)	67.75 ± 29.982	80.25 ± 9.912	0.440
PDR	2 (15.4)	48.00 ± 38.184	52.00 ± 29.698	0.000**
DME	7 (53.8)	51.86 ± 40.288	49.86 ± 29.824	0.539

** p<0.001

There is no apparent relationship between variables such as age, gender, BCVA, DR degree, and GCL thickness before the administration of citicoline with GCL thickness after the administration of citicoline. (table 4)

Table 4. Relationship between age, gender, BCVA, DR degree, and GCL thickness before citicoline administration with GCL thickness after citicoline administration.

Variable	SE	p
Age	13.597	0.409
Gender	2.166	0.920

BCVA	-14.881	0.331
Diagnosis of RD	-13.186	0.178
GCL thickness	0.50	0.861

Ganglion cell layers (GCL) are neural components of the retina that function to transmit and strengthen signals. Signals transmitted from photoreceptor cells going to the brain through its axons extend from the retina to the cerebral cortex.(Mead & Tomarev, 2016) The neural components, especially the GCL, according to a recent study, is a component of the retina that experience early disorders as a consequence of hyperglycemia before it affects the vascular system.(Lynch & Abràmoff, 2017) So that this retinal neuron disorder or retinal degeneration can be used as a way to detect early DR which can ultimately affect the type of DR treatment later in life.(Eggers & Carreon, 2020)

This study found that macular CGL thickness varies for each degree of DR. Macular GCL thickness in early RD stages namely NPDR is greater both at the time before and after administration of oral citicoline if compared to PDR and DME stages. This is in line with the findings of previous studies reported that at the PDR and DME stages, the damage is more severe, and it affects greater loss of ganglion cells and axons resulting in thinning of the macular GCL.

Studies have also found disturbances in both the retinal nerve fiber layer and glial cells that function as retinal structural support which is thought to happen at the beginning of further retinal damage at a more severe stage.(Bhaskaran et al., 2023) Peripapillary retinal nerve fiber layer thickness in patients with severe stages of RD experiences more thinning compared to milder stages.(Bhaskaran et al., 2023; Wan et al., 2021) Other studies that measured total macular thickness state that in the PDR and DME stages, there is an improvement in macular thickness due to improvement in retinal blood vessel permeability, inflammatory process, and retinal *vascular endothelial growth factor* (VEGF).(J. D. Boss et al., 2017; Iyer et al., 2021)

Administration of citicoline showed a significant difference in GCL thickness before and after administration in the PDR group. Average GCL thickness in this group was higher after consuming citicoline than the NPDR and DME groups. This is in line with the function of citicoline as a neuroprotective agent. Citicoline is the main substance in the synthesis of phosphatidylcholine, which is a component of the cell membrane, citicoline works by maintaining stability of the retinal ganglion cell membrane from oxidation reactions that trigger inflammation in ganglion cells which ends in cell death. Additionally, citicoline also works through stimulation of glutathione synthesis as an antioxidant and maintains mitochondria function thereby delaying apoptosis, increasing acetylcholine synthesis, and preventing endothelium dysfunction.

In the NPDR and DME stages, an increase is seen in average macular GCL thickness after citicoline administration although no difference was seen as statistically significant. Animal studies show that citicoline induces antiapoptotic, increasing retinal dopamine levels and inhibiting the depletion of the retinal nerve fiber layer. Studies in humans with glaucoma show there is a decline in damaged retinal ganglion cells after citicoline administration.(Parisi et al., 2018) There was no statistically significant difference possibly caused by not taking into account changes that occurred in the subject during the phase administration of citicoline.(Estrada et al., 2018)

The duration of citicoline administration that has been studied varied widely, ranging from 1 month, 4 months, 1 year to 3 years. This study examined the effect of administration of citicoline for a sufficient time which is 2 weeks which shows there is an effect in the form of improvement of macular GCL thickness in subjects with PDR. Route administration of citicoline which is currently widely used for disease of the eye is through oral, eye drops, and intramuscular routes. No differences were found in terms of the bioavailability of citicoline given either orally or by eye drops. Oral administration has been reported can give better compliance and convenience compared to the use of eye drops in glaucoma populations probably because they already have other topical medications for glaucoma treatment. Oral administration of citicoline can slow down the damage progressiveness in optic nerve fiber in glaucoma.(Lanza et al., 2019)

There are still a limited number of studies using oral citicoline in the prevention of RD progressiveness through measurement of macular GCL thickness, which makes this research a breath of fresh air in the development of RD management, especially at the beginning stage. Further studies are required with larger samples, with controls and strict calculations for confounding factors to strengthen this research results.

This research was conducted at the NTB RSUDP Eye Clinic, which is a referral hospital in NTB province most of the patients who come are in a condition that requires further treatment of RD and its complications such as cataract surgery, retinal laser photocoagulations, anti-VEGF injections, and vitrectomy automatically increase the number of excluded patients. Screening cases from other secondary hospitals and DM patients from other departments such as internal medicine, neurology, or physiotherapy clinics may become the solution to add more patients to participate in further studies.

Conclusion

Oral citicoline (500 mg daily for 2 weeks) improved macular ganglion cell layer thickness in prolipherative diabetic retinopathy (PDR) patients. While no significant changes were observed in the NPDR and DME groups, clinical improvements were still noted in these cohorts. Further research is needed to better assess citicoline as an adjunctive treatment and its potential role in preventing diabetic retinopathy progression.

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