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CORRELATION BETWEEN GANGLION CELL COMPLEX AND RETINAL NERVE FIBER LAYER IN DIAGNOSIS OF GLAUCOMA

By

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ABSTRACT

Background: Glaucoma is a group of diseases of different etiologies with progressive optic nerve degeneration as well as loss of retinal ganglion cells (RGCs) with a subsequent visual impairment. It remains a primary cause of irreversible blindness. So, early diagnosis and treatment of glaucoma has been demonstrated to reduce the rate of disease progression and improve cases's quality of life. Spectral domain-optical coherence tomography (SD-OCT) technologies play an essential role in glaucoma management.

Objective: To determine and compare the use of thickness of macular ganglion cell complex (GCC) and peripapillary retinal nerve fiber layer (ppRNFL) using OCT in diagnosis of glaucoma.

Patients and methods: This was a comparative study carried out on 40 eyes. The eyes were classified into two groups: Group A that represented: 10 normal eyes as a control of the study, and Group B that included 30 eyes with glaucoma classified as regards disease severity into: 10 eyes with early glaucoma, 10 eyes with moderate glaucoma, and 10 eyes with severe glaucoma as regards Hodapp, Parrish, and Anderson (H-P-A) classification. Comprehensive ophthalmic examination, white-on-white perimetry and SD-OCT were done for entire cases. The OCT was done using 3D-OCT (Topcon) 2000 to evaluate the following: RNFL parameters average (total, superior and inferior) thickness and GCC parameters average (total, superior and inferior) thickness.

Results: There was a positive statistically significant association of high probability between the two groups regarding RNFL thickness, GCC thickness, intraocular pressure (IOP), and vertical and linear cup-to-disc ratio ($P<0.001$).

Conclusion: Early diagnosis of glaucoma and initiation of therapy was of high significance, as additional vision loss can be stopped or slowed down. RNFL and GCC measurement with SD-OCT could provide essential data for detection and assessment of glaucoma. GCC thickness and RNFL thickness demonstrated comparable diagnostic value as markers of early, moderate, and severe glaucoma. There was a strong positive association between the RNFL thickness and the GCC thickness in the glaucomatous cases.

Keywords: Glaucoma, ganglion cell complex, retinal nerve fiber layer, optical coherence tomography.

INTRODUCTION

Glaucoma is the primary etiology of irreversible blindness globally *(Davis et al., 2016)*. It represents group of ocular disorders of multifactorial cause characterized by optic neuropathy and neurodegeneration of retinal ganglion cell axons with deformation of the lamina cribrosa *(Casson et al., 2012)*.

Diagnosis of glaucoma is mainly relay on visual field (VF) affection with automated perimetry and glaucomatous

damage of the optic nerve head (ONH), or both. The primary pathological ONH changes are progressive neuroretinal rim thinning and enlargement of the cup/disc ratio, or definite disc cupping in more severe cases *(Michelessi et al., 2015).*

Circumpapillary RNFL (cpRNFL) thickness and macular GCC thickness are efficiently used for diagnostic as well as follow-up *(Kita et al., 2017).*

Prior researches demonstrated that the macular GCC thickness has a similar glaucoma discriminating performance as the ppRNFL thickness, in clinical and in population-based researches *(Schulze et al., 2011*; *Sevim et al., 2013* and *Bambo et al., 2017).* RNFL defect represents one of the first signs that can be found in a patient *(Lisboa et al., 2013* and *Bussel et al., 2014).* Some also say that; the dendritic arbor is the first one affected due to mitochondrial changes, followed by the cell body and lastly the axon *(Moreno et al., 2011*; *Naghizadeh et al., 2014* and *Cennamo et al., 2016).* Another novel research revealed that the GCC/total retinal thickness ratio conducted better than all other RNFL or macular parameters for glaucoma detection *(Kita et al., 2013).*

The aim of the present study was to determine and compare the use of thickness of macular ganglion cell complex (GCC) and peripapillary retinal nerve fiber layer (ppRNFL) using OCT in diagnosis of glaucoma.

PATIENTS AND METHODS

This study was a comparative study that was conducted on 40 eyes. The eyes were classified into two groups: Group A represented 10 normal eyes as a control of the study, and Group B included 30 eyes with glaucoma classified according to disease severity into ten eyes with early glaucoma, 10 eyes with moderate glaucoma, and ten eyes with severe glaucoma as regards H-P-A classification *(Chakravarti, 2017).*

Inclusion criteria: Normal eyes with no prior history of eye diseases, surgeries or laser and with no family history, IOP of 21mmHg or lower by Goldmann applanation tonometery (GAT), normal ONH appearance was depending on stereoscopic examination and with reliable normal VF, glaucomatous optic neuropathy that was defined as either (cup-disc asymmetry between both eyes of more than 0.2, focal thinning, neuro-retinal rim notching, excavation or RNFL defect, disc hemorrhage) on stereoscopic examination by slit lamp and glaucomatous VF Changes on two reliable VF tests.

According to Hoddap parrish Anderson classification which based on standard automated perimetry, the patients were selected to be early glaucoma (MD<-6dB, less than 25% of points are depressed <5% and less than 10 points are depressed <1% on a pattern deviation plot, and all points in the central 5 degree >15dB), moderate glaucoma (MD<-12dB, less than 50% of points were depressed <5% and less than 20 points were depressed <1% on a pattern deviation plot. No points in the central 5 degree can have a sensitivity of 0dB, and only one hemifield may have a point with sensitivity of <15dB within 5 degree of fixation), and severe glaucoma (MD>-12dB, more than 50% of points are depressed <5% or more than 20 points were depressed <1% on a pattern

deviation plot, at least one point in the central 5 degree can have a sensitivity of 0dB, and points within the central 5 degree with sensitivity <15dB in both hemifields).

Exclusion criteria: Cases with any intraocular diseases, previous intraocular surgeries, previous ocular traumas, other optic neuropathies with a neurological field loss, and non-reliable visual fields are described as (a false -ve more than 33%, false +ve more than 33% and fixation losses more than 20%).

All cases were exposed to:

History taking in terms of age, gender, occupation, residency, medical history, prior ocular or systemic diseases and family history for glaucoma.

Comprehensive ophthalmic examination: Best corrected visual acuity (BCVA), IOP evaluation with GAT, slit lamp, gonioscopy using three mirror lens, dilated fundoscopy and stereoscopic ophthalmoscopy of the optic disc with +90D lens.

OCT Imaging: OCT examination was conducted by utilizing Topcon 3D OCT-1000 mark II (Topcon, Tokyo, Japan .(

Visual field testing: Standard VF testing was conducted by utilizing automated static perimetry (Humphery field analyzer with SITA) stander Central 24-2 full threshold test program,2003 Carl Zeiss Meditec), Germany.

Statistical analysis:

Numerical variables were reported as Median and Interquartile Range (IQR) (as Data were not normally distributed), while categorical variables were reported as frequencies and percentages.

Chi squared was used to compare between categorical variables.

Median GCC and RNFL of normal eyes were compared with glaucomatous eyes by Wilcoxon signed rank test. Kruskal-Wallis Test and the post hoc Bonferroni multiple comparisons test was used to compare the different glaucoma severity groups. Mann-Whitney U test was used to determine the P value Receiver operating characteristic (ROC) curves assessed the ability of RNFL and GCC parameters to detect glaucomatous changes in patients with various levels of glaucoma severity. An area under the ROC curve (AUC) value of 1.0 represented perfect discrimination, whereas an AUC of 0.5 represented discrimination that is no better than results obtained by chance. Differences in the diagnostic ability (AUC) of RNFL and GCC were tested for statistical significance by a previously described method. $P \leq 0.05$ was considered statistically significant.

RESULTS

There was no statistically significant difference between normal and glaucoma grades as regards the age and gender. There was a statistically significant difference between normal and glaucoma groups as regards the BCVA and IOP **(Table 1)**.

Table (1): Characteristics of normal and glaucoma grades in the current study

Groups / Parameters		Normal group (n= 10)	Glaucoma group (n= 30)	P	Early group (n= 10)	Moderate group (n= 10)	Severe group (n= 10)	P
Age		56.0 (45.2 to 61.5)	48.0 (33.0 to 59.5)	0.178	48.0 (45.0 to 58.8)	37.5 (26.0 to 50.2)	55.0 (44.2 to 61.0)	0.141
Gender	**Male**	40.0% (4)	33.3% (10)	1	1(10.0%)	4(40.0%)	5(50.0%)	0.226
	Female	66.0% (6)	66.7% (20)		9(90.0%)	6(60.0%)	5(50.0%)	
Best corrected visual acuity (log Mar)		1.0 (1.0 to 1.0)	0.9 (0.7 to 1.0)	**0.002**	1.0 (0.9 to 1.0)	0.9 (0.9 to 1.0)	0.6 (0.5 to 0.7)	**<0.001**
Intra ocular pressure (mmHg)		14.5 (12.2 to 15.8)	26.2 (23.5 to 28.9)	**<0.001**	25.2 (23.1 to 28.1)	24.5 (22.8 to 26.8)	28.5 (25.5 to 30.8)	0.078

There was a statistically significant difference between normal and glaucoma groups as regards, Disc area, Rim area, Vertical cup disc ratio, Linear cup disc ratio, Visual field mean deviation and Pattern standard deviation **(Table 2)**.

Table (2): Optic nerve head and visual field data of normal and glaucoma grades in the current study

Groups / Parameters	Normal group (n= 10)	Glaucoma group (n= 30)	P	Early group (n= 10)	Moderate group (n= 10)	Severe group (n= 10)	p
Disc area	2.1 (2.0 to 2.2)	2.5 (2.2 to 2.7)	**0.011**	2.6 (2.4 to 2.9)	2.3 (2.2 to 2.6)	2.4 (2.0 to 2.7)	**0.032**
Rim area	1.2 (1.2 to 1.8)	1.0 (0.6 to 1.3)	**0.006**	1.3 (1.2 to 1.5)	1.0 (0.9 to 1.1)	0.5 (0.4 to 0.6)	**<0.001**
Vertical cup disc ratio	0.6 (0.3 to 0.6)	0.8 (0.7 to 0.8)	**<0.001**	0.7 (0.7 to 0.7)	0.7 (0.7 to 0.8)	0.9 (0.8 to 1.0)	**<0.001**
Linear cup disc ratio	0.6 (0.3 to 0.7)	0.8 (0.7 to 0.8)	**<0.001**	0.7 (0.7 to 0.7)	0.7 (0.7 to 0.8)	0.9 (0.9 to 1.0)	**<0.001**
Visual field mean deviation	-0.3 (-0.6 to0.1)	-9.4 (-21.9 to -4.3)	**<0.001**	-2.0 (-3.8 to -1.7)	-9.4 (-11.3 to -8.0)	-24.8 (-26.5 to -22.5)	**<0.001**
Pattern standard deviation	1.6 (1.5 to 1.9)	5.3 (2.5 to 10.0)	**<0.001**	1.9 (1.7 to 2.3)	5.3 (4.0 to 6.5)	10.8 (10.1 to 11.4)	**<0.001**

There was a statistically significant difference between normal and glaucoma grades patients as regards mean (superior, inferior, and average) GCC, and mean (superior, inferior, and average) RNFL **(Table 3)**.

Table (3): Testing the difference between normal and total glaucoma (Wilcoxon signed rank test) and between Glaucomatous grades using (Kruskal-Wallis Test)

Groups / Parameters	Normal group (n= 10)	Glaucoma group (n= 30)	P-value	Early Group (n= 10)	Moderate group (n= 10)	severe group (n= 10)	P-value
Superior GCC	106.0 (103.8 to 108.0)	95.5 (78.0 to 103.0)	**<0.001**	102.5 (98.2 to 103.8)	99.0 (91.5 to 104.5)	71.0 (58.8 to 76.8)	**<0.001**
Inferior GCC	109.5 (107.5 to 112.8)	89.5 (67.5 to 101.0)	**<0.001**	97.5 (90.8 to 101.8)	95.0 (90.0 to 102.5)	58.0 (56.5 to 64.0)	**<0.001**
Average GCC	108.0 (105.2 to 110.0)	93.5 (71.0 to 101.8)	**<0.001**	98.5 (95.2 to 103.0)	96.5 (92.2 to 102.0)	61.0 (57.2 to 68.2)	**<0.001**
Superior RNFL	127.5 (120.2 to 135.8)	97.5 (75.2 to 113.5)	**<0.001**	116.0 (99.5 to 125.8)	109.0 (100.8 to 113.2)	65.0 (57.0 to 73.8)	**<0.001**
Inferior RNFL	131.0 (124.0 to 139.0)	97.0 (64.2 to 116.2)	**<0.001**	115.5 (98.2 to 130.2)	99.5 (97.0 to 117.0)	59.5 (49.5 to 64.0)	**<0.001**
Average RNFL	103.5 (100.5 to 105.5)	89.0 (62.0 to 99.8)	**0.002**	97.5 (85.8 to 107.2)	93.0 (90.5 to 100.5)	56.0 (50.8 to 60.0)	**<0.001**

GCC: ganglion cell complex and RNFL: retinal nerve fiber layer.

In early glaucoma, the diagnostic value of average GCC thickness (AUC, 0.895) appeared to be greater than that of Inferior RNFL thickness (AUC 0.77), but there is no significant difference. In moderate glaucoma the diagnostic value of average GCC thickness (AUC 0. 95) appeared to be greater than that of Superior RNFL thickness (AUC 0.91), but there is no significant difference. In severe glaucoma the diagnostic value of GCC thickness (AUC 1) appeared to be equal that of RNFL thickness (AUC 1), but there is no significant difference. GCC and RNFL showed similar diagnostic performance for detecting early, moderate, and severe glaucoma **(Table 4** and **Figure 1)**.

Table (4): Evaluation of OCT Parameters as Diagnostic Tests with the Area under the ROC Curve

Groups / Parameters	Normal versus Early Glaucoma	Normal versus Moderate Glaucoma	Normal versus Severe Glaucoma
Superior GCC	0.78	0.83	1
	(0.71-1)	(0.64-1)	(1-1)
Inferior GCC	0.89	0.87	1
	(0.69-1)	(0.67-1)	(1-1)
Average GCC	0.895	0.95	1
	(0.71-1)	(0.85-1)	(1-1)
Superior RNFL	0.73	0.91	1
	(0.51-0.95)	(0.77-1)	(1-1)
Inferior RNFL	0.77	0.90	1
	(0.54-0.99)	(0.76-1)	(1-1)
Average RNFL	0.65	0.80	1
	(0.37-0.92)	(0.58-1)	(1-1)

Data were the mean area under the ROC curve (95% CI), GCC: ganglion cell complex and RNFL: retinal nerve fiber layer.

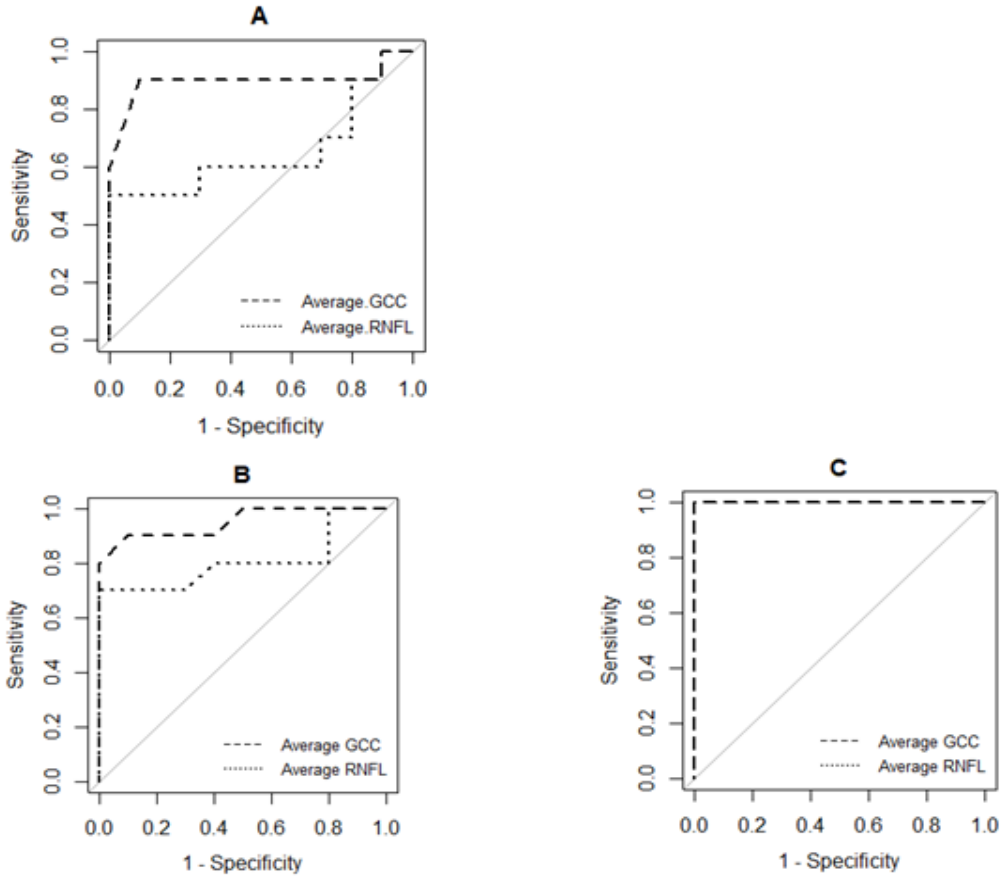

Figure (1): AUCs for average RNFL thickness and average GCC thickness: normal versus early (A), normal versus moderate (B), and normal versus severe (C)

DISCUSSION

Although the diagnostic accuracy of SD-OCT is reasonable in advanced glaucoma, its accurateness in early glaucoma isn't reached the clinical requirements *(Michelessi et al., 2015).* This study showed that the GCC thickness measurements were superior to RNFL thickness values for detection of early glaucoma: However, the two parameters demonstrated a similar performance. These was in the line with *Moreno et al. (2011), Naghizadeh et al. (2014)* and *Cennamo et al. (2016)* demonstrated that GCC parameters to be a better modality in early glaucoma diagnosis compared to RNFL. This finding might have two explanations; first, GCC is a direct measure of RGC integrity as cell body loss could be noticed prior to axonal loss, hypothetically, macular GCC parameters were demonstrated to be an initial predictor in comparison with RNFL ones. Second, as macular GCC scan is done with 7mm×7mm grid centered on the central macula, initial glaucomatous damage that starts in the paracentral region (10°-20°) can easily be detected with this technique *(Kim et al., 2010).*

However, *Mwanza et al. (2014)* and *Akman (2018)* who stated that inferior quadrant of RNFL is considered to be the best RNFL parameters distinguishing glaucoma from normal individuals. *Lisboa et al. (2013)* and *Bussel et al. (2014)* emphasized on the superiority of RNFL over GCC parameters in the detection of early glaucoma as well as glaucoma progression. *Oddone et al. (2016)* demonstrated that RNFL parameters are still favorable to macular parameters for diagnosing manifest glaucoma, however the changes are minimal.

As regards, average GCC thickness (Area under the Curve "AUC" =0.895) is considered a promising predictor for glaucoma in comparison with average RNFL thickness (AUC=0.65), although the AUC difference wasn't significant.

In the line with the present study results, *Vidas et al. (2017)* found that average GCC (AUC=0.957) has a better diagnostic ability in glaucoma detection than average RNFL (AUC=0.906), although the AUC difference has no statistical significance. In contrast with these results, *Sung et al. (2012)* and *Lisboa et al. (2013)* found that average RNFL is better than average GCC in diagnosis of glaucoma.

In accordance with these results, *Cho et al. (2010)* demonstrated that GCC thickness and RNFL could be considered as significant indicators for diagnosis of glaucoma. They reported that there was comparable sensitivity between the macular GCC thickness and the mean sensitivity of peripapillary RNFL thickness. Also, they reported that: the associations of mean sensitivity with GCC global and sectorial (superior& inferior) thicknesses were not significantly different from that of mean sensitivity to global RNFL and sectorial (superior and inferior) peripapillary RNFL thicknesses, on linear regression analysis.

In the same line, *Schulze et al. (2011), Sevim et al. (2013)* and *Bambo et al. (2017)* recorded that macular GCC thickness as well as RNFL thickness demonstrated a comparable diagnostic role as marker for all glaucoma stages. The capability for diagnosis of glaucoma

with macular GCC thickness was similar to that of ppRNFL in high myopic cases *(Kim et al., 2011).*

Moreover, *Barua et al. (2016)* showed that RNFL and GCC thickness have identical capability for diagnosing of moderate and severe glaucoma, inspite the fact that half of the RGCs occupy the macula which makes the diagnostic ability of RNFL parameters superior to GCC in advanced glaucoma.

As regards the demographic data, there were no statistically significant differences as regards age and sex, while, there were a highly statistically significant differences in terms of BCVA, IOP, disc area, rim area, VCDR, LCDR, VFMD and PSD. In agreement, *Soliman et al. (2019)* revealed that there were no statistical significant differences as regards gender, while, there were statistically significant difference as regards IOP, disc area, rim area, VCDR, LCDR, MDVF and PSD, and disagreed with the current study as regards age and BCVA. Moreover, *Hasegawa et al. (2015)* found that VFMD is significantly deteriorated in glaucomatous cases in comparison with controls. In addition, the significant deterioration of visual acuity in glaucoma cases was observed in another research conducted by *Chan et al. (2015).*

CONCLUSION

GCC and RNFL measurement with SD-OCT provided essential information for detection and assessment of glaucoma. There was a strong positive association among the RNFL thickness and the GCC thickness in the glaucomatous cases. Imaging of the GCC has a similar diagnostic ability to RNFL and ONH measurements in the differentiation between cases with glaucoma and healthy subjects.

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العلاقة بين مجمع الخلايا العقدية وطبقة ألياف أعصاب الشبكية في تشخيص المياه الزرقاء

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خلفية البحث: المياه الزرقاء مجموعة من الأمراض ذات المسببات المختلفة يصحبها تنكس تدريجي للعصب البصري بالإضافة إلى فقدان الخلايا العقدية الشبكية (RGCs) مع ضعف البصر، كما تعد السبب الرئيسي للعمى النهائي. لذا، فقد ثبت أن التشخيص المبكر وعلاج المياه الزرقاء يقللان من معدل تطور المرض ويحسن جودة الحياة للمصابين. وتلعب تقنيات التصوير المقطعي التوافقي البصري للمجال الطيفي (SD-OCT) دورًا أساسيًا في تشخيص المياه الزرقاء.

الهدف من البحث: هو تحديد ومقارنة إستخدام سمك مجمع الخلايا العقدية وطبقة ألياف أعصاب الشبكية المحيطة بالعصب البصري باستخدام التصوير المقطعي التوافقي البصري في تشخيص المياه الزرقاء.

المرضى وطرق البحث: تمت دراسة مقارنة أجريت على 40 عيناً، تم تصنيف العيون إلى مجموعتين: المجموعة أ التي تمثل 10 عيون طبيعية كعنصر تحكم في الدراسة، والمجموعة ب التي تضمنت 30 عيناً مصابة بمرض المياه الزرقاء مصنفة من حيث شدة المرض إلى 10 عيون مصابة بمرض المياه الزرقاء المبكر، و10 عيون مصابة بمرض المياه الزرقاء المتوسط و 10 عيون مصابة بمرض المياه الزرقاء الشديدة. وقد تم إجراء فحص شامل للعين، وعمل مجال ابصار وتصوير مقطعي توافقي بصري للحالات كاملة. تم استخدام التصوير المقطعي التوافقي البصري ثلاثي الابعاد لتقييم متوسط سمك طبقة ألياف أعصاب الشبكية (الكلية، العلوية والسفلية)، ومتوسط سمك مجمع الخلايا العقدية (الكلية، العلوية والسفلية).

نتائج البحث: كان هناك ارتباط إيجابي ذي دلالة إحصائية باحتمالية عالية بين المجموعتين فيما يتعلق بسمك طبقة ألياف أعصاب الشبكية وسمك مجمع الخلايا

العقدية، وكان هناك ارتباطاً إيجابياً معتد به إحصائياً لاحتمالية عالية بين المجموعات فيما يتعلق بضغط العين ونسبة الكوب إلى القرص الرأسي والعرضي.

الاستنتاج: التشخيص المبكر للمياه الزرقاء وبدء العلاج لهما أهمية كبيرة، حيث يمكن إيقاف فقدان البصر الإضافي أو إبطائه. ويمكن أن يوفر قياس طبقه أليف أعصاب الشبكية ومجمع الخلايا العقدية باستخدام التصوير المقطعي التوافقي البصري بيانات أساسية للكشف عن المياه الزرقاء وتقييمها"، أظهرت سماكة مجمع الخلايا العقدية وسماكة طبقه أليف أعصاب الشبكية قيمة تشخيصية قابلة للمقارنة كعلامات للمياه الزرقاء المبكرة والمتوسطة والشديدة. ووجد ارتباط إيجابي قوي بين سمك طبقه أليف أعصاب الشبكية وسماكة مجمع الخلايا العقدية في حالات المياه الزرقاء.

الكلمات الدالة: المياه الزرقاء، مجمع الخلايا العقدية، طبقة أليف أعصاب الشبكية، التصوير المقطعي البصري.