Preliminary Study of the Differences in Optic Nerve Head Hemoglobin Measures Between Patients With and Without Childhood Glaucoma

Lucía Perucho-González, MD, PhD; Carmen Dora Méndez-Hernández, MD, PhD; Manuel González-de-la-Rosa, MD, PhD; Cristina Fernández-Pérez, MD, PhD; Federico Sáez-Francés, MD, PhD; Vanesa Andrés-Guerrero, MD, PhD; Julián García-Feijoó, MD, PhD

ABSTRACT

Purpose: To evaluate the effectiveness of quantifying color changes in the optic nerve head in retinal photographs of patients with childhood glaucoma.

Methods: In this observational study, three photographs of the optic nerve head were obtained in 28 patients with childhood glaucoma and 28 age- and sex-matched healthy participants (the childhood glaucoma and control groups, respectively). The Laguna Optic Nerve Head Hemoglobin (ONhE) software (Insoft SL, Tenerife, Spain) was used to determine hemoglobin levels in the optic nerve head. The following parameters were quantified: the hemoglobin levels in the optic nerve head across the whole disc, in 24 sectors (the optic nerve head divided by two concentric rings and eight 45-degree radial sectors), and in the vertical disc diameter (sectors 8 and 20), and the estimated cup–disc ratio and Glaucoma Discriminant Function, which combines the slope of the hemoglobin amount with the mean vertical disc diameter.

Results: Patient ages ranged from 9 to 14 years (median: 11 years) in the childhood glaucoma group, and 7 to 13 years (median: 9 years) in the control group ($P < .061$). Eyes in the childhood glaucoma group showed a significantly higher cup–disc ratio compared to eyes in the control group (0.6 ± 0.2 vs 0.5 ± 0.1, respectively; $P < .0001$). In the childhood glaucoma group, the Glaucoma Discriminant Function was found to be significantly lower than in the control group (-6.5 ± 31.1 vs 9.4 ± 17.1, respectively; $P < .0001$). There were no significant differences in the hemoglobin levels in the optic nerve head across the whole disc between eyes in the childhood glaucoma and control groups (58.2% ± 10.9% vs 58.5% ± 6.7%, respectively; $P = .847$). The Laguna ONhE software showed good reproducibility in measuring percentages of hemoglobin levels in both groups.

Conclusions: The Laguna ONhE software is useful for patients with childhood glaucoma. However, hemoglobin levels in the optic nerve head across the whole disc may have normal values. This method had good reliability and is easy to implement in routine clinical practice.

[*J Pediatr Ophthalmol Strabismus*. 201X;XX(XX):XX-XX.]

From the Departments of Glaucoma (LP-G, CDM-H, FS-F, VA-G) and Ophthalmology (JG-F) and the Methodology Research and Clinical Epidemiology Units (CF-P), Hospital Clínico San Carlos, Madrid, Spain; Instituto de Investigación Sanitaria San Carlos, Hospital Clínico San Carlos, Madrid, Spain (LP-G, CDM-H, FS-F, VA-G, JG-F); the Ocular Pathology National Net (OFTARED), Institute of Health Carlos III, Madrid, Spain (LP-G, CDM-H, FS-F, VA-G, JG-F); the Department of Ophthalmology, University Hospital of La Laguna, Tenerife, Spain (MG-R); and the Department of Ophthalmology, Universidad Complutense, Madrid, Spain (JG-F).

Submitted: January 28, 2017; Accepted: May 8, 2017

The authors have no financial or proprietary interest in the materials presented herein.

Correspondence: Lucía Perucho-González, PhD, Department of Glaucoma, Hospital Clínico San Carlos, Calle del Prof Martín Lagos, 28040 Madrid, Spain. E-mail: luciaperuchogonz@gmail.com

doi:10.3928/01913913-20170801-02

INTRODUCTION

Classically, the pathogenesis of glaucomatous optic neuropathy has been described by two principal theories: mechanical and vascular. According to the mechanical theory, increased intraocular pressure causes stretching of the laminar beams and damage to the retinal ganglion cell axons. The vascular theory of glaucoma considers the neuropathy to be a consequence of insufficient blood supply due to either increased intraocular pressure or other risk factors that reduce ocular blood flow. Conditions including childhood, angle-closure, or secondary glaucomas clearly show that the presence of increased intraocular pressure leads to glaucomatous optic neuropathy. However, observations such as the existence of normal-tension glaucoma cannot be satisfactorily explained by a pressure theory alone. Indeed, most published studies on blood flow report a reduced ocular perfusion in patients with glaucoma when compared to healthy participants.[1] Moreover, the role of reactive oxygen species, excitotoxicity, defective axon transport, trophic factor withdrawal, and loss of electrical activity is increasingly taken into account to increase our understanding of the pathophysiology of glaucoma.[2,3]

In childhood glaucoma, the mechanical theory could be presented as the main pathogenesis mechanism because vascular dysregulation is rare in children.[4,5] In primary congenital glaucoma, the immature angle appearance most likely results from the arrested development of tissues of a neural crest origin in the third trimester, with the severity of abnormality varying according to the stage at which arrested development occurred and whether the site of outflow obstruction is thought to be trabecular.[6]

The glaucomatous damage developed in secondary childhood glaucoma after cataract surgery, glaucoma associated with non-acquired systemic diseases or syndromes (eg, trysomy 21, metabolic disorders, phacomatoses, or congenital rubella), or glaucoma associated with acquired conditions (eg, uveitis, steroid induce, trauma, or tumors) can also be explained by the mechanical theory as opposed to the vascular theory. Subsequently, the differential features found between the glaucoma pathogenesis in adults and children should be taken into account.

Childhood glaucoma is a potentially blinding condition.[5,7] Clinical research suggests that prognosis is largely dependent on early, accurate diagnosis, successful treatment involving control of intraocular pressure to a level at which progression is unlikely, and prevention of amblyopia.[8,9] Because it is uncommon, children with childhood glaucoma are sometimes misdiagnosed or suboptimally treated, thereby allowing irreversible corneal and optic nerve damage to occur. Overall, glaucoma is responsible for 5% of blindness in children worldwide.[10]

The diagnosis of glaucoma in children can be challenging, especially regarding the assessment of any resultant optic nerve damage. Optic nerve head examination is the current gold standard and, in many cases, the only technique that is available for the diagnosis of glaucoma-related optic nerve damage in children because visual field testing is sometimes unreliable. To have some baseline information, it is recommended to obtain retinal photographs of the optic nerve head, which offer helpful objective information for a more accurate evaluation. Additional objective measurements of glaucomatous damage using structural diagnostic devices in patients with childhood glaucoma would be valuable, but obtaining images with an adequate quality to be analyzed is not always possible. This fact is related to the low visual acuity or refractive errors, such as myopia and high astigmatism, that these children often have.[11] Alternatively, all current imaging modalities in glaucoma have a normative database for children younger than 18 years.

The Laguna Optic Nerve Head Hemoglobin (ONhE) software (Insoft SL, Tenerife, Spain) uses a new strategy for measuring the amount of hemoglobin at the optic nerve head level based on the information obtained from conventional retinographies. The potential influence of illumination and the state of the lens is compensated by the program. There is some evidence of the ability of the Laguna ONhE software in detecting early glaucomatous optic nerve heads in adults.[12] However, this technology has not been previously used in the assessment of patients with childhood glaucoma.

The high amount of hemoglobin at the optic nerve head level is responsible for the particular color found when comparing with the rest of the retina. The amount of hemoglobin in different sectors of the optic nerve head might change in accordance with glaucomatous changes induced on the optic nerve head. By quantifying the changes in color, it may be possible to predict the changes that have occurred in the disc as a result of glaucoma.

There is little evidence of any objective and reproducible quantification of blood perfusion

methods applied to the optic nerve head.[13-15] Few strategies for hemoglobin measurement have been published.[16,17]

The main advantage of the Laguna OnhE software is that it is a non-invasive method that is easy to perform and provides quality hemoglobin measurements.[18] In fact, a fundus retinography is usually performed as a regular clinical assessment in patients with glaucoma. Therefore, it is also an inexpensive tool because no further examination would be required.

Children may become tired during assessment in a clinic, which increases the difficulty of exploring the optic nerve in as much detail as in adults. Some patients with childhood glaucoma also have nystagmus and complications maintaining eye fixation. Therefore, performing any of the glaucoma structural imaging modalities is not possible in some cases. However, obtaining a good retinography is feasible most of the time, even in the presence of nystagmus or with poor cooperation. One of the advantages of the Laguna ONhE software is that only good imaging of the optic nerve head is needed and the analysis can be performed afterward. With this technique, we can both evaluate changes at the optic nerve head level using the retinoscopy image and obtain objective parameters that are provided by the Laguna ONhE software. Changes in the optic nerve head can be evaluated at each visit to establish the progression of the patient.

This study was designed to evaluate the effectiveness of quantifying color changes of the optic nerve head as measured by the Laguna ONhE software in patients with childhood glaucoma to collect objective measurements of the eyes of these young patients in whom the visual fields are unreliable and measurements with other structural glaucoma devices are difficult to perform.

PATIENTS AND METHODS

This study protocol was approved by the institutional review board of Hospital Clínico San Carlos, Madrid, Spain, and followed the tenets of the Declaration of Helsinki. Informed consent was obtained from each participant before inclusion in the study.

Participants and Measurement Protocol

For this observational study, we obtained retinographies of 32 eyes of patients with childhood glaucoma (childhood glaucoma group; 28 eyes were included for analysis and 3 eyes were excluded, one for being saturated in red and two because the image quality was not high enough to perform the analysis with the Laguna ONhE software) and 29 eyes of healthy participants (control group; 28 eyes were included for analysis and 1 was excluded due to bad image quality). Only one eye was chosen randomly from each participant unless only one eye met the inclusion criteria.

Patients with childhood glaucoma were consecutively recruited from the Department of Glaucoma, Hospital Clínico San Carlos. Inclusion criteria matched the diagnosis criteria defined for childhood glaucoma during the Consensus Childhood Gaucoma Meeting in 2013.[5] Diagnosis criteria were individuals younger than 18 years of age who presented with two or more of the following: intraocular pressure greater than 21 mm Hg; a progressive increase in cup–disc ratio, cup–disc asymmetry of 0.2 or greater when the optic nerve heads are of similar sizes, or focal rim thinning; Haab striae or a corneal diameter of 11 mm or greater in newborns, greater than 12 mm in children younger than 1 year of age, or greater than 13 mm at any age; progressive myopia or myopic shift coupled with an increase in ocular dimensions unusual for normal growth; and a reproducible visual field defect that is consistent with glaucomatous optic nerve head neuropathy with no other observable reason for the visual field defect.

The healthy age-matched participants were recruited among individuals undergoing a routine eye test or referred for refraction at our hospital. The inclusion criteria for this study group were an ophthalmology examination without abnormal ocular findings and no history of any ocular diseases or surgeries. Individuals with significant lens or corneal opacity impairing optic nerve head visualization or a visual acuity of counting fingers or worse were excluded.

Study Protocol

All participants underwent a full eye examination, including a complete clinical history, visual acuity measurement and refraction, slit-lamp biomicroscopy, Goldmann applanation tonometry, fundus examination, and retinography (Canon CR-DGi Non-Mydriatic Fundus Camera; Canon, Inc., Tokyo, Japan).

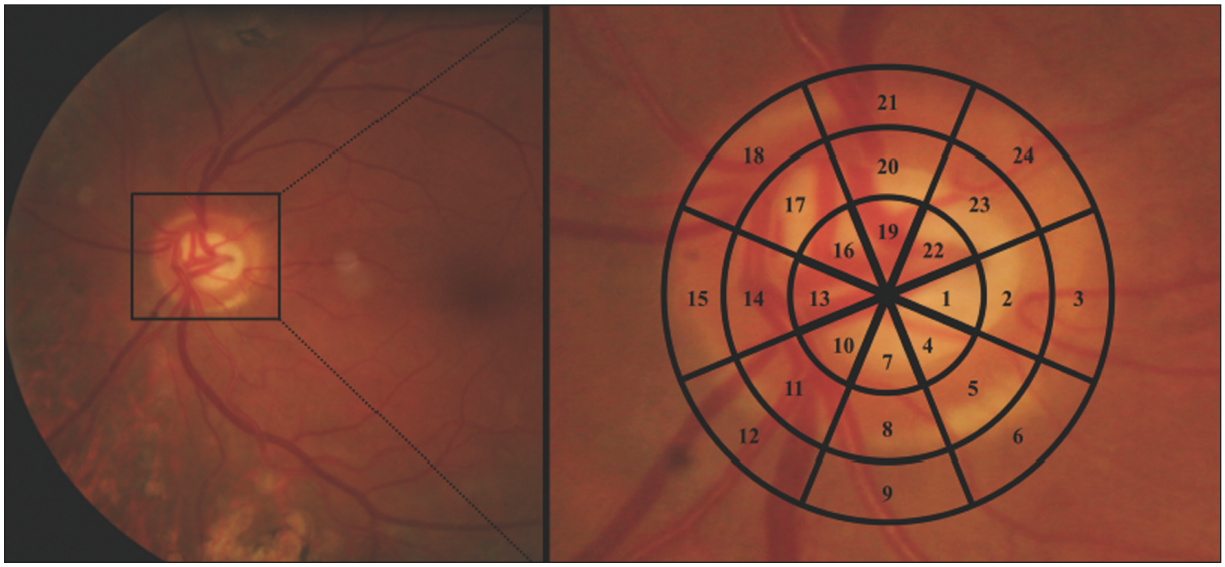

Figure 1. The image of the papilla was divided automatically by the Laguna Optic Nerve Head Hemoglobin (ONhE) software (Insoft SL, Tenerife, Spain) into eight 45-degree radial sectors and two concentric rings using one- and two-thirds of the disc radius. The Laguna ONhE software analyzed the hemoglobin levels in each of these 24 sectors and the average amount of hemoglobin.

Hemoglobin Measurements of the Optic Nerve Head

Zones of the optic nerve head with high hemoglobin levels mainly reflect red light, whereas areas with low hemoglobin levels reflect higher proportions of green and blue light. Using different concentrations or thicknesses of various red blood cell dilutions may help to determine the amount of hemoglobin present through the photographic images obtained with this technique. Both the optic nerve head edges and limits of central retinal blood vessels are automatically identified by the Laguna ONhE software. Therefore, the software provides the hemoglobin levels in the central retinal vessels and optic nerve head tissue separately. Formulas were calculated at the pixels corresponding to the vessels as a whole and for every isolated pixel of tissue. The higher the concentration of hemoglobin, the higher the red light was reflected in that area. Alternatively, when the hemoglobin levels were lower, the proportion of blue and green light reflected increased. The proportions of the three different colors (blue, green, and red) were analyzed by a specific program (MathWorks, Inc., Natick, MA). The strategy used by the Laguna ONhE software has been described in detail previously.[18]

The final result of dividing the papilla with two concentric rings and eight 45-degree radial sectors was a figure with 24 sectors (**Figure 1**). We also obtained the hemoglobin levels across the vertical disc diameter (sectors 8 and 20) because it is widely known that glaucomatous damage principally affects this area of the optic nerve head. The outer ring mostly aligns with the neuroretinal rim, the central ring corresponds to the transition area, which may include the neuroretinal rim and cupping, and the inner rim mainly comprises the cup area. As with other imaging techniques used in the diagnosis of glaucoma, this alignment can be more difficult in tilted discs. On the basis of colorimetric analysis of the papilla, it is known that hemoglobin levels are greater in the more peripheral sectors (ie, the outer ring) in patients both with and without glaucoma. The middle ring tends to show a lower amount of hemoglobin than the outer ring and the innermost ring features the lowest amount of hemoglobin. In other words, tissue hemoglobin levels diminish from the disc periphery toward the center such that the difference between peripheral and central hemoglobin levels can be calculated. By determining color changes in the central disc zone, the Laguna ONhE software estimates the cup and cup–disc ratio. The Laguna ONhE software also uses an index called the Glaucoma Discriminant Function, which combines the hemoglobin slope (obtained through multiple regression analysis of hemoglobin levels estimated for each sector) with the mean hemoglobin amount determined in the vertical disc diameter. The hemoglobin levels in the optic nerve head across the whole disc are expressed as a percentage.

The Glaucoma Discriminant Function is expressed as a whole number from +75 to -90. It de-

TABLE 1

Hemoglobin Levels According to Sector of the Optic Nerve Head[a]

Sector	Childhood Glaucoma Group (%)	Control Group (%)	*P*[b]
Average across all sectors	58.16 ± 10.99	58.46 ± 6.73	.847
Vertical disc diameter (sectors 8 and 20)	60.05 ± 13.05	64.94 ± 7.38	.007
1	63.04 ± 14.57	66.18 ± 9.94	.174
2	63.66 ± 12.53	64.65 ± 7.80	.555
3	59.83 ± 10.73	58.49 ± 8.38	.398
4	61.99 ± 14.95	64.23 ± 10.53	.317
5	62.69 ± 12.09	62.74 ± 8.27	.977
6	59.41 ± 10.25	55.17 ± 8.73	.011
7	57.96 ± 14.72	61.65 ± 10.41	.097
8	59.29 ± 12.97	62.48 ± 8.04	.086
9	58.44 ± 11.15	54.41 ± 8.54	.024
10	55.45 ± 14.70	58.38 ± 9.88	.196
11	63.74 ± 59.81	58.75 ± 7.61	.492
12	56.30 ± 12.64	53.33 ± 7.16	.114
13	54.71 ± 15.89	57.47 ± 10.32	.239
14	56.05 ± 14.04	57.60 ± 8.70	.472
15	56.10 ± 12.24	53.80 ± 7.66	.216
16	54.39 ± 15.12	60.03 ± 10.37	.016
17	57.20 ± 14.81	63.04 ± 8.08	.006
18	58.41 ± 11.41	57.67 ± 7.76	.668
19	58.95 ± 7.76	64.66 ± 15.98	.015
20	60.80 ± 14.19	67.24 ± 7.77	.001
21	60.03 ± 10.39	59.00 ± 10.28	.558
22	61.27 ± 15.05	66.61 ± 10.27	.029
23	64.91 ± 12.99	68.46 ± 7.81	.038
24	61.42 ± 10.15	60.76 ± 8.52	.656

childhood glaucoma group = patients with childhood glaucoma; control group = participants without childhood glaucoma
[a]Values are presented as mean ± standard deviation.
[b]Corrected significant difference ($P < .002$) in Student's *t* test (Bonferroni post hoc) between groups.

scribes the likelihood that the optic nerve disc shows an amount of hemoglobin within the normal range (positive values) or in the range consistent with glaucoma (negative values). Therefore, the relationship is direct in that the further the Glaucoma Discriminant Function is from zero in either direction, the greater is the likelihood that the disc belongs to one diagnostic group or the other.

Statistical Analysis

All statistical analyses were calculated using SPSS (version 20.0; SPSS, Inc., Chicago, IL) and MedCalc (version 9.6.4.0; MedCalc Software, Mariakerke, Belgium) statistical software. Sample distribution was assessed using the non-parametric Kolmogorov–Smirnov test. The normal distribution of average hemoglobin values and hemoglobin values by sectors allowed comparison of the measurements between both groups using a Student's *t* test. Bonferroni correction of the *P* value was performed for multiple comparisons. *P* values less than .002 were considered significant.

RESULTS

A total of 28 eyes in the childhood glaucoma group and 28 eyes in the control group were ex-

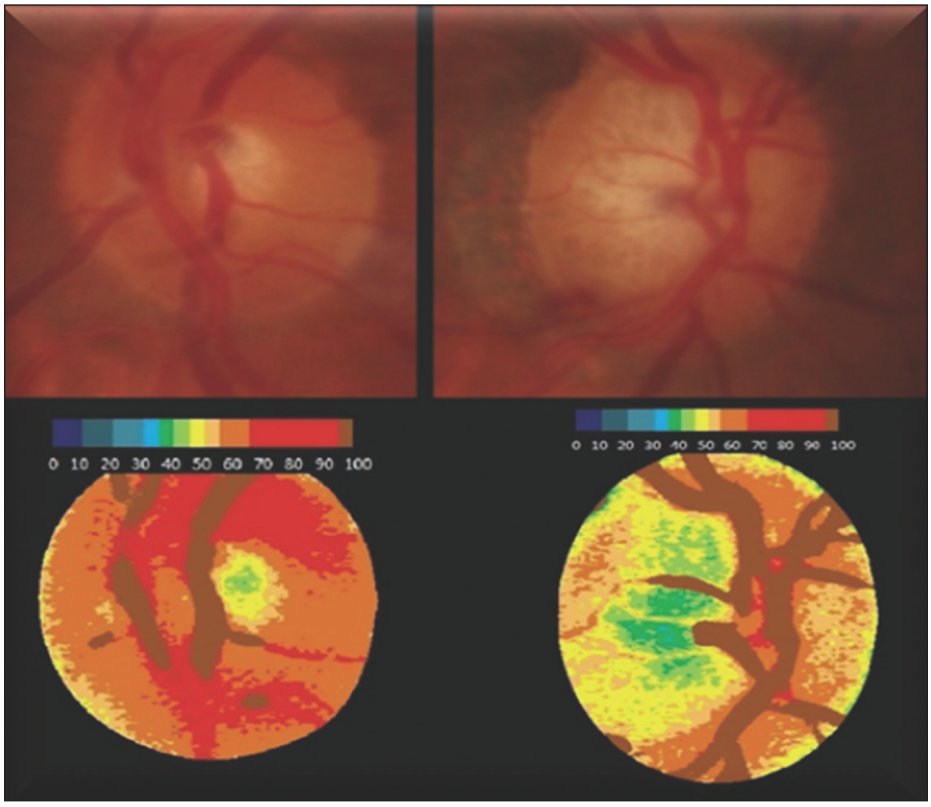

Figure 2. Examples of images of the papilla in a participant without glaucoma (left column) and a patient with childhood glaucoma (right column). Upper images show the color fundus photographs of the optic nerve heads and lower images show the corresponding pseudo-images representing the amount of hemoglobin. A colorimetric scale is shown at the top of the lower images to assess the amount of hemoglobin.

amined. No differences were observed between both groups regarding age (9 to 14 years [median: 11 years] vs 7 to 13 years [median: 9 years] for the childhood glaucoma and control groups, respectively; $P < .061$) and sex (12 males/16 females vs 15 males/13 females for the childhood glaucoma and control groups, respectively; $P = .186$). A P value less than .05 was considered statistically significant.

The cup–disc ratio and Glaucoma Discriminant Function were considered to be pathological if we compared them to the values obtained from the control group ($P < .0001$). The childhood glaucoma group showed a significant reduction in percentages of hemoglobin levels in sector 20 of the optic nerve head (60.80% vs 67.24% in the childhood glaucoma vs control groups, respectively; $P < .001$). In 9 of the 24 sectors analyzed (sectors 6, 8, 9, 16, 17, 19, 20, 22, and 23), the childhood glaucoma group had higher hemoglobin levels than the control group (**Table 1**).

The congruence of the superior disposition of the sectors showing significant differences between both groups is an argument against the interpretation that statistical significances have occurred by chance. Sectors 8 and 20 are usually the most significant in chronic simple glaucoma, but this situation was not repeated in the childhood glaucoma group.

The mean hemoglobin levels of the contiguous superior sectors (sectors 16, 17, 19, 20, 22, and 23) were 59.59% ± 14.87% and 65.01% ± 9.68% in the childhood glaucoma and control groups, respectively ($P < .001$), whereas the average of the remaining sectors were 56.24% ± 9.75% and 56.15% ± 18.03%, respectively ($P = .67$).

Figure 2 shows examples of a normal (left) and glaucomatous (right) papilla from a participant in the control group and a patient in the childhood glaucoma group, respectively. The upper images are the color fundus photographs of the optic nerve heads and the lower images are their corresponding pseudo-images that represent the amount of hemoglobin.

Reproducibility of the studied parameters was assessed by using the coefficient of variation (percentage). A coefficient of variation less than 10% or less than 5% is normally considered to represent high or very high reproducibility, respectively.[19] Moderate coefficients of variation and intraclass correlation coefficients were found in both the childhood glaucoma group (range: 19.2% to 29.8% [coefficients of variation] and 0.661 to 0.865 [intraclass correlation coefficients]) and the control group (range: 5.2% to 14.1% [coefficients of variation] and 0.735 to 0.900 [intraclass correlation coefficients]). Intraclass corre-

TABLE 2

COV and ICC for Repeated Hemoglobin Level Percentages for the Average, Vertical Disc Diameter (Sectors 8 and 20), and Cup–Disc Ratio

	Childhood Glaucoma Group		Control Group	
Parameter	**COV (%)**	**ICC**	**COV (%)**	**ICC**
Average hemoglobin levels (%)	29.8	0.829	5.6	0.883
Vertical disc diameter (%)	30.1	0.661	5.2	0.900
Cup–disc ratio	19.2	0.865	14.1	0.735

COV = coefficients of variation; ICC = intraclass correlation coefficients; childhood glaucoma group = patients with childhood glaucoma; control group = participants without childhood glaucoma

lation coefficient values were considered as follows: slight reliability (between 0 and 0.2), fair reliability (between 0.21 and 0.4), moderate reliability (between 0.41 and 0.6), substantial reliability (between 0.61 and 0.8), and almost perfect reliability (higher than 0.81).[20]

The parameters with the lowest variability were sectors 8 and 20 in the control group (coefficient of variation: 5.2%) and the cup–disc ratio in the childhood glaucoma group (coefficient of variation: 19.2%). We found that the measurement variability was higher in patients with fixation or ocular movement alterations, even when the quality of the photograph was good. Less variability was found in all parameters evaluated in the control group compared to the childhood glaucoma group (**Table 2**).

DISCUSSION

The significant amount of hemoglobin pigment present at the optic nerve head level is responsible for the characteristic color of the optic nerve head. When analyzing a fundus image, the amount of light reflected at different wavelengths (blue, green, and red) is measured. In areas with high hemoglobin levels, the predominant light reflected is red, followed by green and then blue wavelengths. Alternatively, areas with low hemoglobin levels reflect more green and blue wavelengths. Consequently, when an area develops atrophy or becomes poorly vascularized, the proportion of reflected green and blue light increases and the proportion of red light decreases. This may explain the lack of differences between the childhood glaucoma and control groups in hemoglobin levels of the central sectors of the papilla, which is an area that corresponds to the physiological cup.

A reference pattern is required to mitigate both lens absorption and diffusion and any change in the light used to achieve absolute and reproducible results for the optic nerve head. The central retinal vessels are considered to be the reference by the Laguna ONhE software. Measurements of hemoglobin levels at each point of the papilla are calculated using the same formula to describe the chromatic characteristics of the tissue (FT) and the reference vessels (FV). The formula that expresses the amount of hemoglobin at each point is: (FT/FV)Å~100.[21]

The Laguna ONhE software is easier to use than other structural diagnosis devices because good visual acuity or a prolonged stable fixation is not as compulsory as it is in other imaging devices, although the Laguna ONhE software requires little cooperation. The software is also cheaper because it only requires a retinal photograph obtained by a non-mydriatic fundus camera.

Another important advantage of the Laguna ONhE software is that it does not require any normative database. This limitation is one of the main reasons why other imaging devices such as optical coherence tomography are not being adopted for the diagnosis of childhood glaucoma. Moreover, optical coherence tomography requires cooperation, stable fixation, and fairly good visual acuity, with attention and movement remaining issues of perimetry. Consequently, optical coherence tomography imaging is not possible in the presence of nystagmus or significant media opacity.[22]

Obtaining a good retinography does not require good visual acuity or prolonged stable fixation. The good reliability of this new method has been demonstrated and it seems easy to implement in routine clinical practice.[18]

The method used in this study may be applied in all clinical centers because only a good photograph of optic nerve is necessary for image process-

ing with the Laguna ONhE software. The cooperation required is reached in almost every patient, even the youngest of patients, because only a fundus picture using retinography is required.

We found that the hemoglobin levels in more than half of the 24 sectors of the papilla obtained by Laguna ONhE analysis were higher in the control group than in the childhood glaucoma group.

González de la Rosa et al.[12] demonstrated the potential effectiveness of the Laguna ONhE software in adults with glaucoma by comparing the program with different structural and functional tests used for patients with glaucoma. Based on the results obtained by González de la Rosa et al.,[12] the aim of the current study was to investigate the clinical application of this software in evaluating glaucoma damage in patients with childhood glaucoma. The use of the Laguna ONhE software has also been applied in other pathologies such as sleep apnea[23,24] and multiple sclerosis.[25]

In patients with childhood glaucoma, the mechanical theory seems to be the main mechanism of glaucomatous damage. These characteristic features of childhood glaucoma differ from glaucomatous damage in adults and should be considered when analyzing Laguna ONhE results. In adults, the vascular dysregulation that causes a lower blood flow and ischemic damage in the optic nerve head may explain why the results obtained using the Laguna ONhE software in other studies of adult patients with glaucoma had more marked differences than the results obtained in the childhood glaucoma group of the current study.

The Laguna ONhE software is a promising tool for evaluating the eyes of children diagnosed as having childhood glaucoma. Future research should examine the test–retest variability of the Laguna ONhE parameters and the software's ability to evaluate progression in these children.

REFERENCES

1. Flammer J, Orgül S, Costa VP, et al. The impact of ocular blood flow in glaucoma. *Prog Retin Eye Res*. 2002;21:359-593.
2. Chang EE, Goldberg JL. Glaucoma 2.0: neuroprotection, neuroregeneration, neuroenhancement. *Ophthalmology*. 2012;119:979-986.
3. Flammer J, Mozaffarieh M. What is the present pathogenetic concept of glaucomatous optic neuropathy? *Surv Ophthalmol*. 2007;52:S162-S173.
4. Hoskins HD Jr, Shaffer RN, Hetherington J. Anatomical classification of the developmental glaucomas. *Arch Ophthalmol*. 1984;102:1331-1336.
5. Weinreb RN, Grajewski A, Papadopoulos M, Girgg J, Freedman S, eds. *World Glaucoma Association 9th Consensus Meeting: Childhood Glaucoma*. Amsterdam, Netherlands: Kugler; 2013.
6. Anderson DR. The development of the trabecular meshwork and its abnormality in primary infantile glaucoma. *Trans Am Ophthalmol Soc*. 1981;79:458-485.
7. Ho CL, Walton DS. Primary congenital glaucoma. *J Pediatr Ophthalmol Strabismus*. 2004;41:271-288.
8. Richardson KT Jr, Ferguson WJ Jr, Shaffer RN. Long-term functional results in infantile glaucoma. *Trans Am Acad Ophthalmol Otolaryngol*. 1967;71:833-837.
9. Papadopoulos M, Cable N, Rahi J, Khaw PT; BIG Eye Study Investigators. The British infantile and childhood glaucoma (BIG) eye study. *Invest Ophthalmol Vis Sci*. 2007;48:4100-4106.
10. Gilbert CE, Rahi JS, Quinn GE. Visual impairment and blindness in children. In: Johnson GJ, Minassian DC, Weale RA, West SK, eds. *The Epidemiology of Eye Disease*. 2nd ed. Abingdon, Oxford: Taylor & Francis; 2003:260-286.
11. Patil B, Tandon R, Sharma N, et al. Corneal changes in childhood glaucoma. *Ophthalmology*. 2015;122:87-92.
12. González de la Rosa M, González-Hernandez M, Sigut J, et al. Measuring hemoglobin levels in the optic nerve head: comparisons with other structural and functional parameters of glaucoma. *Invest Ophthalmol Vis Sci*. 2013;1:482-488.
13. Delori FC. Reflectometry measurements of the optic disc blood volume. In: Lambrou GN, Greve EL, eds. *Ocular Blood Flow in Glaucoma: Means, Methods and Measurements*. Amsterdam, Netherlands: Kugler: 1989;155-163.
14. Sebag J, Feke GT, Delori FC, Weiter JJ. Anterior optic nerve blood flow in experimental optic atrophy. *Invest Ophthalmol Vis Sci*. 1985;26:1415-1422.
15. Crittin M, Riva CE. Functional imaging of the human papilla and peripapillary region based on flicker-induced reflectance changes. *Neurosci Lett*. 2004;360:141-144.
16. Sugiyama T, Schwartz B, Takamoto T, et al. Evaluation of the circulation in the retina, peripapillary choroid and optic disk in normal-tension glaucoma. *Ophthalmic Res*. 2000;32:79-86.
17. Vilser W, Nagel E, Seifert BU, Riemer T, Weisensee J, Hammer M. Quantitative assessment of optic nerve head pallor. *Physiol Meas*. 2008;29:451-457.
18. Mendez-Hernandez C, Garcia-Feijoo J, Arribas-Pardo P, et al. Reproducibility of optic nerve head hemoglobin measures. *J Glaucoma*. 2016;25:348-354.
19. Hirasawa H, Araie M, Tomidokoro A, et al. Reproducibility of thickness measurements of macular inner retinal layers using SD-OCT with or without correction of ocular rotation. *Invest Ophthalmol Vis Sci*. 2013;54:2562-2570.
20. Sim DA, Keane PA, Mehta H, et al. Repeatability and reproducibility of choroidal vessel layer measurements in diabetic retinopathy using enhanced depth optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2013;54:2893-2901.
21. Dill DB, Costill DL. Calculation of percentage changes in volumes of blood, plasma, and red cells in dehydration. *J Appl Physiol*. 1974;37:247-248.
22. El-Dairi MA, Holgado S, Asrani SG, Enyedi LB, Freedman SF. Correlation between optical coherence tomography and glaucomatous optic nerve head damage in children. *Br J Ophthalmol*. 2009;93:1325-1330.
23. Mojon DS, Hess CW, Goldblum D, Böhnke M, Körner F, Mathis J. Primary open-angle glaucoma is associated with sleep apnea syndrome. *Ophthalmologica*. 2000;214:115-118.
24. Roberts TV, Hodge C, Graham SL, Burlutsky G, Mitchell P. Prevalence of nocturnal oxygen desaturation and self-reported sleep-disordered breathing in glaucoma. *J Glaucoma*. 2009;18:114-118.
25. Bambo MP, Garcia-Martin E, Perez-Olivan S, et al. Diagnostic ability of a new method for measuring haemoglobin levels in the optic nerve head in multiple sclerosis patients. *Br J Ophthalmol*. 2013;97:1543-1548.