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Quantification of optic nerve cross sectional area on MRI: A novel protocol using Fiji software
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1 **TITLE:**
2 **Quantification of optic nerve cross sectional area on MRI: A novel protocol using Fiji software**
3
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17
18 **KEYWORDS:**
19 MRI; Optic nerve; Glaucoma; ImageJ; Fiji; White matter
20
21 **SUMMARY:**
22 We provided a detailed protocol for a standardized method of optic nerve assessment and
23 quantification using MRI, utilizing a widely available imaging sequence, and open access software
24 for image analysis. Following this standardized protocol would provide meaningful data for
25 comparison between different patients and different studies.
26
27 **ABSTRACT:**
28 Optic nerve assessment is an important aspect of glaucoma diagnosis and follow-up. This project
29 describes a protocol for a unified methodology of optic nerve cross-sectional assessment and
30 quantification using 3 T MRI for image acquisition and ImageJ's Fiji software for image processing
31 quantification. Image acquisition was performed using 3 T MRI, with proper instructions for the
32 patient to ensure straight fixation during imaging. A T2-weighted fat suppressed sequence was
33 used. A coronal cut taken 3 mm behind the globe and perpendicular to the optic nerve axis should
34 be uploaded to the software. Using the threshold function, the white matter area of the optic
35 nerve is selected and quantified, thus, eliminating inter-individual measurement bias. We also
36 described the normal limits for the optic nerve cross-sectional area according to age, based on
37 previously published literature. We used the described protocol to assess optic nerve of a
38 suspected glaucoma patient. The optic nerve cross-sectional area was found to be within the
39 normal limits, a finding further confirmed via optical coherence tomography of the optic nerve.
40
41 **INTRODUCTION:**
42 Glaucoma is an optic neuropathy that is considered to be the most common cause of irreversible
43 blindness [1]. Despite that, it is still poorly understood in terms of its pathophysiology and
44 diagnosis, with no single standard reference for establishing the diagnosis [2]. According to the

National Institute for Health and Care Excellence (NICE) the diagnosis of primary open-angle glaucoma (POAG) requires the assessment of multiple domains, including optic disc assessment on fundus exam or optical coherence tomography (OCT) imaging, visual field assessment, and intraocular pressure measurement [3]. The idea behind diagnosing glaucoma is establishing the presence of progressing optic neuropathy, which can be done quantitively on OCT [4]. In this regard, MRI can also be used for optic nerve assessment and quantification of its white matter area [5], but for this to be clinically meaningful, the protocol used in optic nerve white matter quantification needs to be standardized. Moreover, a protocol should also accommodate inter-individual variation, a factor that might affect accuracy in different diseases [6].

Optic nerve assessment in glaucoma is optimally assessed via ophthalmic imaging, including OCT, where the most anterior part of optic nerve (e.g., optic disc) is assessed. On the other hand, the use of MRI for optic nerve assessment usually assesses the retrobulbar part of optic nerve at various distances from globe. Several studies found a strong correlation between optic disc assessment using OCT and MRI [7, 8]. However, there is still no unified protocol for optic nerve assessment and quantification on MRI. Outlining the optic nerve border on MRI has been used to quantify its cross-sectional area [5]. However, this method has considerable inter-rater variability, as it needs to be done by an experienced rater and requires considerable time for outlining. The aim of the current project was to provide a protocol for a unified methodology for optic nerve cross sectional assessment and quantification using 3 T MRI for image acquisition and ImageJ's Fiji software for image processing and quantification.

PROTOCOL:

The following study was approved by the research committee and institutional review board of the University of Jordan Hospital. The following protocol will describe the imaging technique used to acquire MRI images, followed by image processing and optic nerve quantification using Fiji software.

1.   MRI image acquisition

NOTE: MR image acquisition was done using a 3 Tesla (3 T) MRI to perform multiplanar T2-weighted fat suppression sequence (**Table of Materials**).

1.1.   Fully explain the examination to the patient. The following include instructions and explanations that need to be mentioned to the patient.

1.1.1.   Explain to the patient that they will need to change clothes and wear a special gown for imaging.

1.1.2.   Have patients remove any worn eyeliner as it can produce artifacts (especially at 3 T) due to electrical conductivity of the titanium oxide pigment.

1.1.3.   Ensure that the patient does not have any contraindications to perform MRI imaging[9]:

89

90   1.1.3.1.   Ask the patient about any metallic materials, which might include face masks,
91   piercings, artificial limbs, magnetic dental implants, cerebral artery aneurysm clips.

92

93   1.1.3.2.   Ask the patient about metallic intraocular foreign bodies. For this, ask the patient
94   if they have welded without proper protective gear.

95

96   1.1.3.3.   Ask the patient about any implantable devices might be incompatible with MRI,
97   including pacemakers and insulin pumps, analgesic drugs, or chemotherapy pumps. In addition
98   to this, cochlear implants/ear implant, Implantable neurostimulation systems, Implantable
99   neurostimulation systems, Catheters with metallic components, are all contraindicated.

100

101   1.1.3.4.   Ask the patient about metallic foreign body left inside their body. This includes
102   bullets, shotgun pellets, and metal shrapnel

103

104   1.1.3.5.   Ask the patient about any surgical clips or wire sutures, joint replacement or
105   prosthesis, inferior vena cava (IVC) filter, ocular prosthesis, stents, or intrauterine device.

106

107   1.1.3.6.   Ask the patient if they have gotten a tattoo in the last 6 weeks.

108

109   1.1.3.7.   Ask the patient if they have undergone a colonoscopy procedure in the last eight
110   weeks.

111

112   1.1.3.8.   Due to the confined space of the MRI machine, ask the patient if they have
113   claustrophobia.

114

115   NOTE: Difficulty might be found with patients having high body mass index (BMI).

116

117   1.1.4. Explain to the patient that the exam is expected to take 15 minutes, where the patient
118   needs to stay still.

119

120   1.2.   After completing the instructions and making sure the patient fully comprehends the
121   exam, obtain a signed consent.

122

123   1.3.   During MRI image acquisition, lay the patient supine in the MRI machine, and fixate on a
124   straight target during imaging without any head movement. For patients with poor visual acuity,
125   use a sound stimulus to optimize fixation. More comprehensive methods for fixation involve
126   shutting one eye, use of a fixation target centrally in the form of an LCD screen that changes
127   colors, and use of ocular lubricants.

128

129   1.4.   Ensure that the patient is aware that there is a squeeze button that can be pressed if they
130   need anything while in the MRI machine. While a head coil can be used, an eye coil and an orbital
131   coil may be more suited for ophthalmic imaging.

132

133 1.5.   Input the following parameters for image acquisition: A T2-weighted fat suppression
134 sequence (TR = 3000 milliseconds; TE = 90 milliseconds; TE = 100; field of view = 16 cm×16 cm;
135 matrix = 296*384; slice thickness = 3 mm; slice gap = 0.3 mm). The final image analyzed was an
136 oblique coronal image 3 mm behind the globe. It is important to note that while T2-weighted fat
137 suppression sequence is generally used for optic nerve imaging, other sequences can be used,
138 including T2 fast spin echo imaging.
139
140 1.6.   Take a coronal cut of the optic nerve orthogonal (i.e., perpendicular) to the nerve 3 mm
141 posterior to the globe. Use scout images in the transversal and oblique sagittal planes to ensure
142 optimal optic nerve direction and optic nerve-globe junction positioning.
143
144 1.7.   Assess the quality of gaze fixation by CSF distribution around the optic nerve, where it
145 should be uniformly distributed around the optic nerve with almost equal thickness at all sides.
146
147 1.8.   Repeat the process to image the optic nerve for the other side.
148
149 **2.   Image analysis**
150
151 2.1.   Download Fiji image processing package from (https://imagej.net/Fiji).
152
153 2.2.   Upload the coronal image of optic nerve to ImageJ Fiji software for analysis by clicking
154 **File** from the menu bar, followed by **Open** button. Choose the coronal image to be processed.
155 Transfer the images to Fiji software without losing image quality during transfer, as image quality
156 loss will lead to unreliable image analysis results.
157
158 2.3.   Standardize the scale by specifying the number of pixels per a unit of length by drawing a
159 straight line on the map scale. Then choose **Set Scale** from the **Analyze** menu bar. Specify the
160 length of the line as appears on the map scale with the proper unite of length (i.e., mostly mm).
161
162 2.4.   Convert the image into a grayscale using the image menu, and then choosing **Type** and **8-**
163 **bit**.
164
165 2.5.   Quantify the range of intensity of white matter pixels.
166
167 2.5.1.   Using the Lasso selection tool (**Plugin | Segmentation | Lasso tool**), select a sufficient
168 area of white matter, making sure not to include gray matter area during selection. We found
169 that a total selected white matter area of around 1000 pixels is enough. Use **Analyze** and
170 **Measure** tool to quantify the selected area.
171
172 2.6.   Show the **Histogram** tool from **Analyze** menu, which shows the distribution of pixels
173 intensity in the white matter area selected. Click on the **Live** box to make sure the histogram
174 assesses the selected area. The graph on the histogram should show a normal distribution of
175 intensity.
176

177   2.7.   Calculate white matter intensity range as follows:

178   Lower limit = mean intensity - (3* standard deviation)

179   Upper limit = mean intensity + (3* standard deviation)

180

181   2.8.   Open the **Threshold** tool from **Image** menu, followed by the **Adjust** function. Specify the

182   range calculated from the previous step. Tick only **dark background** function and specify black

183   and white annotation **B&W** from drop list, then click apply. The mask for white matter present

184   within the optic disc will appear.

185

186   2.9.   Using the Lasso selection tool (**Plugin | Segmentation | Lasso tool**), select the black area

187   representing the optic disc.

188

189   2.10.   Use the **Measure** function from the **Analyze** menu bar, which will calculate the area

190   marked by the threshold function in mm.

191

192   **REPRESENTATIVE RESULTS:**

193   The cup to disc ratio for a 30-year-old male patient presenting for a checkup ophthalmology exam

194   was 0.8 (**Figure 1A**), which is suspicious and might be suggestive of glaucoma. Upon performing

195   an optical coherence tomography for nerve fiber layer thickness, we found that the nerve

196   thickness was within the normal limits for age (**Figure 1B**). The patient was scheduled for an orbit

197   MRI, where a coronal cut for optic nerve assessment was ordered and performed as per the

198   abovementioned protocol.

199

200   We obtained a coronal MRI cut, 3 mm behind the optic disc. The white matter mean intensity

201   was 94.372 (SD 7.085), which resulted in white matter intensity range of:

202   Lower limit = 94.372 – 21.255 = 73.117

203   Upper limit = 94.372 + 21.255 = 115.627

204

205   **Figure 2** shows the coronal image (**Figure 2A**), the coronal image after applying the white matter

206   threshold using the calculated upper and lower limits (**Figure 2B**), and the optic nerve white

207   matter for quantification (**Figure 2C**). The cross-sectional area for the white matter of the left

208   optic nerve was 6.9 mm^2 (0.069 cm^2), which is within the normal limits for his age, as shown in

209   **Table 1**.

210

211   **FIGURE AND TABLE LEGENDS:**

212   **Figure 1:** Fundus image showing high cup to disc ratio, which might be suggestive of glaucoma

213   (**A**). An optical coherence tomography for the nerve fiber layer (NFL) showing a within normal

214   limits NFL (**B**).

215

216   **Figure 2:** Coronal T2 -weighted fat suppressed MRI image obtained perpendicular to the optic

217   nerve 3 mm behind the optic disc (**A**). The same coronal cut after applying a pre-calculated

218   threshold range (**B**). Optic nerve white matter (**C**).

219

220   **Table 1 shows the normal range of optic nerve cross sectional diameter using MRI 3mm from**

221   **globe, as found by previous studies.**
222
223   **DISCUSSION:**
224   We described a protocol to assess and quantify optic nerve white matter that might be used for
225   glaucoma patient assessment. The protocol uses widely available imaging sequences for image
226   acquisition, and it uses the open-source Fiji software for image analysis. We standardized the
227   image parameters that were previously found to be most accurate and highly reproducible in
228   optic nerve image acquisition, including asking the patient to fixate straight ahead, using T2 with
229   fat suppression sequence, and capturing the cross-sectional area 3 mm behind the globe. In
230   addition, we described a detailed image analysis method that eliminates manual segmentation
231   and corrects for signal variability between patients. The importance of this protocol is that it
232   eliminates the variation in region of interest (ROI) segmentation by the radiologist, which is
233   usually the main source of error in optic nerve assessment on MRI [12]. While we tried to provide
234   normative data for optic nerve cross sectional area with **Table 1**, further data using the standard
235   protocol described is needed for the use and comparison in clinical settings. Such data need to
236   accommodate different age groups due to the age variation in optic nerve size, as shown in **Table**
237   **1**. Such variation is not evident between genders [15], but was recently suggested to be present for
238   refractive error [16].
239
240   Previous studies applied different methodologies for optic nerve white matter quantification,
241   and they mostly used the software present in their workstation for image analysis. Initial studies
242   on optic nerve assessment adopted a quantification approach based on cross sectional area,
243   using manual segmentation by technicians or radiologists [12, 17]. Wang et al. also used manual
244   segmentation of optic nerve cross-sectional area at different distances from the globe for
245   correlation with OCT [11]. Omodaka et al. used the average cross-sectional area on the coronal cut
246   and the length of the optic nerve from the disc to the optic chiasm on the axial cut through
247   manual annotation to extract indicators of the optic nerve for correlation with OCT [8]. Despite
248   being correlated with OCT, reproducibility of this method might not yield the required accuracy
249   for longitudinal assessment of optic nerve. Ramli et al. quantified the volume of the optic nerve
250   through manual segmentation of the isointense signal at all axial sections [5], an approach that
251   might miss the optic nerve matter not captured by the axial sections themselves, human error
252   during image manual segmentation, or even in the determination of the optic nerve length to be
253   included in the quantification assessment.
254
255   While different studies used the assessment of the cross-sectional area of the optic nerve, they
256   differed in the distance of measurements from globe. Wang et al. assessed 3 mm, 9 mm, and 15
257   mm behind the globe, and found that the 3 mm cross-sectional assessment had the highest
258   correlation with intraocular pressure [11]. Bäuerle et al. analyzed the reproducibility of assessment
259   of the optic nerve on MRI at 3 mm and 5 mm behind the globe, and they found a good assessment
260   for both cases [10]. Lagrèze et al. measured the cross-sectional area 5 mm, 10 mm, and 15 mm
261   behind the globe and found that the cross-sectional assessment was most accurate in the 5 mm
262   cross sectional area compared to measurements further from the globe [17]. In this protocol, we
263   used a 3 T MRI for images acquisition, where its use in optic nerve assessment was previously
264   found to be superior to 1.5 T MRI [18, 19]. The increasingly utilized 7 T MRI might also provide

265 superior results but will also require its normative values. Regarding MRI sequence used, we used
266 T2 fat suppression sequence, mainly due to its wide availability and its inherent ability to
267 delineate the optic nerve surrounding CSF after eliminating the surrounding intraconal fat.
268 Previous studies used other sequences with reliable results, including the half-Fourier single-shot
269 turbo spin-echo (HASTE) sequence and Diffusion Tensor Imaging (DTI) sequence [7, 12], which might
270 not be widely available.
271
272 An important aspect to consider during image acquisition is making sure that the patient is
273 fixating on a straight target, as fixating on a non-straight target during imaging will yield a non-
274 accurate optic nerve quantification [12]. Fixation in OCT is monocular on a close target, requiring
275 the patient to have good visual acuity in the eye to be assessed to see the near target with one
276 eye, whereas for MRI the target is further away, the fixation is binocular, and requires fewer
277 visual demands. However, fixation might still be an issue for patients who have high refractive
278 error or poor vision. While the use of MRI to assess and follow patients with glaucoma might not
279 be feasible in the presence of low cost, simpler imaging techniques, including the OCT, MRI can
280 be useful in special situations where OCT do not provide conclusive data, or OCT itself cannot be
281 obtained, such as in the presence of significant ocular medial opacity. Furthermore, the described
282 protocol can be used when MRI imaging is warranted in cases of unexplained optic neuropathy
283 to exclude secondary causes [20, 21].
284
285 One of the main limitations of this protocol is the inability to assess patients who cannot properly
286 fixate, including patients with poor visual acuity in both eyes. In this regard, the use of sound
287 stimulus will improve the quality of fixation during image acquisition [22]. Moreover, as a new
288 methodology, future studies are needed to depict normal values for MRI-based cross-sectional
289 areas for optic nerve white matter. The importance of establishing normal values is further
290 stressed by the fact that optic nerve is also composed of significant amount of connective tissue
291 [23], a tissue that does not have similar functional abilities as nerve fibers. While quantification of
292 optic nerve fiber layer thickness in OCT might provide a false impression of remanent nerve tissue
293 due to the inclusion of connective tissue in the quantification process [24], such a false impression
294 is not present in this MRI-based quantification method. Motion artifacts might also lead to blur
295 in images, especially upon eye movement during exam. While it should be avoided during
296 imaging, establishing white matter range will reduce the impact of such artifacts on the accuracy
297 of optic nerve white matter quantification, as the changes caused by the motion artifact on
298 cerebral white matter is almost like the optic nerve's white matter.
299
300 The main strength of the current protocol is the elimination of inter-individual differences during
301 optic nerve quantification, even when performed by non-specialized medical doctors or
302 technicians. In addition, it used a widely available open-source software for image analysis. While
303 it is not feasible to do a dedicated MRI imaging for optic nerve quantification, especially in the
304 presence of the OCT, it is recommended to perform this protocol during MRI imaging done for
305 other purposes, including the exclusion of secondary causes of optic neuropathy and glaucoma.
306
307 **ACKNOWLEDGMENTS:**

313

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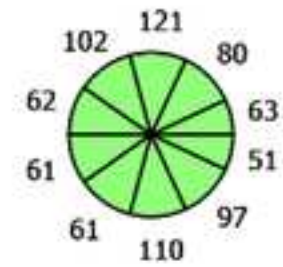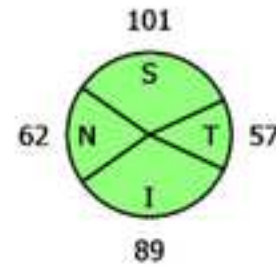381   10.1136/bjophthalmol-2014-305745 (2015).
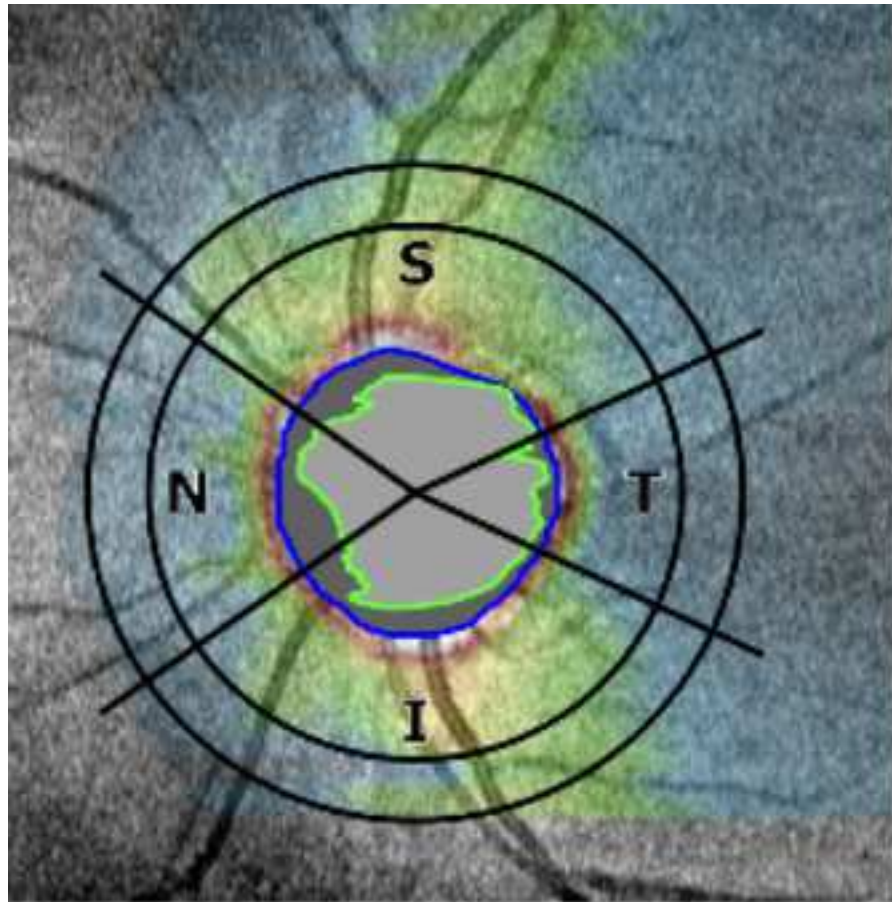
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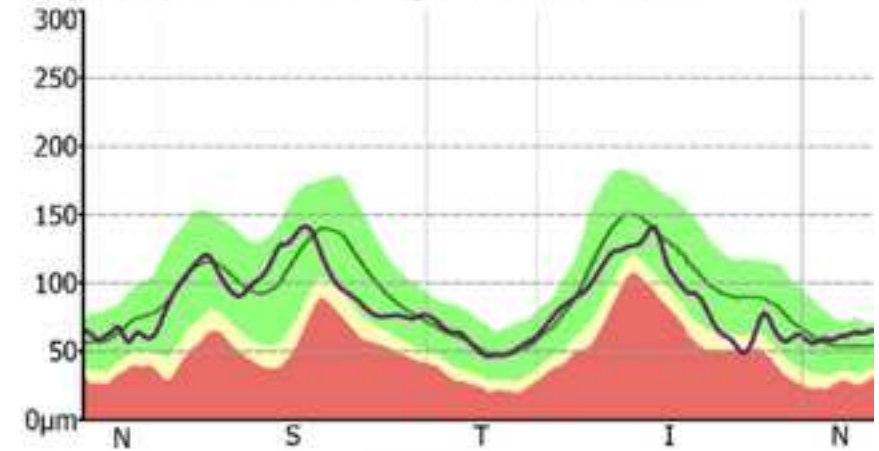
B

Figure 2

Click here to access/download;Figure;Figure 2.jpeg ±

A   **B**   **C**

Table 1
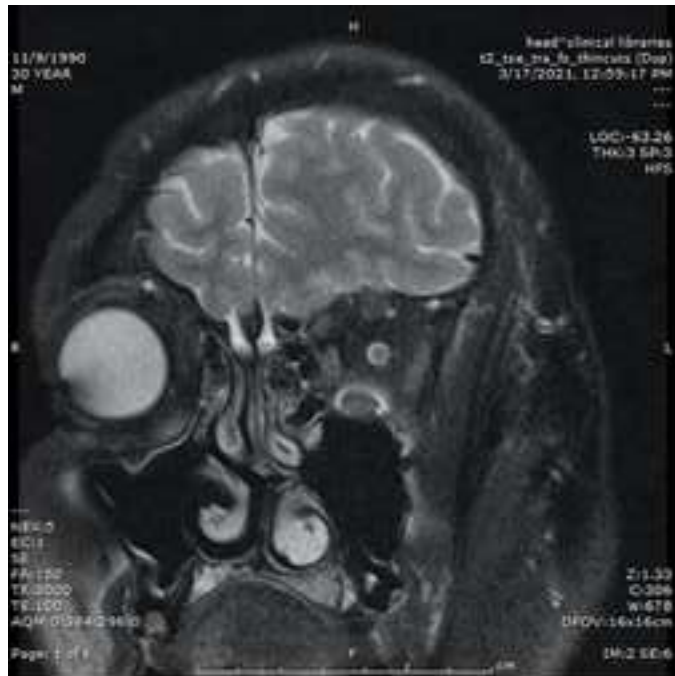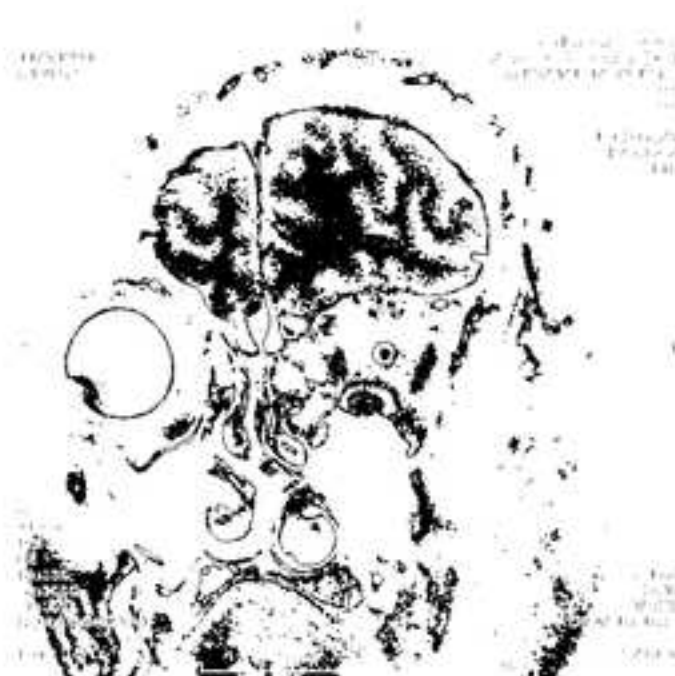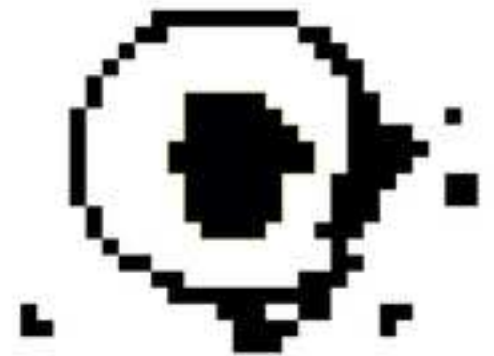
Study	Sample size	Age (Years)	Mean cross sectional area (mm^2)	Imaging sequence
Bäuerle, 2013. [10]	15	Mean (SD) 24.5 ± 0.8	5.69 ± 0.77	T2-weighted turbo spin echo (TSE) sequence
Wang 2012. [11]	21	Mean (SD) 51.6±12.0	5.03 ± 0.35	T2-weighted fast recovery fast spin echo (FRFSE) sequence
Weigel, 2006. [12]	32	Mean (range) 25 (22–39)	5.7 ± 0.6	T2-weighted turbo spin echo (TSE) sequence
Yiannakas, 2013. [13]	8	Mean (range) 31 (29–33)	6.2 (1.3)	T2-fat-suppressed
Al-Haddad, 2018. [14]	211	Median (interquartile) 8.6 (3.9–13.3)	4.0 ± 0.20 *	T1-weighted inversion recovery sequence

*Calculated using the provided optic nerve diameter.

Table of Materials

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Table of Materials
JoVE_Table_of_Materials.xlsx

Response to reviews

Editorial and production comments:
Changes to be made by the Author(s) regarding the written manuscript:
None.
Response: Thank you.

Changes to be made by the Author(s) regarding the video:
1. Please include a human ethics title card after the introduction with the name of the insitutions review board that have verified the protocol as ethical. The card should say the following: The following study was approved by the research committee and institutional review board of the University of Jordan Hospital.
2. 8:26 - Conclusion is spelled wrong.
Response: We made the changes on the uploaded video.