Retinal nerve fibre layer and ganglion cell layer changes in children who recovered from COVID-19: a cohort study

Barbara Burgos-Blasco,¹ Noemi Güemes-Villahoz,¹ Laura Morales-Fernandez,¹ Ignacio Callejas-Caballero,¹ Pilar Perez-Garcia,¹ Juan Donate-Lopez,¹ Jose Tomas Ramos-Amador,¹ Julian Garcia-Feijoo¹,²

ABSTRACT

Objective To investigate the optic nerve and macular parameters of children who recovered from COVID-19 compared with healthy children using optical coherence tomography (OCT).

Design Cohort study.

Setting Hospital Clinico San Carlos, Madrid.

Patients Children between 6 and 18 years old who recovered from COVID-19 with laboratory-confirmed SARS-CoV-2 infection and historical controls were included.

Interventions All patients underwent an ophthalmological examination, including macular and optic nerve OCT. Demographic data, medical history and COVID-19 symptoms were noted.

Main outcome measures Peripapillary retinal nerve fibre layer thickness, macular retinal nerve fibre layer thickness, macular ganglion cell layer thickness and retinal thickness.

Results 90 patients were included: 29 children who recovered from COVID-19 and 61 controls. Patients with COVID-19 presented an increase in global peripapillary retinal nerve fibre layer thickness (mean difference 7.7; 95% CI 3.4 to 12.1), temporal superior (mean difference 11.0; 95% CI 3.3 to 18.6), temporal inferior (mean difference 15.6; 95% CI 6.5 to 24.7) and nasal (mean difference 9.8; 95% CI 2.9 to 16.7) sectors. Macular retinal nerve fibre layer analysis showed decreased thickness in the nasal outer (p=0.011) and temporal inner (p=0.036) sectors in patients with COVID-19, while macular ganglion cell layer thickness increased in these sectors (p=0.001 and p=0.015, respectively). No differences in retinal thickness were noted.

Conclusions Children with recent history of COVID-19 present significant changes in peripapillary and macular OCT analyses.

INTRODUCTION

COVID-19, caused by SARS-CoV-2, was first detected in Wuhan, China, in December 2019 and has since become a worldwide pandemic. COVID-19 may range from an asymptomatic infection to a severe form with respiratory failure.¹

Although the most common symptoms in adults are respiratory, there are increasing reports of neurological manifestations. Headaches and dizziness are the most predominant central nervous system symptoms, ranging from 10% to 20% of patients.² Other more serious neurological manifestations include seizures, cerebrovascular events, encephalitis and neuromuscular disorders. These may implicate direct central nervous system viral invasion or parainfectious complications of the disease.³ ⁴

There are fewer studies in children, and those available suggest that they are less likely to become severely ill than older adults, with children accounting for only 1%–5% of COVID-19 cases. More than 80% of paediatric cases are asymptomatic or mild cases with better prognosis than adults.² ⁵⁻⁹ Neurological manifestations in paediatric population are less frequent and have been less thoroughly investigated than in adults, accounting for only 1%–5% of COVID-19 cases.¹ ¹⁰⁻¹³

Optical coherence tomography (OCT) is a rapid, non-invasive technique which quantifies retinal layer thickness with excellent reproducibility. This technique has been successfully used to monitor changes in a number of ophthalmological and neurological diseases and could give insight into neurological involvement in COVID-19. In

What is already known on this topic?

▸ Neurological manifestations in paediatric COVID-19 are less frequent and have been less thoroughly investigated than in adults.

▸ Recent reports reveal optic nerve changes in patients who recovered from COVID-19 and suggest that the retinal nerve fibre layer may be affected in these patients.

What this study adds?

▸ Children with recent history of COVID-19 present increased peripapillary retinal nerve fibre layer thickness.

▸ Macular optical coherence analysis reveals decreased retinal nerve fibre layer thickness as well as increased ganglion cell layer thickness in some sectors.
SARS-CoV-2 infection, recent reports suggest that retinal nerve fibre layer (RNFL) may be affected, but studies are scarce and only adult COVID-19 patients have been evaluated.14

Significant differences in COVID-19 characteristics in adults and children due to their different immune response may also cause different RNFL involvement. Hence, the present study was designed to analyse the optic nerve and macular parameters of children who recovered from COVID-19 compared with healthy children, this being the first study to quantify retinal changes in paediatric patients who recovered from COVID-19.

METHODS

Patient selection

We designed a cohort study at Hospital Clinico San Carlos (Madrid, Spain). For the case group, patients between 6 and 18 years old with laboratory-confirmed SARS-CoV-2 infection from 15 August to 30 November 2020 were considered. A historical control group was also included.

Patients included were retrieved from the paediatrics department and consisted of children who were treated in the hospital’s paediatric emergency department between the mentioned dates and tested positive for SARS-CoV-2 by reverse transcriptase PCR from nasopharyngeal swab. All children who tested positive for SARS-CoV-2 in the hospital’s paediatric emergency department were given a follow-up appointment and it was on the day of the appointment with the paediatric department that the ophthalmological examination was performed by two ophthalmologists (NG-V and BB-B). Patients’ sociodemographic data (age, sex and race), medical history and clinical parameters of infection were retrieved from the hospital’s records.

The control group consisted of healthy subjects between 6 and 18 years of age which had been recruited for a normative database in 2018. A historical control group was decided in this study due to the difficulty of being certain of no history of SARS-CoV-2 infection in children, a population with a particularly high prevalence of asymptomatic cases. The controls included had undergone the same ophthalmological examination with the same device and software used in this study.

For both groups the same exclusion criteria were applied. Subjects with psychiatric, neurological or ophthalmological diseases were excluded. Individuals with previous diagnosis or diagnosis made during the examination of optic nerve head disease (including glaucoma, optic neuritis and congenital optic nerve head abnormalities), macular disease, retinal vascular disorders, high myopia (refractive error greater than 6 dioptres) or uveitis were excluded, as well as those with previous ophthalmic procedures.

Patients’ parents provided written informed consent. In children aged 12 or older, written informed assent was also obtained.

Ophthalmological examination

At the time of the study, all patients were asymptomatic, had been discharged from the hospital if they had been admitted and were not on quarantine.

Without pharmacological mydriasis, a fundus colour photograph, a macula-centred high-definition OCT (dense macular cube protocol) and an optic nerve-centred high-definition OCT were performed in all study participants. The equipment used in all patients was the Eidon true colour confocal scanner camera (Centervue, Padova, Italy) and the Spectralis OCT (Heidelberg Engineering, Heidelberg, Germany).

Macular OCT analysed the central 6×6 mm2 area. Layers’ thickness included in the present study were retinal thickness (between the inner limiting membrane (ILM) and the Bruch’s membrane), RNFL (between the ILM and the ganglion cell layer (GCL)) and GCL (between the RNFL and the inner plexiform layer), which were automatically provided by the device’s software. Furthermore, optic nerve analysis with OCT provided peripapillary RNFL thickness (globally and in six quadrants: superior temporal, temporal, inferior temporal, superior nasal, nasal, inferior nasal) as a result of automated segmentation.

The images of the fundus and tomographic scans were independently evaluated for presence of haemorrhages or exudative lesions by two different ophthalmologists (NG-V and BB-B).

All examinations were carried out by experienced physicians and only data from the right eye were included for analysis. Subjects with media opacity or whose images were considered of poor quality (signal strength level below 7/10) were excluded from both groups.

Statistical analysis

Statistical analyses were performed with the SPSS V25.0 software. Continuous variables are presented as mean and standard deviation, while numbers and percentages are used for categorical variables. Differences in age and sex between groups were compared using the χ² test and Student’s t-test. Quantitative variables were studied with the Student’s t-test. Statistical significance was set at 0.05.

RESULTS

The total study population comprised 90 subjects: 29 patients who recovered from COVID-19 and 61 healthy controls. Demographic and clinical characteristics are summarised in Table 1. There were no statistically significant differences between groups in terms of age, sex and refractive error.
Ophthalmological examination of children who recovered from COVID-19 was unremarkable, with none of them presenting visible optic disc oedema/swelling, retinal haemorrhages or other related clinical features. None of the children referred visual alterations, visual field loss or other visual symptoms during the acute phase of the infection and thereafter up to the date of evaluation. The mean days from PCR-confirmed diagnosis to ophthalmological examination were 38.8 ± 13.3 days.

Comparison of optic nerve and macular OCT parameters between groups is portrayed in tables 2 and 3. Patients with COVID-19 presented a statistically significant increase in RNFL global thickness and in the superior temporal, inferior temporal and nasal sectors (figure 1). OCT data on the macular region also revealed differences in GCL and RNFL thickness (figure 2). No differences in macular thickness were noted between the groups.

OCT data from patients with COVID-19 with headache, anosmia or ageusia were compared with that from healthy controls. No differences were detected in any of the sectors of the layers evaluated.

**DISCUSSION**

As the COVID-19 pandemic progressed, clinicians encountered numerous patients with severe neurological complications from acute COVID-19 infection. Neuroimaging manifestations have even been reported in asymptomatic children with SARS-CoV-2 infection. Our analysis of the optic nerve using OCT reveals an increase in peripapillary RNFL thickness, along with a decrease and an increase in macular RNFL and GCL, respectively, in children who recovered from COVID-19.

The first reported ophthalmological examinations in patients with COVID-19 qualitatively evaluated retinal abnormalities such as haemorrhages and exudates. Marinho et al.15 were the first to report hyper-reflective lesions at the GCL and inner plexiform layer using OCT in 12 adults 11–33 days after COVID-19 symptom onset. Four patients presented subtle cotton wool spots and micro-haemorrhages along the retinal arcade on fundus examination. However, these findings have been strongly questioned by other authors.17 18 Lani-Louzada's19 group evaluated severe patients during their hospital stay, finding retinal changes in 12% (3 of 25) of patients, but they believe they are most likely secondary to clinical intercurrences or comorbidities and not a result of direct viral damage. Retinal findings in patients with COVID-19 include haemorrhages, cotton wool spots, dilated veins and tortuous vessels.16 20 Our research team has conducted several studies in COVID-19, finding no fundus abnormalities in 80 laboratory-confirmed recovered patients (160 eyes).21 In the current study no alterations such

**Table 2** optic nerve optical coherence tomography analysis showing retinal nerve fiber layer (RNFL) thickness measured in micrometres

| RNFL thickness | Control group (n=61) | COVID-19+ (n=28) | P value | Mean difference | 95% CI |
|----------------|----------------------|------------------|---------|----------------|-------|
| Mean SD        | Mean SD              |                  |         |                |       |
| RNFL G         | 101.6 9.3            | 109.3 9.8        | 0.001**| 7.7            | 3.4   |
| RNFL I         | 73.9 12.0            | 77.8 14.8        | 0.228  | 3.9            | −2.5  |
| RNFL TS        | 142.3 18.7           | 153.3 15.6       | 0.005**| 11.0           | 3.3   |
| RNFL TI        | 141.7 21.8           | 157.3 18.9       | 0.001**| 15.6           | 6.5   |
| RNFL N         | 73.7 12.9            | 83.5 15.9        | 0.006**| 9.8            | 2.9   |
| RNFL NS        | 112.8 24.1           | 114.3 22.3       | 0.788  | 1.4            | −9.1  |
| RNFL NI        | 121.0 28.7           | 126.6 24.2       | 0.339  | 5.6            | −6.1  |

**GCL** T2 38.0 5.1 39.8 3.9 0.067 1.8 −0.1 3.8
**GCL** T1 47.2 5.6 50.0 4.7 0.015* 2.8 0.6 5.1
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**Layers’ thickness is measured in micrometres.**

**Table 3** Macular optical coherence tomography (OCT) analysis of children with COVID-19 compared with healthy controls

| Macular OCT | Control group (n=61) | COVID-19+ (n=28) | P value | Mean difference | 95% CI |
|-------------|----------------------|------------------|---------|----------------|-------|
| Mean SD     | Mean SD              |                  |         |                |       |
| Macular thickness | 273.8 42.6          | 272.6 28.1       | 0.809  | −1.2           | −11.2 |
| RNFL S1     | 23.7 3.7             | 23.8 3.3         | 0.877  | 0.1            | −1.7  |
| RNFL S2     | 36.8 6.7             | 38.5 4.4         | 0.167  | 1.7            | −0.7  |
| RNFL N1     | 20.7 3.7             | 19.6 2.9         | 0.123  | −1.1           | −2.5  |
| RNFL N2     | 49.6 9.8             | 45.2 5.9         | 0.011* | −4.4           | −7.7  |
| RNFL N1     | 25.6 13.4            | 22.0 3.7         | 0.055  | −3.6           | −7.3  |
| RNFL N2     | 41.5 19.0            | 37.0 6.8         | 0.112  | −4.5           | −10.0 |
| RNFL T1     | 16.7 1.7             | 16.1 1.1         | 0.036* | −0.6           | −1.3  |
| RNFL T2     | 19.1 4.6             | 17.9 1.3         | 0.064  | −1.2           | −2.5  |
| GCL S1      | 52.7 6.0             | 54.1 5.3         | 0.291  | 1.3            | −1.2  |
| GCL S2      | 37.1 4.7             | 37.1 3.5         | 0.999  | −0.1           | −1.8  |
| GCL N1      | 52.7 5.2             | 54.0 4.3         | 0.213  | 1.3            | −0.8  |
| GCL N2      | 39.3 3.9             | 42.1 3.6         | 0.001**| 2.8            | 1.1   |
| GCL T1      | 51.6 6.2             | 52.2 7.6         | 0.696  | 0.6            | −2.6  |
| GCL T2      | 36.5 4.5             | 37.4 6.2         | 0.470  | 0.9            | −1.7  |
| GCL T1      | 47.2 5.6             | 50.0 4.7         | 0.015* | 2.8            | 0.6   |
| GCL T2      | 38.0 5.1             | 39.8 3.9         | 0.067  | 1.8            | −0.1  |

**Layers’ thickness is measured in micrometres.**

**Table 3** Macular coherence tomography (OCT) analysis of children with COVID-19 compared with healthy controls

| Layers | Control group (n=61) | COVID-19+ (n=28) | P value | Mean difference | 95% CI |
|--------|----------------------|------------------|---------|----------------|-------|
| Mean SD| Mean SD              |                  |         |                |       |
| Mean  | SD                   |                   |         |                |       |
| GCL T2 | 38.0 5.1             | 39.8 3.9         | 0.067  | 1.8            | −0.1  |
| GCL T1 | 47.2 5.6             | 50.0 4.7         | 0.015* | 2.8            | 0.6   |
| GCL T2 | 38.0 5.1             | 39.8 3.9         | 0.067  | 1.8            | −0.1  |
as the previously described by other groups were present, which could be due to a milder form of the disease in children.

Furthermore, the effect of SARS-CoV-2 on retinal vascularisation has been investigated in adult patients with COVID-19 using optical coherence tomography angiography (OCTA). Zapata et al. evaluated the retinal vascularisation of 96 patients with COVID-19 using OCTA. No alterations nor ischaemic zones or vascular dilations were observed in the vascular architecture of the superficial or deep plexus at macroscopic scale. However, patients with moderate and severe SARS-CoV-2 pneumonia had decreased central retinal vascular density as compared with that of asymptomatic/paucisymptomatic cases or control subjects. These alterations could be due to persistent hypoxaemia or immune-related mechanisms. Similar results were obtained by Abrishami et al. in 31 patients with COVID-19 compared with healthy controls, the former presenting lower vascular density of the superficial and deep capillary plexus in the foveal and parafoveal regions.

Regarding peripapillary vascularisation, Savastano et al. compared radial peripapillary capillary plexus (RPCP) perfusion density and flow index in post-COVID-19 patients with those in healthy controls. RPCP perfusion density was decreased in patients who recovered from COVID-19 compared with controls, systemic arterial hypertension and age correlating with some parameters. Also, RNFL average thickness was linearly correlated with RPCP flow index and perfusion density in the study group. Therefore, impairment in the blood supply to the optic nerve may relate to peripapillary RNFL.

In an initial case series of our study group, patients with optic nerve OCT examinations prior to SARS-CoV-2 infection showed an increase in peripapillary RNFL thickness (mean: 4.3 µm) compared with previous examinations in seven of the eight eyes included. The only patient’s eye that showed a decrease had glaucoma, which accounts for the thinning. These findings were later supported by a large case-control study we conducted on 90 patients with COVID-19 and 70 healthy individuals, with patients with COVID-19 presenting increase in global RNFL thickness as well as in the nasal superior and nasal inferior sectors of the peripapillary RNFL. Macular RNFL showed decrease in volume, superior inner, nasal inner and nasal outer quadrants in patients with COVID-19. In addition, patients who recovered from COVID-19 presented increased GCL thickness in volume, superior outer, nasal outer and inferior outer quadrants. These results were closely related to the presence of nervous system symptoms, which we did not find in the current study. This is probably because children present a milder form of the disease, and symptoms are vague and are more difficult to determine in the paediatric population.

No similar studies to the previously described evaluating optic nerve structural changes or in vascularisation have been performed in paediatric COVID-19. Our results are in accordance with those obtained by our group in adults, supporting these findings. The same trend in differences with healthy subjects as in adults were observed, although not in the exact same sectors. In adults, increases in global peripapillary RNFL (mean difference 4.3; 95% CI 0.8 to 7.7) along with several sectors were found. Paediatric patients with COVID-19 also presented increases in global RNFL (mean difference: 7.5; 95% CI 2.9 to 12.0), but the superior temporal, inferior temporal and nasal sectors were the sectors with increased RNFL thickness. As for macular RNFL thickness, a decrease similar to the adult sample was found, although the nasal outer and temporal inner were the sectors affected in the current study. Children who recovered from COVID-19 also showed increased GCL thickness in the nasal outer (mean difference 2.9; 95% CI 1.2 to 4.6) and temporal inner sectors, while adults had increased thickness measured in the superior outer, nasal outer (mean difference 2.5; 95% CI 1.1 to 4.0) and inferior outer quadrants. The similarity in these results in both adult and paediatric populations further endorses our findings. No differences in macular thickness were noted between groups, which highlights the superiority of having different layer values.

Neurological complications are rare in children suffering from COVID-19, although up to 17% may present neurological manifestations, mostly non-specific. In multisystem inflammation syndrome in children related to COVID-19, the incidence of neurological manifestations is unexpectedly high, around 15%. Specific neurological complaints like seizures, encephalopathy and meningeval signs are probably more common in children with severe illness (9.0%). Nonetheless, nervous system

![Figure 1](https://example.com/figure1.png)

**Figure 1** Peripapillary retinal nerve fibre layer analysis comparing children with COVID-19 with healthy controls. Layers’ thickness is measured in micrometres. ** indicates p<0.01 compared with the control group. G, global; N, nasal; NI, nasal inferior; NS, nasal superior; T, temporal; TI, temporal inferior; TS, temporal superior.

![Figure 2](https://example.com/figure2.png)

**Figure 2** Macular optical coherence tomography analysis evaluating differences in RNFL and GCL thickness. Layers’ thickness is measured in micrometre. *P<0.05, **P<0.01. 1, inner; 2, outer; GCL, ganglion cell layer; I, inferior; N, nasal; RNFL, retinal nerve fibre layer; S, superior; T, temporal.
involvement is not always clinically manifested, as Lindan et al.\(^\text{11}\) reported. In all phases of COVID-19, the most prevalent neuroimaging manifestations observed in children resembled an immune-mediated parainfectious pattern of disease, with ophthalmic features also showing imaging abnormalities. Neuritis, defined as enhancement of the cranial and spinal nerves or the cauda equina, was reported in 12 (32%) patients, 4 of them in the asymptomatic group.\(^\text{13}\) These findings are consistent with our results of subclinical optic nerve involvement.

Multiple mechanisms for neurological involvement have been postulated, including direct viral invasion, blood circulation pathway, neuronal pathway, hypoxic injury, immune injury and ACE2, among other possible mechanisms. Studies have shown the presence of ACE2 receptors in the nervous system, making these neurological manifestations possible, also due to direct cytopathic effect. SARS-CoV-2 RNA has been detected in cerebrospinal fluid and retinal biopsies, confirming the viral presence and activity in the central nervous system.\(^\text{24, 25}\) Hypoxic injury and the immune response may also be responsible for neural damage. In this regard, SARS-CoV-2-related neurological manifestations mostly occur in severe cases, in which there is an increased immune response with a “cytokine storm.”\(^\text{26}\) In a series of paediatric patients with multisystem inflammatory syndrome with neurological involvement, patients showed acute splenial lesions on neuroimaging that are thought to represent focal oedema secondary to inflammation.\(^\text{12}\) Thus, the increased peripapillary RNFL thickness found in our series could be explained by neuronal cell oedema secondary to neuroinflammatory injury, similarly to oedema in other parts of the nervous system.

This study has several limitations. First, our study included a relatively small sample size and could be improved by performing an analysis during the symptomatic phase of the disease. This was avoided due to the emergency situation and risk of contagion. In addition, longitudinal testing with repeat imaging at fixed intervals could provide valuable information regarding both the short-term and long-term effects of COVID-19 on RNFL. Children included presented mild or asymptomatic forms of the disease, with no blood tests being performed in most patients. Hence, no inflammatory markers are available and differences according to disease severity could not be analysed. Finally, the clinical relevance of our findings is unclear as are any associations with visual outcomes, as the patients were all asymptomatic and thus the need for routine OCT testing in children with COVID-19 is yet to be determined.

In conclusion, our study demonstrated significant RNFL and GCL alterations in children with recent history of COVID-19. The potential involvement of the optic nerve by COVID-19 warrants a further larger-scale study of patients infected with COVID-19 worldwide.

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ORCID iD Barbara Burgos-Blasco http://orcid.org/0000-0003-2178-6164

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