ORİJİNAL ARAŞTIRMA ORIGINAL RESEARCH

DOI: 10.5336/ophthal.2022-91360

Evaluation of the Thicknesses of Macula, Retinal Nerve Fiber and, Ganglion Cell Complex in COVID-19 Patients Who Develop Anosmia or Ageusia: Cross-Sectional Study

Tat veya Koku Kaybı Gelişen COVID-19 Hastalarında Makula, Retina Sinir Lifi ve Ganglion Hücre Kompleksi Kalınlıklarının Değerlendirilmesi: Kesitsel Çalışma

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ABSTRACT Objective: To evaluate whether the presence of anosmia or ageusia associates with the thicknesses of the macula, peripapillary retinal nerve fiber layer (pRNFL), and ganglion cell complex (GCC) in patients diagnosed with coronavirus disease-2019 (COVID-19) who had fully recovered. **Material and Methods:** This study was conducted on 123 eyes of 123 participants. Only the right eyes of all subjects were included in the study. Group 1 consisted of 35 eyes of 35 patients with anosmia or ageusia who had recovered from COVID-19 and Group 2 consisted of 31 eyes of 31 patients without anosmia or ageusia who had recovered from COVID-19. Group 3 consisted of 57 eyes of 57 healthy subjects who were age matched with the study groups. The macula, pRNFL, and GCC thicknesses measured by optical coherence tomography were recorded and compared between groups. One-Way ANOVA test and Independent sample t-test were used to compare examinations between all groups. **Results:** Inferior pRNFL and average and inferior GCC values in Group 1 were significantly higher than Group 3 (p=0.001, p=0.044, p=0.017, respectively). There was no difference in macula thickness between all groups. **Conclusion:** In this study, we found that the GCC and pRNFL thicknesses in patients with anosmia or ageusia who had recovered from COVID-19 were significantly higher than those of healthy individuals. Anosmia or ageusia symptoms may be associated with increased pRNFL and GCC thickness in COVID-19 patients.

Keywords: Anosmia or ageusia; COVID-19; retina; retinal nerve fiber layer; ganglion cell complex

ÖZET Amaç: Koronavirüs hastalığı-2019 [coronavirus disease-2019 (COVID-19)] tanısı konmuş ve tamamen iyileşmiş hastalarda tat veya koku kaybı varlığının makula, peripapiller retina sinir lifi tabakası (pRSLT) ve ganglion hücre kompleksi (GHK) kalınlıkları ile ilişkili olup olmadığını değerlendirmek. **Gereç ve Yöntemler:** Bu çalışma, 123 katılımcının 123 gözü üzerinde yapılmıştır. Tüm deneklerin sadece sağ gözleri çalışmaya dâhil edildi. Grup 1, COVID-19'dan iyileşen, tat veya koku kayıplı 35 hastanın 35 gözünden ve Grup 2, COVID-19'dan iyileşen ve tat veya koku kayıpsız 31 hastanın 31 gözünden oluşuyordu. Grup 3, yaşları çalışma gruplarıyla eşleştirilmiş 57 sağlıklı bireyin 57 gözünden oluşturuldu. Optik koherens tomografi ile ölçülen makula, pRSLT ve GHK kalınlıkları kaydedildi ve gruplar arasında karşılaştırıldı. Tüm gruplar arasındaki muayeneleri karşılaştırmak için tek yönlü ANOVA testi ve bağımsız örneklem t-testi kullanıldı. **Bulgular:** Grup 1'de alt pRSLT, ortalama ve alt GHK değerleri Grup 3'ten anlamlı derecede yüksekti (sırasıyla p=0,001, p=0,044, p=0,017). Tüm gruplar arasında makula kalınlığında fark yoktu. **Sonuç:** Bu çalışmada, COVID-19'dan iyileşen, tat veya koku kaybı olan hastalarda GHK ve pRSLT kalınlıklarının sağlıklı bireylere göre anlamlı derecede yüksek olduğunu bulduk. Tat veya koku kaybı semptomları, COVID-19 hastalarında artmış pRSLT ve GHK kalınlığı ile ilişkili olabilir.

Anahtar Kelimeler: Tat veya koku kaybı; COVID-19; retina; retina sinir lifi tabakası; ganglion hücre kompleksi

Coronavirus disease-2019 (COVID-19) can infect the lung, brain, kidney, intestine, as well as retinal endothelial cells via the angiotensin-converting enzyme (ACE) 2 receptor.[1]

Coronavirus has been shown in post-mortem retinal biopsies.[2] For this reason, coronavirus may have an ischemic and neurodegenerative effect on retinal tissue with immune-mediated inflammation

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Peer review under responsibility of Turkiye Klinikleri Journal of Ophthalmology.

Received: 17 May 2022 ***Accepted:*** 04 Jul 2022 ***Available online:*** 01 Aug 2022

after a direct viral invasion or systemic involvement. In addition, histopathological studies have shown the viral effect on endothelial cells of arterial and venous circulation.[3] Inflammation of endothelial cells can lead to microvascular dysfunction, edema and, thrombosis. Retinal vascular changes such as microhemorrhages and small infarcts have been reported in COVID-19 patients.[4] Optical coherence tomography (OCT) and OCT-angiography (OCT-A) have been used in many studies to show this effect. Decreased retinal vessel density without thickening of the ganglion cells has been reported.[5-7]

Ganglion cells are localized in the ganglion cell layer of the retina. The axons of these cells form the optic nerve fiber layer around the optic nerve head, and then these fibers combine to form the optic nerve. After the optic nerve follows the path of the optic chiasm, the optic tract and the lateral geniculate bodies, it finally terminates in the occipital cortex. Therefore, the optic nerve is considered as an extension of the central nervous system (CNS).[8] Neurological involvement can be seen in 17.3% to 36.4% of patients who have COVID-19.[9] The most common initial symptoms of CNS involvement of COVID-19 were reported as headache, confusion, agitation, dysexecutive syndrome, anosmia, and ageusia.[10]

This study aimed to evaluate whether the presence of anosmia or ageusia (A/A) associated with the thicknesses of macula, peripapillary retinal nerve fiber layer (pRNFL) and ganglion cell complex (GCC) in COVID-19 patients and had recovered from the disease.

MATERIAL AND METHODS

This study was cross-sectional and observational study. It conducted in the accordance with the principles of the Declaration of Helsinki and was approved by the Research Protocol and Haydarpaşa Numune Training and Research Hospital Ethics Committee (date: February 8, 2021, no: HNEAH-KAEK 2021/KK/3). Informed written consent was obtained from all patients.

Patients who had a definite diagnosis of COVID-19 with polymerase chain reaction (PCR) minimum two weeks ago and maximum 3 months ago were included in the study. Participants with systemic diseases were not included in this study. None of the participants had a history of ocular disease (glaucoma, corneal or retinal disease), ocular inflammatory disease, eye surgery. None of the participants had visual acuity below 8/10 (decimal system) with Snellen chart and refractive errors above ±3 diopters as spherical equivalent.

This study was conducted on 123 eyes of 123 participants. Only the right eyes of all participants were included in the study. Group 1 consisted of 35 eyes of 35 participants with A/A who had recovered from COVID-19 and Group 2 consisted of 31 eyes of 31 patients without A/A who had recovered from COVID-19. Group 3 consisted of 57 eyes of 57 healthy subjects who were age matched with the study groups.

Group 1 and Group 2 consisted of healthcare workers and their relatives. The time from the first PCR test positivity to the examination data was recorded. Detailed clinical history of all cases was taken in which the presence of pneumonia was confirmed by lung tomography, fever was present, hospitalization was necessary, and loss of taste and smell occurred.

Group 3 was made up of healthy individuals who had no COVID-19 symptoms and had no close and/or distant contact with people with COVID-19 infection.

A complete ophthalmologic examination was performed on each participant. This examination was including best-corrected visual acuity (BCVA), measurement of intraocular pressure (IOP) with Goldmann applanation tonometry, central corneal thickness measurements and detailed fundus examination. Macular thickness (MT), pRNFL, GCC measurements were made with Fourier-domain OCT (FD-OCT; RTVue-100, Optovue, Fremont, CA). Measurements of low signal strength and not focusing on optic disc or fovea were not evaluated.

The average thicknesses of fovea, parafovea, and perifovea were evaluated. The average, temporal, superior, nasal, and inferior RNFL measurements were recorded. The superior, inferior, and average GCC measurements were recorded.

SPSS Version 22 (IBM SPSS, Türkiye) was used for statistical analysis. The data were tested initially whether the distribution of variables was normal with the Kolmogorov-Smirnov test. One-Way ANOVA test and independent sample t-test were used to compare examinations between all groups. Pearson's chi-square test was used to compare the categorical variables in different groups. A p value <0.05 was considered statistically significant.

RESULTS

The mean age was 40.82±10.87, 40.77±10.77 and, 40.31±11.34 years in Group 1, 2, and 3 respectively (p=0.971). The BCVA of all patients was 10/10 (decimal system) with Snellen chart. There was no significant difference between Group 1 (14.85±1.85 mmHg), Group 2 (14.77±2.64 mmHg), and Group 3 (15.24±2.74) in terms of IOP (p=0.633). None of the patients had glaucomatous optic disc parameters. None of the patients had pathological findings in fundus examination such as retinopathy, retinal hemorrhages, retinal or macular edema at the time of examinations.

The prevalence of olfactory or taste deficiency in COVID-19 patients was 53.03%. Subject' characteristics, mean values of age, sex and body mass index, PCR duration, and COVID-19 symptoms are shown in Table 1.

The mean pRNFL, GCC, and MT values of Group 1, Group 2, and Group 3 are shown in Table 2. The inferior pRNFL and average and inferior GCC measurements were significantly higher with the A/A group than Group 3 (p=0.001, p=0.034, p=0.008, respectively). There was no difference in terms of MT measurements between all groups.

DISCUSSION

This study compared the macula, GCC, and pRNFL thicknesses of patients with or without A/A who had recovered from COVID-19 to those of healthy individuals. To our knowledge, this is the first study in the literature to report retinal findings in COVID-19 patients who develop A/A.

In this study, inferior RNFL and average and inferior GCC values were found to be significantly higher in patients with A/A who recovered from COVID-19 than in healthy subjects. Taste and smell disorders are very common in COVID-19 patients.[11] Worldwide, the prevalence of olfactory or taste deficiency in COVID-19 was reported as 49.0%.[12] In our study, this rate was 53.03%. In a review towards how the COVID-19 virus affects the olfactory nerve, it is stated that ACE2 and TMPRSS2, which are the cell entry proteins of the virus, are not expressed by most of the olfactory receptor neurons, therefore, loss of smell may be due to the involvement of sustentacular

TABLE 1: Subject characteristics in patients with (Group 1) and without (Group 2) anosmia or ageusia who had recovered from COVID-19, and healthy individuals (Group 3).

	Group 1 $\bar{X}$±SD	Group 2 $\bar{X}$±SD	Group 3 $\bar{X}$±SD	p value
n (%)	35 (28.45)	31 (25.20)	57 (46.34)	
Age (year)[1]	40.82±10.87	40.77±10.77	40.31±11.34	0.971
Female (60)	39.71±11.27	41.75±10.63	40.83±11.12	0.848
Male (63)	42.50±10.59	40.15±11.26	39.43±11.28	0.633
Female/Male (%)[3]	21/14 (60/40)	12/19 (38.7/61.3)	27/30 (47.36/52.64)	0.216
BMI[1]	25.93±3.78	25.48±4.07	25.38±3.71	0.969
PCR test duration (days)[2]	53.71±23.38 (Minimum 15/Maximum 90)	52.09±28.97 (Minimum 15/Maximum 90)		0.805
Hospitalization (%)[3]	2 (40%)	3 (60%)		0.442
Fever (%)[3]	12 (57.1%)	9 (42.9%)		0.498
Pneumonia (%)[3]	8 (72.7%)	3 (27.3%)		0.162

[1]One-Way ANOVA test; [2]Independent sample test; [3]Chi-square test; p<0.05; SD: Standard deviation; BMI: Body mass index; PCR: Polymerase chain reaction.

TABLE 2: The comparison of RNFL, GCC, and macular thickness between patients with (Group 1) and without (Group 2) anosmia/ageusia who had recovered from COVID-19 and healthy individuals (Group 3).

	Group 1 $\bar{X}$±SD n=35	Group 2 $\bar{X}$±SD n=31	Group 3 $\bar{X}$±SD n=57	p value
RNFL (µ)				
Average	110.77±9.86	107.51±9.09	106.36±7.41	0.060
Temporal	84.91±10.74	81.93±10.63	85.05±11.38	0.410
Superior	137.80±18.66	133.32±15.79	135.94±13.32	0.509
Nasal	80.51±9.90	80.03±9.22	76.96±9.07	0.146
Inferior	139.62±15.14	134.61±16.09	127.33±33	0.001*
				1-2 0.486
				1-3 0.001*
				2-3 0.105
GCC (µ)				
Average	104.92±7.90	101.98±10.26	100.30±7.79	0.044*
				1-2 0.342
				1-3 0.034*
				2-3 0.652
Superior	104.13±7.98	101.24±10.15	100.13±8.49	0.109
Inferior	105.78±8.17	101.91±10.49	100.13±8.49	0.017*
				1-2 0.277
				1-3 0.008*
				2-3 0.88
MT (µ)				
Fovea	249.48±18.30	240.70±25.20	241.19±19.26	0.126
Parafovea	317.82±18.56	318.83±17.02	318.26±13.31	0.968
Perifovea	281.57±11.05	279.43±12.38	277.92±10.21	0.309

One-Way ANOVA test *p<0.05; RNFL: Retinal nerve fiber layer; GCC: Ganglion cell complex; SD: Standard deviation; MT: Macular thickness.

cells in the olfactory epithelium. The return of taste and smell defect in a short time in COVID-19 patients support the involvement of these sustentacular cells rather than the olfactory nerve. However, it may also be possible for the virus to reach the brain through cranial nerves in the nasal cavities such as the trigeminal nerve, olfactory nerve, vagal nerve, as well as cells that cross the blood-retina barrier or cavities containing spinal fluid. Since the taste defects occur in the same time period as olfactory defects, it was predicted that support cells may be responsible, as in the loss of smell. However, there are no studies yet available on this subject.[12] In our study, thicknesses of retinal nerve fiber and GCC layer were higher in cases whose sense of smell or taste was affected. In this case, it can be assumed that the virus affects the brain tissue in cases with an impaired sense of smell or taste. Although the virus does not directly reach the brain, the resulting inflammatory mediators may affect the retinal nerve fiber and ganglion cell layer. Therefore, we hypothesize that pRNFL and GCC thickness could be affected in COVID-19 patients with neurological involvement.

However, COVID-19 disease can also affect the retina independent of neurological effects. ACE-2 receptors have been found in the inner nuclear layer at the photoreceptors of the retina, suggesting that the COVID-19 may have direct retinal invasion.[13] Endothelial cell dysfunction associated with apoptosis caused by direct viral invasion or immune-mediated damage of endothelium was reported.[3] In addition, one study reported that edema and acute peripheral arterial thrombosis were the results of hypercoagulability state seen in COVID-19 infection.[14] Microvascular damage in the retina of COVID-19 patients may be associated with these thromboembolic events. A decrease in the density of the retinal plexus vessel has been shown in studies that include OCT-A of

COVID-19 patients.[5-7] Although a few studies showed no significant decrease in retinal vessel density with OCT-A, this did not exclude the possibility of micro-thrombus, as the micro-occlusions in vessels that are smaller than 20 µm may not be detected in OCT-A.[15] In our study, we also hypothesized that the significant increase of values of the GCC and pRNFL in COVID-19 patients may be caused by edema resulting from micro-thrombus. In addition, autoimmune reactions and inflammation may continue in the retinal layers, even though the individuals become PCR negative and clinically recover from the disease.

Örnek et al. reported focal retinal nerve fiber loss after COVID-19 infection but did not specify how long after the PCR test the study was performed.[16] Another study that was conducted on patients with COVID-19 reported that perfusion density and flow index were linearly correlated with the mean pRNFL thickness in the evaluation of radial peripapillary capillary plexus with OCT-A.[17] Over the long term, pRNFL loss seems possible as a result of micro-circulation disorder and the formation of autoantibodies against retinal nerve fibers.[18] However, in contrast to these studies, we found an increase in pRNFL values in COVID-19 patients. In one other study, although the result was not statistically significant in patients with COVID-19, an increase in pRNFL thickness was reported in all quadrants.[19] This study supports our work.

To our knowledge, this is the first study in the literature to report retinal findings in COVID-19 patients who develop A/A. Therefore, we could not compare the data of our study with any other similar study. Also, this study was a cross-sectional study, and the sample size was not large. In addition, the patients recovered from the infection and had only mild symptoms. However, the strong point of this study was that the control group was matched to the study group in terms of age. In addition, since it is a prospective study, we strictly applied the inclusion criteria in case selection.

CONCLUSION

In the current study, we found that the GCC and pRNFL thicknesses in patients with A/A who had recovered from COVID-19 were significantly higher than those of healthy individuals. A/A symptoms may be associated not only with the involvement of the olfactory nerve but also with neurological involvement therefore it may affect the retinal layer thickness. Further prospective studies with a larger sample could be more informative.

Source of Finance

During this study, no financial or spiritual support was received neither from any pharmaceutical company that has a direct connection with the research subject, nor from a company that provides or produces medical instruments and materials which may negatively affect the evaluation process of this study.

Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

All authors contributed equally while this study preparing.

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