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Assessment of retinal vessels and corneal endothelium alterations in acute and post-COVID-19

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The COVID-19 pandemic, caused by SARS-CoV-2, has shown significant systemic effects beyond the respiratory system, including ophthalmologic complications. The ***purpose*** *of the study is to assess alterations in retinal vessels and corneal endothelium in patients with acute and post-COVID-19 infection.* ***Material and methods.*** *A prospective observational study was conducted at Al-Hussain Teaching Hospital in Iraq at two years 2021–2022, including 50 adult patients (56 % male) aged 61.7 ± 12.5 years with confirmed COVID-19 by PCR testing. Common comorbidities included hypertension (30 %), smoking (26 %), and diabetes mellitus (16 %). Comprehensive ophthalmologic examinations were performed, including retinal imaging and specular microscopy. Clinical and laboratory data were collected and analyzed using SPSS version 26.0.* ***Results.*** *Significant laboratory changes were observed between acute and post-acute phases, indicating an inflammatory response during acute infection. Retinal alterations improved significantly post-acute COVID-19, with reductions in retinal hemorrhage, vessels tortuosity, and veins dilatation. Specular microscopy showed improvements in cell density, average cell area, coefficient of variation, and hexagonality.* ***Conclusion.*** *COVID-19 has significant impacts on retinal and corneal health, with notable improvements post-infection. These findings highlight the need for on-going ophthalmologic monitoring in COVID-19 patients to manage and mitigate long-term ocular complications.*

Keywords: COVID-19; retinal vessels; corneal endothelium; ophthalmologic complications; SARS-CoV-2
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Оценка изменений сосудов сетчатки и эндотелия роговицы при остром COVID-19 в постковидном периоде

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Пандемия COVID-19, вызванная SARS-CoV-2, ассоциирована со значительными системными эффектами за пределами дыхательной системы, в том числе с офтальмологическими осложнениями. ***Цель*** *работы — оценить изменения сосудов сетчатки и эндотелия роговицы у пациентов при остром COVID-19 и в пост-ковидном периоде.* ***Материал и методы.*** *Проспективное наблюдательное исследование, проведенное в больнице Аль-Хуссейн в Ираке в течение 2021–2022 гг., включало 50 пациентов (56 % мужчин) в возрасте 61,7 ± 12,5 года с подтвержденным с помощью ПЦР-тестирования COVID-19. Сопутствующие заболевания включали гипертонию (30 %), курение (26 %) и сахарный диабет (16 %). Проведено комплексное офтальмологическое обследование, включая ретинальную визуализацию и зеркальную микроскопию. Клинические и лабораторные данные собраны и проанализированы с использованием SPSS версии 26.0.* ***Результаты.*** *В период между острой и пост-острой фазами наблюдались значительные лабораторные изменения, указывающие на воспалительную реакцию. В постковидном периоде состояние сетчатки значительно улучшилось, уменьшились ретинальные кровоизлияния, извилистость сосудов и расширение вен. Зеркаль-*

ная микроскопия показала улучшение в плотности и средней площади клеток, коэффициента вариации и гексагональности. ***Заключение.*** *COVID-19 оказывает значительное влияние на состояние сетчатки и роговицы, при этом наблюдается заметное улучшение после инфицирования. Эти данные подчеркивают необходимость постоянного офтальмологического мониторинга пациентов с COVID-19 для диагностики и минимизации долгосрочных офтальмологических осложнений.*

Ключевые слова: COVID-19; сосуды сетчатки; эндотелий роговицы; офтальмологические осложнения; SARS-CoV-2
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The COVID-19 pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in late 2019 in Wuhan, China, and rapidly spread worldwide, leading to unprecedented global health and economic challenges [1]. While initial studies primarily focused on the respiratory system, it became evident that the virus affects multiple organ systems, including the eyes. As the pandemic progressed, understanding the full spectrum of COVID-19's effects became crucial for comprehensive patient care.

A growing body of research has highlighted concerns regarding COVID-19-related ophthalmologic health problems [2]. Theories explaining virus transmission to the eyes include conjunctival droplet inoculation, migration of upper respiratory tract infection through the nasolacrimal duct, and hematogenous involvement of the lacrimal gland [3].

The angiotensin-converting enzyme 2 (ACE2) cellular receptor and transmembrane protease serine 2 (TMPRSS2) are expressed in the human cornea, retina, and conjunctival epithelium, facilitating the binding of SARS-CoV-2. This binding explains the emergence of ocular manifestations in COVID-19 infection [4, 5].

Ocular symptoms can develop shortly before the systemic symptoms of COVID-19, such as fever and cough [6]. Noted ophthalmologic symptoms of COVID-19 infection include dry eye, foreign body sensation, itching, blurred vision, conjunctivitis, chemosis, and photophobia [7]. Further ocular symptoms of COVID-19 infection include episcleritis, uveitis, retinal vascular changes, cotton wool spots, optic neuritis, cranial nerve palsy-related ocular motility imbalances, and transient accommodation imbalances [8, 9].

A Canadian study linked conjunctivitis to corneal subepithelial infiltrations, corneal epithelial defects, the development of tender preauricular lymphadenopathy, and conjunctival follicular response in COVID-19 cases [10].

This study aims to assess the alterations in retinal vessels and corneal endothelium in patients with acute and post-COVID-19 infection. By understanding these alterations, we hope to provide better diagnostic and therapeutic approaches for managing ophthalmologic complications associated with COVID-19.

MATERIAL AND METHODS

Study Design. This is a prospective observational study conducted to assess alterations in retinal vessels and corneal endothelium in patients with acute and post-COVID-19 infection. The study was carried out in the COVID-19 isolation unit at Al-Hussain Teaching Hospital in Iraq. The study was conducted from early January 2021 to the end of October 2022.

Population. Adult patients (18 years or older) who were confirmed to have COVID-19 infection.

Inclusion criteria. Adult patients (18 years or older), confirmed COVID-19 infection by PCR testing, underwent a chest computer tomography (CT) scan, and provided written informed consent to participate in the study.

Exclusion criteria. Patients under the age of 18, patients not confirmed to have COVID-19 infection by PCR testing, patients who did not undergo a chest CT scan, patients with unavailable clinical and laboratory data, patients with a history of ophthalmologic disorders (such as glaucoma, uveitis, amblyopia, strabismus, dry eye, keratoconus, corneal opacity) before COVID-19 infection, and patients using topical ophthalmic drops.

Clinical and laboratory data collection. Full history was taken from all participants, including gender and age, as well as past medical history, which encompassed major illnesses and comorbidities. A detailed clinical examination was performed, and COVID-19 patients' laboratory parameters were collected.

Retinal imaging and analysis. Participants underwent pupil dilation of both eyes using mydriatic drops (Tropicamide 1 %) 15 minutes before the acquisition of retinal images. Two pairs of fundus photos were taken for each participant, one for each eye, using the Digital Retinography System (DRS) fundus camera. Each set consisted of four photos with a 45° to 40° field of view and a resolution of 48 pixels/degree, with two centered on the macular area and two on the optic nerve head. The quality of fundus images was evaluated by two specialists. Retinal alterations and abnormalities were assessed using an automated retinal image analyzer.

Ophthalmologic examination. All subjects in the study underwent corrected visual acuity determinations, as well as anterior and posterior segment examinations. The number of corneal endothelial cells, cell area variation coefficient, and hexagonal cell percentage were evaluated and analyzed.

Specular microscopy. A non-contact specular microscope (Topcon SP1-P, Tokyo, Japan) was used to evaluate the central endothelium of the cornea. Measurements were taken by a single examiner. The procedure for specular microscopy involved taking three panoramic images of the central cornea in panoramic mode, which the device then combined to obtain a complete analysis of the central corneal endothelium.

Statistical analysis. For all statistical analyses, SPSS version 26.0 (Chicago, IL, USA) was used. Descriptive statistics for continuous variables included the mean, standard deviation (SD), and ranges, as appropriate. The percentage prevalence of demographic qualitative variables was noted.

RESULTS

The study population consisted of 50 participants with a mean age of 61.7 ± 12.5 years, ranging from 24 to 93 years. Gender distribution showed a slight male predominance, with 56 % males and 44 % females. The prevalence of comorbidities among the participants is summarized in Table 1, highlighting hypertension as the most common (30 %), followed by smoking (26 %), and diabetes mellitus (16 %). Dyslipidemia was present in 12 % of the population, while heart disease and severe

COVID-19 were observed in 6 % and 8 % of participants, respectively. These demographic and clinical characteristics, detailed in Table 1, align with existing literature, underscoring the higher vulnerability of older adults and the significant role of comorbidities in COVID-19 severity.

Table 1. Demographic data and clinical features of the study population
Таблица 1. Демографические данные и клинические характеристики исследуемой популяции

Variables Показатели		Frequency Частота	Percentages Процент
Age, years Возраст, лет Mean ± SD		61.7 ± 12.5	
Sex Пол	Male Мужской	28	56.0
	Female Женский	22	44.0
Comorbidities Сопутствующие заболевания	Hypertension Гипертония	15	30.0
	Diabetes mellitus Сахарный диабет	8	16.0
	Dyslipidemia Дислидемия	6	12.0
	Heart disease Заболевания сердца	3	6.0
	Smoking Курение	13	26.0
	Severity of COVID-19 Тяжелый COVID-19	4	8.0

Table 2. Laboratory results of the study group in acute and Post-COVID-19
Таблица 2. Лабораторные результаты исследуемой группы в остром периоде и после COVID-19

Variables Показатели	Acute COVID-19 Острый COVID-19	Post-acute COVID-19 Пост-острый COVID-19	p-value
WBC Лейкоциты (4–11 × 10^9 L)	5.51 ± 1.75	106.10 ± 603.56	0.022
Lymphocyte count Лимфоциты (1.5–3.5 × 10^9 L)	1.31 ± 0.66	0.98 ± 0.43	< 0.001
Neutrophil count Нейтрофилы (2.5–7.5 × 10^9 L)	4.07 ± 1.19	6.64 ± 5.10	< 0.001
Platelets Тромбоциты (150–450 × 10^9 L)	246.30 ± 69.87	243.88 ± 93.62	0.828
PT Протромбиновое время (9.4–12.5 S)	11.88 ± 1.39	11.07 ± 1.71	< 0.001
PTT Частичное тромбопластиновое время (25–37 S)	30.91 ± 3.83	29.40 ± 1.17	< 0.001
CRP С-реактивный белок (< 6 mg/L)	52.51 ± 42.08	16.64 ± 14.80	< 0.001
Serum ferritin Сывороточный ферритин (20–250 mg/L)	506.94 ± 190.19	240.23 ± 152.10	< 0.001
D dimer D димер (>)	0.90 ± 2.01	0.35 ± 0.14	< 0.001
Serum creatinine Сывороточный креатинин (0.7–1.2 mg/dL)	1.1 ± 0.50	0.70 ± 0.30	< 0.001

Laboratory results showed significant changes between acute and post-acute COVID-19 phases. Notably, there were significant differences in WBC, lymphocyte count, neutrophil count, PT, PTT, CRP, serum ferritin, D-dimer, and serum creatinine levels, indicating a marked inflammatory response during acute infection that subsided post-acute phase (Table 2). These findings highlight the increased vulnerability of older adults, the impact of comorbidities, and dynamic changes in laboratory markers throughout the disease.

Retinal alterations improved significantly from acute to post-acute COVID-19 phases (Table 3). Retinal hemorrhage, vessels tortuosity, and veins dilatation decreased, and cotton wool spots disappeared. Mean artery and vein diameters also significantly decreased, indicating recovery and normalization of retinal features.

Specular microscopy showed significant improvements post-acute COVID-19 (Table 4). Cell density (CD) slightly increased ($p = 0.023$), average cell area (AVG) decreased ($p = 0.01$), coefficient of variation (CV) reduced ($p = 0.034$), and hexagonality improved ($p = 0.039$). Central corneal thickness (CCT) showed no significant change ($p = 0.21$).

DISCUSSION

The mean age of our study population was 61.7 ± 12.5 years. This aligns with a retrospective analysis indicating that middle-aged and older adults with COVID-19 are more vulnerable to the disease and have a higher mortality rate. In older populations, anatomical and physiological changes in the lungs and reduced defense barrier function are contributing factors [11].

Regarding gender distribution, 56 % of our study population were males. This finding is consistent with A. Invernizzi, et al. [12], where 68.7 % of the study population were men. However, B. Modjtahedi, et al. [13] reported opposite results, with 53.6 % of their study population being women, while exploring retinal vascular occlusions after COVID-19 infection.

Epidemiological studies worldwide have shown that males have higher illness and mortality rates than females. Females are generally more resistant to infections than males, possibly due to factors such as sex hormones, higher expression of ACE2 receptors in men, and lifestyle habits like higher smoking and drinking levels in men [14].

Our study results showed that hypertension (30 %), smoking (26 %), and diabetes mellitus (16 %) were the most common comorbidities. This is in agreement with J. Yang, et al. [15] meta-analysis, which

Table 3. Retinal alterations of the study group in acute and post-COVID-19
Таблица 3. Изменения сетчатки в исследуемой группе при остром COVID-19 и в постковидном периоде

Variables Показатели	Acute COVID-19 Острый COVID-19 n = 92	Post-acute COVID-19 Постковидный период n = 92	p-value
Retinal hemorrhage Ретинальные кровоизлияния	19 (20.6 %)	2 (2.17 %)	0.0009
Cotton wool spots Белые ватообразные очаги	3 (3.26 %)	0 (0.0 %)	0.5
Vessel's tortuosity Извитость сосудов	15 (16.3 %)	6 (6.52 %)	0.03
Veins dilatation Расширение вен	19 (20.6 %)	6 (6.52 %)	0.007
Mean artery diameter Средний диаметр артерий	93.43 ± 1.22	89.21 ± 3.34	< 0.001
Mean vein diameter Средний диаметр вен	139.01 ± 4.14	134.86 ± 5.35	< 0.001

Note. n — number of eyes.
Примечание. n — число глаз.

Table 4. Specular microscopic results of the study group in acute and post-COVID-19
Таблица 4. Результаты зеркальной микроскопии исследуемой группы при остром COVID-19 и в постковидном периоде

Variables Показатели	Acute COVID-19 Острый COVID-19	Post-acute COVID-19 Постковидный период	p-value
CD, cells/mm^2 Плотность клеток, клеток/мм^2	2424 ± 369	2446 ± 363	0.023
AVG, μm^2 Площадь средних клеток, мкм^2	423 ± 132	356 ± 64	0.01
CV, % Коэффициент вариации, %	38.45 ± 18.60	30.55 ± 13.86	0.034
Hexagonality, % Гексагональность, %	70.15 ± 25.32	77.28 ± 104.71	0.039
CCT, μm Центральная толщина роговицы, мкм	531 ± 39	522 ± 39	0.21

Note. CD — mean cell density, AVG — mean cell area, CV — mean coefficient of variation, CCT — central corneal thickness.

found hypertension (21.1 %) and diabetes (9.7 %) to be prevalent comorbidities.

Smoking has long been known to harm lung health and increase susceptibility to infections. N. Çetin Kargin [16] found that 72.03% of COVID-19 patients were current smokers.

According to P. Wu, et al. [17], ocular symptoms are more prevalent among patients with severe systemic disease who have abnormal blood and inflammatory measurements. SARS-CoV-2 viral RNA has been found in the retina [18], and alterations in retinal vessels can reflect systemic vascular changes [12]. P. Marinho, et al. [19] first reported retinal changes associated with COVID-19, specifically hyper-reflective changes in the inner retina. L. Pereira, et al. [20] in Brazil observed peripheral retinal hemorrhages, macular hyperpigmentation, retinal sectoral pallor, peripapillary flame-shaped hemorrhages, hard exudates, and cotton wool spots in 55.6 % of their patients.

Hypercoagulability, a documented effect in COVID-19 patients, may contribute significantly to retinal findings. Coagulopathy in COVID-19 has been linked to direct viral infection and endothelial cell damage, complement-mediated endothelial injury, microangiopathy, and local thrombus formation. In our study, cotton wool spots were recorded in 3 out of 92 eyes. M. Landecho, et al. [21] found similar results, with six out of 27 asymptomatic participants with previous COVID-19 infection having cotton wool spots.

M. Abrishami, et al. [22] observed increased retinal thickness in COVID-19 patients, although their comparison to age-matched controls did not reach statistical significance. They also reported changes in retinal microvasculature in patients who recovered from COVID-19 [23].

At baseline, mean artery diameter (MAD) and mean vein diameter (MVD) were significantly higher in COVID-19 patients compared to unexposed subjects ($p < 0.0001$). Both MAD and MVD significantly decreased in COVID-19 patients at follow-up ($p < 0.0001$) [12]. M. Pirraglia, et al. [24] found no retinal manifestations in COVID-19 patients, except for one patient diagnosed with probable fungal retinitis. A. Invernizzi, et al. [25] compared 54 COVID-19 patients to 133 controls and found higher rates of retinal hemorrhages and cotton wool spots, as well as increased MAD and MVD in COVID-19 patients.

N. Patel, et al. [26] reported retinal hemorrhage in 27.1 % (n = 13) of 48 eyes that underwent color fundus photography, optic disc edema in 2.1 % (n = 1), and no cotton wool spots. R. Sim, et al. [27] found retinal microvascular signs in 11.6 % of young COVID-19 patients.

Our results align with A. Invernizzi, et al. [12], who found that most retinal changes disappeared six months after the disease resolved, suggesting a possible link with the generalized inflammatory and pro-coagulant status seen in acute COVID-19.

L. Jiang, et al. [28] presented a case of acute bilateral corneal decompensation caused by COVID-19 pneumonia and prolonged ICU ventilation. SARS-CoV-2 enters via the ACE2 receptor, which is downregulated in COVID-19. Human ocular surface cells, including the cornea, contain ACE2 receptors. Upregulation of inflammatory cascades in ACE2-deficient mouse models results in corneal haze, edema, and ocular surface inflammation.

The higher central corneal thickness (CCT) observed in the COVID-19 group in S. Erdem, et al. [29] could be attributed to impaired endothelial cell function caused by immune dysregulation and the pro-inflammatory effects seen in these patients. Their results agreed with our findings, showing a reduction in the number of endothelial cells and hexagonal cells (polymorphism), as well as an increase in the cell area variation coefficient (polymegatism) and the average cell area.

The cross-sectional design of the research, conducted in a single center on a relatively limited group of participants, is a limitation of this study. These results should be investigated further in future large-scale, multi-center, long-term studies.

CONCLUSION

This study highlights significant alterations in retinal vessels and corneal endothelium in patients with acute and post-COVID-19 infection. The findings indicate an inflammatory response during the acute phase, with marked improvements post-acute phase. Retinal hemorrhage, vessels tortuosity, and veins dilatation decreased significantly,

and improvements in specular microscopic results were observed. These results underscore the importance of continued ophthalmologic monitoring and care for COVID-19 patients to address potential long-term ocular complications. Future research should focus on larger, multi-center studies to further validate these findings and explore the underlying mechanisms.

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