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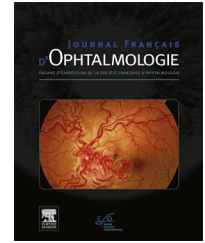


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ORIGINAL ARTICLE

The importance of tears stability in SARS-CoV-2 transmission: COVID-19 prevalence in dry eye patients

Importance de la stabilité du film lacrymal dans la transmission du SRAS-CoV-2 : prévalence du COVID-19 chez les patients atteints de sécheresse oculaire

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Summary

Objective. – To investigate whether dry eye disease (DED) is a risk factor for COVID-19.

Method. – In this retrospective cohort study, patients who were diagnosed with DED by an ophthalmologist and whose Schirmer test was less than 5 mm were identified. Patients who missed follow-up examinations, patients with malignancy, Human Immunodeficiency Virus patients and patients having undergone bone marrow transplantation were excluded. Among the DED patients, patients with positive SARS-CoV-2 PCR tests were identified on October 11, 2020. Subsequently, patients were divided into four age groups (25–49; 50–64; 65–79; and 80+). The COVID-19 prevalence per 100,000 people was determined for each age group, and risk analysis was performed by comparing this with the general population in Turkey.

Results. – In total, 10,023 DED patients were identified and included in the study. Among these, the PCR test was positive in 359 patients. The COVID-19 prevalence per 100,000 population in DED patients was calculated as 3581.7, while according to the Ministry of Health data, it was 524.7 in the general Turkish population. The odds ratio of DED patients versus the general population was 6.62 ($P < 0.001$) (7.66 in the 25–49 group; 6.59 in the 50–64 group; 6.23 in the 65–79 group; and 7.24 in the 80+ age group).

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Conclusions. – The present study showed a high COVID-19 prevalence in DED patients compared to the general population. These findings support the concept that the ocular surface may be a gateway for SARS-CoV-2 and that the tear film is important part of the immune system.

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MOTS CLÉS

Infection par coronavirus ;
COVID-19 ;
Maladie des yeux secs ;
Patients souffrant de sécheresse oculaire ;
SRAS-CoV-2

Résumé

Objectif. – Étudier si la maladie de l'œil sec (DED) est un facteur de risque de COVID-19.

Méthode. – Dans cette étude de cohorte rétrospective, les patients qui ont reçu un diagnostic de DED, par un ophtalmologiste et dont le test de schirmer était inférieur à 5 mm, ont été identifiés. Les patients absents aux examens de contrôle, les patients atteints de tumeur maligne, du virus de l'immunodéficience humaine et ceux ayant subi une greffe de moelle osseuse ont été exclus. Parmi ces patients atteints de DED, des patients présentant des tests PCR positifs au SARS-CoV-2 ont été identifiés le 11 octobre 2020. Plus tard, les patients ont été divisés en quatre groupes d'âge (25–49 ; 50–64 ; 65–79 ; et 80+). La prévalence du COVID-19 pour 100 000 personnes a été déterminée pour chaque groupe d'âge et l'analyse du risque a été effectuée en la comparant à la population générale en Turquie.

Résultats. – Au total, 10 023 patients DED ont été identifiés et inclus dans l'étude. Parmi ceux-ci, le test PCR a été positif chez 359 patients. La prévalence du COVID-19 pour 100 000 habitants chez les patients atteints de DED a été calculée à 3581,7, tandis que selon les données du ministère de la Santé, elle était de 524,7 dans la population turque en général. Le risque relatif des patients atteints de DED par rapport à la population générale était de 6,62 ($p < 0,001$) (7,66 chez les 25–49 ans ; 6,59 chez les 50–64 ans ; 6,23 chez les 65–79 ans ; et 7,24 chez les groupes d'âge de 80 ans et plus).

Conclusions. – La présente étude a montré une prévalence élevée de COVID-19 chez les patients atteints de DED par rapport à la population générale. Ces résultats suggèrent que la surface oculaire peut être une porte d'entrée pour le SARS-CoV-2 et que le film lacrymal participe aux modes de défense immunitaire.

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Introduction

Coronavirus (CoV) is a single-stranded RNA virus that typically causes respiratory and enteric infections in humans and wild animals [1,2]. The latter two CoVs (SARS-CoV in 2002 and MERS-CoV in 2012) caused in severe lower respiratory tract infection, rapidly proceeding to pneumonia, and have resulted in thousands of people to get infected and hundreds of deaths in about 30 countries [3,4]. In December, 2019, another outbreak of highly contagious pneumonia caused by a new coronavirus (SARS-CoV-2) appeared in Wuhan, China. Subsequently, SARSCoV-2 has grown into a global health threat within weeks. The disease caused by CoV was named Coronavirus Disease 2019 (COVID-19) [2,3]. As of October 16, 2020, COVID-19 has been confirmed in nearly 40 million people and resulted in more than 1 million deaths [5,6].

The tear film provides nutrients and oxygen to the avascular cornea, helps to remove metabolic wastes and debris, protects the ocular surface by carrying antimicrobial agents (enzymes and proteins), and provides wetness to the ocular surface [7,8]. The ocular surface structure of the tear film consists of an aqueous gel with a gradient of mucin content decreasing from the ocular surface to the undersurface of the outermost lipid layer [7,9,10]. Reduced aqueous

secretion due to decreased production or increased evaporation leads to Dry Eye Disease (DED) [8]. The tear film in patients with DED is unstable and incapable of maintaining the protective qualities that are necessary for its structure and function [7,9–11]. Therefore, microbial agents can survive longer on ocular surface and attach more comfortable to the host cell [11].

Even though the eye is considered to be one of the important entry gates for respiratory viruses [4,12,13], there is not enough evidence about the three CoVs. This is the first study of susceptibility to CoVs infection in DED patients to our knowledge. The purpose of this study is to calculate COVID-19 prevalence in DED patients and to investigate whether DED is a risk factor for SARS-CoV-2 by comparing the prevalence of COVID-19 with the general population.

Methods

This retrospective cohort study was approved by COVID-19 Scientific Research Evaluation Commission of Ministry of Health of the Republic of Turkey and local medical ethics committee and followed the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE)

reporting guideline. Data were analyzed and interpreted by the authors. The study adhered to the tenets of the Declaration of Helsinki. In the Ophthalmology Clinic of S.B.U. Konya Education and Research Hospital, the electronic medical records of the patients over 18 years who were diagnosed with dry eye disease (DED) after 2008 by an ophthalmologist and who had an ocular lubrication drug use report (hydroxyethylcellulose, lanolin, polyacrylic acid, sodium hyaluronate, polyvinylpyrrolidone (PVP), sodium chloride, or potassium chloride) were retrospectively reviewed. In order for a patient to have a drug use report, the scheimer test must be 5 mm or below, as well there is no copayment for patients with a drug use report in Turkey. The reason of drug use report in Turkey is that The Ministry of Health aims to facilitate free access to the drug in patients who should always use the drug. Patients, who have not come for control examinations in the previous year before the pandemic began; patients with a malign tumor; patients with bone marrow transplantation; or HIV positive patients were excluded from the study. The age and sex of each patient were recorded.

DED patients who met the study criteria were identified. Afterwards, the patients who applied to the COVID-19 outpatient clinic with suspected COVID-19 infection among these patients until February 11 October 2020 were determined. We obtained the medical records and compiled data for these patients. A confirmed COVID-19 case according to WHO was defined as a positive result Real-Time Reverse-Transcriptase–Polymerase-Chain-Reaction (RT-PCR) assay of nasal and pharyngeal swab specimens [14]. Only laboratory-confirmed cases were included in the analysis. In order to a PCR test to be performed in our hospital, the patient must have at least two symptoms of fever, shortness of breath, novelization, diarrhea, cough, headache, loss of taste and smell.

Ministry of Health of Republic of Turkey divides the coronavirus patients into 6 groups in terms of age (under 15; 15–25; 25–49; 50–65; 65–80; and 80 and above). The age groups under 15 and 15–25 according to this classification were excluded, because the patients under the age of 18 were not included in the study. The patients then were divided into 4 age groups (25–49; 50–64; 65–80; and 80 and above).

Statistical analysis

Data were collected and analyzed using SPSS version 22.0 (SPSS, Inc., Chicago, IL, USA) for all statistical analyses. All DED patients and general population of Turkey were stratified by age groups and PCR test result. The prevalence of COVID-19 cases per 100,000 people were determined for each age group. The numbers of people in each group were compared using chi-square statistics. The correlation among variables was evaluated by using Pearson correlation analysis.

Results

In the medical data recording system, a total of 12,916 patients whom an ocular lubrication drug report was prescribed were detected. Of these patients, 10,023 patients

(2350 male, 7673 female) over the age of 25 who met the study criteria were identified as shown in the Table 1. Among all those patients, 2213 of them were aged 25 to 49 years (22.1%), 3409 of them were aged 50 to 64 years (34%), 3387 of them were aged 65 to 79 years (33.8%), and 1014 of them were aged 80 years and older (10.1%). In total, 739 of patients (208 male, 531 female) applied to COVID-19 polyclinic with the suspicion of COVID-19 and the PCR test of 359 patients (101 male, 251 female) were positive. Ninety-five of the patients whose PCR test were positive were aged 25 to 49 years, 116 of them were aged 50–64, 106 of them were aged 65–79, and 42 of them were aged 80 and above.

According to the weekly situation report of the Turkish Ministry of Health COVID-19 dated 05-11/10/2020, the demographic distribution of the patients was as shown in Table 2. Among all those notified patients, 166,402 persons were aged 25 to 49 years (49.4%), 63,278 persons were aged 50 to 64 years (18.8%), 29,575 persons were aged 65 to 79 years (8.8%), and 8294 persons were aged 80 years and older (2.5%). The age is unknown for 1 notified patient [15].

According to Turkish Statistical Institute (TUIK) data, the population of Turkey is 83,154,997. There is a total of 50,986,980 individuals aged 25 and over. There are 30,816,114 people in the 25–49 age group, 12,620,139 in the 50–64 age group, 6,047,884 people in the 65–79 age group, 1,502,846 people over 80 (Table 2) [16].

In persons over 25 years of age, COVID-19 prevalence per 100,000 DED patients were 3581.7; while in the general Turkish population it was 524.7. COVID-19 prevalence per 100,000 by age groups in DED patients were 4292.8 in the 25–49 age group, 3402.7 in the 50–64 age group, 3129.6 in the 65–79 age group, and 4142 in the 80 and above age group. In the general Turkish population, these rates were 539.9 in the 25–49 age group, 501.4 in the 50–64 age group, 489 in the 65–79 age group, and 551.8 in the 80 and above age group (Fig. 1) The difference between the two groups were statistically significant ($P < 0.001$).

The risk ratio of DED patients getting COVID-19 disease was evaluated in all age groups (aged 25 and above) in general, as shown in the Table 3. The risk of DED patients (aged 25 and above) getting COVID-19 disease compared to the general population was 6.62. It was 7.66 in 25–49 age group, 6.59 in 50–64 age group, 6.23 in 65–80 age group, and 7.24 in the 80 and above age group. The results were statistically significant for all groups (Pearson χ^2 0.00, $P < 0.001$).

Discussion

More detailed information about the SARSCoV-2 is urgently needed to take under control its pandemic spread. SARS-CoV-2 principally spread by virus-containing respiratory droplets expelled by infected individuals and direct contact with virus-contaminated fomites [4]. During close contact with infected people and contaminated surfaces, the eye can be easily exposed anatomically to droplets and fomites that include viruses. Although liquid in the surface of the eye is partially absorbed by the cornea and conjunctiva, it mostly drained through the nasolacrimal duct and then transported toward the respiratory tract [17]. For these reasons, conjunctiva can be considered to be an important entry gate for respiratory viruses, as well conjunctival

Table 1 Number of DED patients and their PCR data by age groups.

Age of group	25–49	50–64	65–79	80+	Total (25+)
COVID-19 suspect DED patients	224	242	202	71	739
PCR +	95	116	106	42	359
PCR –	129	126	96	29	380
Total DED patients	2213	3409	3387	1014	10 023

PCR: polymerase-chain-reaction; DED: dry eye disease.

Table 2 Total number of COVID-19 patients in Turkey and general Turkish population by age groups.

Age groups	25–49	50–64	65–79	80+	Total (25+)
Number of total COVID-19 patients in Turkey	166,402	63,278	29,575	8294	267,549
Turkey population	30,816,114	12,620,139	604,7884	150,2843	50,986,980

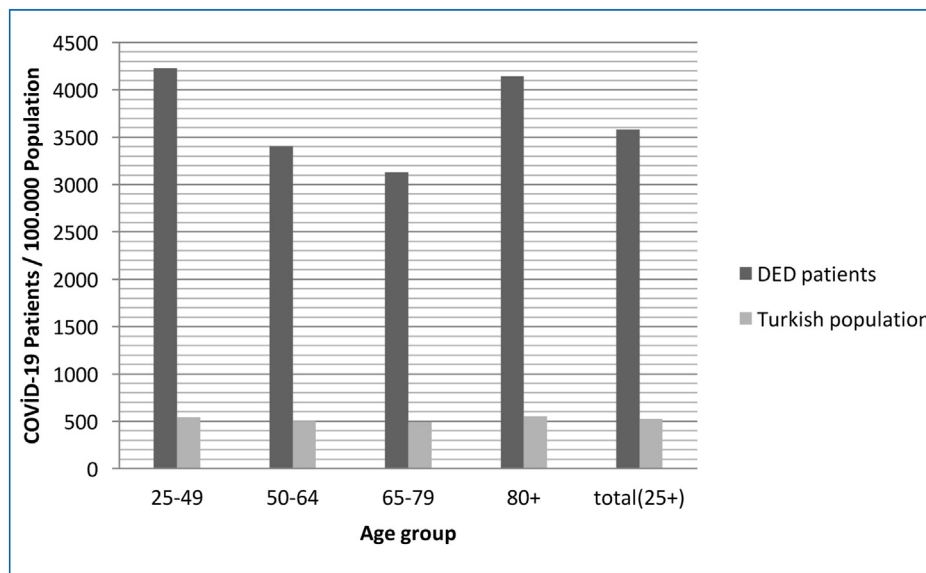


Figure 1. COVID-19 patients per 100,000 by age group in DED patients and general Turkish population. DED: dry eye disease.

Table 3 The risk ratio and 95% confidence interval of DED patients compared to general population of Turkey.

Age group	Risk ratio	95% confidence interval	
		Lower	Upper
25–49	7.66	6.29	9.33
50–64	6.59	5.51	7.89
65–79	6.23	5.16	7.52
80+	7.24	5.38	9.75
Total(25+)	6.62	5.98	7.33

secretions and tear may contain viruses and spread viral infections [4,12].

The invasiveness of the virus, target receptors on the host cell membrane, and the immune conditions of the host are the main factors for viral entrance efficacy into host cells [18,19]. ACE 2 acts as the key cell surface receptor for SARS-CoV-2, which binds the viral spike protein, and TMPRSS2, which provides viral entry following viral spike

protein binding to ACE2, is an important cell surface protease [20,21]. After immunohistochemical analysis, ACE2 expression was shown in conjunctiva, limbus and cornea, especially with pronounced staining on the superficial conjunctiva and corneal epithelial surface and also confirmed by Western blot analysis of ACE2 and TMPRSS2 expression in human corneal epithelium, thus the human eye surface has been shown to be susceptible to SARS-CoV-2 infection [22].

Detailed investigations have revealed that Heparan Sulfate Proteoglycans (HSPGs) facilitated further binding of SARS-CoV to ACE2 [23]. The viruses are continuously eliminated on the ocular surface with the contribution of antimicrobial agents including lactoferrin (by preventing the adhesion of SARS-CoV to its attachment receptor, HSPGs) [24] and secretory IgA (by helping to kill both bacteria and viruses) in tears and constant tear rinsing [13]. In addition, IgA binds to the SARS-CoV-2 spike protein and blocks the interaction of the host with the ACE2 receptor and finally lysozyme and lipocalin may have some effect on preventing SARS-CoV-2 from entering the body via the ocular surface [25].

The studies done so far have found that, compared to the tracheal inoculation, conjunctival inoculation of COVID-19 resulted in a mild lung infection [26] and (kerato) conjunctivitis is a rare manifestation of COVID-19 [4,27,28]. Also, SARS-CoV-2 RNA was detected in only five per cent of ocular surface swabs (9/178) [29]. The fact that coronaviruses caused mild infection by conjunctival inoculation and that detection of low amounts of sars-cov-2 RNA on the ocular surface in coronavirus patients were attributed to the protective effect of tears [18].

At the time of writing, this study was the only research to assess the association between DED and COVID-19 infection to our knowledge. It was determined that people involuntarily touch their eyes about 10 times per hour [30]. It is also known that ACE-2 and TMPRSS2 are present on the ocular surface [31]. In DED patients, it was found to be a weakening of the physical barrier of the eye (epithelium damage, tear instability) and a decrease of the amount of antimicrobial agents (slgA, lysozyme, lactoferrin etc.) in tears [9,11,32]. Also, Dr. Li Wenliang, who was the first to raise the alarm about the coronavirus, had caught the COVID-19 virus from an asymptomatic glaucoma patient [33]. Lastly, commonly used dry eye treatments do not seem to contribute to reducing the risk of infection [11]. Aforementioned facts support our hypothesis on DED and COVID-19 infection risk.

We found that 359 persons out of 10,023 DED patients were diagnosed with COVID-19. The COVID-19 prevalence per 100,000 population of DED patients was 3581.7. When this rate is compared to the data of the Turkish Ministry of Health, the risk of COVID-19 is 6.62 times higher in DED patients than the general population. It was found to be important that the risk rate (7.66) higher in the 25–49 age group with the highest number of COVID-19 cases in 100,000 people (539.9) and the risk rate (6.23) was lower in the 65–79 age group with the least number of COVID-19 cases in 100,000 people (489). These findings show that risk of COVID-19 is also increased in DED patients in age groups with a high rate of COVID-19 in general population ($P < 0.05$). In other words, while the risk is lower in age groups with fewer cases in the general population, the risk is higher in age groups with more cases (Table 3). These findings support that the ocular surface is one of the important transmission routes in the COVID-19 outbreak, especially in DED, in which ocular immunity is weakened.

In conclusion, this study found that the risk of SARS-CoV-2 transmission was 6.62 times higher in DED patients compared to general population, suggesting that DED is associated with more susceptibility to COVID-19 infection. These findings support that the eye may be an entry gate for SARS-CoV-2; moreover, tear stability, the most important part of ocular immunity, is also an important part of systemic immune system. DED patients should take extra prevention like frequent hand hygiene and appropriate personal protection to avoid SARS-CoV-2 transmission during the current pandemic.

Ethics

All procedures performed in studies involving human participants comply with the ethical standards of the institutional and/or national research committee and the 1964 Declaration of Helsinki and its subsequent amendments or

comparable ethical standards. Informed consent was not required for this study and this study was approved by Necmettin Erbakan University Ethics Committee.

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Disclosure of interest

The authors declare that they have no competing interest.

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