ORIGINAL PAPER



The impact of prolonged face mask use on ocular surface health during COVID-19 pandemic: a clinical, and conjunctiva impression cytology study

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Received: 9 December 2022 / Accepted: 19 October 2023 © The Author(s), under exclusive licence to Springer Nature B.V. 2024

Abstract

Purpose To investigate the relationship between prolonged face mask use and ocular surface health utilizing conjunctival impression cytology, the Schirmer test, the tear break-up time (TBUT) test, and the ocular surface disease index (OSDI) questionnaire.

Methods In this cross-sectional prospective study, individuals who used face masks for at least eight hours per day for at least six months were compared to healthy volunteers who used face masks for no more than one hour per day. Each participant completed an OSDI questionnaire. The Schirmer test (under anesthesia), the TBUT test, and conjunctiva impression cytology analysis according to the Nelson classification method were performed on each participant.

Results This study included 102 (49 male, 53 female) face mask users with an average age of 33.29 ± 7.71 years and 110 (60 male, 50

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female) healthy controls with an average age of 32.96 ± 7.10 years (p = 0.746). The total OSDI score was significantly higher in face mask users than the control group $(25.18 \pm 3.54 \text{ vs } 9.46 \pm 2.13, p < 0.001)$. The mean Schirmer test value and TBUT were significantly lower in the study group than the control group (p < 0.001, p < 0.001). There was a statistically significant difference between the two groups in total score and stage of the Nelson classification system (p < 0.001, and p = 0.024, respectively). All conjunctiva impression cytology assessments, including cellularity, cell-cell contact, nucleus/cytoplasma ratio, goblet cell amount, and metaplasia, revealed statistically significant deterioration in the study group compared to the control group (p < 0.001, p = 0.025,p < 0.001, p < 0.001, and p < 0.001, respectively).

Conclusion The prolonged use of face masks leads to dry eyes. The findings of conjunctiva impression cytology indicate the role of inflammation in the pathogenesis of mask associated dry eye.

 $\label{eq:constraint} \begin{array}{l} \textbf{Keywords} \quad Face \mbox{ mask } \cdot \mbox{ Dry } eye \cdot \mbox{ Conjunctiva} \\ \mbox{ impression } cytology \cdot \mbox{ Tear } break-up \mbox{ time } \cdot \mbox{ Schirmer } \cdot \\ \mbox{ OSDI } \end{array}$

Introduction

The tear film layer performs critical functions such as improving visual quality by enhancing corneal transparency, hydrating the eye surface, providing

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nutritional support, protecting ocular stem cells, and providing antimicrobial activity [1]. A stable tear film layer is crucial for maintaining ocular surface health, ensuring comfort, and optimizing vision quality. Both internal and environmental factors can disrupt the balance between tear production and evaporation, ultimately resulting in dry eyes [2]. Environmental factors such as allergens, medications, the use of contact lenses, air conditioners, and low-humidity conditions may cause hyperosmolar tears and commence an inflammatory response [3, 4]. Inflammatory cytokines disturb the tear film layer by triggering apoptosis in the cornea and conjunctival epithelium, leading to squamous metaplasia, goblet cell loss, and disruption in mucin production [5–7].

The use of face masks in public areas has become prevalent as a precaution against the severe acute respiratory syndrome coronavirus-2 (SARS CoV-2) pandemic. An increase was recorded globally in the number of patients presenting to the ophthalmology department with dry eye complaints such as burning, stinging, ocular irritation, allergic symptoms, foreign body sense, and visual fluctuation during the pandemic [8, 9]. This disorder was described as "maskassociated dry eye (MADE)" [10]. Although there are several studies investigating MADE, most of them only used surveys or tear function tests. We aimed to evaluate the relationship between face mask use and ocular surface health by utilizing ocular surface disease index (OSDI) scores, tear function tests, and conjunctival impression cytology in our study.

Methods

Ethical approval

Current study was conducted in compliance with the norms of the Helsinki Declaration after getting permission from the ethics committee at Sureyyapasa Chest Diseases and Thoracic Surgery Training and Research Hospital (Approval number: 115/2021). Informed consent was obtained from each participant at the beginning of the research.

Study population

This cross-sectional comparative study includes patients who applied to the Ophthalmology

Department of Sancaktepe Sehit Prof. Dr. Ilhan Varank Training and Research Hospital between January 2022 and March 2022, when the use of masks was mandatory due to the pandemic. The study included participants aged eighteen and above who used face masks for an average duration of eight hours or more per day (study group) and participants who used face masks for one hour or less per day (control group) during a minimum period of six months.

In accordance with pandemic protocols, the wearing face masks in communal spaces became obligatory. However, using a mask at home was not mandatory. The healthy control group comprised individuals who often engage in activities within their residence during the pandemic, such as homemakers or those who are unemployed. Individuals who reported engaging in screen activities for more than 2 h per day were excluded from both groups. All participants in the study were using a standard face mask made of the same material and design.

The exclusion criteria were as follows: history of ocular and eyelid surgery or trauma; history of blepharal and periorbital skin disease; conjunctiva or eyelid disorder such as ptergium, entropion, and ectropion; ocular diseases such as glaucoma, uveitis, and strabismus; history of dry eye; herpes zoster infection; and wearing contact lenses; wearing glasses; refractive error greater than 1 diopters; bestcorrected visual acuity (BCVA) less than 20/20; systemic disorders such as diabetes, rheumatism, psoriasis, and rosacea; medications; smoking; pregnancy; breastfeeding; daily screen activities for excess of two hours.

Ophthalmological examination

A detailed ophtalmological examination, including measures of spherical equivalent, best-corrected visual acuity (BVCA), biomicroscopic anterior segment, and fundus examination was performed for each participant by an experienced ophthalmologist (S.K.). The right eye of each participant was assessed.

The OSDI (Allergan, Irvine, CA, USA) questionnaire was then administered to each participant to assess their symptoms. The OSDI consists of 12 items divided into three categories: ocular symptoms, reactivity to environmental factors, and visual functions. Each item's response is scored on a scale from 0 (never) to 4 (always). The total OSDI score is measured from 0 to 100. A higher OSDI score indicates more severe symptoms.

Following the OSDI questionnaire, Schirmer tests (under anesthesia) and tear break-up time (TBUT) tests were used to assess tear functions. Basal tear secretions were assessed by applying the 35×5 mm filter paper strip (Clement Clark International Ltd., UK) of Schirmer test to the outer onethird of the inferior fornix for five minutes under topical anesthetic (0.5% proparacaine HCl, 0.5% Alcaine, Alcon) in all cases. The paper strip was prevented from touching the cornea. It was considered moderate dry eye if the wetness on the Schirmer test paper was less than 10 mm, and severe dry eye if it was less than 5 mm [1, 4]. Fluorescein-impregnated sheets were moistened and touched to the inferior fornix to measure TBUT. The fluorescein was dispersed throughout the ocular surface after the participants were told to blink. The tear film layer was then examined using a biomicroscope with a cobalt blue filter. Duration was recorded from the last blink until the first sign of corneal dryness was observed. Less than 10 s of TBUT were defined as dry eye [1, 4]. Both the Schirmer test and the TBUT test are non-invasive procedures that pose no risk to the individuals being tested.

Histopathological examination

A Conjunctival impression cytology specimen from each case was obtained in the ophthalmology department using the Egbert et al. technique [11]. The conjunctival impression cytology examination is a non-invasive diagnostic technique that involves obtaining a swab from the individual's conjunctiva. The cellulose asetat filter paper (11,107–47 N; Sartorius, Gottingen, Germany, pore size 0.20 μ m) was cut into 4×5 mm pieces, and placed in the superior bulbar conjunctiva 5 mm away from the limbus with a smooth ended forceps under topical anesthesia (0.5% proparacaine HCl, 0.5% Alcaine, Alcon). After gently pressing for 5 s, the filter paper was carefully removed from the bulbar conjunctiva and put in a sterile container with 95% ethyl alcohol solution.

The fixed specimens were stained using the Papanicolaou technique, washed in xylene (Merck KGaA, Darmstad, Germany), fixed in Entellan (Merck KGaA, Darmstadt, Germany). The specimens were then simultaneously evaluated under a light microscope (Axio Lab.A1, Carl Zeiss, Gottingen, Germany) by an experienced, blinded pathologist (F.B.) using the Nelson classification method for the staging of squamous metaplasia of the epitelial cells [12]. According to the Nelson classification method, each specimen was examined under 5 subheadings, including cellularity, intercellular contact, nucleus/cytoplasm ratio, quantity of goblet cells, and metaplasia, and scored from 0 to 3 for each subsection. A total score was calculated for each specimen, and staging was conducted. The total cytology score was categorized as stage 0 with a score of 0-3; stage 1 with a score of 4-5; stage 2 with a score of 6-9; and stage 3 with a score of 10 or above. A higher stage indicates a more severe disorder.

Statistical analysis

The statistical analysis was performed using IBM SPSS Statistics for Windows, version 23.0 (Statistical Package for Social Sciences, Chicago, Illinois, USA). The Kruskal–Wallis test was performed to analyze the normality of the variables' distributions. The variables with a normal distribution were analyzed using an independent sample Student's *t* test and expressed as mean \pm standard deviation, while variables with a non-normal distribution were analyzed using the Mann–Whitney U test and expressed as median (25–75%). The categorical variables were assessed using Fischer's exact test and described as numbers and percentages.

Results

This study included 102 (49 male, 53 female) face mask users with an average age of 33.29 ± 7.71 years and 110 (60 male, 50 female) healthy controls with an average age of 32.96 ± 7.10 years. There was no statistically significant difference in terms of age and gender between the two groups (p=0.746, and p=0.209, respectively). According to the Schirmer test results, 26 subjects (25.5%) had moderate dry eyes, 24 subjects (23.5%) had severe dry eyes, and 52 subjects (51%) had no dry eyes in the face mask user group. However, 22 subjects (20%) had moderate dry eyes, 2 subjects (1.8%) had severe dry eyes, and 86 subjects (78.2%) had no dry eyes in the control group. The mean Schirmer test value in the study group was 7 (3–10) mm, while it was 17 (12–18) mm in the control group (p < 0.001). The TBUT in the study group was 5.64 ± 1.32 s and 11.81 ± 1.8 s in the control group (p < 0.001). OSDI score was measured as 25.18 ± 3.54 in the study group and 9.46 ± 2.13 in the control group (p < 0.001). The demographic and clinical characteristics of both groups are represented in Table 1.

Histopathological findings of two groups are displayed in Table 2. The total Nelson classification score was 7.55 ± 2.02 in the study group, and 3.00 ± 1.13 in the control group. There was a statistically significant difference between the two groups in total score and stage of the Nelson classification system (p < 0.001, and p = 0.024, respectively). All subgroup assessments, including cellularity, cell-cell contact, nucleus/cytoplasma ratio, goblet cell amount, and metaplasia, revealed statistically significant deterioration in the study group compared to the control group (p < 0.001, p = 0.025, p < 0.001, p < 0.001, andp < 0.001, respectively). Furthermore, we detected inflammatory cells in the specimens of 64 subjects in the study group but only in the specimens of 12 subjects in the control group.

Discussion

In the current study, we discovered that using a face mask for an extended period of time had negative impacts on OSDI score, tear function tests, and impression cytology when compared to an age- and gender-matched control group.

According to the OSDI survey results in our study, face mask users have greater ocular irritation symptoms, resulting in impaired visual functions and lower quality of life. The higher OSDI score of face mask users in our study was consistent with previous studies in the literature [13–18]. Schiffman et al. [19] reported that the OSDI is a highly valid, accurate, sensitive, and specific test for determining the frequency of dry eye symptoms and visual functions related to quality of life.

In our study, Schirmer test findings revealed that 25.5% of the face mask users exhibited moderate dry eye, while 23.5% had severe dry eye, resulting in a total prevalence of 49%, whereas dry eye was diagnosed in 21.8% of subjects in the control group, with

 Table 2
 Histopathological findings of the two groups based on Nelson classification system

Variables	Study group ($n = 102$)	Control group $(n=110)$	р
Cellularity			
0	12 (11.8%)	70 (63.6%)	< 0.001**
1	42 (41.2%)	30 (27.3%)	
2	45 (44.1%)	10 (9.1%)	
3	3 (2.9%)	0 (0.0%)	
Cell-cell cont	act		
0	3 (2.9%)	10 (9.1%)	0.025**
1	75 (73.5%)	84 (76.4%)	
2	24 (23.5%)	16 (14.5%)	
Nücleus/ cytoplasma ratio	0.82 ± 0.15	0.00 ± 0.00	< 0.001*
Goblet cell			
1	15 (14.7%)	90 (81.8%)	< 0.001**
2	36 (35.3%)	20 (18.2%)	
3	51 (50%)	0 (0.0%)	
Metaplasia			
0	6 (5.9%)	60 (54.4%)	< 0.001**
1	33 (32.4%)	50 (45.5%)	
2	57 (55.9%)	0 (0.0%)	
3	6 (5.9%)	0 (0.0%)	
Total score	7.55 ± 2.02	3.00 ± 1.13	< 0.001*
Nelson grade			
0	37 (36.3%)	50 (45.5%)	0.024**
1	24 (23.5%)	36 (32.7%)	
2	23 (22.5%)	13 (11.8%)	
3	18 (17.6%)	11 (10.0%)	

* Independent sample student t test

** Fisher's exact test

20% exhibiting moderate dry eye and 1.8% experiencing severe dry eye. The results of the TBUT test similarly supported the findings of the Schirmer test and revealed a high rate of dry eye in the study group. Results of tear function tests suggest that prolonged face mask use hampers the production of all three components of the tear film layer, including aqueous, mucin, and lipid. The worsening in TBUT and Schirmer test results due to the mask use was in line with previous research [13–15]. Since these studies were conducted during a period when face mask use was mandatory in public places and outdoors, they were unable to examine a control group of healthy

Variables	Study group $(n = 102)$	Control group $(n = 110)$	р
Age (year)	33.29±7.71	32.96±7.10	0.746*
Male	49 (48%)	60 (54.5%)	0.209**
Spherical equivalent (D)	-0.15 ± 0.47	-0.03 ± 0.48	0.058*
Schirmer (mm)	7 (3–10)	17 (12–18)	< 0.001***
TBUT (s)	5.64 ± 1.32	11.81 ± 1.8	< 0.001*
OSDI score	25.18 ± 3.54	9.46 ± 2.13	< 0.001*

Table 1 Demographic and clinical findings of the two groups

TBUT tear break up time, OSDI ocular surface disease index

* Independent sample student t-test

** Fisher's exact test

*** Mann whitney U test

people who did not wear masks. For this reason, they compared the findings of individuals before and after using masks on the same day. But we compared the outcomes of individuals who wore masks for over six months to those of a healthy control group that did not wear masks. Using a better control group, we produced safer test findings and corroborated the literature results.

White et al.[10] described MADE, for the first time, a novel ocular disease associated with the coronavirus pandemic. Several studies in the literature indicate that prolonged mask use increases the frequency and severity of dry eye [8, 9, 13–15]. However, most of the studies assessing the association between face mask use and ocular surface alterations often included only online surveys, questionnaires, Schirmer, and TBUT tests. There is a limited amount of research that includes comprehensive examinations for the etiopathogenesis of MADE. In the majority of studies, it has been proposed that enhanced evaporation owing to air leakage from the mask may play a role in the etiology of MADE. In a more comprehensive study, Mastropasqua et al.^[20] assessed in vivo confocal microscopy, conjunctival impression cytology, fluorescein staining, and lissamine staining test results, in addition to tear function tests, and questionnaire, in people who wore face masks at baseline, the first month, and the third month. He discovered an increase in fluorescein and lissamine staining, a decrease in corneal dendritic cell density using in vivo confocal microscopy, and an increase in HLA-DR expression using conjunctival impression cytology in the first and third months of face mask users. These findings indicate the existence of inflammation in the etiology of MADE [21–23]. However, he found no change in goblet cell density using in vivo confocal microscopy. On the other hand, we discovered a reduction in goblet cell density in mask wearers after performing a more comprehensive conjunctival impression cytology analysis using the Nelson classification technique. Conjunctival impression cytology is more sensitive than confocal microscopy at detecting changes in the number of goblet cells when triggers are present [12]. In addition, Mastropasqua et al. [20] assessed the effects of mask use for a maximum of three months, but we evaluated the effects of mask use for over six months.

Previous studies often attribute the etiology of MADE to increased evaporation caused by exhaled air escaping from the face mask. The findings of Schirmer and TBUT tests in our study suggest that MADE may affect the lipid, aqueous, and mucous layers of tears. However, in evaporation-induced dry eye, the lipid layer is primarily affected. It is also well documented that inflammation is the primary cause of dry eye etiology [1, 2]. In our study, the findings of conjunctiva impression cytology of face mask users, including loss of goblet cells, decreased cell connections, deterioration in cell morphologies, squamous metaplasia, and presence of inflammotory cells, indicate that inflammation is a significant factor in the etiology of MADE [24]. Mastropasqua et al. [20] also emphasized the role of inflammation in the pathogenesis of MADE. D'Souza et al.[17] reported that tear-soluble factors, including various cytokines, interleukins, interferons, and inflammatory cells are increased in face mask users. She claimed that CO2 stress caused by hypercapnia in face mask users might be the source of inflammation [25]. Park et al.[26] stated that using a face mask for six hours induces changes in skin temperature, redness, and hydration. Previous studies have revealed an association between the use of masks and the development of allergic skin responses and dermatitis [27]. We also think that an allergic skin reaction to the mask's synthetic material might produce inflammation. This inflammatory response might extend to the periocular area via local propagation, subsequently impacting goblet cells. Moreover, the act of often touching the face and periocular region as a result of wearing a mask might potentially elicit an inflammatory response.

The limitation of our study is that we did not perform a correlation analysis between conjunctiva impression cytology findings and other test findings. Besides, it may be beneficial to make a gender-based assessment. In addition, more extensive research is required to explain the mechanism of the inflammatory process in the pathogenesis of MADE. Furthermore, it could be beneficial to explore the functions of meibomian glands in individuals with MADE.

Conclusion

In the current study, we discovered an increase in OSDI scores, worsening in the Schirmer and TBUT tests, and dry eye-related abnormalities in conjunctiva impression cytology in long-term face mask users. To the best of our knowledge, this is the first study on MADE that has utilized conjunctiva impression cytology according to the Nelson classification technique in the literature. The conjunctiva impression cytology findings of our study indicate the importance of inflammation in the pathogenesis of MADE in addition to evaporation. In addition, one of the advantages of our study is that the control group consists of healthy individuals who do not wear face masks.

Author contributions All authors contributed to the study conception and design. Material preparation, data collection and analysis were performed by SK, FB, and ASK. The first draft of the manuscript was written by SK, and all authors commented on previous versions of the manuscript. All authors read and approved the final manuscript.

Funding Each author declares that no funds, grants, or other support were received during the preparation of this manuscript.

Declarations

Conflict of interest Each author declares that there is no conflict of interest on this study.

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