Objectives: For a definitive diagnosis of COVID-19, respiratory tract samples are evaluated by polymerase chain reaction (PCR). In our study, PCR using a tear sample was used to diagnose COVID-19, and it was questioned whether it was a screening method. Unlike the general practice, Schirmer strips were used instead of a swab for tear sample collection in this study. In addition, the diagnostic values of serum procalcitonin (PCT), C-reactive protein (CRP), and Neutrophil (NEU) count in predicting COVID-19 disease from tears were also questioned.

Methods: A total of 94 patients who were positive for COVID-19 by PCR test were included in this study. Tear samples were obtained from patients with Schirmer strips, commonly used in eye examination, and studied with the PCR technique. CRP, PCT value, and NEU count were also compared between the positive and negative groups of the PCR. The obtained data were analyzed using the R Studio software, and the results were considered statistically significant for p<0.05.

Results: Of these patients, 61 (64.9%) tear PCR was negative, and 33 (35.1%) tear PCR was positive. The mean age was 61.72 ± 17.62 years. The patients were divided into two groups: tear PCR positive and negative. There was no significant age difference between these groups. As a result of ROC Analysis; When serum PCT, CRP, and NEU % values were examined in predicting COVID-19 disease from tears, it was seen that CRP (p=0.027) and especially PCT (p=0.003) values of patients with PCR-positive were significantly higher.

Conclusions: PCR study on tears collected with Schirmer strips is a different and non-invasive method, but it was concluded that the proposed method could not be used as a screening test. In addition, significantly higher serum PCT values were found in patients with COVID-19 positivity in tears (p<0.05).

Keywords: novel coronavirus disease; PCR; SARS-CoV-2 virus; schirmer strip; tear.

Introduction

The new SARS-CoV-2 (Severe acute respiratory syndrome coronavirus-2), is an enveloped, single-stranded RNA virus belonging to beta-coronaviruses that can cause disease in many vertebrates, including humans [1]. Human coronaviruses (HCoV) usually cause mild upper respiratory tract and enteric infections, while the strain known as SARS-CoV-2 causes a severe respiratory illness called COVID-19 [2]. Past studies indicate that the genetic makeup of SARS-CoV-2 is similar to that of SARS-CoV-1, which caused the 2002 pandemic [3, 4]. Like SARS-CoV-1, SARS-CoV-2 is also the angiotensin-converting enzyme agent in varied cell types and tissue, including the conjunctiva. It enters the system by recognizing enzyme 2 (ACE2) as its host receptor [5–7]. Prolonged shedding of SARS-CoV-2 was also detected. By real-time reverse transcriptase-polymerase-chain reaction
(RT-PCR) assay, feces samples of infected individuals and isolated from mucous membranes. In addition, indirect contact with bodily fluids of infected patients, such as saliva, feces, urine, and tears, may be effective [8–10].

Regarding the eye, the virus can be detected by RT PCR testing in conjunctival swab samples from patients infected with SARS-CoV-2. Infectious aerosols in HCoV-infected individuals may cause ocular complications and respiratory tract infections following ocular exposure [11, 12]. Detection of SARS-CoV nucleic acid in the tears of HCoV patients has been associated with the disruption of the blood-retinal barrier, such as conjunctivitis, retinal vasculitis, retinal degeneration, and retinal disorders [13–15].

The technique of detecting various viruses and infections in tears using PCR is widely used by ophthalmologists [16, 17]. The study performed by collecting tears from SARS-Cov patients with the conjunctival swab technique is the first recorded case series for detecting SARS coronavirus from tears. This work significantly impacts ophthalmology and medical practice [18, 19].

Similarly, in this study, SARS-CoV-2 was investigated by PCR from tear samples in patients with positive COVID-19 PCR tests in nasopharyngeal swabs. The new and different aspect of our research was using Schirmer strips, which are less traumatic and less invasive than the conjunctival swab technique. In addition, C-reactive protein (CRP), procalcitonin levels, and neutrophil count were studied as markers of infection/inflammation in the blood. Also, the relationship between the clinical picture and the test results from tears was investigated.

Materials and methods

Sampling

This prospective case series involved 94 COVID-19 patients hospitalized in the COVID-19 clinic of the Republic of Turkey Ministry of Health Gülhane Training and Research Hospital providing tertiary care between 30 June and 10 September 2021 and whose PCR test was positive. In this study, adult patients with positive COVID-19 tests, whose hospitalization did not exceed 20 days, were used. The reason for this is that PCR positivity rarely lasted more than 20 days in past studies. Since a sufficient number of patients could not be reached simultaneously, samples had to be taken on different days. Laboratory findings (including PCT, NEU, and CRP) were retrospectively obtained from the clinical records. Patients under 18 years of age with any ocular results such as conjunctivitis, epiphora, conjunctival hyperemia, and eye irritation, and patients treated in the intensive care unit were excluded from the study lastly, the patients treated in the intensive care unit were excluded from the study. All patients were verbally informed about the study in conformity with the rules of the Declaration of Helsinki. Approval for this prospective study was obtained from the Health Sciences University Gülhane Scientific Research Ethics Committee (17.06.2021 decision no. 2021/293).

Procedure

Sampling was done by the same ophthalmologist on different days in four separate visits in total and avoided contamination using Schirmer paper. For each patient whose tear PCR sample was collected, the corresponding date of hospital admission was recorded. Each strip of Schirmer paper (no. 41 Whatman filter paper, 5 × 35 mm) was folded one millimeter, and the folded portion was inserted into the lower lid in the outer third of both eyes. As with routine eye examination, after filter paper insertion, the patients were asked to keep their eyes open, and wink as usually as possible. The strips were removed after 3 min. Tears samples were obtained from the lower fornix using Schirmer strips. After sampling, Schirmer strips were placed into 1.5 mL capacity microcentrifuge tube containing vNAT® Viral Nucleic Acid Buffer (Bioeksen R&D Technologies Incorporated Company, Turkey) and stored at 2–8 °C until further steps for PCR assays.

RNA extraction was performed on the day of sampling. Viral RNA was extracted using Bosphore® Viral RNA Extraction Spin Kit (Anatolia Geneworks®, Turkey). Firstly, an extraction mixture consisting of 40 µL proteinase K, 10 µL carrier RNA, and 5 µL internal control was prepared for each sample and transferred to microcentrifuge tubes. Then, the tear samples in the vNAT® buffer were vortexed for 1 min. After vortexing, 400 µL of the sample was mixed with the extraction mixture, and RNA extraction was performed according to the manufacturer’s instructions. Lastly, RNA was collected in a 60 µL elution buffer. If the extracted RNA was to be used immediately for quantitative reverse transcription PCR (RT-qPCR), it was kept at 4 °C. Otherwise, it was stored at −20 °C for further analysis.

RT-qPCR was performed using the CFX96 TouchTM Real-Time PCR System (Bio-Rad, USA). Using the SARS CoV-2 Double Gene Rt-qPCR Kit (Bioeksen R&D Technologies Incorporated Company, Turkey), the RT-qPCR process was applied with a total reaction volume of 20 µL containing a 15 µL mixture and 5 µL of template nucleic acid for each sample. The amplification curves obtained in the FAM channel for SARS-CoV-2 (N) and SARS-CoV-2 (ORF1ab) genes and the HEX channel for the human RNase P gene were evaluated to the recommendations of the manufacturer. Non-sigmoidal curves were recorded as negative. Ct values of ≥38 in the FAM channel were defined as positive.

CRP and PCT levels of the patients were determined from blood samples taken into vacuum blood collection tubes. Neutrophil counts were studied in whole blood samples collected in ethylenediaminetetraacetic acid (EDTA) tubes. The neutrophil count was determined with the Beckman Coulter Unicel DxH 800 device within 1 h after collecting diagnostic blood samples. CRP levels were measured by an immunoturbidimetric method using Beckman Coulter OSR6147 reagent on a Beckman Coulter AU 680 autoanalyzer. Using the Access PCT Immunoassay Systems kit, PCT levels were also studied on a Beckman Coulter Dxl 600 analyzer. Inflammatory biomarkers (including CRP, PCT, and NEU) were compared between two groups with positive and negative PCR in tears.

Statistical analysis

All statistical analyses were done using R Studio Version 3.6.2. The Shapiro-Wilk test was used for normal distribution. To compare two
groups, the Mann-Whitney U test was utilized. We used binary logistic regression to test the factors’ efficacy on tear PCR positivity.

The diagnostic decision-making properties of serum PCT, CRP, and NEU% values to predict COVID-19 disease were analyzed by Receiver Operating Characteristics (ROC) curve analysis. In the evaluation of the area under the curve, the cases where the Type-1 error level was below 5% were interpreted as the diagnostic value of the test was statistically significant.

**Results**

A total of 94 patients, 49 men, and 45 women, whose PCR tests are (+) and who were hospitalized in the COVID-19 services of Gülhane Training and Research Hospital between 30 June and 10 September 2021, were included in our study. The average the patients’ age was 61.72 ± 17.62 years. Of these patients, PCR tests were negative for 61 (64.9%) people, and PCR tests for the rest of the patients were found to be positive (33 people [35.1%]). The patients were separated into two groups as tear PCR positive and negative. No significant differences in ages were detected. CRP, PCT value, and NEU count were also compared between PCR positive and negative groups. It was seen that the CRP (p=0.027) and PCT (p=0.003) values of the patients with positive PCR were significantly higher. There was no significant difference in terms of neutrophils (Table 1). It was also found that the sampling day for PCR was noticeably earlier in these patients than in those who were negative (Figure 1).

The diagnostic decision-making properties of serum PCT, CRP, and NEU% values to predict COVID-19 disease were analyzed by ROC curve analysis. In the presence of significant cut-off points, these limits’ sensitivity, specificity, positive predictive, and negative predictive values were calculated.

As a result of the evaluation made with ROC analysis, the diagnostic values of serum PCT, CRP values, and NEU % count in predicting COVID-19 disease from tears were determined and are shown in Figure 2.

PCT was found to have a diagnostic value compared to other tests (p<0.05) (Table 2).

Binary logistic regression was utilized to study the impact of CRP, PCT values, and NEU count on tear PCR positivity on the day of PCR. None of these factors could be shown to significantly impact tear PCR positivity (p=0.441).

The mean PCR examination day was 3.55 ± 3.01 days in the group with positive tear samples, while it was 5.11 ± 3.72 days in the negative group. The day of sampling of tears for PCR testing was significantly earlier in these patients compared to those who were negative.

**Discussion**

This study detected 35.1% positivity by PCR test performed on tear samples of COVID-19 patients whose nasopharyngeal swabs were positive. Our study found a higher positivity in the tears of COVID-19 patients compared to many of the past reports. Although the method we used is less traumatic and easy, when we look at the positivity rate obtained, we see that it is not appropriate to apply this method for screening purposes.

**Table 1**: Distribution of CRP, Procalcitonin, and Neutrophil results according to the PCR results of the participants.

|                          | Positive | Negative | Z     | p-Value* |
|--------------------------|----------|----------|-------|----------|
| Day of PCR (n=94)        | 3.55 ± 3.01 | 3 (0–11) | 5.11 ± 3.72 | 4 (0–22) | −2.309 | 0.021 |
| CRP, mg/L (n=94)         | 68.44 ± 61.41 | 65 (1.82–248.4) | 46.17 ± 49.79 | 27 (0.8–183.32) | −2.216 | 0.027 |
| Procalcitonin, ng/mL (n=82) | 0.27 ± 0.37 | 0 (0.03–1.66) | 0.22 ± 0.66 | 0 (0.01–4.65) | −2.991 | 0.003 |
| Neutrophil, % (n=94)     | 75.54 ± 18.74 | 80 (3.1–96) | 75.89 ± 14.15 | 79 (41.9–94.3) | −0.429 | 0.668 |

*Mann-Whitney U Test.

**Figure 1**: The boxplot of the values that differ between groups.
Various studies have shown that the SARS-CoV-2 virus infects primarily through respiratory droplets. Apart from this main transmission, many studies show transmission through body secretions. The eye can be an essential gateway for some respiratory viruses, such as SARS-CoV-2, through the nasolacrimal canal due to the anatomical link between the upper respiratory tract and the mucosa of the ocular surface sharing the same entrance [20]. The first information about the virus was recorded by an ophthalmologist, Dr. Li Wenliang, who worked in an eye clinic, and later died from this disease [21]. In this study, we conducted viral isolation and quantitative RT-PCR analysis. We tried to describe the probability of diagnosis through tears by evaluating the collected samples. Nasopharyngeal swab samples were routinely examined during the beginning of the hospitalization of the patients, while tear samples were taken for research purposes only at random times. Tears were sampled by an ophthalmologist using a Schirmer test strip at time points ranging from 3 to 20 days after the hospitalization. Precautionary measures were taken to prevent the contamination of samples. The number of ocular samples was doubled by collecting tears from both eyes. Although conjunctival swab has been generally accepted as the main method for assessment of viral RNA, we used Schirmer test

![Figure 2: Calculation of the sensitivity and specificity values of these limits in the presence of serum PCT, CRP, and NEU% values.](image)

| Variables | AUC     | Std. error | Cut-off | p-Value<sup>a</sup> | %95 CI     | Positive predictive value | Negative predictive value |
|-----------|---------|------------|---------|---------------------|------------|--------------------------|---------------------------|
| PCT, ng/mL| 0.703   | 0.064      | >0.03   | 0.0016              | 0.592–0.799| 44.4                     | 89.3                      |
| CRP, mg/L | 0.624   | 0.068      | >11.64  | 0.0665              | 0.510–0.729| 41.7                     | 90.9                      |
| NEU (%)   | 0.533   | 0.069      | >87.8   | 0.669               | 0.40–0.644 | 47.6                     | 72.1                      |

<sup>a</sup>Receiver operating characteristics (ROC) curve analysis.

Table 2: Diagnostic value of serum PCT, CRP, and NEU% values in predicting COVID-19 in tears.
strips for tear collection as it is less traumatic and practical. The Schirmer strip tear collection method has been previously validated in other studies [19]. The role of the ocular surface has been extensively investigated as a possible portal of entry for SARS-CoV-2 RNA, a reservoir for replication, and a mediator for its delivery [21–23]. To our knowledge, either precomeal tear film from only one eye was tested in the published literature, or tear samples from both eyes were transferred to separate VTMs (Viral Transport Media) [24]. In our study, tear samples from both eyes at the same time are transferred to the same VTM to increase the amount of tears. In light of the literature, tear samples were taken, usually in the early stages of the disease (3–20 days) and not exceeding 20 days, when viral fragments could be detected [25, 26]. Viral load decreases in the second and third weeks of symptoms. The detection rate of viral RNA in tears taken usually varies between 0 and 10% [27]. However, in Hanege’s study positivity rate is found to be 55.3% (21 people out of 38 people in total); this is the highest rate in the literature. In our study, a higher rate compared to most of the studies may have been found due to the above reasons. The past studies marked that the conjunctival swab remains as the main method for tear sampling for RT-PCR testing [26].

In the most recent studies reported, it is reported that conjunctival swabbing for tear sampling remains the main method for RT-PCR testing.

CRP, Procalcitonin values, and Neutrophils count were also compared between tear PCR positive and negative groups. The ROC analysis examined the diagnostic values of serum PCT, CRP values, and NEU count in predicting COVID-19 disease from tears. CRP (p=0.027) and especially PCT (p=0.003) values were significantly higher in patients with positive results for the tear samples’ PCR tests. It was observed that the CRP and PCT values of the patients whose tears tested positive in PCR were significantly higher. There was no significant difference in terms of NEU count. This was interpreted as the fact that the acute phase reactants in the patients were high, parallel to the severity of the clinical condition. There is a relationship between PCT and CRP levels in systemic infections. Although there are studies suggesting CRP in the follow-up of such diseases because of its lower cost [28], we observed that PCT has a more significant diagnostic value in predicting COVID-19 disease than other tests in our study (p<0.05). In addition, as expected, the tear PCR sampling day was detected earlier in the positive group. However, significant heterogeneity of up to 80.3% may be due to differences in techniques and sample collection time between different studies, patients’ clinical conditions, and test administration methods [27]. We observed a slight increase in the rate of positive tear RT-PCR testing for SARS-CoV-2 compared to previous studies. We collected samples from both eyes during the early days of hospitalization when the viral load appeared to be high. This may explain our high rate.

There may be some limitations in our study. When the tests of SARS-CoV-2 positive patients became negative in the nasopharyngeal sample, it would have been much more meaningful to take tears and search for the virus with PCR. But the study could not be done because it was planned on inpatients and during hospitalization. Again, due to time constraints, the ability to take a tear sample once should be added to the limitations as a weak point of the study. In addition to possible short-term viral shedding with tears, the presence of a small amount of viral RNA in conjunctival secretions may have been insufficient to detect the virus in ocular samples by RT-PCR [29]. In addition, one-time sampling from patients and different sampling days were the factors limiting our study. In such studies, providing a sufficient amount of tear samples is necessary. It may be better to study with a larger sample size in a multicenter group by sampling multiple tears during the disease. Another limitation was the inability to perform simultaneous PCR of the nasopharyngeal and tear samples. And finally, the last limitation of this study is the absence of a nasopharyngeal PCR negative group. A population-based cross-sectional study can be designed in larger groups by adding such a group.

Conclusions

In the literature, there are various studies with different positivity rates in diagnosing COVID-19 in tears. Although SARS-CoV-2 virus positivity in the tears of COVID-19 patients was found to be higher (35.1%) in our study compared to past studies, it does not seem appropriate to recommend this method as a screening test.

In addition, in our study, we aimed to draw attention to the fact that tear sampling using Schirmer strips is less traumatic and easy to apply. Larger samples should be used for more meaningful results. Our study is important because it raises new questions about the methods that can be used in the diagnosis of COVID-19. We also want to signify the importance of investigating such different diagnostic methods in the future.

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