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Original Research Article

Conjunctival Swab Testing for COVID: Worth the Effort?

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Abstract:

Purpose: To evaluate the presence of COVID-19 viral RNA in conjunctival secretions of moderate to severely ill hospitalized patients with COVID-19 and to find its association with clinical and laboratory characteristics of patients.

Methods: Total of 98 subjects from the COVID-19 admission unit with laboratory-confirmed SARS-CoV-2 infection was included. Presence of any ocular manifestations were noted and asked for via a questionnaire and RT-PCR conjunctival swab testing for Corona virus was done by on duty ophthalmologist.

Results: 51(52.04%) out of 98 subjects were males. 11 patients (11.2%) had viral RNA detected by RT-PCR, 4(4.08%) patients had inconclusive results and remaining were negative. In the positive group males significantly outnumbered females [p=.008], 5(45.45%) had some co-morbid condition, 2(18.18%) of them had conjunctivitis and the TLC (total leukocyte count) and NLR (neutrophil lymphocyte ratio) were significantly lower in this group [p=.01]. Mortality rate in conjunctival swab positive patients was 54.54% (6 out of 11), while it was 24.13% (21 out of 87) in swab negative patients.

Conclusion: Lower TLC and NLR along with appreciably higher mortality rates in the positive group tells us that the severity of the disease cannot only be judged by the laboratory parameters but also by the wide dissemination of the virus in other tissues like conjunctiva and thus conjunctival swab testing can be used as an additive tool and serve as a marker of prognostic value.

Keywords: Conjunctival swab testing, coronavirus, TLC, NLR.

Introduction

A novel coronavirus (CoV) named severe acute respiratory syndrome coronavirus–2 (SARS-CoV-2) emerged from China in December 2019. It is a highly transmissible, potentially fatal disease which transmits through infected person's respiratory droplets [1,2]. Symptoms can appear within 14 days of exposure but mostly symptoms are seen after about 5 days.

The main symptoms of COVID-19 are fever, cough, fatigue, dyspnoea, sore throat, headache, and gastrointestinal complaints like diarrhoea. Many complications in severe cases such as respiratory failure, pneumonia, shock and multi organ failure can also occur [3,4]. Regarding the ocular involvement, the references in the medical literature of this disease are scarce so far, but some case reports have highlighted the presence of conjunctivitis [5]. Since the diagnosis of COVID-19 patients cannot be only based on symptoms, till date the most accepted way for testing for Coronavirus has been reverse transcriptase-polymerase chain reaction (RT-PCR) which is a simple and reliable molecular test on respiratory samples (throat swab/ naso pharyngeal swab/oropharyngeal/ endo tracheal aspirates and broncho-alveolar lavage). Some authors have suggested testing of saliva as an alternative as it is non-invasive and non-aerosol generating and has

good sensitivity and sensitivity [6,7,8]. This motivated us to evaluate the presence of COVID-19 viral RNA in conjunctival swabs of moderate to severely ill hospitalized patients with COVID-19 and to find its association with clinical and laboratory characteristics of patients.

Material and Methods

This study was conducted at a tertiary care centre by a resident ophthalmologist posted in the COVID wards from 11th May to 20th May, 2021. This is a cross sectional study in which moderate to severely ill, nasopharyngeal/oropharyngeal swab RT-PCR confirmed hospitalized patients were included.

The inclusion criteria were as follows: over 18 years of age, in moderate to severely ill condition (according to CURB-65 score) and ability to give consent. Patients who were admitted to the intensive care unit, unable or unwilling to give consent, unable to adequately report previous eye symptoms due to general health status, COVID-19 suspect and having mild disease were excluded. Signed consent was also obtained for each sample collection.

The patients were made to fill a questionnaire regarding their ocular complaints in simple yes/no question format. Information was also collected from the hospital records consisting of demographic details, exposure history, systemic symptoms, systemic illness, radiological findings, laboratory tests, ocular symptoms, and ocular signs. Ocular examination was done using a torch light and conjunctival swabs were taken by the doctor during ward rounds by maintaining at least 1m distance and wearing proper PPE.

Eyelid was everted and sample was obtained by sweeping the inferior fornices of either of the two eyes with sterile nylon flocked swabs without topical anaesthesia. The details of the ocular examination were recorded. Conjunctival swabs were sent to the Microbiology department for RT-PCR testing for novel Coronavirus. Laboratory work-up included the total leukocyte count, neutrophil lymphocyte ratio, LFT (liver function test), RFT (renal function test) and other relevant investigations. The data collected was entered into a Microsoft Excel Sheet and the statistical analysis was performed using EPI-INFO Statistical software. A p value of <0.05 was considered significant.

The study was conducted after approval from the Ethical Committee Board of the State Medical College.

Results

The study was carried out on patients present in the Covid wards of the hospital from 11th May to 20th May, 2021. Out of 212 patients present there, 98 patients met the inclusion criteria and were included in the study. 11 patients out of 98 tested positive for COVID-19 in conjunctival swab by RT-PCR, 4 had inconclusive reports and 83 were negative. Male to female ratio was almost similar (52.04% v/s 47.96%). 15 out of 98 patients had at least one co-morbid condition (Table 1). Mean age was slightly higher in conjunctival swab positive group than the negative group (60.9±10.76 v/s 50.7±14.33) [p value = 0.094]. Males significantly outnumbered females in positive group (10:1) whereas ratio was reverse in the negative group where females were more than males (10:1 v/s 41:46)[p value = 0.008]. Significantly higher number of patients had at least one co-morbid condition in positive group compared to negative group (45.45% v/s 11.4%) [p value = 0.012].

In the positive group 2 (18.18%) patients had Type 2 DM (Diabetes Mellitus) and 1 (9.09%) patient each had Hypertension, COPD (chronic obstructive pulmonary disease), chronic kidney disease and pancytopenia respectively whereas in the negative group (negative plus inconclusive), 6 (7.22%) had Type 2 DM, 4 (4.81%) were hypertensive, 2 (2.40%) had hypothyroidism and 1 (1.20%) patient each had COPD, morbid obesity and 8 months pregnancy respectively. 4 (36.36%) and 3 (27.27%) had ocular complaints and signs respectively in the positive group compared to 29 (33.33%) and 44 (50.57%) respectively in negative group. Higher number of patients had conjunctivitis in the positive group compared to negative group (18.18% v/s 8.04%) [p value = 0.266]. In laboratory parameters, the TLC (Total leukocyte count) was 7.25 ± 2.56 in the positive group compared to 11.21 ± 3.21 in the negative group [p value = 0.01] whereas NLR (neutrophil to lymphocyte ratio) was 9.72 ± 2.12 and 15.81 ± 4.12 in the positive and negative group respectively [p value = 0.01][Table 2].

Significantly higher number of patients died in the positive group compared to negative group (54.54% v/s 24.13%) [p value = 0.066].Mean hospital stay in the positive group was 9.9 ± 7.05 days compared to 12.59 ± 7.14 days in the negative group [p value = 0.990] [Table 3].

Table 1: Socio-demographic profile of study subjects

Age in Yrs	51.84±14.35 Yrs
Male : Female	51:47
Patients with co-morbid conditions	15 out of 98

Table 2: Association between Conjunctival swab result and clinical and laboratory parameters

	Negative (n=87)	Positive (n=11)	P value
Mean age	50.70±14.33	60.90±10.76	0.094
Sex (M:F)	41:46	10:1	0.008
Religion (Hindu : Muslim)	79:8	10:1	1.000
Co-morbid conditions	10 (11.49%)	5 (45.45%)	0.012
Ocular complaints	29 (33.33%)	4 (36.36%)	1.000
Ocular signs	44 (50.57%)	3 (27.27%)	0.204
Conjunctivitis	7 (8.04%)	2 (18.18%)	0.266
TLC	11.21±3.21	7.25±2.56	0.01
NLR	15.81±4.12	9.72±2.12	0.01

Table 3: Association between duration of hospital stay and mortality with Conjunctival swab result

	Negative (n=87)	Positive (n=11)	P value
Mean Hospital stay	12.59±7.14	9.90±7.05	0.990
Mortality	21(24.13%)	6(54.54%)	0.066

Discussion

The mucosa of the conjunctiva is directly exposed to infectious droplets which are expelled by the patients during close contact and when there is hand-eye contact via contaminated hands. Moreover, the conjunctiva and the mucosa of the upper respiratory tract is connected by nasolacrimal duct, and both share the same entry receptor of SARS-CoV-2 which is angiotensin converting enzyme 2 (ACE2), on the membranes of the host cell. Hence, it is reasonable to point out that conjunctiva can act as a route of transmission as it can be easily involved in SARSCoV-2 infection [9].

Initially, none of the reports suggested ocular transmission of COVID-19. A study conducted at Zhejiang University evaluated the conjunctival secretions of 30 confirmed cases (60 eyes) of COVID-19. In only one of these patients which had conjunctivitis, conjunctival secretions were tested positive for the virus by RT-PCR [10]. Another study by Chen et al. detected SARS-CoV-2 in the conjunctival sac of 3 patients out of 67 COVID-19 positive cases [11]. These studies do show that SARS-CoV-2 can be detected in conjunctival sac, but only in a small percentage of COVID-19 positive patients. In our study, out of 98 confirmed positive patients of COVID-19, coronavirus was detected through conjunctival secretions of 11 patients and 4 had inconclusive results.

In our study, we compared the conjunctival swab positive and negative groups for various clinical and laboratory parameters. Though the mean age of positive group was slightly higher compared to negative group but the difference was not significant. In positive group males significantly outnumbered females [p=.008] which was consistent with findings of Kumar K1 where single confirmed case was a male and Chen et al [11] where out of 3 positive and probably positive cases two were males. Probably the reason behind this male preponderance is that males are more social and outgoing compared to females and so are more exposed to crowded places where infectious droplets can enter conjunctiva. Similar percentage of patients had ocular complaints in both the groups. RT-PCR positivity from patients having conjunctivitis was not significantly higher compared to patients not having it. The finding is consistent with the findings of Kumar K1 and Chen et al [11] where none of the RT-PCR positive patient had conjunctivitis. In the study by Xia J et al [10] single positive patient had conjunctivitis.

Significantly higher number of patients in the positive group had co-morbid conditions compared to negative group [p=.012] which seems logical as patients having co-morbid conditions have low immunity leading to more viral proliferation and dissemination.

Multiple earlier studies have reported that higher TLC and high NLR are associated with severe COVID-19 [12,13,14,15,16,17]. In our study the positive group had significantly low mean TLC and NLR compared to negative group [p=.01]. We could not find any other study which tested this association during literature search. Mortality in the positive group (54.54%) was appreciably more than the negative group (24.70%) though it was not statistically significant. May be wide viral dissemination to many body parts is a better marker of severity of the disease as compared to laboratory parameters. Duration of hospital stay was lesser in the positive group compared to the negative group which may be explained by the more mortality in the positive group thereby leading to lesser mean stay.

There are several limitations in this study such as single centre study with a relatively small sample size which lacks inclusion of mild cases, lack of detailed ocular examination due to difficulty in safe access and requirement of additional resources in the covid wards, sampling done only once from the

eyes of each patient which can increase the prevalence of false-negatives, poor preparation of the patients as conjunctival swab testing was done without topical anaesthesia which can be a painful procedure.

Conclusion

Possibility of conjunctival transmission of COVID-19 is still controversial and has considerable public health consequences. Good number of patients in our study had RT-PCR positivity in conjunctival secretions. This may be because of either conjunctiva acting as portal of entry or because of viral dissemination in various body secretions. In our study, the conjunctival swab positive group consisted of less severe patients (based on TLC and NLR) but had a higher mortality rate while the negative group had more severe patients as per blood counts but had a lesser mortality rate. Hence, it can be said that severity can not only be judged on the basis of blood counts and HRCT scores, but also by wide dissemination of the virus into other tissues of the body like conjunctiva. And so, conjunctival swab RT-PCR testing may possibly be used as an additive tool in moderate to severe patients of Covid-19 to assess severity. Also, positive result's relation to mortality gives us a better understanding of the disease.

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