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Original Research Article

Conjunctival Swab Positivity for SARS-Cov-2 Viral Nucleic Acid in Nasopharyngeal Swab rRT-PCR Confirmed COVID-19 Cases in a Tertiary Care Hospital

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Abstract: ***Background***: India was a part of Covid-19 pandemic & experienced a massive surge of cases during second wave. Tears & conjunctival secretions were also thought to be source of infection. Our study aimed to estimate the prevalence of conjunctival swab positivity for SAR-COV-2 by rRT-PCR and to find an association between ocular symptoms & conjunctival swab (CS) positivity in patients with nasopharyngeal Swab (NPS) confirmed Covid-19 disease. ***Material & Methods***: It was a prospective, international study on patients with NPS rRT-PCR Confirmed covid -19 disease. Complete history, Systemic & ocular examination along with basic parameters were documented. Clinical information about hospitalization & respiratory aid was recorded. Within 2 days of NPS, CS was taken. Statistical Analysis: Data was analyzed by Microsoft Excel SPSS (Chicago Inc) Software for window & open epi software (version 3). Yates corrected Chi-square test was used to test association & prevalence was reported as a point estimate with 95% CI. ***Results:*** Out of 76 admitted patients. 72 NPS confirmed Covid -19 positive cases was included. Prevalence of CS positivity was 5.56% (4 patients) Mean age of sample was 64.11+- 20.408 years. Prevalence of CS positive in males was 10% & in females 2.38%. Prevalence of CS positivity in isolation ward was 1.61% where as in ICU it was 30%. Prevalence of CS positivity was 1.59% in Patients on high flow oxygen therapy, 25% in patients on BiPAP and 66.67% in patients on ventilator. Study showed OR-17.4, i.e. patients with ocular symptoms had 17.4 times more risks of CS positivity as compared to patients with no ocular symptoms. The association between ocular symptoms & CS positivity is statistically significant. ***Conclusion***: Over all Prevalence of CS positivity is 5.6%. With increase in severity of disease, the prevalence of CS positivity increases and there is positive association between ocular symptoms & CS positivity.

Keywords: Covid-19 pandemic, SARS-Cov-2, rRT-PCR, CS positivity.

INTRODUCTION

India was a part of world wide pandemic of Corona virus disease 2019 (COVID-19) caused by severe acute respiratory syndrome Corana virus 2 (SARS COV -2). On January 2020 the World Health Organization (WHO) characterized the outbreak as a Public health emergency of international concern. The first case of covid -19 disease in India was reported on 30th January 2020 and then it spread rapidly. In September 2020 the cases declined but again in march 2021 India experienced a massive surge in covid-19 positive cases and deaths were much larger than the first wave as the daily count was double of the first peak on April 2021. As per WHO in April 2021, one in every three new cases globally was reported in India. Soon India became second largest in case load globally [1]. With all new variants in circulation which were spreading at a very fast pace, we needed to rule out any spread through body fluids like tears . The virus can be transmitted through various body fluids but insufficient data is available to validate the presence of virus in human tears [2]. The first case series with detection of

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SARS coronavirus from tears was by Loon SC *et al*., in 2004 [3]. A close contact of an infected patient may get inoculated with virus by droplet transmission [4].

During the recent outbreak of the coronavirus2019, a large number of ophthalmologist were reported to be infected [5]. Viral particles have also been detected in conjunctival secretions of SARS-CoV-2 patients who present with conjunctivitis. So tearsand conjunctival secretions of infected patients can also be a source of infection [6, 7].

There is limited data available on ocular sampling from patients with COVID-19.This study was done to find prevalence of conjunctival swab(CS) positivity amongst the nasopharyngeal swab(NPS) positive patients and to find an association between ocular symptoms and CS positivity.

MATERİAL AND METHODS

This was a prospective, interventional study was conducted at a peripheral Government medical college, a New Government Medical College, from May-2021 to February 2022 after getting approval from the institutional ethical committee (IEC). The study was conducted adhering to principles of Declaration of Helsinki.

Out of 76 covid 19 positive patients 72 patients were taken who fulfilled the inclusion criteria .The inclusion criteria included all admitted patients with at least one nasopharyngeal swab positive for SARS Cov 2 with or without ocular symptoms. Patients less than 5 years old were excluded from study. Patients diagnosed or suspected of mucormycosis were excluded. All other admitted patients suffering from non COVID-19 respiratory illness were excluded from this study.

Informed consent was taken from the patients in written as well as vernacular language before taking history and conjunctival swab sample collection. SOPs were followed and adequate precautions were taken before taking complete systemic as well as ocular history and collection of sample.

Presenting symptoms and ocular manifestations occurred during the disease in positive patients were noted. Basic parameters including body temperature, oxygen saturation (Spo_2), respiratory rate and pulse rate were documented at the time of hospitalization and examination findings of the physician were also recorded. Baseline investigations included a complete blood profile and X-ray chest findings were noted.

Before the conjunctival swab procedure, an ophthalmologist examined the status of eyelids, conjunctiva, and cornea. Eye examination was done at the bedside. Clinical information about hospitalization timing, results of diagnostic and serological examinations, and type of respiratory device was recorded. We also documented results of the last nasopharyngeal swab for each patient. Within two days of nasopharyngeal swab, conjunctival swab taken. The sampling procedure was performed at the bedside by the same ophthalmologist in both eyes. The samples were obtained without topical anesthesia and after sufficient time had elapsed since lacrimal substitutes had been used.

Procedure for Sampling of Tears: The swab was leaned on the caruncula for 5 seconds. It was then slowly moved across the exposed inferior fornix conjunctiva to the external fornix within 5 seconds. The samples were obtained without topical anesthesia and after sufficient time had elapsed since lacrimal substitutes had been used. In all patients, the conjunctival swabs were performed in the right eye first.

Subsequently, the tips of the swab sticks were broken off after collecting the sample and placed into a viral transport medium and were transferred to the microbiology department of this institute without any interruption to the cold chain for further analysis where real-time RT-PCR was performed to detect the viral RNA genome and virus load in each samples of spread of infection from one patient to another. Real-time RT-PCR was carried out using commercial, Indian Council of Medical Research approved SARS-CoV 2 RT-PCR kit.

Statistical Analysis:

All the collected data was entered in the microsoft excel sheet and analyzed by using the Microsoft Excel SPSS (Chicago INC) software for window and open epi software (version 3). The qualitative data and quantitative data were reported as proportions/percentage and (mean ± sd) respectively. Yates corrected Chi-square test was used to test the association of Conjunctival Swab Results with clinical symptoms and ocular symptoms. Prevalence was reported as a point estimate along with 95%confidence interval. A p-value less than 0.05 was considered as significant. All p-values were two tailed.

Observations:

Out of 76 admitted covid-19 patients, 72 confirmed positive cases of COVID-19 by at least one nasopharyngeal swab with or without ocular symptoms were included in the study. 2 patients were below 5 years and 2 patients were discharged before conjunctival swab sampling, so total 4 patients were excluded from the study.

Out of 72 patients, 4 patients were conjunctival swab rRT PCR positive. The prevalence of conjunctival swab rRTPCR positivity in the sample was 5.56%.

Out of 72, the age of the patients ranged from 10 years to 85 years, mean age being 64.11±20.408 years. Out of 72 patients, 30 were males and 42 patients were females. Baseline mean systolic blood pressure of the patients was 120.53± 84.113mmHg and mean diastolic blood pressure was 71.61± 10.639mmHg. mean SPO_2 was 91.60±5.673%.Mean respiratory rate was found to be 21.90± 4.685per minute. The mean body temperature of patients was 99.19± 1.667°F. Mean baseline pulse rate was 89.38± 11.299 per minute (Table 1).

Table 1: Distribution of different parameters of the subjects studied

Parameters	N	Mean	Std. Deviation	Minimum	Maximum
Age	72	46.11	20.408	10	85
SBP	72	120.53	18.113	94	177
DBP	72	71.61	10.639	36	110
SpO2	72	91.60	5.673	77	99
R.Rate	72	21.90	4.685	16	35
Fever(F)	72	99.19	1.667	90	102
Pulse Rate	72	89.38	11.299	61	112

Out of 4 conjunctival swab positive cases 3 were males 1 was female. The prevalence of conjunctival swab positive in males was 10.00% and in females was 02.38% (Table 2).

Table-2: Gender Distribution of subjects

Gender	No. with Conjunctival swab positive /total no. of RT-PCR positive	Prevalence (95% C I)
Males	3/30	10.00(3.46 – 27.68)
Females	1/42	02.38(0.42 - 12.32)

Out of 72 hospitalized patients 62 were admitted in the isolation ward and 10 in ICU. The prevalence of conjunctival swab positive in the Isolation ward was 01.61 (1 patient out of 62) whereas in ICU the prevalence of conjunctival swab was 30% (3 out of 10).

Out of 72 admitted patients 63 patients were on high flow Oxygen therapy, 4 were on BiPAP (Bilevel positive airway pressure) 2 were on CPAP (Continuous positive airway pressure) and 3 were on ventilators.

The Prevalence of conjunctival swab positivity in patients on high flow oxygen therapy was 1.59%, on BiPAP therapy was 25%, on CPAP therapy was 0 and on ventilator was 66.67% (Table-3).

Table-3: Prevalence of Conjunctival Swab positive according to different variables

Variables	No.with Conjunctival Swab positive /total no. of RT-PCR positive	Prevalence (95% C I)
Isolation Ward	1/62	01.61(0.29 – 8.58)
ICU	3/10	30.00(10.78 - 60.32)
High Flow Oxygen	1/63	01.59(0.28-8.46)
BIPAP	1/4	25.00(4.56-69.93)
CPAP	0/2	0
Ventilator	2/3	66.67(20.77-93.85)

The patients presented commonly with symptoms of fever (60%), sore throat (50%), cough (36%), breathlessness (30%), nausea (6%), and vomiting (4%) (Table-4). There was an overlap of symptoms in some patients.

Table 4: Statistical association between systemic symptoms and conjunctival swab results

Clinical Symptoms	Conjunctival Swab Results		Total	P-Value
	Positive	Negative		
Fever	4	28	32	0.396
Sore throat	2	27	29	0.133
Breathlessness	4	11	15	0.436
Nausea	0	3	3	*
Vomiting	0	2	2	*
Cough	2	19	21	0.487

* Cannot be calculated because of the presence of zero.

Out of 4 conjunctival swab positive patients 3 had ocular symptoms {Conjunctivitis (2 patients) and Foreign body sensation (1 patient)}. Patients with ocular symptoms have 17.4 times more risk of conjunctival swab positivity as compared to the patients who have no ocular symptoms (OR-17.4). The association between ocular symptoms and conjunctival swab positivity is statistically significant (P<0.05) using Fischer`s exact test (Table-5).

Table 5: The association between the proportion of patients with ocular symptoms and the results of conjunctiva swab

Ocular Symptoms	Conjunctival Swab Results		Total	Odds Ratio(95% C I)	P-Value
	Positive	Negative			
Positive	3	10	13	17.4 (1.64-184.4)	0.017
Negative	1	58	59		
Total	4	68	72		

DISCUSSION

In our study the overall prevalence of conjunctival swab positivity in the nasopharyngeal swab rRT-PCR confirmed COVID-19 patients was 5.56% (4 out of 72). In a study by Kaushik J *et al*., [4], out of 30 enrolled patients only two (6.7%) were positive for rRT-PCR SARS-COVID-19 in conjunctival swab. In a study on 102 patients by Zhang X *et al*., [8], out of 72 laboratory confirmed COVID-19 patients only two patients (2.78%) had conjunctivitis, of these two patients, one patient tested positive via rRT-PCR from conjunctival swab .Another study by Wu P *et al*., [9], stated a prevalence rate of 5.2%, 95% CI, 0.6-17.8 of SARS CoV-2 nucleotide in conjunctival specimens of patients with COVID-19. In a prospective study by Xia J *et al*., [5], out of 30 enrolled patients only one patient was conjunctival swab rRT-PCR positive. Loon *et al*., [3], found the prevalence of conjunctival swab positivity to be 37.5%while in our study the prevalence is 5.56%. In another study by Zhou Y et al [10], of all the 67 cases enrolled in the study, one was positive for the conjunctival swab rRT-PCR. In a study by Seah *et al*., [11], 64 samples of tears of COVID positive patients, all showed negative RT-PCR result.

The mean age of our study group was 46±20.408 years which corresponds with a study by Kaushik *et al*., [4] 44.80±15.37 years while the mean age(SD) in a study Azzolini C *et al*., [2] was 58.7(14.2) years. While in the study by Wu P *et al*., [9] it was 65.8±16.6 years. The mean age in a study by Meduri A, *et al*., [12] was 77.1±12.6 years (range 44-92years). In our study the prevalence of conjunctival swab positive in the Isolation ward was 01.61% (1 patient out of 62) whereas in ICU the prevalence of conjunctival swab was 30%.ICU admission can be related to severity of disease and thus our study showthat with increase in severity of diseases the prevalence of CS positivity increases. In a study by Azzolini C *et al*., [2], the percentage of conjunctival swab positive in patients with covid admitted in ICU was 17.3%, while 46.2% in high intensity Medicine department and 36.5% in infectious disease department.In our study the prevalence of conjunctival swab positivity in patients on high flow oxygen therapy was 1.59%, on BiPAP therapy was 25%. In a study by Ralli M *et al*., [2], they stated that invasive maneuvers and respiratory devices like continuous positive airway pressure masks or helmets may increase the risk of viral diffusion by creating a closed environment around the head. A study by Azzolini C *et al*., [2] found no association between respiratory device use and conjunctival swab results.

In a stidy by Seah *et al*., [11], out of17 patients none demonstrated ocular symptom, though one developed conjunctival congestion and chemosis during the stay in hospital. Zhou *et al.*, [10] noted ocular findings in eight patients of a group of 121 with confirmed Covid-19 disease and documented a correlation between ocular involvement and the severity of the covid-19 disease. But only one of them showed positive results for CS. 2 patients without ocular symptom showed positive CS. A study by Meduria *et al*., [12], shows that a significant correlation exist between ocular sign and symptoms and severity and the duration of disease however none of the patient for tear film analysis showed a positive result for SARS-CoV-2. In a study Kaushik *et al*., [4], the co-relation between clinical symptom and CS positivity was not statisticslly significant. Where as in our study, the association between ocular symptoms and conjunctival swab positivity is statistically significant (P<0.05). This conjunctival infection can be from air borne droplets as well as from particles diffused in atmosphere or from body fluids [14-16].

Conjunctival infection can be ffrom air borne droplets asa well as fromparticles diffused in atmosphere [13, 14]. This study has few limitations: we could not determine the infectivity of the viral material detected and thus the definitive clinical relevance. Other limitations include the cross-sectional design of the study, which lacks long-term prospective evaluation of the patients.The limited number of negative conjunctival swab in the presence of a positive nasopharyngeal test in patients with COVID-19 may be due to difficulties in patients' tear sampling or sampling time lag.

CONCLUSION

Overall Prevalence of conjunctival swab positivity in nasopharyngeal swab positive Covid -19 Patients was 5.56%. We found a positive association between ocular symptom and conjunctival swab positivity.With the increase in severity of diseases the Prevalence of conjunctival swab positivity increased.

Conflict of Interest: The authors share no conflict of interest, of any form.

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