RESEARCH ARTICLE

Red eyes: COVID-19 or Microbial infection

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Abstract

Objective: To identify the source of eyes infections during coronavirus disease-2019 pandemic.

Method: The cross-sectional study was conducted at the Al-Basrah Teaching Hospital, Iraq from March 2 to September 24, 2020, and comprised eye swabs from patients having confirmed diagnosis of coronavirus disease-2019. The swabs were subjected to microbiological and molecular examinations. They were cultivated on blood agar and savoured dextrose agar plates to detect the types of microbes and then confirmed by genetic analysis using polymerase chain reaction. Chi-square was used to identify the differences between samples who had symptoms or those who had no symptoms $p \leq 0.05$ was considered significant.

Results: Of the 213 patients quarantined with coronavirus disease-2019, there were 98(46%) males and 115(54%) females. Of them, 22(10.3%) had red eyes; 10(45.45%) males and 12(54.54%) females, with overall age ranging 18-74 years. Streptococcus pneumonia was detected in 8(36.6%) patients, followed by staphylococcus aureus and candida albicans 5(22.7%) each, and haemophilus influenzae 3(13.6%).

Conclusion: Red eyes of some patients with coronavirus disease-2019 may have been caused by secondary infection.

Key Words: Agar, Staphylococcus aureus, Photophobia, Polymerase chain reaction, Glucose, Pruritus.

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Introduction

Since the beginning of the coronavirus disease-2019 (COVID-19) in Wuhan, China there were well over a hundred million confirmed cases and over a couple of million deaths reported worldwide.[1] A coronavirus member, called the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), was found to be the causative agent of the pandemic.[2] There have been several reports of eye redness and irritation in COVID-19 patients, both anecdotal and published, suggesting that conjunctivitis may be an ocular manifestation of SARS-CoV-2 infection.[3]

A study conducted during the 2003 SARS outbreak detected SARS-Covid in tear samples in SARS patients in Singapore.[4,5]

The scientists mobilised their efforts to understand the symptoms and the route of the infection. A specialist in ophthalmology in Wuhan was the first scientist who noticed strange SARS-like pneumonia cases, and, unfortunately, he died of it a month later.[6]

The virus was highly contagious and could use nose, mouth and eyes to infiltrate the body.[6] A red or pink eye was considered to be one of the symptoms related to COVID-19. However, the newness of the disease and conflicting information meant no one was sure whether the eye redness was caused by the virus itself, or was it a secondary infection.[7] Some studies reported that the redness appeared in the early stage of COVID-19 infection[8], while another said that the symptom showed at the mid-stage.[9] Chinese researchers reported that about 1% of patients had developed conjunctivitis,[5] whereas other reports observed eye symptoms in a majority of COVID-19 cases.[6]

Symptoms like eye redness, dryness, itchiness, burning and grittiness, foreign body sensation, light sensitivity with tears, and conjunctiva swelling were detected in COVID-19 patients in different stages of the infection.[7,8]

A study tried to isolate SARS-CoV-2 from the tears of COVID-19 patients, and found that just 1 out of 30 patients showed positive ribonucleic acid (RNA) of SARS-CoV-2 in their tears that were collected within 2-3 days and were tested using reverse transcription polymerase chain reaction (RT-PCR).[9]

A study reported that the epithelial of conjunctiva might be the potential route of SARS-CoV-2 entry.[3] It is still a controversial matter whether the eyes are the site of infection, or the virus reached the eyes through lacrimal glands or nasolacrimal duct of the nasal canal, which was commonly considered a site of virus entry.[10]

The World Health Organisation (WHO) continuously kept a watch on the signs and symptoms of COVID-19 patients, and reported that fever and dry cough were the most

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common symptoms.[11] Other studies reported that just about 1% of patients would develop congestion of conjunctiva and, according to the results, the SARS-CoV-2 was hard to get through the eyes.[12,13] The presence of SARS-CoV-2-related receptor called angiotensin converting enzyme 2 (ACE2) in retina may make the ability of virus to infected eyes much more acceptable.[14] In addition, SARS-CoV-1 which had emerged in March 2003 in Guangdong province of China, followed by Hong Kong, was also found to have infected the eyes and used the ACE2.[15]

The red-eyes were seen resulting from either a COVID-19 infection or a secondary infection.[16]

The current study was planned to identify the source of eyes infections during the COVID-19 pandemic.

Patients and Methods

The cross-sectional study was conducted at the Al-Basrah Teaching Hospital, Iraq, from March 2 to September 24, 2020, and comprised eye swabs from patients having confirmed diagnosis of COVID-19 who had been quarantined. Another group of infected persons with no redness in the eyes were enrolled as controls. COVID-19 patients not having red eyes were excluded.

The sample was raised using non-probability sampling technique.[16]

All patients were asked to look up, and the lower lid was gently pulled down, exposing the conjunctiva. The swab stick was swept along the lower fornix from inner to outer canthus. Each swab was placed directly into the carrier media tube.

Eye swabs were taken by ophthalmologists and patients with redness were given serial numbers to maintain anonymity. Two-transport medium with sterile swabs were performed on each patient to get swabs from each eye. Swab samples were cultivated in the microbiology laboratory of Al-Basrah Teaching Hospital. Swabs were cultivated on blood agar (BA) (OXOID, Cat No. CM0055, UK) and savoured dextrose agar (SBA), (OXOID, Cat No. CM0041, UK) plates by swabbing them on the surface of the media plate.

For biochemical detection, the bacterial isolations that appeared on culture were subcultured and analysed through standard laboratory procedures. Colony morphology, blood haemolysis, gram staining and biochemical reactions like optochin susceptibility, haemin X factor / nicotinamide adenine dinucleotide V factor, bile solubility coagulase, and catalase tests were performed to detect suspicious microbial species[17] by biochemical kit (RSL, Cat No. 36873, India)

For genetic analysis, suspected bacterial species were confirmed by using standard polymerase chain reaction (PCR).[18-22] Bacterial sample genome deoxyribonucleic acid (DNA) extraction was performed using a commercial kit (Dongsheng Biotech N1112, China) as per the manufacturer's instructions. Candida genome extraction was done using a quick yeast genomic DNA extraction kit (Dongsheng Biotech N1162, China) as per the manufacturer's instructions. The detection by PCR was performed using pairs of primers (Table 1).

Agarose gel electrophoresis was performed to analyse PCR amplified products. A horizontal gel containing 1.3% weight-over-volume (w/v) agarose gel which was dissolved in 1X tris-borate-ethylenediaminetetraacetic acid (EDTA) (TBE) solution which was composed of 0.09M Tris, 0.09M Boric acid and 2.0Mm EDTA; adjusted to potential of hydrogen (pH) 8.3. It also had 0.5µl DNA safe dye (GeneDireX, Novel Juice, China) for staining the DNA. Undiluted amplified PCR products 30µl were mixed with 10µl sample buffer composed of 0.4X TBE, 50% glycerol and 0.025% bromophenol blue. Then 10µl of each sample was loaded to each well of the gel along with 20µl of 5kbp ladder (Dongsheng Biotech DS™ 5000, M1111, China). The system was run in 1X TBE buffer at 160V for about 3h. Band results were then visualised using a trans-illuminator and were photographed.[18]

For PCR, DNA amplification was performed in 50µl. The solution reaction contained 5µl of 10X PCR buffer with 0.2mM deoxynucleotide triphosphates (dNTP) and 3.5mM magnesium chloride (MgCl2). Forward and reverse primers were added in a volume of 0.2µl each. Tag DNA polymerase (Promega, Cat No. D6001, USA) was performed by adding 2.5U to each reaction. A part of microbial colony was taken directly from the surface of the media plate by a sterile tip to serve as a DNA template. Thermocycler (Bioneer, Korea) was performed to run the PCR reaction.[23,24] The first denaturation included 3min at 94°C followed by 30 cycles of amplification, which included 40s at 94°C denaturation, 60°C as annealing and 2min for extension of the reaction at 72°C. This was followed by another cycle of 40s at 94°C and another extension step at 72°C for 12min. Positive and negative controls were used, and the process was applied in PCR cabinet to prevent contamination.[23]

The data were analysed by SPSS version 26, the data were presented as frequencies and percentage, and the association was measured by Chi square test (when the expectation frequencies less than 5%). The data value more than 0.05 was considered as not significant.

The study was approved by the ethics review committee of Al-Zahraa College of Medicine, University of Basrah, Iraq, and informed consent was obtained from each patient.

Results

Of the 213 quarantined COVID-19 patients assessed, there were 98(46%) males and 115(54%) females. Of them, 22(10.3%) had red eyes; 10(45.45%) males and 12(54.54%) females, with overall age ranging 18-74 years (Table 2). Of the patients with red eyes, 16(72.7%) complained of itching, pain and redness, while 6(27.3%) had no other symptoms except red eyes. There were no significant differences in symptoms among patients in terms of age and gender (Table 2).

There was a correlation of eye redness with symptoms and duration of illness. Patients who had a disease duration of 1-4 days developed symptoms like dryness, tears, itching, pain and photophobia. The symptoms were not identified after the 4th day. Statistical analysis showed there was no significant differences between the time of the infection and appearance of symptoms. $p \leq 0.083$

The diagnosis of S. aureus was confirmed by positive catalase and coagulase results (Table 3). Streptococcus (S.) pneumonia was detected in 8(36.6%) patients, followed by staphylococcus (S.) aureus and candida (C.) albicans 5(22.7%) each, and haemophilus (H.) influenzae 3(13.6%). The infected patients with red eyes were divided into those with and without associated symptoms (Table 4-5). No infectious isolation was detected in 3 samples, in patients' number 15, 16 and 17.

In the control group having 20 subjects, the main isolate was S. aureus 4(20%) and 2(10%) C. albicans.

PCR confirmed 3(60%) S. aureus isolates through the detection of nun a gene which appeared around 966bp, H. influenza was not detected in the absence of Hip6 gene at 273bp, 2(40%) isolates of C. albicans were detected by PCR and showed a band at about 700bp, while S. pneumonia was detected in 8(50%) in the symptomatic group, with bands showing at 1000bp, which is equivalent of the size of ply gene of S. pneumonia (Figure).

Table-1: Forward and reverse primers used.

Organism	Forward primer	Reverse primer	Gene	Ref
H. influenzae	5′-ACTTTTGGCGGTTACTCTGT-3′	5′-TGTGCCTAATTTACCAGCAT-3′	HiP6	20
S. pneumonia	5′-ACCCCAGCAATTCAATTCAAGTGT-3′	5′- TACGCACTAGTGGCAAATCG-3′	ply	21
S. aureus	5′-GCGATTGATGGTGATACGGTT-3′	5′-AGCCAAGCCTTGACGAACTAAAGC-3′	nuc A	22
C. albicans	5′-CGGAGATTTTCT CAATAAGGACCAC 3′	5′-AGTCAATCTCTGTCTCCCCTTGC 3′	KER1	23

H: Haemophilus, S: Streptococcus, S: Staphylococcus, C: Candida.

Table-2: Patient data and duration of their symptoms.

Patient Serial No.	age	sex	Duration (days)	Symptoms	*p*-value <0.05
1	74	Male	7	NO	0.083
2	71	Female	3	YES	
3	69	Male	2	YES	
4	64	Male	8	NO	
5	64	Female	3	YES	
6	62	Male	4	YES	
7	60	Female	3	YES	
8	58	female	1	YES	
9	55	Male	4	YES	
10	55	Female	7	NO	
11	48	Male	1	YES	
12	47	Male	4	YES	
13	47	Female	2	YES	
14	38	Male	2	YES	
15	32	Female	5	NO	
16	30	Female	7	NO	
17	29	Male	7	NO	
18	29	Female	2	YES	
19	27	Male	1	YES	
20	23	Female	1	YES	
21	21	Male	2	YES	
22	18	Male	4	YES	
		Total /Yes		16	
		Total /NO		6	

In specimens 15, 16 and 17, no infection was detected.

Table-4: Type of isolates detected in eyes of symptomatic patients.

Patient Serial No.	Species			
1	S. aureus			
2		S. pneumonia		C. albicans
3		S. pneumonia		
4	S. aureus	S. pneumonia	H. influenza	C. albicans
5		S. pneumonia		C. albicans
6				
7	S. aureus		H. influenza	C. albicans
8		S. pneumonia		
9			H. influenza	
10		S. pneumonia		
11	S. aureus	S. pneumonia		
12		S. pneumonia		C. albicans
13	S. aureus		H. influenza	

H: Haemophilus, S: Streptococcus, S: Staphylococcus, C: Candida.

Table-5: Type of isolates detected in the eyes of asymptomatic patients.

Patient Serial No.	Species			
17	S. aureus	H. influenza	S. pneumonia	
18			S. pneumonia	
19				C. albicans
20	S. aureus			
21				
22	S. aureus			C. albicans

H: Haemophilus, S: Streptococcus, S: Staphylococcus, C: Candida.

Table-3: Gram stain and Biochemical test for microbial diagnosis.

Microbes	Media	Gram stain	Blood haemolysis	Optochin susceptible	X factor /V factor	Bile solubility	Catalase	Coagulase
S. aureus	BA	+	+	-			+	+
S. pneumonia	BA	+	+	+		+		
H. influenza	BA	-	-	-	+/+			
C. albicans	SDA	+	-					

H: Haemophilus, S: Streptococcus, S: Staphylococcus, C: Candida, BA: Blood agar, SDA: Sabouraud dextrose agar.

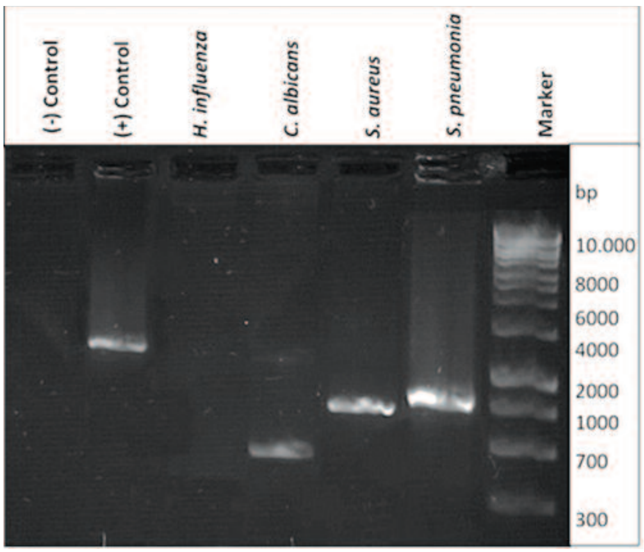

Figure 3: Gel electrophoresis showing bands of the isolates depending on their base pairs. H: Haemophilus, S: Streptococcus, S: Staphylococcus, C: Candida.

Discussion

The study focussed on the correlation between microbial community and eye redness during COVID-19 infection.

The absence of eye redness in most patients is not completely understood. Some studies have suggested that a very small proportion (<1%) of COVID-19 patients may develop eye redness.[5,25]

The current study found that several microbes present in the eyes may cause the redness and the other symptoms as a secondary infection. S. pneumonia has been accused of being the most common cause of conjunctivitis, keratitis and red eyes.[26,27] Staphylococcus is considered a causative agent of keratitis in combination with S. pneumonia (28,29).[28,29] In addition, C. albicans has also been mentioned in this context by several studies.[30,31]

On the other hand, there are some viral infections, like the respiratory syncytial virus (RSV), which causes red eyes and uses eyes as a gateway to move to the lungs and cause pneumonia.[32]

The current study has several limitations, as biomicroscopic slit-lamp examination was not carried out because of the risk to healthcare workers. Besides, the conjunctival swabs were collected at only one time point. In an experiment conducted on animals, there was some evidence that the presence of SARS-COV-2 virus in conjunctiva may be transient after ocular conjunctival inoculation.[33]

Other limitations included the fact that the sample size was not calculated, which could influence the power of the study. The sample was relatively small as only one sample of a tear swab and conjunctival scraping was taken from each patient. The presence of virus in the tear secretion could not be ruled out, but it was clear that conjunctival swabs and conjunctival scrapings were not useful samples for confirming or excluding the diagnosis.

Conclusion

Symptomatic and asymptomatic redness in the eyes of COVID-19 patients may have been because of the virus itself or/and because of microbes related to eye infection.

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