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Porphyrin as a Biomarker for the detection of COVID-19 using fluorescence spectroscopy on human body fluids: an analogical approach

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Abstract:

COVID-19 is an inter-human communicable, contagious acute respiratory disease caused by novel coronavirus (SARS-COV-2). Coughing & sneezing being the primary sources of transmission through close contact (<1.5 m) with the affected individual; SAR-COV-2 can also spread via human body fluids such as saliva, spit and mucus. We present an analogical approach to investigate the potential of saliva as a diagnostic medium to detect novel corona virus on three groups of head and neck cancer patients using fluorescence spectroscopy. The observed results indicate significant difference in fluorescence intensities among the groups. Receiver operating characteristic analysis (ROC) differentiated among the groups with accuracies of 92.15%. 85.71% and 67.92 % respectively.

Keywords – COVID-19, head and neck cancer, saliva, porphyrins, fluorescence spectroscopy, receiver operating characteristic analysis.

Introduction

COVID-19 is a contagious disease caused by novel coronavirus SARS-CoV-2, and inter-human communicable through respiratory droplets (diameter~5-10 μm) released while coughing, or sneezing. The affected individuals may be symptomatic or asymptomatic depending on the viral load, immunity and internal metabolism [1]. SARS-CoV-2 is a positive single strand RNA nucleus (+ss RNA), having four structural proteins namely, ORF4 or Envelope (E), ORF5 or Membrane (M), ORF9 or Nucleocapsid (N) and ORF2 or Spike (S). The spike proteins of SARS-CoV-2 attach itself to "*Angiotensin-Converting Enzyme* 2 (ACE-2)" receptors of the infected individual, as shown in Figure 1. In absence of any effective pharmaceutical interventions, more than 242 millions of people contracted the disease worldwide, out of which ~5 millions succumbed till date, resulting into a pandemic [2, 3, 4, 5]. The transmission pathways suggest that human body fluids would detect the presence of the virus. Human saliva consists of several important biological markers such as nucleic acids (DNA and RNA), amino acids (tryptophan, tyrosin etc.), proteins, amylase, coenzymes (NADH, FAD etc.), porphyrins, metabolites etc. [6].

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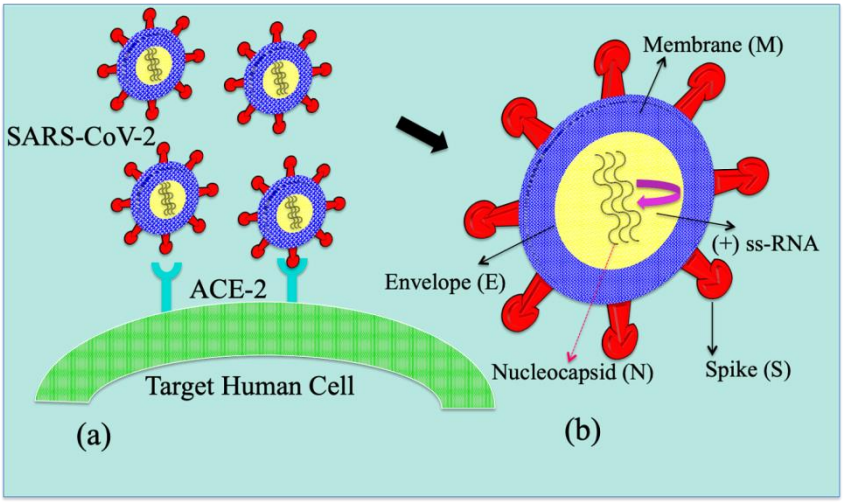

Figure. 1 (a) Schematic illustration of SARS-CoV-2 entering the target human cell through ACE-2 receptors, (b) Different structural proteins of SAR-CoV-2.

Its extensive production (~1 to 1.5 liters per day in an adult human) and ease in sample collection (collected multiple times from patients and volunteers of any age group) makes it a potential candidate for disease detection in non-invasive manner. The optically active porphyrin, at a particular excitation wavelength, gives fluorescence near orange (590-620 nm) to red (620-750 nm) regions of the spectrum [7, 8]. Saliva has been studied by many research groups for forensic purposes such as sexual assault cases, to examine the drug abuse cases and to diagnose HIV infected patients using various spectroscopic probing techniques [9]. It has also been examined for oral, breast and lung cancer detection [10, 11]. It has shown potential in the detection of respiratory viruses, including coronavirus too [12]. According to a study by Liu et al. [13], porphyrin accumulation increases in coronavirus patients with disease progression, therefore, spectral range (605-770 nm) may be a good choice to discriminate among symptomatic, asymptomatic and control groups with higher accuracies. In this article, we have shown the applicability and effectiveness of fluorescence spectroscopy on human saliva samples of head and neck cancer patients, with an aim to explore the plausibility of novel coronavirus detection by the similar approach.

Materials and Methods

Samples were collected from 31 SCC patients, 22 dysplastic patients and 20 control groups. The mean age of SCC, dysplastic and control groups were 49±12, 42±8, and 35±7 years respectively. Sample collection was done in Hallet hospital affiliated to GSVM Medical College Kanpur. A few milliliters of sample (~1 to 2 ml) from each patients and volunteers were collected in the sterile containers. To conduct the study, ethical clearance was obtained

with IEC communication number IITK/IEC/2015-16/2/10 and the CTRI number CTRI/2017/10/010102.

For the fluorescence measurements, an in-house developed compact system was used. This system consists of a laser diode of 375 nm and optical components such as collimating lens (CL), long-pass filter (LPF 400 nm)) and optical fibers. Cuvette filled with the saliva was placed inside the sample holder and laser light was irradiated onto it through a collimating lens. Fluorescence signals were recorded by the spectrometer with 1s integration time. Data acquisition was done using Ocean Optics Spectra Suit software.

Results and Discussion

Averaged fluorescence spectra recorded from SCC (n=31), dysplastic (n=22) and control (n=20) groups in the scan range of 400 to 800 nm are shown in Figure 2 (a). In the fluorescence spectra, band of FAD near 500 nm and porphyrin bands near 634, 674 and 701 nm are observed. Minor bands near 403 and 437 nm are also observed, likely to be Raman bands due to presence of water. The averaged spectra indicate significant difference in fluorescence intensities among all the three groups. The presence of porphyrin bands was observed in 25 SCC patients and 20 dysplastic patients and 15 control groups respectively. Intensity of porphyrin bands in SCC cases was higher than the dysplastic and control groups. Receiver operating characteristic (ROC) analysis was employed in the area values of the spectra in the spectral range of (605-770 nm). ROC applied on the binary data sets (SCC to normal, dysplasia to normal and SCC to dysplasia) compute the sensitivity, specificity, accuracy and cut-off values. ROC analysis generated the ROC curves as shown in Figure 2 (b). SCC to normal, dysplasia to normal and SCC to dysplasia are differentiated with sensitivities of 96.77%, 72.73%, 48.39% and specificities of 85%, 100%, and 95.45% with the overall accuracy of 92.15% (47/51), 85.71% (36/42) and 67.92% (36/53) respectively.

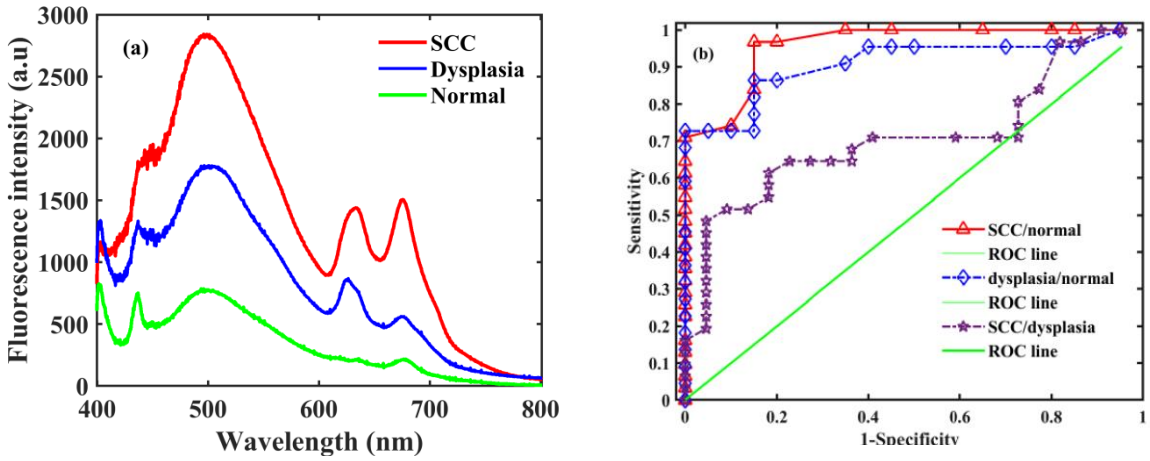

Figure. 2 (a) Averaged spectra of SCC, dysplastic and normal saliva samples (b) ROC curve.

Conclusion

Fluorescence spectroscopy employed on human saliva gives rise to FAD and porphyrin bands. FAD bands were dominant in all the three groups, whereas, porphyrin bands were prominent in SCC and dysplastic groups. ROC applied on the area values of the spectra was able to differentiate the groups with higher values of sensitivity and specificity. This study reveals that fluorescence spectroscopy on human saliva may be useful for coronavirus detection in non-invasive manner with efficient testing capacity.

Declaration of conflict of interest

Authors have no conflict of interest to declare.

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