Orf 1 and n gene among covid 19 positive healthcare workers and comparison of level of exposure for covid 19 among various departments they work in.

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Abstract

Objective: The current hospital-based retrospective descriptive study seeks to analyse the ORF1 and N genes of covid positive healthcare workers, taking into account the Ct values of both genes and comparing the level of covid 19 exposure in various departments where they work.

Study design & methodology: CT values from 93 covid positive health care workers were analysed retrospectively at different time intervals. Swabs were taken on day 6 - 9 of the illness. All health-care employees were included in the study, but they were separated into two groups for a better comparison of exposure levels: those who were directly exposed and those who were indirectly exposed.

Result: HCWs who were indirectly exposed had lower ORF1ab CT values than HCWs who were directly exposed, implying a larger viral load in indirectly exposed HCWs. When taken department as parameter, it backed it up, with highly exposed people being indirectly exposed to the patient (i.e office). The paediatric department was shown to be more affected among those who were directly exposed.

Conclusion: When compared to directly exposed HCWs (such doctors and nurses), HCWs in indirect contact with the patient were shown to be more infected with COVID 19. People working in healthcare offices were

found to be the most infected with covid 19. Using age as a criterion, those who were directly exposed were generally infected in their second decade, and those who were directly exposed were in their third and fourth decades.

Introduction

In December of 2019, Wuhan, Hubei Province, China, became the epiCentre of a pneumonia outbreak. Chinese scientists discovered new coronavirus 19 in January 2020. (1). During the first wave, India reported its first case in March 2020, and was among the top most impacted countries in the global pandemic, according to daily news reports, with infectivity rates declining by September 2020.

The dramatic increase in the number of illnesses due to COVID 19 in India from April 2021 has been dubbed the second pandemic wave, with daily cases exceeding 400,000 for the first time. HCWs' health has suffered as a result of the sudden surge in hospital admissions and increased demand for medical attention. According to government data, 87,000 HCWs have been infected and 573 HCWs have died in the month of August 2020 in India during the first wave. According to another ICMR study, due to a shortage of frontline HCWs, 5% of frontline HCWs may contract hospital-acquired illnesses (2). Data suggests that hospital support employees, as well as doctors, are more susceptible to infection than the general population.

The genome structure of CoVs' single-stranded non-segmented positive-sense RNA includes two-thirds of RNA that encodes viral polymerase RNA-dependent RNA polymerase (RdRp), RNA synthesis materials, and two large nonstructural polyproteins that are not involved in host response modulation, open reading frames (ORF1a-ORF1b). The remaining one-third of the genome is made up of four structural proteins; spike (S), envelope (E), membrane (M), nucleocapsid (N), and other helper proteins (10)

In India, the ICMR-National Institute of Virology in Pune used a two-step RT-PCR technique to diagnose covid 19. The rtRT-PCR (Reverse Transcriptase Real Time Polymerase Chain Reaction) is a gold standard molecular diagnostic method for detecting a wide spectrum of viruses. It was used to detect the presence of SARS CoV-2 in the upper respiratory tract (3,4) by collecting nasopharyngeal and oropharyngeal swabs.

The E (envelope) gene peculiar to the Sarbeco sub-genus was initially screened using primers and probes from two separate techniques combined. A confirmatory test was performed on samples that tested positive in the screening test, targeting two genes: one SARS-CoV-2 specific RdRp (RNA dependent RNA polymerase) gene and another Sarbeco subgenus ORF-1b-nsp14 gene. Positive samples for SARSCoV-2 infection was verified by either of the two genes (3)

When the sample fluorescence exceeds a predetermined threshold above the computed background fluorescence, the Ct is calculated. The lower a gene's Ct value is, the more the gene is present in the sample. (5) Few research have looked at the relationship between Ct values and symptom severity at presentation, and there isn't enough evidence to support this. Several studies have suggested that there is no relation between severity of disease and ct value (7)

However, in this study, we are looking at the ct values (> 35, deemed positive) of healthcare employees who work as front-line workers or are directly exposed to patients, as well as healthcare workers who are profoundly committed to healthcare but do not interact with patients. As they are assigned to different settings in the

healthcare system, the level of exposure is also established.

Though the ct value for identifying illness severity is debatable, it could be utilised by doctors to guide clinical and patient care decisions, such as isolation for COVID-19 patients, as well as infection control, public health, and occupational health decisions (6)

Materials & methods

Study design

The current study focused on healthcare personnel at Rajarajeshwari medical college and hospital, which is a dedicated covid care Centre in Bangalore, Karnataka, India. Since the beginning of the epidemic, the hospital has been active in Covid testing and treatment. Age, gender, and department of employment were all taken into account. Other factors like day of illness, source of infection, medications and comorbidities were not taken into consideration for this study.

The COVID-19 positive confirmation was carried out in accordance with the Indian Council of Medical Research (ICMR) norms. The RT-PCR profiling was carried out on healthcare workers who were examined between days 6 and 9 of their sickness. Ct values of both the ORF1 gene and the N gene were determined, with a threshold of 35 being considered positive for both genes.

Sample collection

Throat swab and deep nasal swab samples were taken from HCWs for SARS-CoV-2 detection using sterile swab sticks, and both swabs were then placed in ICMR-approved viral transport medium (VTM) vials (Microxpress).

RT-PCR for SARS-CoV-2 detection

One Step Reverse Transcriptase Real Time Polymerase Chain Reaction (rtRT-PCR) was performed to detect the presence or absence of ORF1ab and N gene. Kit used for RT-PCR was Meril& extraction kit of Taurs was used, standard operating procedures of the respective kits were followed.Only single kit was considered for this study ,due kit to kit variation and issue of efficiency. The reaction mixture preparation and amplification programwas used as per manufacturer recommendation. The result interpretation was made according to manufacturer instruction considering threshold cycle value (Ct≤35) for both ORF1ab & N genes and Internal Control (IC).

Statistical analysis: Qualitative variables were summarized by frequency and percentage, whereas continuous variables were summarized by mean and SDas most of them didn't show any sign of asymmetric shape. The CT values corresponding to N gene and ORF1 gene were compared between directly and indirectly exposed to patient groups by independent t-test. The distributions of them were visualized by box-whisker plots. Age and sex were also compared between those two groups by independent t-test and chi-square test of independence and visualized by box-whisker and multiple bar plots. The distribution of infection across different departments by bar diagram. P-value less than 0.05 was considered as statistical significance. The data was analyzed by statistical software R version 4.1.0 (R Core Team, 2021, Vienna, Austria).
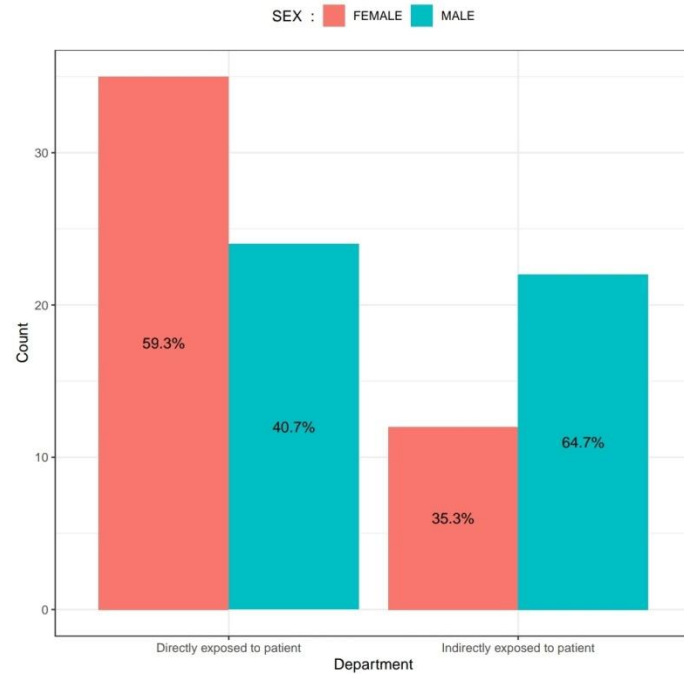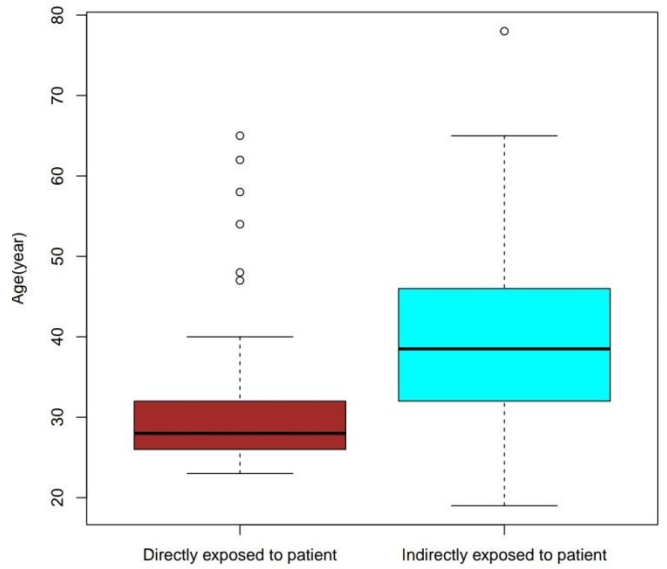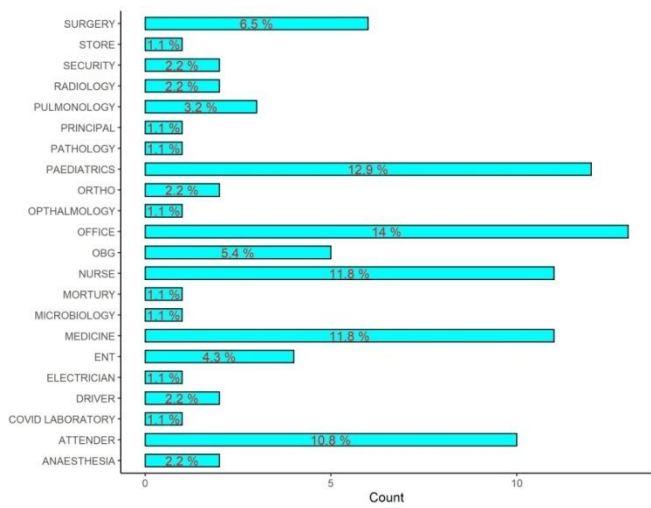
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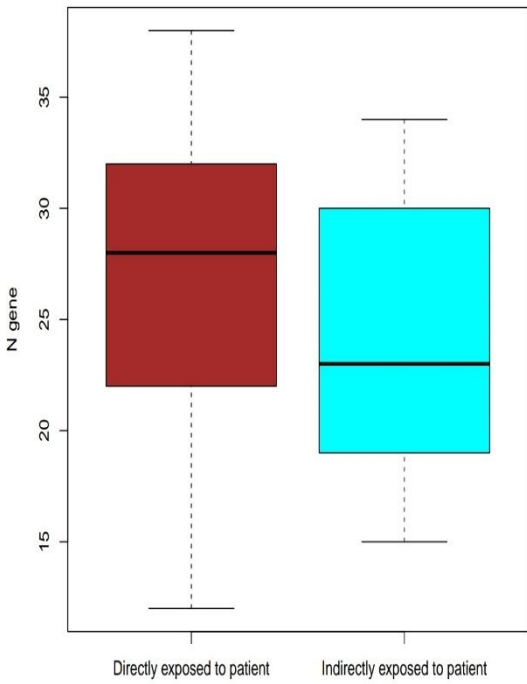
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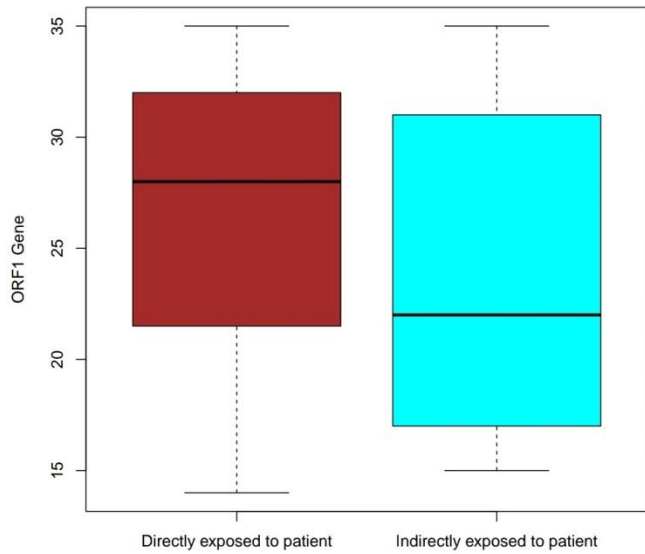
Graph 2:

Graph 3:

Graph 4:

Graph 5:

To study the dynamics of ORF1ab and N gene in COVID-19 positive HCWs in RRMCH from Nasopharyngeal and oropharyngeal swab, we have considered 93 HCWs in this retrospect study.

In our study we collected the data retrospectively from the month of April end to may end,in the second wave of pandemic.From the data collected In our hospital, the study group consists of 50.54%(n=47) female & 49.46% male(n=46), 63.4% being directly exposed (n=59)& 36.56% being indirectly exposed (n=34).

The most common department infected with Covid 19 was office personnel (14%), followed by paediatrics (12.9%), general medicine and nurses (11.8%), and attenders (10.8%).

The N gene in HCWs who are directly exposed the mean CT value was 26.56±6.11 whereas those indirectly exposed mean ct value was 24.18±6.16 however statistically not different (P value = 0.076)

The ORF1ab gene in directly exposed the mean CT value was 26.36±6.43, and those indirectly exposed the mean CT value 23.09±6.85 , ORF1 gene CT value being significantly lower (suggesting high viral load) in those indirectly exposed when compared to HCWs directly exposed.(P value = 0.027).Which could explain HCWs who are directly exposed to covid 19 may be more conscientious about following cautious precautions than those who are indirectly exposed.

When taken age as a parameter In HCWs who were directly exposed the mean 31.29±9.29 and in indirectly exposed the mean age was 39.68±12.05 .As the age in indirectly exposed is higher than those who are directly exposed, that can be a factor for higher CT value in HCWs who are indirectly exposed.

Discussion

This study suggests that HCWs who were indirectly exposed (office workers, attendants, laboratory workers, etc.) were exposed to a higher viral load than HCWs who were directly exposed, when both ORF1 gene and N gene CT values were taken into account, as many studies have shown that CT value is inversely proportional to viral load (11-15).HCWs in close contact with infected patients are not at higher risk of infection than other staff members, according to Charlotte et al., which could be attributed to good compliance and protection provided by personal protective equipment (PPE) (16). This could also explain why HCWs who are in contact with patients indirectly do not properly observe preventive protocols and are exposed to higher viral loads.

Furthermore, we must consider the possibility of infection from contacts outside of the hospital. It was also discovered in this study that there is a surge in positive instances in people working in department, such as paediatrics, at a specific time, indicating that their exposure is high because they work in high-risk environments, such as hospitals.: casualty , ICUs , wards & OPD.Anaesthesia, on the other hand, is considered to be the department with highest risk in this pandemic as there is less man force and requires round the clock

availability, In our study 2.2 % of HCWs from this departMent were exposed, this least number could be owing to proper usage of protective equipment (donning, doffing) and preventative measures. The high number of HCWs with positive cases is related to COVID 19's rapid transmission, which explains why they should follow distancing recommendations when dealing with their peers in the workplace.

Only high Ct values were found among HCW (especially indirectly exposed) who were tested for SARS-CoV-2, which could indicate that testing was delayed after exposure or symptom onset. As a result, education efforts could be stepped up to encourage HCW to get tested for SARS-CoV-2 as soon as possible (17)

Males have a greater mortality and morbidity rate than females, according to general population research, and gender is a risk factor for higher severity and fatality in COVID-19 patients, regardless of age or susceptibility (18-20). Our research found that males were more affected than females among indirectly exposed HCWs, whereas females were more affected among directly exposed HCWs.

In our study, health care workers in our hospital from third to fourth decade were most infected with covid 19 , to be specific HCWs who are directly exposed were of younger age groups with mean age group of 31.29 years when compared to those who are indirectly exposed , with mean age group of 39.68years, which may indicate that younger age groups are being frontliners to fight this pandemic when compared to the older ages .

Conclusion

HCWs who were indirectly exposed had lower ORF1ab CT values than HCWs who were directly exposed,

implying a larger viral load in indirectly exposed HCWs. Directly exposed HCWs may have lower virus loads as a result of protective measures taken.The department also backed it up, with highly exposed people being indirectly exposed to the patient (i.e office). When both directly and indirectly exposed age groups are considered, those in their third and fourth decades are heavily exposed. Drawbacks observed were COVID Testing was done at multiple time intervals, (but within a span of month), CT scan wasn't considered in this study to further be supported for assessment of severity. Protection which is provided by vaccination wasn't taken into account which could be owing to low CT values in directly exposed HCWs.

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