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A CORRELATION BETWEEN COVID-19 CASES AND DEATH DURING FIRST, SECOND AND THIRD WAVES OF COVID-19 IN LATA MANGESHKAR HOSPITAL (LMH) WITH TOTAL CASES IN INDIA

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ABSTRACT

Background: Now it has been more than 2 years since the first cases of the new Corona virus variant SARS Cov2 have been detected in China. The first wave of the pandemic hit a lot of countries hard and numerous patients died. Not enough dedicated equipment and limited information of the disease added to the severity of this first and second phase. **Aim and Objective:** This study is done with an aims to correlate COVID-19 cases and death during first, second and third waves of Covid-19 in LMH with total cases in India. **Methodology:** The Covid-19 data from all three Covid-19 waves was retrieved from COVID-19 Data Repository by the CSSE, JHU, USA. The retrieved data was analysed to find out the correlation between all RT- PCR COVID-19 confirmed positive cases and death during in three waves in, LMH with total positive cases in India. Out of 2935 COVID-19 cases 2410 cases tested positive from outside labs and 525 cases was confirmed with RT-PCR. **Result:** During the first, second and third waves of Covid-19 in LMH the mortality rate were 8.59%, 14.22% and 8.51%. While in these three waves of Covid-19 in India the mortality rate was 1.43%, 1.28% and 0.78% respectively. A moderate positive linear correlation was found between average COVID 19 case and death from cumulative data of three waves from LMH and India with R value: 0.444 ($p<0.05$) and R value: 0.491 ($p<0.05$). **Conclusion:** The COVID-19 positive case and death of LMH are found to be moderate positive linear correlating with COVID-19 positive case from India. The mortality rate found to be increased in the second waves in LMH while mortality rate found to be increased in the first waves in India.

KEYWORDS: Waves of Covid-19, Mortality rate, Correlation.

INTRODUCTION

Now it has been more than 2 years since the first cases of the new Corona virus variant SARS Cov2 have been detected in China.[1] Since then five SARS-CoV-2 variants have been designated as variants of concern by the World Health Organization (WHO): the Alpha(α) (B.1.1.7, Q.1-Q.8),[2] Beta(β) (B.1.351, B.1.351.2, B.1.351.3),[3] Gamma(γ) (P.1, P.1.1, P.1.2),[4] Delta(δ) (B.1.617.2 and AY.1 sublineages)[5] and Omicron(o) (B.1.1.529).[6,7]

In India during the first wave of SARS-CoV-2 the variant with spike protein D614G 226 mutation had dominated. Most common lineages observed in second wave were Delta.[8] While Omicron (B.1.1.529), a highly mutated SARS-CoV-2 variant, has emerged in the south of African continent leads the third wave in India.[9]

The first wave of the pandemic hit a lot of countries hard and numerous patients died. Not enough dedicated equipment and limited information of the disease added to the severity of this first phase. We all learned from our mistakes made during this first and second wave of the pandemic and due to that we were nutralised the third wave and the confidence to be able to manage possible fourth wave a lot better. Nevertheless, much higher infection numbers, more patients in ICUs and in India also more deaths were seen during the second and third wave, therefore, there is a need to study all three waves.

METHODOLOGY

In this study analysed data was collected from three COVID-19 waves LMH and total cases in India. The study was carried out in VRDL, Dept of Microbiology, NKP Salve Institute of Medical Sciences and Research Centre (NKP SIMS&RC) and Lata Mangeshkar Hospital

(LMH), Nagpur, India. The ethical clearance obtained from the Institute Ethical Committee (IEC).

Data collection

Data was retrieved from COVID-19 Data Repository by the Center for Systems Science and Engineering (CSSE) at Johns Hopkins University (JHU), USA. The retrieved data was analyses to find out the correlation between all RT- PCR COVID-19 confirmed positive cases and death during in three waves in, LMH with total positive cases in India.

Selection criteria

The 2935 cases of COVID-19 positive from OPD/IPD, LMH, Nagpur were included for the study. The COVID-19 cases data for India from JHU, USA also included in the study. The cases from outside, LMH, OPD/IPD, Nagpur were excluded from the study.

Diagnostic testing for COVID-19

Out of 2935 COVID-19 cases 2410 cases tested from outside labs and 525 cases tested by RT- PCR at VRDL, Dept of Microbiology, NKP SIMS & RC and LMH.

Collection of samples

Combined nasopharyngeal and oropharyngeal swabs from positive patients will be used. The Samples was transported in a 3 ml viral transport media (VTM). Stability of samples is enhanced by cooling therefore samples should be kept at 2-8°C. The samples are processed immediately after collection so that the viral load in the sample is same.

Directions

1. The pouch was cut to remove the swab.

Nasopharyngeal swab. Dry swab was inserted in to nostril and back to the nasopharynx. It leaved in place for a few seconds. The swab was removed slowly with a rotating motion.

Oropharyngeal swab. Patient was asked to open his/her mouth. The back of the throat was swabbed near the tonsils thoroughly.

2. The swab break near the break point and inserted into the tube containing viral transport medium and cap closed tightly.
3. The sample labeled correctly with the name of the patient and time and date of collection. The samples were transported immediately to the VRDL, Dept of Microbiology, NKPSIMS &RC and LMH for extraction for processing.

RNA Extraction.

1. 140 µl of pooled VTM was added to Collection Tube, Polypropylene (2.0 ml).
2. 560 µl of Carrier RNA-Lysis Solution (HRL) was added to the pooled VTM. Mix by pulse vortexing for 15 seconds.
3. The samples were incubated for 10 minutes at room temperature (15-25°C).
4. The samples were centrifuged for 10 seconds to remove droplets formed inside the cap of collection tubes.
5. The 560 µl of ethanol (96-100%) was added to the sample, mix well by gentle aspiration.
6. The samples were centrifuged for 10 seconds to remove droplets formed inside the cap of collection tubes.
7. The lysate obtained was transfer onto the HiElute Miniprep Spin Column. Centrifuged at 8,000 rpm for 1 min and the flow-through were discarded after the spin. This step repeated with the remaining sample.
8. The 500 µl of Prewash Solution (RW1) was added to the HiElute Miniprep Spin Column (Capped). Centrifuged at 8,000 rpm for 1 minute. The flow-through was discarded after the spin and the collection tube reused.
9. 500 µl of diluted Wash Solution (WS) was pipetted out. The tube was gently closed and centrifuged for 3 minutes at 14,000 rpm to wash the column. The flow-through was discarded. Centrifuged for 1 minute at 14,000 rpm to dry the membrane.
10. The HiElute Miniprep spin column (Capped) was transfer to a new 2 ml capped collection tube. 60µl Elution Solution (RNase-Free Water) was Pipeted directly onto the HiElute Miniprep Spin column (Capped).
11. The tube was incubated for 1 minute at room temperature (15-25°C). The tube was gently closed and centrifuged for 1 minute at 8,000 rpm.
12. The eluate contains pure RNA, it was stored at -80°C.

RT-PCR.

RNA samples obtained after extraction were used for PCR.

A. Protocol for PCR Master Mix Preparation

1. In the Master Mix Preparation room, all kit components was we thawed on ice, mixed by inverting thetubes and centrifuged the reagents for several seconds. Kept on ice for later use.
2. Based on the number of specimens to be tested (N), including the PTC and NTC, calculated the volume of the components to be added as N volume of 1X

Table 1: Protocol for 1X PCR Master Mix Preparation.

Sr.No.	Components	Volume (µL) to be added for 1X (for a 25 µL reaction)
1.	RT Buffer	5
2.	10X Solution H	2.5
3.	M-MuLV Reverse Transcriptase	1
4.	nCoV Multiplex Primer-Probe Mix	4
5.	Test – Extracted Sample RNA	5
6.	Positive Control/Test Sample/Negative Control	-
7.	Water	7.5
	Total volume	25

3. 1.5 mL Nuclease free centrifuge tubes used for the preparation of the reaction system. After all the reagents were added, mixed them thoroughly and centrifuged for several seconds.
4. 20 µL of master mixture was loaded into the 0.2 mL PCR reaction strips then, 5 µL of the negative control was added.
5. In the Template Addition Room, nCoV Multiplex Positive Control and extracted test RNA was added intothe strip.
6. The strips were tightly caped and mixed.
7. The strips were placed in Real-time PCR machine as per the PCR program.

B. PCR program recommended for Reaction

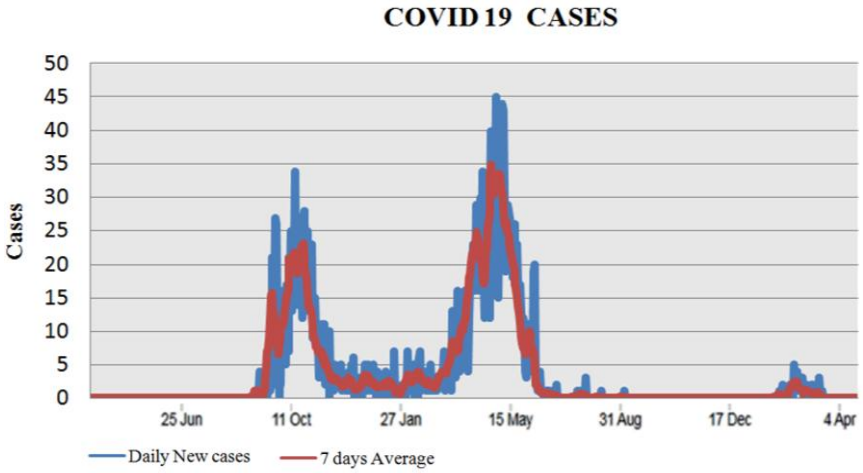
1. cDNA Synthesis: 50°C for 15 minutes
2. Initial denaturation; 95°C for 3 minutes
3. Denaturation: 95°C for 15 seconds
4. Annealing: 58°C for 30 seconds (Plate Read) No. of cycles: 40
5. Channel: FAM/JOE#/TexasRed/Cy5
6. Hold: 4°C for ∞

The 5 µl of that RNA was taken for the PCR. PCR was performed in the same run, keeping the entire conditions uniform. Results was saw on the InstaQ 96 PCR software and each reaction was analysed for N gene, E gene and RdRP gene with Ct value ≤ 35 cut off.

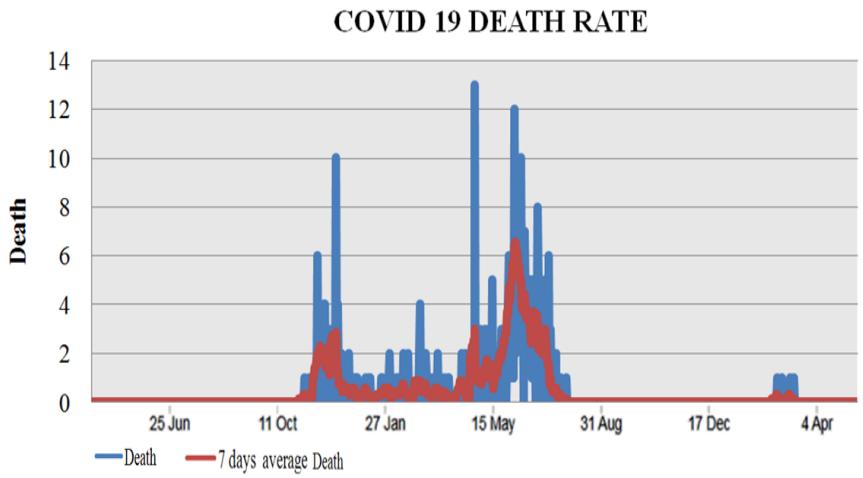
RESULT

Out of 2935 COVID-19 cases 2410 cases tested from outside labs and 525 cases confirmed by RT- PCR at VRDL, Dept of Microbiology, NKP SIMS & RC and LMH.

During the first, second and third waves of Covid-19 1,067, 1,825 and 43 patients admitted in LMH respectively, out of which 92, 260 and 4 patients expired in each wave. These three waves of Covid-19 in LMH the mortality rate was 8.59%, 14.22% and 8.51% respectively.

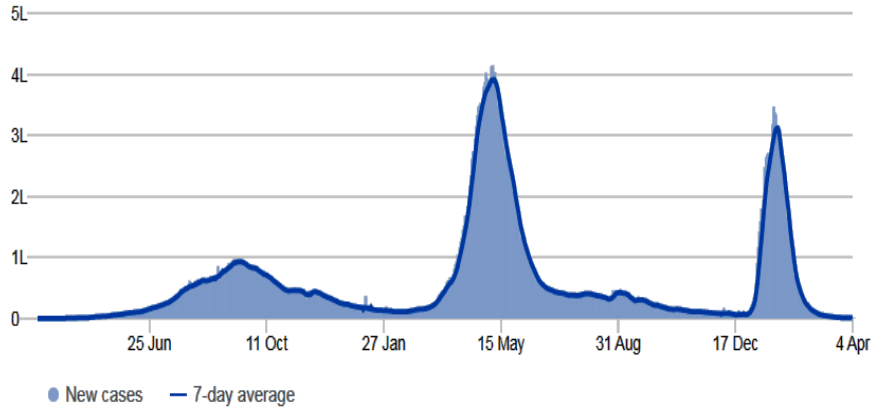


Graph 1: The cases of COVID-19 positive during first, Second and Third COVID-19 waves in OPD/IPD, LMH, Nagpur.

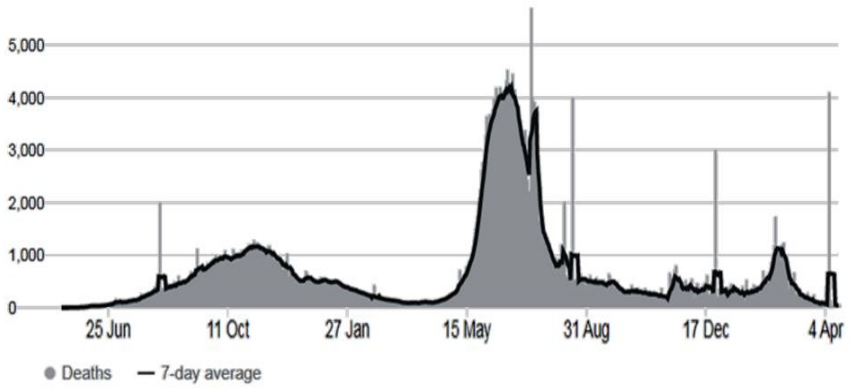


Graph 2: The Death of COVID-19 positive during first, Second and Third COVID-19 waves in OPD/IPD, LMH, Nagpur.

The three waves of Covid-19 which last long for 3.1.2020 to 6.2.2021 first wave, 7.2.2021 to 16.10.2021 second wave and 17.10.2021 to 11.4.2022 third wave during this time 1,08,14,314, 2,32,39,269 and 89,82,559 patients observed in India respectively, out of which 1,54,918, 2,97,062 and 69,711 patients expired in each wave. These three waves of Covid-19 in India the mortality rate was 1.43%, 1.28% and 0.78%.respectively.



Graph 3: The cases of COVID-19 positive during first, second and third COVID-19 waves in India.[10]



Graph 4: The cases of COVID-19 Deaths during first, second and third COVID-19 waves in India.[10]

Statistical analysis

The statistical Analysis of first, second and third waves in India and LMH was studied. The date with minimum number of average deaths in India was taken as end of wave. The average case and death data calculated from daily new COVID 19 case data from the LMH and India.

The Parsons correlation Cofficient (R) was calculated from data using Epi Info v0.6 Statistical analysis software with $p \leq 0.05\%$ significance level. A moderate positive linear correlation was found with R value: 0.444 ($p<0.05$) between average COVID 19 case cumulative data from three waves from LMH and India, The

cumulative data cumulative death data from three waves from LMH and India also found to be moderate positive linear correlate with R value: 0.491 ($p<0.05$).

R value 0.803, 0.44 and -0.228 found in average COVID 19 case data from first, second and third waves from LMH and India respectively, While R value 0.574, 0.376 and 0.584 found in average COVID 19 death data from each wave from LMH and India respectively. All three waves' data are found to be positively correlated, except average COVID 19 case data from third waves.

DISCUSSION

Many countries have experienced multiple waves of coronavirus outbreaks. In the first wave absence of vaccines and antiviral drugs, social distancing was the only way to suppress the spread of SARS-CoV-2. Presence of multiple vaccines in the second and third waves India able to tackle spread of SARS-CoV-2. In this study, correlation between the first, second and third waves in India and LMH was studied. The date with minimum number of average deaths was taken as end of wave. Unlike the first and second wave the social distancing policies were strengthened rapidly, a large number of COVID-19 cases occurred in the third wave within a short time.

Hence, this study found significant differences between the three waves of the COVID-19 pandemic in LMH and India. Our results designate that the third wave is more serious than previous waves, which may be due to a lack of strict social distancing strategy and public health involvements. Our result coincide with Indian COVID-19 data studies shows that the first and second wave of COVID-19 pandemic had the most harmful impact on community health. In contrast, the third wave demonstrates more constant evolutionary dynamics.[11] In addition, sufficient epidemiologic investigations and contact tracing could not be performed during the second and third wave, and there was a marked increase in the proportion of unknown routes of transmission.[12,13] The reason for the clear differences across phases and waves is not yet known, although it has been suggested that Delta (δ) B.1.617.2 and AY.1 sublineages variant of COVID-19 emerged in India in the December of 2021 responsible for second wave, and transmission to the general population was replicated across the India.

The third waves of India's COVID-19 infections led by the Omicron(o) B.1.1.529 variant which saw a sharp rise in COVID-19 cases in January-February 2022, the variant is already in community transmission and hospitals were saw more patients despite a decline in cases in major cities in India. The most cases of the Omicron variant were mild, although hospitalisations and cases in intensive care were increasing.

If the early reaction is delayed, the COVID-19 pandemic can extend volatility in local communities, making it complicated to manage using consequent social distancing approach.

CONCLUSION

The COVID-19 positive case graph and death graph of LMH are found to be coinciding with COVID-19 positive case from India. The mortality rate found to be increased in the second waves in both India and LMH. All three waves' data form cases and deaths are found to be positively correlate except average COVID 19 case data from third waves which might be due to maximum number of patients are treated in home isolation.

Conflict of interest

None to declare

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