ORIGINAL RESEARCH

Genome Analysis for Sequence Variants in Sars-Cov -2 in Symptomatic Individuals at Tertiary Care Hospital

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ABSTRACT

Background: COVID-19 is an acute viral illness caused by severe acute respiratory syndrome corona virus 2(SARS-CoV-2). Since the onset of the SARS-CoV-2 pandemic, multiple new variants of concern have emerged which are associated with enhanced transmissibility and increased virulence? It also highlights the role of the clinical interprofessional teams, public health agencies, and community participation in improving patientcare. An analysis of genomic sequencing variants of SARS-CoV-2 in symptomatic patients during 2nd and 3rd wave of pandemic by next-generation sequencing (NGS).

Material and Methods: A total of 200 symptomatic patients, throat/nasopharyngeal swab were collected for real-time reverse transcription-polymerase chain reactions (RT-PCR) at tertiary care hospital, Ongole. The specimens were transported under cold chain according to guidelines to Centre for Cellular & Molecular biology (CCMB), Hyderabad, for genome sequence analysis by next generation sequencing (NGS). Study period – 2ndwave i.e., MARCH 2021 –NOVEMBER 2021 & 3rdwave i.e., DECEMBER 2021 –MARCH 2022 according to WHO.

Results: Out of 200 samples analysed, 132 samples of 2nd wave & 68 samples in 3rd wave. Out of 132 samples, 57 Delta (B.1.617.2), 75 Delta sub-lineages. Out of 68 samples 41 Omicron (B.1.1.529), 11 Omicron lineages (BA.1), 16 Omicron (BA.2).

Conclusion: During the 2ndwave the symptomatic patients were detected with more delta and delta sub lineages showing high mortality rate. During 3rdwave omicron and omicron sub lineages were detected more than delta showing very high transmissibility and less mortality. Continuous monitoring and analysis of the sequence variants to understand the genetic heterogenicity.

Keywords: COVID-19, RT-PCR, Genetic Heterogenecity, Pandemic.

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INTRODUCTION

In early December 2019, a case of pneumonia with unknown aetiology was identified in Wuhan city, China, which was identified as virus. The virus is isolated and sequenced through next generation sequencing (NGS), it is identified as Novel Cov, named as 2019nCoV, later named as SARS-CoV2 based on Simplot analysis.[1] SARS-CoV2 pandemic has effect over 23 million population with more than 0.8million deaths in globally over 200

countries.[2] This pandemic had severely disrupted the health care system and halted socioeconomic status.COVID-19 is an acute viral illness caused by severe acute respiratory syndrome corona virus 2(SARS-CoV-2). The route of transmission of SARS-CoV2 by air droplets, and close contact, which leads to large outbreaks at workplaces, health care institution and crowded areas.[3] During the ongoing pandemic, as the public concern, the appearance of genomic variants as a natural result the evolution of the virus. SARS-CoV2 and some other RNA viruses have shown high mutation rate which is $8x10^{-4}$ expected substitution per titre per year. Over one million SARS-CoV2 genome variants, heterogenicity noted worldwide, have been filed in GISAID (Globally Initiative on Sharing all Influenza Data).[4] These variants are routinely monitored through epidemiological investigations, sequencing based surveillance and laboratory studies. According to SIG (SARS-CoV2 interagency group) these variants are classified into four classes like variant being monitored (VBM), variant of interest (VOI), variant of concern (VOC), variant of high consequence (VOHS).[5] According to WHO and PANGO lineage, under variant of being monitored (VBM),Alpha (B.1.1.7and Q lineages), Beta (B.1.351and descendent lineages), Gamma (P.1 and descendent lineages). Delta (B.1.617.2 and AY lineages), epsilon (B.1.427 and B.1.429) were identified. Under variant of concern (VOC) omicron (B1.1.529), BA1, BA2, BA1.1, BA3, BA4, BA5 lineages. Till now no variant of interest (VOI) and variant of high consequence (VOHS) noted globally.[5]

MATERIALS & METHODS

An analysis was conducted on total of 200 symptomatic patients of covid 19, who were admitted at tertiary care hospital Ongole, during 2nd wave and 3rd wave. According to WHO,2nd wave i.e., from march 2021to November 2021 and 3rd wave i.e., from December 2021 to march 2022.[2] One sample of Throat swab and one nasopharyngeal swab samples into a sterile tube of 3mlof viral transport medium (VTM)was collected by a trained health care professional. Real time reverse transcriptase polymerase chain reaction (RT-PCR) test performed at Department of Microbiology by Bio-Rad CFX 96 platform according to protocol provided by manufacturer. These samples were reported positive if both ORF1ab and N gene with cut-off CT value ≤35 and with sigmoid amplification curves according to protocol provided by Meril COVID19 One Step RT-PCR Kit. The covid 19 positive samples were triple packed and transported under cold chain (4°C) according to guidelines to Centre to Cellular Molecular Biology (CCMB), Hyderabad for whole genome sequence by Next generation sequencing (NGS).[6]

RESULTS

Total of 200 samples collected, 132 samples were collected during 2nd wave and 68 samples were during 3rd wave. Out of 132 samples of 2ndwave, 85(65.9%) were male, and 45 (34.09%) were females, out of 68 samples 3rd wave, 45(66.17%) were male and 23(33.82%) were females. During the 2ndwave, out of 132 samples 57 (43%) were delta (B.1.617.2), 75(57%) were delta sub lineages.[8,12] Out of 75 the delta sub lineages are 2(3%) is AY100, 1(1%) is AY101, 4(5%)AY102, 6(8%)AY103, 1(1%), 6(7%)AY11, 1(1%)AY101, 2(3%)AY122, 8(11%)AY127, 1(1%)AY16.1, 4(5%)AY20, 2(3%)AY23, 1(1%)AY26, 6(8%)AY33, 7(9%)AY4, 4(5%)AY42, 2(9%)AY43, 7(9%)AY44, 2(3%)AY75, 1(1%)AY78, 2(3%)AY84, 1(1%)AY88, 2(3%)AY92.2, 1(1%)AY99.2.[12] 132 covid 19 symptomatic patients during 2ndwave were followed up 43(32%) were expired, 33(25%) severe infection of covid19 with respiratory distress requiring mechanical ventilatory (either invasive or non-invasive) respiratory rate more than 30/min, spo2 <90% on room air, 38(29%) moderate

infection with pneumonia with no signs of severe disease, respiratory rate >24/min, spo2 <94% un room air ,18(14%) were mild with fever and upper respiratory tract infection.

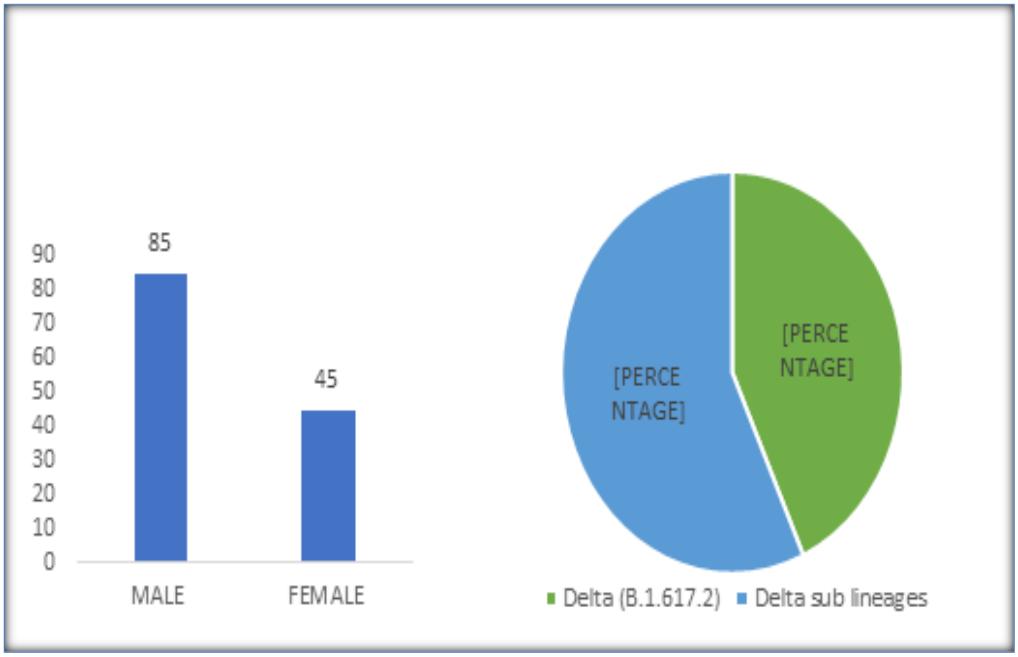

Figure 1, 2: A) Gender distribution of 2nd wave symptomatic patients. B) Variants during 2nd wave

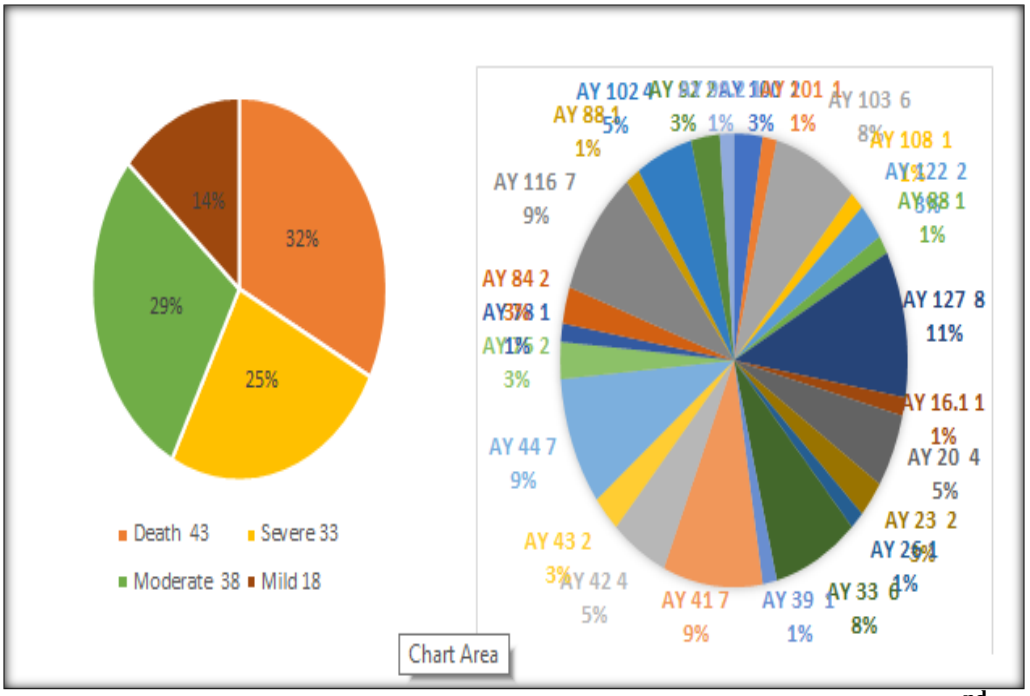

Figure 3, 4: c) AY descendent of delta (plus) sub lineages D) outcome of 2nd wave

During the 3rd wave, out of 68 samples 41(60%) were omicron (B.1.529), 11(16%) were B.A.1, 16(24%) were BA.2. Follow up of these 68 covid 19 symptomatic patients showed 2(3%) were expired, 12(27%) were severe, 20(29%) were moderate, 28(41%) were mild.[10,12]

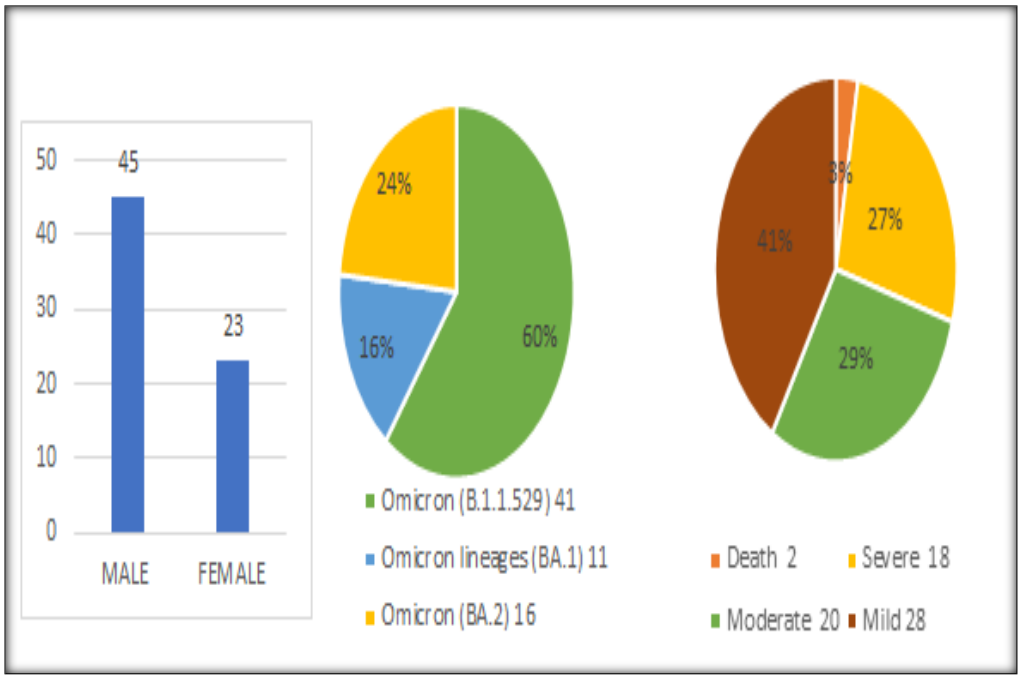

Figure 5, 6, and 7: E) gender wise distribution of 3rd wave symptomatic patients F) variants in 3rd wave G) outcome of 3rd wave

Gender wise distribution of variants

Out of 132 sample during 2nd wave, 57 (43%) were delta (B.1.617.2), 75(57%) were delta sub lineages. Out of 57 delta variant 33 were males and 24 were females, and out of 75 delta sub lineage 45 were male and 30 were female. Out of 68 sample during 3rdwave, 41(60%) were omicron (B.1.529), 11(16%) were B.A.1, 16(24%) were BA.2. Out of 41omicron (B.1.529), 25 were male and 16 were females. Out of 11 omicron lineage (B.A.1), 5 were male and 6were female, out of 16 (BA.2) lineage 12 were male and 4 were female. Male predominance is more as the compared the study conducted by savitesh et.al.[7]

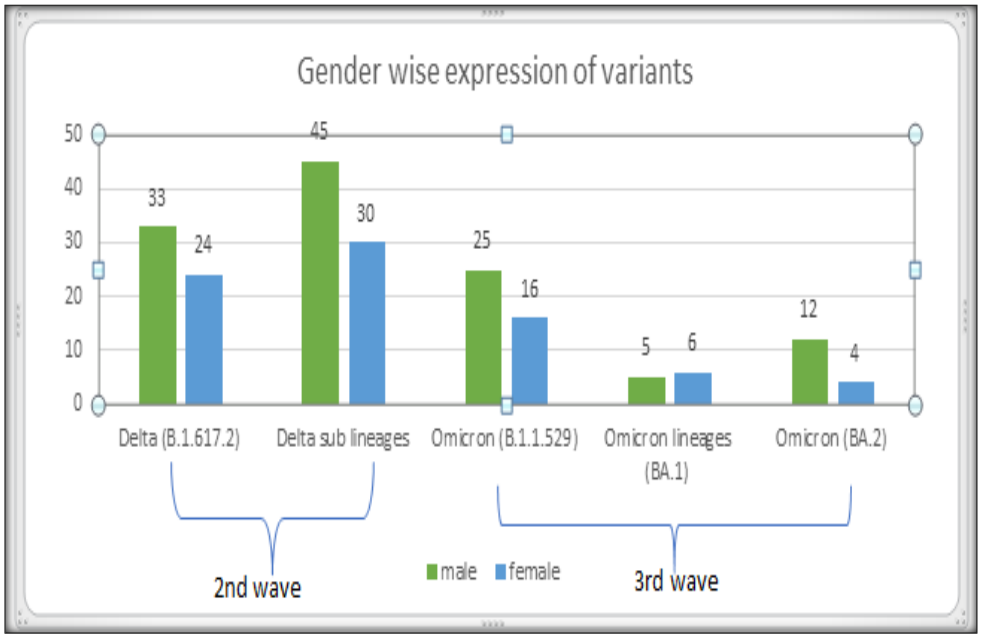

Figure 8: Gender wise distribution of variants

DISCUSSION

The SARS-CoV2 can infect humans irrespective of gender and age. Inthe present study a total of 200 symptomatic admitted patients were analysed form web-based portal (IHIP) to determine gender and genomic variants of SARS-CoV2. In this study male predominance was identified compared to the female population. The researchers considering the higher rate of smoking, exposure, prior respiratory illness, androgens, increased level of plasma ACE2 concentration. These ACE2 receptors are necessary forcellular entry of SARS-CoV2.[7]

Several mutations occur within the receptor binding domain (RBD) of S PROTEIN, these mutations affecting the residue, which play a major role in the angiotensin converting enzyme 2(ACE2) and antibody may not able to recognize the newer antigen, these are associated with increased transmission and infectivity.[9] During the 2nd wave the appearance of highly infectious delta variant (B.1.617.2) is of great concern.[8] These SARS CoV -2 variants had 13 different types and sites of mutation of which 7 mutations occurred inS protein. The delta variant is able to escape some neutralizing antibodies which were identified as highly infectious.[9] In the present study delta and delta sub lineages were predominant with high infectivity and mortality during 2nd wave.

During the 3rdwave, omicron (B.1.529) was the predominant of SARS-CoV2 variant.[10,11] Most of the Omicron variant can evade neutralizing antibodies, either form the vaccination or natural infection, thus there is the greater possibility of breakthrough the infections.[10] Despite of high transmission and less hospitalization and low mortality rate with high case recovery rate in the 3rd wave is significantly higher than 2nd wave. In the present study, omicron and omicron sub lineages were predominant with very high transmissibility and less mortality when compared to 2ndwave.[10]

CONCLUSION

Continuous monitoring and analysis of sequencing of variants to understand type of variant prevalent in that particular area, the genetic heterogenicity,characterization of disease pattern and outcome of disease. During 2nd wave, delta and delta sub lineages were predominant showing high infectivity, hospitalization, more complication with increased mortality rate. During the 3rd wave omicron and omicron sub lineages were predominant showing high transmissibility, less hospitalization and complications and high recovery rate.

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