Original Research Paper

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A STUDY ON OMICRON VARIANT BY GENE SEQUENCING, IN AND AROUND GUNTUR IN A TERTIARY CARE HOSPITAL.

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ABSTRACT **Background:** Severe acute respiratory syndrome coronavirus 2 (SARS-CoV2) variant Omicron (B.1.1.529) has ushered panic responses around the world due to its contagious and vaccine escape mutations. The essential infectivity and antibody resistance of the SARS-CoV-2 variant are determined by its mutations on the spike (S) protein receptor-binding domain (RBD). **Aim & Objectives:** The present study aims to determine the variants of omicron by genomic sequence. **Materials And Methods:** A retrospective study was conducted at State Level Viral Research Diagnostic Laboratory (VRDL) in Department of Microbiology, Guntur Medical College, Guntur for a period of 6 months. 519, RT-PCR positive samples were analyzed by Next clade and CT values of <25 were processed by Illumina COVIDSeq assay. **Results:** Out of 519 samples, Females were 51.8% and Males were 48.1 %. Mean age for females were 25 years and males were 35 years. Hospitalized were 78.2% and Quarantined were 21.7%. 56.2% belongs to Rural population and 43.7% were Urban population. 30.4 % population were reinfected following 1st wave of covid infection. 60.4% patients were diagnosed with co-morbidities. All 519 samples were Omicron positive, CT value ranged from 10 to 25 processed of which CT value of 10 - 0.38%, 11 to 15 - 21.6%, 16 to 20 - 37.2%, 21 to 25-26.2%. By Covid lineage, BA.2- 26.9%, BA 5.2.1- 9.05%, BA 2.10 and BA 5.2 - 8.4% , BA 2.75-8.09%, BA.1- 0.19%. By Next Clade analyzes it showed 21L omicron- 40.2%, 22 B omicron-24.2%,22 D omicron-20.2%,23 B omicron -9.63%, 22 F omicron -3.08%, 22 A omicron-0.38%, 21 K, 22 L, 23 A, Delta 21J and 21 B kappa-0.19%. P value is <0.001 which shows significant association. **Conclusion:** Omicron variant of SARS COV-2 is a global pandemic. By analyzing, the gene sequencing 40.2% were 21L omicron subvariant followed by 22 B omicron- 24.2% in this region. Sequencing helps in determining the exact rate of transmission and severity of this VOC (including the symptoms) and the treatment available.

KEYWORDS : COVID-19, SARS-CoV-2, Epidemiology, Cycle threshold, Omicron, RT-PCR, Illumina COVIDSeq.

INTRODUCTION:

SARS-COV 2 responsible for global pandemic is mutating highly, the variant Omicron(B.1.1.529) first case was reported on 11 NOV 2021 at Bostwana and it spreads worldwide. WHO designated it as a Variant of concern (VOC) on 26 NOV 2021. In, India first case was reported in Kerala on 12th December 2021. SARS COV-2 is a RNA virus of 30 kb nucleotides that encodes 29 proteins, 23 of them localized in spike protein which bound to mutate and the efforts should be aimed for prevention. In Omicron, 30 mutations occurred in the Spike protein that leading to reduced affinity of antibody binding to S-protein and is 10 times more contagious and has higher chance of reinfection in those who had COVID-19 disease. Though previous VOCs emerged in which natural immunity from COVID-19 infections was common but in this, VOC which has emerged at a time when vaccine immunity is increasing in the world. Omicron has reduced vaccine effectiveness against infection to 33% from 80% for Delta. By PCR, "S" genes dropout or "S" genes failure was observed, hence Sequencing was introduced.

MATERIALS AND METHODS

Study Design:

Retrospective study was conducted at State Level Viral Research Diagnostic Laboratory (VRDL) in Department of Microbiology, Guntur Medical College, Guntur. Institutional Ethical committee approval was taken before the commencement of the study.

Study Period: 6 months

Sample Type: 519, RT PCR positive COVID 19 samples

Study Procedure:

- A total of 519 samples were selected for sequencing with(Cycle threshold, CT<25).
- Viral nucleic acid was extracted using the QIAampviral RNA minikit.
- First strand cDNA synthesis was done using the GoScript reverse transcriptase system.
- Enrichment was done using COVIDSeq primer pool 1 and 2 (CPP1 and CPP2, respectively).
- The library was prepared using IlluminaCOVIDSeq assay kit according to guidelines.
- Prepared libraries were purified and pooled and then quantified with a double-stranded DNA (dsDNA) high-sensitivity (HS) assay kit.
- Libraries were normalized to a 4 nM concentration.
- Pooled normalized libraries were denatured and finally diluted to a 1.2 nM concentration according to the IlluminaMiniseq system denature and dilute libraries.
- The libraries were then loaded and run on an IlluminaMiniSeq instrument following the standard protocol for 150-bp paired-end reads.
- The Illumina DRAGEN COVID Lineage app v3.5.8 was used to process and assemble the raw reads as well as for variant calling and lineage determination.

RESULTS:

Out of 519 samples, Females were 51.8% and Males were 48.1 %. Mean age for females were 25 years and males were 35 years. Hospitalized were 78.2% and Quarantined were 21.7%. 56.2% belongs to Rural population and 43.7% were Urban population. 30.4 % population were reinfected following 1st wave of covid infection. 60.4% patients were diagnosed with co-morbidities. All 519 samples were Omicron positive, CT value ranged from 10 to 25 processed of

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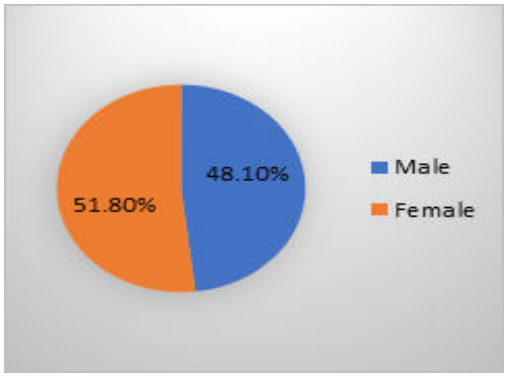

Figure 1: Genderwise distribution

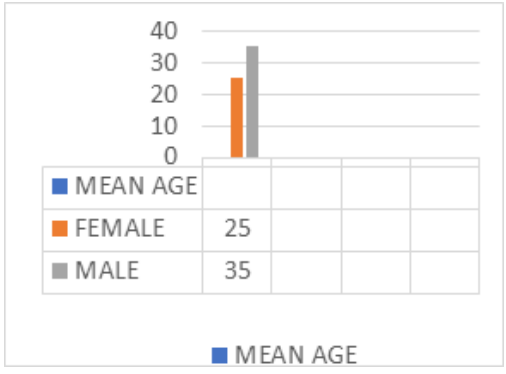

Figure-2: Mean age distribution

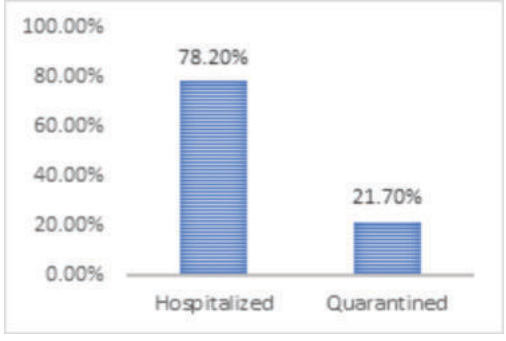

Figure 3: No of patients Hospitalized / Quarantined

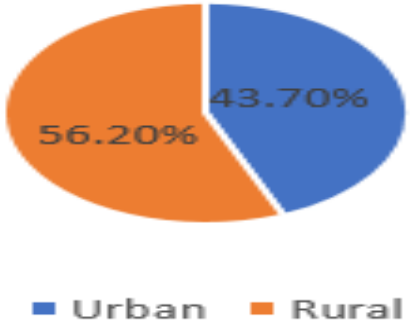

Figure 4: Population

Table 1 - CT value

10	0.38%
11-15	21.6%
16-20	37.2%
21-25	26.2%

Table 2- Covid lineage

BA.2	26.9%
BA 5.2.1	9.05%
BA 2.10	8.4%
BA 5.2	8.4%
BA 2.75	8.09%
BA 2.38.1	5.5%
BA 2.75.1	5.2%
BA 2.76	4.8%
BA.1	0.19%

Table 3 : NEXT CLADE analysis

21L omicron	40.2%
22B omicron	24.2%
22 D omicron	20.2%
23 B omicron	9.63%
22 F omicron	3.08%
22 A omicron	0.38%
23 A omicron	0.19%
21 K omicron	0.19%
22 L omicron	0.19%
21 J delta	0.19%
21B kappa	0.19%

DISCUSSION:

Omicron VOC of SARS-CoV-2 sequences shows many mutations on S-gene, followed by ORF1ab, N-gene, M-gene. ORF3a, E-gene, ORF6, and ORF7b were the lowest mutated genes. The most mutated S-gene, which encodes a structural protein, functions as a viral binding protein to the host cell receptors and determines the host range.

- Average age range of cases were between 20 and 49 years whereas, this study shows age range of 25-35 years old.
- Females were significantly affected more.
- According to Guo et al. it is a dominant strain on a worldwide scale, contributing for 99.7% of registered sequences.
- Guo et al., suggested that effective reproduction number of BA.2 was approximately 1.26 times more than that of BA.1.
- Karim et al., proposed in cases which are recounted, the virus might have lingered in an immune-compromised person for a longer length of time.
- Qassim et al. found that among those with a prior infection, Ct values were 1.30 cycles higher than in those without prior infection.
- Soa et al., proposed that Ct values can reflect the current epidemiological trends and dynamics of a certain region.
- Ito et al., suggested that, substantial increase in Omicron instances is likely to be in near future due to its significant advantage of increased transmissibility.

CONCLUSION:

Omicron variant of SARS COV-2 is a global pandemic. VOCs have highlighted the importance of vaccination in combination with existing public health prevention measures such as masks, hand hygiene as a pathway to viral endemicity.

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