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An Epidemiological Analysis of the 3rd Wave of SARS CoV-2 Genome Sequencing: A Peripheral Hospital-Based Study

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Abstract
SARS-CoV2 virus, a positive sense enveloped single standard RNA virus encoded with 29 proteins and host derived membrane. Structural proteins help virus to package its RNA and other proteins help in viral replication by enabling its entry into the host. Among structural proteins few important proteins are S which is the spike protein, resemble 33s like a crown, M, which is a hydrophobic membrane protein, E which is the envelope protein and N is the nucleocapsid protein works as RNA binding protein. Through the constant mutation virus changes its emergence profile. Single mutation is not always advantageous for virus, but combination of mutations causes increased transmissibility and increase receptor binding capacity thereby changes the surface structure. Whole genome sequencing is an important tool to assess the mutations and helps to understand the changing trends of the virus. Here we are reporting the genome sequencing of those patients who were already positive for RTPCR Tests. The aim, of the present study, is to identify the different lineages circulating in West Bengal as well as the epidemiological analysis of the set of patients to control the disease severity. During the (JULY 2021-AUG 2022). of COVID-19, there were mostly 2 strains delta and omicron. The epidemiological genome sequencing study helps to highlight on the coming and changing viral trends and helps in prevention of the newly arrived variants.
Keyword: *SARS- COV2, Epidemiology, Mutation, Variant.*

Introduction

SARS-CoV2 virus, a positive sense enveloped single standard RNA virus-encoded with 29 proteins and host-derived membrane[(1)]. Structural proteins help the virus to package its RNA and other proteins help is viral replication by enabling its entry into the host[(1)]. Among structural proteins few important proteins are S which is the spike protein, resembles a crown, M, which is a hydrophobic membrane protein, E which is the envelope protein and N is the nucleocapsid protein works as an RNA binding protein[(2-3)]. Through constant mutation, the virus changes its emergence profile. A single mutation is not always advantageous for the virus, but the combination of mutations causes increased

transmissibility and increased receptor binding capacity there by changing the surface structure.

Whole-genome sequencing is an important tool to assess the mutations and helps to understand the changing trends of the virus. Here we were reporting the genome sequencing of those patients who were already positive for RTPCR Tests. This study was to investigate the changing trends of SARS- COV-2 as well as the mutational status of different lineages of West Bengal over a period of 13months (JULY 2021-AUG 2022). Analysis of the results will help us with the identification of lineage which is circulating in the specific area of West Bengal as well as the prevention of the mutant virus. The aim, of the present study, was to identify the different lineages circulating in specific part of West Bengal as well as the epidemiological analysis of the patients to control the disease severity.

Materials and Methods

Nasopharyngeal and oropharyngeal swabs of SARS COV -2 were taken and samples were tested for RT-PCR. Positive samples of SARS COV-2 were sent to the Regional VRDL laboratory for sequencing. Patient Samples from different districts of West Bengal who were reported at VRDL of peripheral Medical College and Hospital were taken. Samples were stored at -80◦C and then samples were packed in triple layer packaging method. All packed samples were sent to the regional laboratory for sequencing. 216442 Samples were tested; 383 samples were sent for genome sequencing. RNA extraction and sequencing- viral nucleic acid were extracted from nasopharyngeal and oropharyngeal swab .200 µL of the sample were taken from VTM and RNA extraction was performed by Magmax Viral extraction kit (Thermo Fisher Scientific). 50 µL of elution buffer was used to elute RNA. Real-time RTPCR was performed selecting the ORF and N quality. For 96 well one positive control (CPC) and one negative control were selected demographic information were analyzed for all tests who were tried positive for SARS COV-2 were communicated through phone for the subtle elements of inoculation status, history of prior COVID disease, co morbidity status, the onset of sickness, hospitalization status. Sequence analysis- The results of whole-genome sequencing were analyzed. The patient's age, address, variant of the gene, gene mutation of the samples was analyzed using statistical software.

Result

216442 samples were tested for SARS COV2. 383 samples had details of all demographic criteria and were selected for whole genome sequencing. 8 samples were failed for genome sequencing and 13 samples were rejected. 323 samples processed for genome sequencing.

Overall, 48.70% of cases were symptomatic and 51.30% of cases were asymptomatic. The patient presented with cough 69.2% cases, low-grade fever in 78%, sore throat 52%, breathless 13%, body ache 21%, nausea 10%, loss of smell, and test 88% of the cases. Patients presented with co morbidity such as 6.3% Diabetic, 12% Asthmatic, hypertensive, renal disease. 24.34% developed COVID after vaccination.

The mutations which were observed were Delta (B.1.617.2), Delta Sub-Lineages, Omicron other Sub-Lineages, Omicron (BA.2).

Delta sub lineages are AY.4,AY.127, AY.122, AY.98.1, AY.89, AY.84, AY.120, AY.102, AY.7.2, AY.34, AY.23, AY.103, AY.46.1, AY.46, AY.4.6, AY.86, AY.43, AY.20, AY.39, AY.75, AY.44, AY.45, AY.102, AY.61, AY.19, AY.7.2, AY.7.1, AY.16, AY.111, AY.24, AY.9.2, AY.100.

Omicron subvarients are BA.2.75.1, BA.5.2, BJ.1, BA.2.75, BA.5.2.1, BA.2.38.1, BA.2.75.2, BA.2.76, BA.5.2, BA.2.

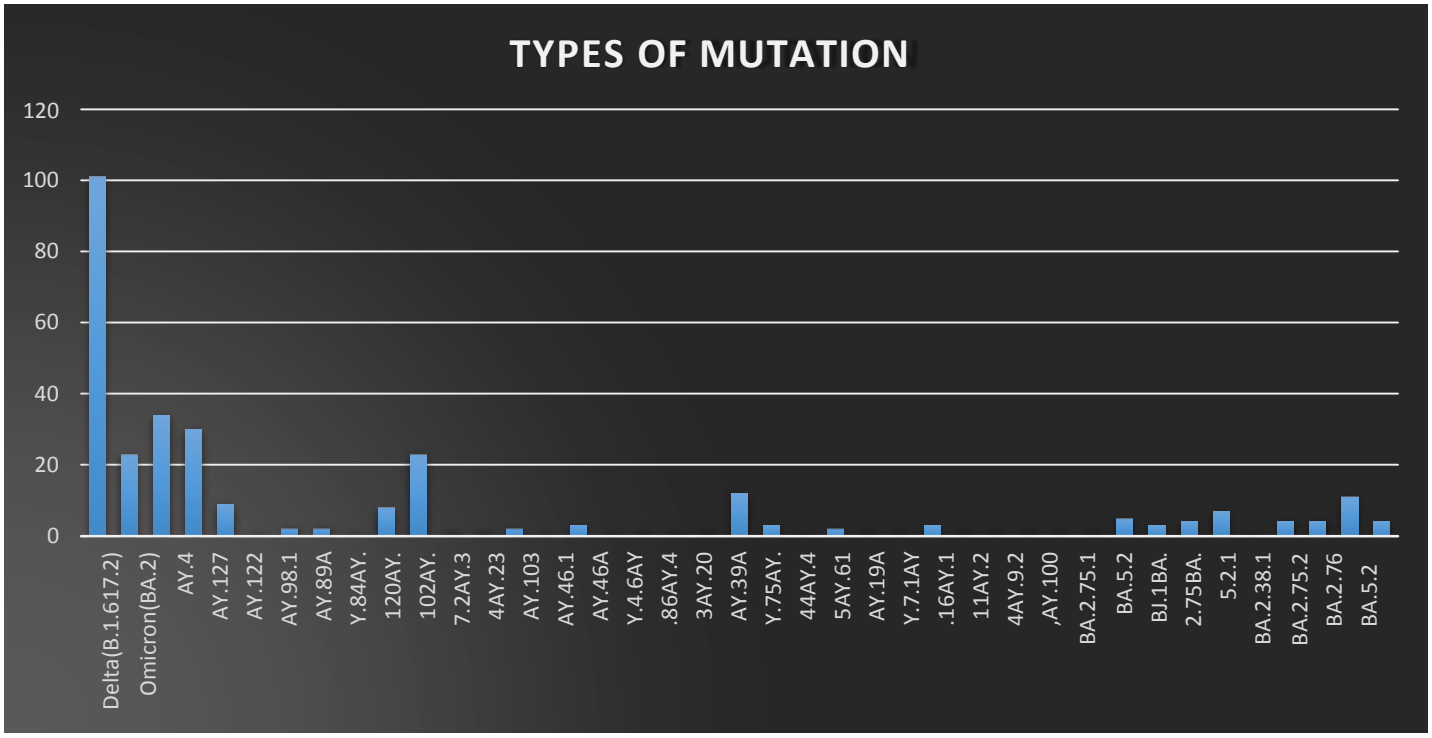

Fig 1: variants and Subvarient of sarscov2

Delta variants has more male predominance and in contrast omicron variant has more female predominance.

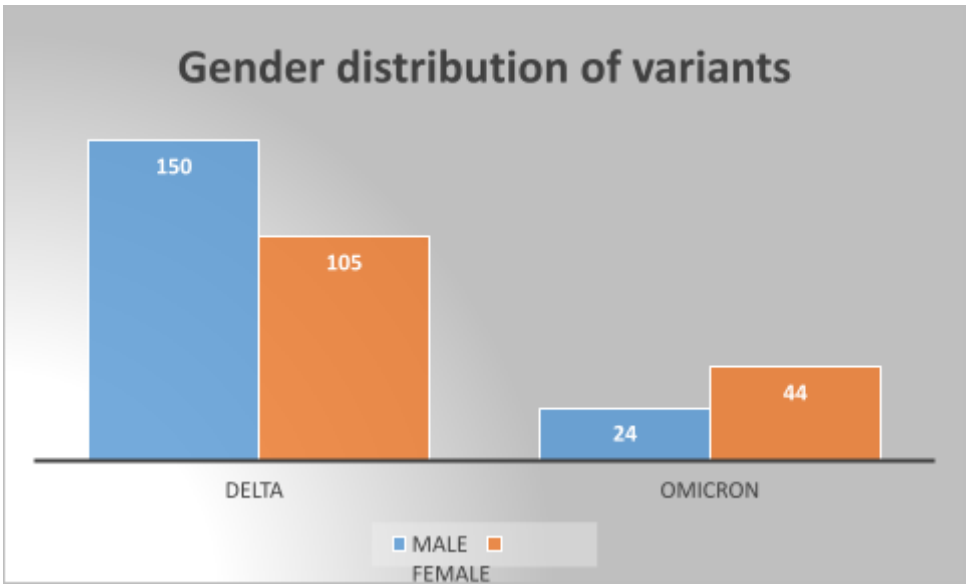

Fig 2: Gender distribution of delta and omicron variant

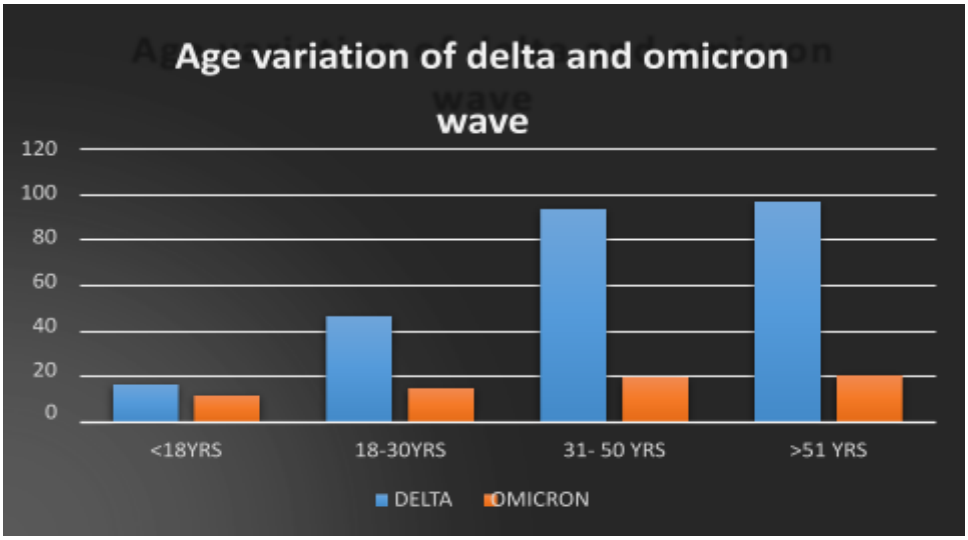

Fig 3: Age wise distribution of mutant variant

Discussion

Whole-genome sequencing helps in the identification of new trends, their prevalence in a specific area, and prevention[4-6]. During the period of (JULY 2021-AUG 2022) of COVID-19, there were mostly 2 strains Delta and Omicron. Delta sub variants are AY.4, AY.127, AY.122, AY.98.1, AY.89, AY.84, AY.120, AY.102, AY.7.2, AY.34, AY.23, AY.103, AY.46.1, AY.46, AY.4.6,

AY.86, AY.43, AY.20, AY.39, AY.75, AY.44, AY.45, AY.102, AY.61, AY.19, AY.7.2, AY.7.1, AY.16, AY.111, AY.24, AY.9.2, AY.100.

The most common type of mutation was noticed was Delta (B.1.617.2), followed by sub variant of delta AY.4 AND AY.127.

There are various subfamilies of delta variants. B.1.617.2 Lineage was observed in a maximum no of cases. Among the 323 samples, 101 samples were B.1.617.2. B.1.617.2 may induce cell-cell fusion in the respiratory tract and possibly higher pathogen city even in vaccinated individuals with neutralizing antibodies[7]. This variant was identified in December 2020 in India. The transmissibility of this variant was 40% to 60% higher than the other variant[8]. Campbell[9] stated that Delta variants spread rapidly across different continents, with increased distribution observed compared to alpha variants.

The Delta Plus Variant (AY.4) - The Delta plus variant was first found in India in April 2021. Additionally, the variation has been spread to nine additional nations: The United States, United Kingdom, Portugal, Switzerland, Japan, Poland, Nepal, Russia, and China. The variation contains an extra transformation called K417Non the spike protein. It has been suggested that this change slightly reduces binding affinity to ACE2.

Right now, it is dubious whether this extra change is causing improved seriousness, transmissibility, and safe avoidance of this Delta. Additionally, variation compared to the Delta variation. 34 cases were detailed positive for this variation[10].

The sub variant of Delta (AY.127)

Four distinct non-silent mutations that are reproducible in both the human and hamster infections that were researched in Hong Kong were discovered using AY.127 viral sequences from humans as references. Three of these mutations were in the spike viral protein, with two mutations in the N-terminal domain (NTD; Leu18Phe and His49Tyr) and one mutation in the receptor binding domain (Asp427Gly) in the S1 region. The Leu18Phe mutation can affect the binding of some NTD- specific antibodies and the His49Tyr mutation can enhance viral entry. As p427Gly is not located in the receptor binding motif that direct interacts with host ACE2, and its impact on ACE2 receptor binding and other biological functions require further investigation[11].

Sub variant to delta (AY.39) - frequency is currently low. vaccine effectiveness does not seem to be different when compared to another delta variant. 12cases were reported among the 323samples.

The third most common variant after AY.127 is the omicron variant (BA.2). Omicron is subdivided into BA.1 and BA.2. BA.2 appears to be more transmissible by nature. The new BA.2 sub variant is 30% easier to spread than the original omicron variant. Among those who are infected, headache, sore/itchy throat, sneezing, runny nose, and general body soreness are the most prevalent symptoms. However, and two more symptoms of Omicron BA.2 sub variant: dizziness and weariness.

During the study period omicron variant was observed 23 out of 323 samples.

Mlcochova, P. et al[8], hypothesized that B.1.617.2 Raw virus particles contained spikes that were cleaved at a higher rate than B.1.1.7, which was assumed to be involved in the mechanism of increased infectivity. They also observed that B.1.617.2 has more replication and spike intrusion This may explain the advantages of B.1.617.2.

Gender distribution among the various lineages: The study evaluated that among 323 cases 150 cases of B.1.617. 2 (delta variant) was positive in male patients and 105 cases were positive for female cases. Among the 68 cases of omicron variants 24 were male and 44 cases were female. This results show that delta variant has slight male predilection where in contrast omicron variant has more female predilection.

Gin gian et al[12] concluded that according to the clinical severity classification, men were more likely to develop more severe cases than women and among the deceased patients, the number of men is 2.4 times that of women. Men and women had the same susceptibility, but men were more dying.

Age distribution among various lineage

The present study showed that omicron affects the pediatric population than the delta variant. Younger adults and older adults group has similar ratio of infectivity in both the variants.

Even older age group has similar infectivity rate among the two different variants.

In conclusion, the epidemiological genome sequencing study helps to highlight on the coming and changing viral trends and helps in prevention of the newly arrived variants.

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