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Case Report

Emergence of Delta Plus (AY.1) and Kappa (B.1.617.1) Variants of SARS-CoV-2 in Second Wave of India: A Case Series from Eastern Uttar Pradesh

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Abstract

Prognosis of a COVID-19 caused by Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) is determined by how the host and virus variant interact with each other. The second wave was spreading at a breakneck pace in India, eventually and became a "tsunami by spreading of delta (B.1.617.2) variant. To determine the cause of this condition, randomly chosen positive SARS-CoV-2 samples were sequenced for the detection of variants responsible for this miserable situation. We have found mainly delta variants in clinical samples. Surprisingly, first time two delta plus (AY.1) and one kappa (B.1.617.1) variants of SARS-CoV-2 were also reported on July 2, 2021 from our centre of Uttar Pradesh. Unfortunately, among these, two non-vaccinated cases, person infected with delta plus and another with kappa variants had loss of their life admitted in ICU COVID-19 ward with severe conditions. Remaining, one case infected with delta plus was recovered who had already taken the vaccination of both doses. This finding suggests that SARS-CoV-2 genome variations have a substantial influence on COVID-19 outcome, resulting in an increase in reproduction number (R_0) in the second wave. However, our findings point to a possible role for vaccination against the more lethal double-mutated delta plus variant. This primary report also opens a door towards large-scale gene sequencing of SARS-CoV-2 samples in order to develop precautionary measures against subsequent waves.

Keywords: SARS-CoV-2; Delta Plus Variant; Kappa Variant; Sequencing; COVID-19

Introduction

Coronavirus is one of the most common viruses that attack the human respiratory system. The current pandemic, Coronavirus Disease-19 (COVID-19), began in December 2019 in Wuhan, China, and has destroyed the whole World. The first COVID-19 patient was identified on January 30, 2020, in India, and on April 27, 2020, in

Gorakhpur, Uttar Pradesh [1,2]. At the time of writing, 3,17,26,507 persons in India have reported laboratory confirmed infection with the Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2), with 4,25,195 (1.3%) of them deceased [3]. India is one of the country which is most affected by the COVID-19 pandemic; especially the second wave was spreading at a phenomenal speed than first wave. Nobody knows how far it will spread or how long it will last, or what the future of this sickness will be in different parts of the world.

The SARS-CoV-2 contains several variations, which differs owing to their potential for increased transmissibility, pathogenic capacity, or decreased vaccination efficacy. Mutations are crucial in the ongoing evolution and appearance of new SARS-CoV-2 variants [4]. Variations in the SARS-CoV-2 genome have already been observed in various geographical regions. Whereas the "A" (ancestral type, such as the bat and pangolin coronavirus strains) and "C" subtypes are common in the United States and Europe, the "B" subtype has been found to be common in East Asia [5]. The B-type mutated into further B.1, which is the parent of the major worldwide variations of concern, designated alpha, beta, gamma, and delta by the WHO in 2021 [6]. Especially, delta variant is more transmissible than the original virus, and its first outbreak was emerged in October 2020 in India, now designated as variant of concern labelling as VOC-21APR-02 on 6 May 2021 [7]. Its notable mutations are L452R, T478K, P681R and also show the evidence of re-infection [8].

In this present study, we have described three cases of Covid-19 infection with two delta plus and one kappa variant of covid-19 virus person presenting with fever, cough, shortness of breath and altered taste and smell too positive for COVID-19. This case study provides the information for research monitoring purpose and especially the management of delta plus and kappa variant patients with different risk levels/complications.

Methodology and follow up

Suspected SARS-CoV-2 patients were diagnosed by COVID-19 Viral Research Diagnostic Laboratory (VRDL) having Bio Safety Level-3 (BSL-3) standard molecular facility in the Department of Microbiology, Baba Raghav Das Medical College Hospital Gorakhpur in northern India from May 17, 2021 to June 12, 2021. The inclusion criteria of suspected COVID-19 used in this study are similar with orientation recommended by Indian Council of Medical Research under the Ministry of Health and Family Welfare; Government of India [9]. 30 samples were chosen at random for sequencing in order to identify SARS-CoV-2 variants based on severity of cases and cycle thresholds (CT)-values ≤25. The demographic and clinical data were obtained by a hospital data management system.

Diagnosis of SARS-CoV-2 virus

The naso- and oro-pharyngeal swab samples were collected from the patients' referrals according to standard protocol while wearing a personal protective equipment (PPE) kit [10]. There were two stages to the test of SARS-CoV-2 Virus; (1) RNA extraction was done through Zybio nucleic acid extraction kit (magnetic Bread Method, batch no. 5104038) under standard biosafety guidelines, (2) The RT-PCR for SARS-CoV-2 was performed with a single tube multiplex assay by QuantoStudio™5 Real-Time PCR system (Thermofisher, USA) through the GB SARS-CoV-2 RT-PCR kit. The PCR conditions were as follows: uracil-DNA glycosylases enzyme (mixed in COVISure RT master mix to prevent laboratory carryover contamination) incubation at 25°C for 2 minutes, reverse transcription at 50°C for 15 minutes, and activation at 95°C for 3 minutes. Denaturation for 40-45 cycles at 95°C for 10 seconds and annealing/extension at 60°C for 30 seconds were used in PCR amplification. The CT-value ≤ 25 was considered significant enough to send the material to be sequenced.

Blood sample collection for biochemical markers

Biochemical assessment was done by using an aseptic technique and the machine used was fully automatic batch analyzer for serum or plasma preferred samples. These were the routine process among hospitalized patients recommended by clinicians, who were positive for the SARS-CoV-2. All the clinical and laboratory parameters like biochemical profile, especially CRP, serum ferritin, D-dimer, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), procalcitonin (PCT) and serum bilirubin were measured.

Genome Sequencing process of SARS-CoV-2

We have tested 171810 out of which 382 found SARS-CoV-2 positive during 17th May- 12th June 2021. Then, we have selected for SARS-CoV-2 variant detection randomly 30 samples especially CT values were less than 25. We have sent 1 ml aliquot of each sample by using triple layer packaging for gene sequencing in Institute of

Genomics and Integrated Biology (IGIB), New Delhi. The samples were sequenced on orthogonal platforms as well as (shotgun and amplicon) using ONT and Illumina platform [11].

Results

We have found 27 SARS-CoV-2 delta variants in out of 30 samples. Surprisingly, first time two delta plus (AY.1) and one kappa (B.1.617.1) variants of SARS-CoV-2 were also reported at July 2, 2021 from Uttar Pradesh (U.P.). The cases were further observed as newly found variants of our centre as mentioned below.

Cases with delta plus variant (B.1.617.2 double mutant or AY.1)

We have found two cases with SARS-CoV-2 Delta plus variant through sequencing and their demographic, clinical, biochemical and follow up are mentioned in table 1.

Case 1

In among of them, a 23 years old female case from Lucknow, U.P. was recovered through medication and home isolation, recognised as case-1 (table 1). She has already taken the both the doses of Covishield® vaccine before the detection of SARS-CoV-2 infection. After 90 days, she had faced COVID-19 symptoms as severe chill, cough, alter taste and smell, and then presented in emergency COVID-19 ward from BRD Medical college Gorakhpur U.P., where oro-pharynx and naso-pharyngeal sample was collected and tested. There sample was detected as RT-PCR positive (CT value; RdRp:20, E-gene:19.1). Her biochemical tests suggestive of CRP, D dimer, serum ferritin and serum bilirubin were measured and mentioned in table 1. The sample was further sequenced and identified as delta plus variant of SARS-CoV-2 with presence of mutations as reported in table 2. She had no foreign travel history. However, she was posted in ICU COVID-19 ward for her duties. Onset of symptoms, she developed fever (101.7°F), fatigue, myalgias, and mild respiratory distress with SpO2 of 89-92% with respiratory rate of 18 per minute. She become home isolate and started on oxygen via nasal cannula and on 1-gram doxycycline intravenously every 8 hours and tablets 500 mg azithromycin, 650 mg paracetamol and 12 mg ivermectin orally daily. After ten days, she has again found RT-PCR positive (CT value; RdRp: 27). Being a mild infection, she was treated with oral azithromycin, doxycycline, 1000 mg Vitamin C, 100 mg zinc and levocetirizine plus montelukast, for five days. Again RT-PCR repeated after 8 days of illness and the test was negative for SARS-CoV-2.

Case 2

A second case was a male with 66 years old from the district of Deoria, U.P. He had not taken any COVID-19 vaccine before the infection. However, he had no any foreign travel history and did not have any direct contact with COVID-19 confirm cases. He was admitted as category 1 (symptomatic influenza like illness [ILI] patient) case in ICU COVID-19 ward with symptoms of COVID-19 with sPO_2 - 80%, and further detected SARS-CoV-2 positive (May 17, 2021) as mentioned in table 1 as case-2. Patient's condition was deteriorated 2 days after admission to our facility, and he became hypoxic to 75% oxygen saturation while on nasal cannula. Then he was kept on ventilator with oxygen support 15 litre/minutes, remdesivir, injectable antibiotics (azithromycin, ceftazidime/avibactum), bronchodilators, systemic steroids, vitamin C etc. The clinical symptoms started with high grade fever and shortness of breath, later on anorexia, abdominal pain, sore throat, chest pain observed. Different biochemical test were done at the time of admission, which showed raised/high value of C reactive protein, D dimer, serum ferritin and procalcitonin and were continuously monitored during treatment. Patient could not survive after 12 days of treatment on May 29, 2021. His computed tomography finding was suggestive of showing extensive bilateral ground glass appearance throughout the lung with predominance in the peripheral lower lobes concluding as extensive viral pneumonitis with CORDAS score of 18/25 rapidly landing into acute respiratory distress syndrome (ARDS). The mutations of SARS-CoV-2 detected by sequencing are listed in table 2.

SN.	Parameters	Case-1	Case-2	Case-3
1.	Variant (Lineage)	Delta plus AY.1	Delta plus AY.1	Kappa B.1.617.1
2.	Age (in years)	23	66	65
3.	Gender	Female	Male	Male
4.	Vaccination status	Taken both doses	Unvaccinated	Unvaccinated

5.	Clinical history	Fever Breathlesness fatigue, myalgias Nausea	Fever Breathlesness Bodyachae Nausea	Fever Breathlesness Fainting/choking attack Nausea, Vomiting
Biochemical parameters				
	CRP (Normal value: > 10 mg/L)	24	37.6	43.65
	D-dimer (Normal value : < 0.4 mcg/ml	2.35	3.26	4.74
	Serum ferritin (Normal value : 20-250: Male and 10-120: Female, ng/ml)	480	725	824
	SGOT (Normal value: 0-40 IU/L)	-	58.2	56.4
	SGPT (Normal value: 0-40 IU/L)	-	61.8	60.3
	PCT (Normal value: < 0.05 ng/mL)	-	0.09	0.10
	Serum Bilirubin (Normal value: 0.3-1.4 mg/dL)	1.20	1.38	1.55
	RT-PCR (CT value; RdRp gene)	20	18.3	22
	Hospitalization	Home quarantine	Admitted in ICU ward of COVID-19	Admitted in ICU ward of COVID-19
	sPO^2 at admission/diagnosis	90-95%	75-80%	75-85%
	sPO^2 on follow up	80%	60%	66%
	Remdesivir/tocilizumab status	Not given	Remdesivir	Tocilizumab
	Severity status	Moderate	Critical	Critical
	Outcome	Recovered	Decease	Decease

Table 1: Comparison of firstly detected cases of delta plus and Kappa variant of SARS-CoV-2 from Uttar Pradesh**.**

Case with Kappa variant (B.1.617.1)

A 65 years known case of diabetic male from the district of Santkabirnagar, (U.P.) was admitted on June 12, 2021 in ICU COVID-19 ward with worse clinical symptoms like hypoxia, high grade fever, chest pain, unconsciousness and values of biochemical tests mentioned in table 1. SpO_2 was 75-80% at time of positive for SARS Co-V-2 with CT value was 22 mentioned as case-3 in table 1. He was on required 100% fraction of inspired oxygen (FiO2) and a positive end-expiratory pressure (PEEP) of 14 to maintain an oxygen saturation of >90%. Patient was given injectable antibiotics, vitamin C, systemic steroids and other life saving medicines along with one dose of 8 mg per kg (567 mg) of tocilizumab, an anti-interleukin-6 monoclonal antibody on the day of admission. But due to late presentation of ARDS and co-morbidity, patient was died on June 14, 2021. His sample was sent for sequencing and mutations detected and labelled as case of kappa variants as mentioned in table 2.

Cases	VOI	VOC	Mutations
Case-1 (delta plus)	No	Yes	210:G:T, 241:C:T, ORF1a:F924F, ORF1a:A1306S, ORF1a:P2046L, ORF1a:P2287S, ORF1a:D2907D, ORF1a:V2930L, ORF1a:T3255I, ORF1a:T3646A, 11332:A:G, ORF1b:P314L, ORF1b:G662S, ORF1b:P1000L, ORF1b:A1918V, S:T19R, S:T95I, S:G142D, S:K417N, S:L452R, S:T478K, S:D614G, S:P681R, S:D950N, ORF3a:S26L, M:I82T, ORF7a:V82A, ORF7a:T120I, ORF8:A51A, 28270:TA:T, N:R203M, N:G215C, N:D377Y, 29742:G:T

Case-2 (delta plus)	No	Yes	210:G:T, 241:C:T, ORF1a:T866T, ORF1a:F924F, ORF1a:A1306S, ORF1a:P2046L, ORF1a:V2930L, ORF1a:T3255I, ORF1a:T3646A, 11332:A:G, ORF1b:P314L, ORF1b:-G662S, ORF1b:P1000L, ORF1b:P1548T, ORF1b:A1918V, S:T19R, S:K417N, S:L452R, S:T478K, S:D614G, S:P681R, S:D950N, ORF3a:S26L, M:I82T, 28270:TA:T, N:R203M, N:G215C, N:S327S, 29742:G:T
Case-3 (kappa)	Yes	No	210:G:T, 241:C:T, ORF1a:F924F, ORF1a:Y1064Y, ORF1a:V1446I, ORF1a:T1567I, ORF1a:P3371S, ORF1a:T3646A, ORF1b:P314L, ORF1b:K469N, ORF1b:G1129C, OR-F1b:M1352I, ORF1b:K2310R, ORF1b:S2312A, S:T95I, S:D111D, S:G142D, S:E154K, S:L452R, S:E484Q, S:D614G, S:P681R, S:Q1071H, ORF3a:S26L, M:F53F, M:I82S, ORF7a:V82A, ORF7a:P84S, 28270:TA:T, N:D144H, N:R203M, 29742:G:T

Table 2: Description of different mutation pertaining to delta plus and kappa variants.

Abbreviation used: VOI: Variant of Interest, VOC: Variant of Concern, ORF: Open Reading Frame Highlighted-as Common Mutations Observed.

Discussion

We have found two delta plus (AY.1) and one kappa (B.1.617.1) variants except delta variants of SARS-CoV-2 were also reported in our study. Unfortunately, among these, two non-vaccinated cases, person infected with delta plus and another with kappa variants had loss of their life admitted in COVID-19 ICU with severe conditions. Remaining, one case infected with delta plus was recovered who had already taken the vaccination of both doses.

Aside from SARS-CoV-2 genomic variants, host and host-agent interactions may affect their outcomes. Patients' age, gender, severity status, existence of co-morbidity, variance in the gut flora, and previous childhood vaccinations (particularly Bacillus Calmette-Guerin) are all possible host variables [12,13]. When the genotype of SARS-CoV-2 variations will compared across symptoms, co-morbid illness, and vaccination status of COVID-19 patients, a clustering of unique viral genome variants in newly emerging variants was find out and helpful for patients care as well as controlling new wave of COVID-19.

Our findings of new emerging cases of Delta plus was also cause for concern, as it indicated a quick spread and the likelihood of diminished vaccination efficacy, as well as an elevated risk of re-infection of this geographic region for the second wave of COVID-19. Public Health England refers to the delta variation as "Delta with K417N," and it consists of two clades that correspond to Pango lineages AY.1 and AY.2 [14]. From "Delta plus K417N," it was given the moniker "Delta plus" [15]. The mutation's name, K417N, alludes to the substitution of lysine (K) with asparagine (N) at position 417 [16]. After 22 cases of the "Delta plus" variation of SARS-CoV-2 were detected in our country, India's Ministry of Health and Family Welfare labelled it a Variant of Concern (VOC) on June 22, 2021 [17]. However, the leading scientists and virologists argued that this variant was identified in limited number of individuals as well as insufficient evidence to warrant labeling the variation as a unique variety of concern after the release [18].

The kappa variant was detected in India for the first time in Dec 2020 and accounted for more than half of the sequences submitted from India at the end of March 2021 [19,20]. Public Health England identified it as a variation under investigation (VUI-21APR-01) on April 1, 2021 with L452R, E484Q, and P681R are noteworthy mutations [21,22]. This first case finding of kappa (sub-lineage of B.1.617) variant has given the idea that our population are also affected with of new emerging mutations of SARS-CoV-2. Other factors might include difficulties in implementing and adhering to public health as well as social gatherings (such as large gatherings during cultural and religious festivities and elections). To understand the relative contributions of these variables, more research is needed. The different host factors may include stage of disease, the degree of immune response against the virus due to difference in degree of T regulatory response, presence of co-morbid illness, and variation in the gut microbiota [23]. This primary report also opens a door towards large-scale gene sequencing of SARS-CoV-2 samples in order to develop precautionary measures against subsequent waves.

Conclusion

In conclusion, the new genotypic mutation of SARS-CoV-2 virus across symptomatic and asymptomatic COVID-19 patients in eastern Uttar Pradesh suggests that SARS-CoV-2 genome variations have a substantial influence on COVID-19 outcome, resulting in an increase in reproduction ratio (R_0) in the second wave. Except Delta variant, our findings point to a possible role for vaccination against the more lethal double-mutated delta plus variant. Apart from SARS-CoV-2 genome variations and mutations, agent-host interactions may influence the outcome of SARS-CoV-2 infection. We would like to emphasize on the potential importance of the mutations observed in all three cases of the symptomatic patients. We conclude that the virus genotype variations show a strong relationship with COVID-19 severity and significant role of viral genome variations in COVID-19 symptoms, presentation and outcome.

Conflict of Interest

There is no financial benefit or conflict of interest to be reported for this case series.

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