IDENTIFICATION OF 3 KEY MUTATIONS IN SPIKE PROTEIN ASSOCIATED WITH THE SARS-COV-2 DELTA VARIANT (B.1.627.2 LINEAGE) IN COVID-19 PATIENTS IN BAC GIANG PROVINCE

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Summary

***Background**: Emergence of the SARS-CoV-2 Delta variant is rapidly spreading worldwide. Identification of mutations associated with the SARS-CoV-2 Delta variant facilitated the genomic surveillance and control of the COVID-19 pandemic. **Objectives**: We used a simple and effective-cost method for detection of key mutations associated with the SARS-CoV-2 Delta variant from clinical samples of Vietnamese patients. **Subjects and methods**: A total of forty-eight nasopharyngeal samples were collected from patients infected with SARS-CoV-2 from 5/2021 to 6/2021 in Bac Giang province. RNA was extracted and SARS-CoV-2 was detected by in-house molecular methods. The sequence analysis obtained for detection of its key mutations and phylogenetic tree was constructed for identification of the SARS-CoV-2 Delta variant. **Results**: In this report, 44/44 (100%) nasopharyngeal samples of COVID-19 patients were detected two key mutations at the L452R, T478K in the receptor binding region of spike protein associated with SARS-CoV-2 Delta variant (B.1.617.2 lineage). Of those, 42/44 samples had D614G mutation in Spike protein. **Conclusion**: In the present study, we showed three key mutations at L452R, T478K, and D614G associated with the SARS-CoV-2 Delta variant in the population of COVID-19 patients in Northern Vietnam.*

** Keywords: SARS-CoV-2; Delta variant; Mutations.*

INTRODUCTION

COVID-19 pandemic caused by SARS-CoV-2 remains a big challenge and unsolved problems in many countries. According to WHO's COVID-19 Weekly Epidemiological Update, there were more than 216 million people infected with SARS-CoV-2, and the cumulative number of deaths is 4.5 million in 145 countries and territory regions [2]. Although there were several vaccines approved for emergency use authorization by the WHO and FDA, however, new cases are still increasing for months from many regions in the world. The emergence of novel variants of SARS-CoV-2 has been shown to be associated with a rapid transmission of SARS-CoV-2 and breakthrough infection

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in vaccinated individuals [3, 4] The WHO declares that several variants designated as “variant of concern” and SARS-CoV-2 Delta variant is responsible for the majority of new cases in many countries. Data indicated that the Delta variant is 40 - 60% more transmissible than Alpha and almost twice as transmissible as the original Wuhan strain of SARS-CoV-2. In addition, patients infected with Delta variant had a higher viral load than those infected with other variants [5]. Diagnosis of COVID-19 is based on real-time RT-PCR assay for confirmatory detection of SARS-CoV-2 in clinical samples [6]. However, several real-time RT-PCR kits failed to detect SARS-CoV-2 associated with the presence of mutations in primer and probe binding regions [7], therefore genomic surveillance of circulating variants have facilitated the detection and tracking of VOC (Variant of Concern) and identification of key mutations of SARS-CoV-2 Delta variant play important roles in the effective control of COVID-19 pandemic. The most common approach for identifying of SARS-CoV-2 variants is full genome sequencing, but it requires technical expertise, modern equipment, and turnaround time [7]. Therefore, this methodology is no available to most resource-limited countries. Recently, several real-time PCR assays have become available for the detection of specific mutations of VOC, however, it only identifies known mutations of VOC, and novel mutations are not available [8]. In this study, we used a simple and effective-cost method for identification of key mutations associated with the Delta variant in clinical samples of Vietnamese patients based on Sanger sequencing from a fragment of Spike gene of SARS-CoV-2.

SUBJECTS AND METHODS

1. Subjects

Clinical samples: A total of 48 nasopharyngeal samples collected from patients of COVID-19 in acute phase of illness visited at the Field Hospital from May to June, 2021 in Bac Giang province. Ethical approval was obtained from the local authorities for all samples of the study.

2. Methods

SARS-CoV-2: SARS-related Coronavirus 2, isolate USA-WA1/2020 (Catalog No. NR-52286, a kind gift provided by the National Institute of Vaccine Control and Biologicals) that has been inactivated by heating to 65°C for 30 minutes using for the extraction of RNA for the validation of real-time RT-PCR for detection of SARS-CoV-2. The viral RNA was extracted from 140 µl of cell culture supernatant of infected cells inactivated and nasopharyngeal samples of COVID-19 patients using QIAamp Viral RNA Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer’s instruction. The RNA was finally eluted in a final volume of 60 µl of AE buffer and was stored at -80°C until use.

Real-time RT-PCR: An in-house real-time RT-PCR targeting the E and N gene for detection and quantification of SARS-CoV-2 assay was developed in our laboratory. The positive control using RNA extracted from isolate USA-WA1/2020 (Catalog No. NR-52286). Primers and probes are in table 1. Briefly, all amplifications were performed in a total volume of 20 µl containing 10 µl of 2X Luna® Universal Probe One-Step RT-qPCR Kit (Biolab, UK), 1.2 µl each of the 5 µM forward and reverse primers, and the 0.2 µl of 5 µM probe, 7.2 µl of

RNase/DNase-free water and 5 µl of RNA template. All reactions were run on a RotorGene Q 5plex Dx (Qiagen, Germany) using the following thermal cycling conditions: 50°C for 2 min, followed by 45 cycles of 90°C for 15 min, 94°C for 15 sec, and 58° for 60 sec.

* *PCR and sequencing:* A fragment of 725 bp of Spike gene was amplified by one-step RT-PCR (Qiagen, Germany) using primer sequences previously described [10]. PCR reactions were performed on Mastercycler (Eppendorf, Hamburg, Germany) with the following thermal cycle: The initial denaturation step at 95^0 C for 5 min was followed by 35 cycles at 95^0C for 30 sec, 58^0C for 30 sec, and 72^0C for 30 sec, with the final extension step at 72^0C for 7 min. Amplification products were run on a 1.5% agarose gel electrophoresis after staining with Ethidium Bromide 10 mg/mL and visualized under UV light, and then purified and sequenced using a 3130 XL sequencer.

Table 1: Primers and probe sequences used in this study.

Primers and Probes	Sequences
S-Fm	5'-AGGGCAAACTGGAAAGATTGCT-3'
S-Rm	5'-CAGCCCCTATTAAACAGCCTGC-3'
VE1-Pr	TEXAS-RED-5'-AACCGACGACGACTACTAGCGTGCCTT-3'-BHQ1
VE6-F	5'-CGGAGTTGTTAATCCAGTAATGGA-3'
VR6-R	5'-GTTCGTACTCATCAGCTTGTGCTT-3'
qVN-F	5'-GGTCCAGAACAAACCCAAGGA-3'
qVN-R	5'-GACATTCCGAAGAACGCTGAA-3'
qVN-Pr	FAM-5'- ATTGCACAATTTGCCCCCAGCG -3' BHQ1

* *Phylogenetic analysis:* Our sequenced amplicons obtained were aligned with reference sequences from different countries retrieved from the GISAID (reference sequences in figure 1) using Bioedit 7.0 (www.mbio.ncsu.edu/BioEdit/bioedit.html) and MEGA 7.0 software (www.megasoftware.net). The phylogenetic tree was constructed using the neighbor-joining method, and significance level was estimated with 1000 bootstrap replicates.

* *Statistical analyses:* By using SPSS 20.0 (IBM, Armonk, New York, United States). Mutation frequencies were obtained by direct counting, and statistical analysis was performed by Mann-Witney U test, and a p-value less than 0.05 was considered statistically significant.

RESULTS AND DISCUSSION

Among 48 nasopharyngeal samples analyzed from COVID-19 patients, there were 18 males (37.5%) and 30 females (62.5%), with a mean age of 38.08 ± 14.9 years. Ct values ranged from 14.05 to 31.05 for N gene and 14.08 to 30.29 for gene E. Partial sequencing of Spike gene was performed successfully in 44 out of 48 samples and the obtained sequences were compared with the reference sequence – NC_045512.2, there were 11 mutations detected in this population. Note that all sequences possessed two key mutations in the receptor binding domain region (RBD) of SARS-CoV-2 at the L452R, T478K, which were specific for

B.1.617.2 lineage. Of those, 42 samples had the D614G mutation, identified in the first wave of the COVID-19 epidemic in Vietnam [1]. In addition to two specific mutations of L452R, T478K for B.1.617.2 lineage, there were 4 samples with two additional mutations E620Q, D627H and three samples with V620F mutation within the spike but outside the RBD region, and other Spike mutations observed at a lower frequency (table 2). Of note, we indicated no samples carrying the E484Q Spike gene mutation reported to be associated with the immune escape ability of virus.

Table 2: Frequencies of Spike Mutations of SARS-CoV-2 detected in this study.

Nucleotide location	Reference	Mutation	Amino acid	Frequency, n (%)
22917	T	G	L452R	44/44 (100)
22995	C	A	T478K	44/44 (100)
23403	A	G	D614G	42/44 (95.45)
22992	G	C	S477T	1/44 (2.27)
23417	G	C	E619Q	4/44 (9.09)
23420	G	T	V620F	3/44 (6.82)
23441	G	C	D627H	4/44 (9.09)
22985	G	T	A475S	1/44 (2.27)
23088	G	T	R509I	1/44 (2.27)
22923	G	T	R454I	1/44 (2.27)
23481	C	T	S640F	1/44 (2.27)

For the phylogenetic analysis, 16 SARS-CoV-2 sequences of Spike gene were retrieved from GISAID and Wuhan reference sequence NC_045512.2 from GenBank, along 44 sequences obtained used in this study. The results detected in all sequences were clustered into B.1.617.2 branch (figure 2).

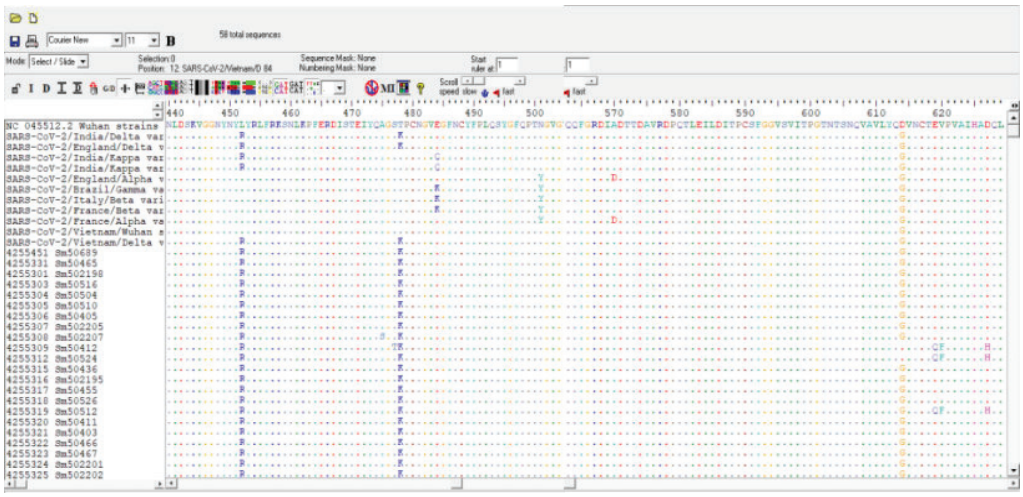

Figure 1: Location of three key mutations at L452R, T478K and D614G in partial Spike gene of SARS-CoV-2 (n.22798-23522) associated with SARS-CoV-2 Delta variant.

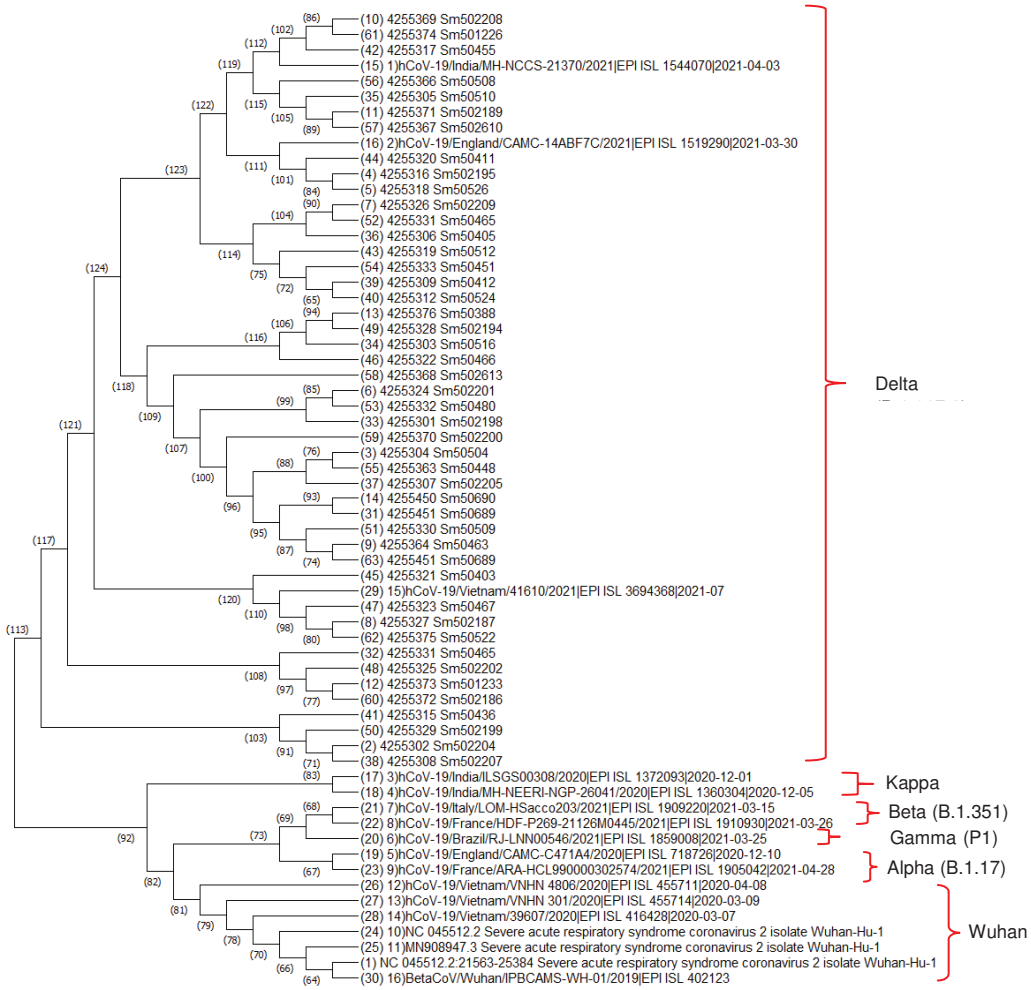

Figure 2: Phylogenetic tree of SARS-CoV-2 Delta variant based on the partial Spike gene fragment (n.22798-23522) obtained in this study and 16 reference sequences of SARS-CoV-2 Wuhan strains retrieved from Genebank and GISAID (MN908947.3, NC_045512.2, EPI_ISL_402123, EPI_ISL_416428, EPI_ISL_455714, EPI_ISL_455711) and SARS-CoV-2 Alpla (EPI ISL 718726, EPI ISL 1905042), Beta (EPI ISL 1910930, EPI ISL 1909220), Gamma (EPI ISL 1859008), Kappa (EPI ISL 1360304, EPI ISL 1372093) variant using CLUSTAL_W with Kimura's correction.

CONCLUSION

In the present study, we used a simple and effective-cost method for molecular detection of key mutations at L452R, T478K, and D614G associated with SARS-CoV-2 Delta variant in COVID-19 patients in Northern Vietnam.

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Conflicts of interest

The authors have no conflicts of interest to disclose

Author contributions

H.X.S, N.T.X, L.C.T: conceived and designed, supervised study, drafting of the manuscript and critical revision of the manuscript.

N.T.T.L, D.T.T.H, N.V.N.M, N.H.B: performed experiments, acquisition of data, analysis and interpretation of data.

L.C.T, L.Q.H, N.T.X, H.X.C: material support, acquisition of data and commented on manuscript.

H.A.S, N.M.P, N.T.L, T. V.T, H.V.L, N. T.G, D.Q: critical revision of the manuscript.