Original Article

SPIKE GENE TARGET FAILURE AMONG RT-PCR POSITIVE SARS-COV-2 SAMPLES IN DHAKA CITY DURING EARLY VARIANT WAVES

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ABSTRACT

Background: Real time RT-PCR is a widely used method for detecting SARS-CoV-2, the causative agent of COVID-19 infection. A phenomenon termed 'spike gene target failure' (SGTF) has been reported in certain SARS-CoV-2 lineages, in which there is failure to detect the spike (S) gene target in samples that are positive for other RT-PCR targets, which occurs due to a deletion of six nucleotides in the spike gene (69-70del). The SGTF trait has been validated as a reliable surrogate marker for presence of 69-70del in the viral genome. We retrospectively determined the prevalence of SGTF trait among COVID-19 positive samples in Dhaka city.

Methods: The study was conducted in the department of Virology at National Institute of Laboratory Medicine and Referral Center (NILMRC), Dhaka between November 2020 and June 2021. A total of 5,259 nasopharyngeal and oropharyngeal swab samples that tested positive for SARS-CoV-2 with TaqPath COVID-19 Combo PCR kit, were screened for the SGTF marker. SGTF was defined as the non-detection of S-gene-target in samples that were positive for both N and ORF1ab targets with Ct values ≤30.

Results: Among 5,259 PCR-positive samples, 144 (2.74%; 95% CI: 2.30%, 3.20%)) showed SGTF trait in our study. The peak SGTF detection rate was observed in February 2021 (10.77%; $p<0.0031$), which corresponded with the widely-reported circulation of Alpha variant in Bangladesh that characteristically displays 69-70del in the spike gene. Sequencing was not performed, which limited lineage confirmation.

Conclusion: An abrupt increase in SGTF marker detection at PCR laboratories should be investigated thoroughly to enable the prompt identification of emerging SARS-CoV-2 lineages carrying 69–70 deletion.

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INTRODUCTION

Since its emergence in Wuhan, China in December 2019, the Severe Acute Respiratory Syndrome Coronavirus 2 (SARS-CoV-2) has caused an astounding number of infections – over 550 million, and more than 6 million deaths worldwide as of April 2022.[1] Real-time reverse transcription PCR (RT-PCR), a primer-probe based assay, has been used extensively for SARS-CoV-2 genome detection.[2] However, many viruses undergo substantial genetic mutation, which can over time result in mismatches between the primer-probe and the viral gene target, often manifesting as diagnostic failure in PCR.[3] One such mutation in the SARS-CoV-2 genome involves deletion of six nucleotides in the spike protein (S)

gene at position 21765–21770, resulting in the loss of two amino acids: histidine and valine at positions 69 and 70 respectively (69-70del). The deletion has led to failure of amplification of the S-gene target in an otherwise positive PCR test while using certain commercial three-target PCR assays including TaqPath COVID-19 Combo kit (ThermoFisher Scientific, USA). This occurrence, termed 'spike gene target failure' (SGTF) or 'spike gene drop out', interestingly was not associated with diagnostic failure as the amplification of the two remaining targets namely nucleocapsid (N) and open reading frame 1ab (ORF1ab) remained unaffected.[4-5] Researchers subsequently used this finding to their advantage and validated SGTF result in a PCR test as a reliable surrogate marker for the presence of 69-70del within the viral genome.[6]

SGTF trait was first detected November 2020 in England.[7] Whole genome sequencing of these strains revealed that they belonged to a novel lineage of SARS-CoV-2, termed B.1.1.7 by PANGO lineage system and Alpha variant by WHO. Separate studies conducted in England and the USA during known waves of B.1.1.7 strains reported 99.6% concordance between SGTF marker and the presence of 69-70del in the viral genome.[1,7] Thereafter, SGTF was utilized as a screening tool for early detection and monitoring of the spread of B.1.1.7 variant in multiple countries of the world, including Portugal[4], France[5], Canada[8], and Pakistan[9]. Later on, in November 2021, this marker was again effectively employed for the prompt identification of the emerging B.1.1.529 lineage (Omicron variant) in South Africa.[10] Clearly, it has the potential to identify and track any variant harbouring the 69-70del that may arise in the course of the pandemic, provided that a subset of samples is validated by sequencing.[1,6]

While sequencing is the gold standard to identify circulating strains, the capacity for routine genomic surveillance is inadequate in many countries including Bangladesh.[11] Underdeveloped laboratory infrastructure, high operational and maintenance costs, and a limited pool of trained laboratory and bioinformatics personnel collectively impede large-scale sequencing initiatives.[12] SGTF marker has been deemed a ready-made, simple tool for screening variants carrying 69-70del in resource limited settings.[8] As a leading COVID-19 testing center of Bangladesh, National Institute of Laboratory Medicine and Referral Center, Dhaka has access to a large COVID-19 RT-PCR data set. Our study aimed to perform a retrospective analysis of the data to determine the prevalence of SGTF trait and the potential circulation of 69-70del lineages in Dhaka city.

METHODS

Study design

This retrospective, observational, cross-sectional study was conducted at the Department of Virology of National Institute of Laboratory Medicine and Referral Center (NILMRC), located in Dhaka, the capital city. Our institute operates a well-equipped biosafety level-2 (BSL-2) molecular laboratory, which served as a government-designated COVID-19 testing facility during the pandemic. Between November 2020 and June 2021, a total of 40,409 nasopharyngeal and oropharyngeal swab samples from suspected COVID-19 patients were tested by RT-PCR using TaqPath COVID-19 Combo PCR kit (ThermoFisher Scientific, USA). The samples were received from various hospitals, homes and collection booths in Dhaka city.

The inclusion criteria comprised all samples testing positive for SARS-CoV-2 by RT-PCR, using TaqPath assay within the given time frame, irrespective of the patients' age and gender. Samples with missing demographic information or amplification data were excluded. Out of 40,409 specimens, 5,259 (13.01%) satisfied the eligibility criteria, and were purposively selected for inclusion in our study.

Reverse transcription RT-PCR

Viral RNA was extracted using MagMax Viral/Pathogen Nucleic Acid Isolation Kit (ThermoFisher Scientific, USA), followed by amplification with TaqPath kit. The assay employs three primer-probe sets targeting three different genomic regions of SARS-CoV-2 RNA, namely ORF1ab, N and S. As per the manufacturer's instructions, a positive RT-PCR test was defined as the amplification of at least two of the three gene targets with cycle threshold (Ct) values ≤35.

Operational definition of SGTF

For our study, the positive RT-PCR results were retrospectively screened for the presence of spike gene target failure (SGTF). SGTF was defined as the failure to detect S-gene target in otherwise RT-PCR positive samples that showed amplification of both N and ORF1ab targets with cycle threshold (Ct) values ≤30.[4] Samples with low overall viral burden can erroneously exhibit the SGTF trait,[10] so nine results showing weak amplification of N and ORF1ab (Ct value ≥30) were excluded from our analysis.

Data analysis

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Amplification-related data were obtained from the PCR instruments, and corresponding demographic information (age and gender) of the individuals was retrieved from test reports. These data were analyzed using the Statistical Package for the Social Sciences (SPSS) software, version 27.0 (International Business Machines Corporation, New York, USA). Independent samples t-test was performed to assess differences in mean values between groups. 95% confidence intervals for proportions were calculated by one-sample binomial test. Pearson's chi-square test was applied to compare categorical values between groups. To compare proportion across months, chi-square test of homogeneity was performed, followed by post-hoc analysis. Differences were considered statistically significant for p value <0.05.

Ethical clearance

Our research was approved by the Institutional Review Board of NILMRC (NILMRC/Ethical Com/Viro-2020:12).

RESULTS

Out of 5,259 RT-PCR-positive samples, 144 (2.74%; 95% CI: 2.30%, 3.20%) showed spike gene target failure, i.e., they were positive for N and ORF1ab targets but negative for S gene. Among 5,115 non-SGTF RT-PCR positive samples, 5,112 (97.20%) displayed amplifications of all three targets while the remaining three (2.80%) were positive for only N and S genes.

Out of 144 SGTF-positive cases, 96 (66.67%) were male while 48 (33.33%) were female. Non-SGTF RT-PCR positive cases comprised 3296 (64.48%) male and 1819 (35.56%) female participants. No significant association was observed between gender and SGTF status (chi-square test, p=0.581). The mean ages of SGTF and non-SGTF groups were 38.56 ± 14.382 years and 39.85 ± 14.295 years respectively, with no statistically significant difference observed (independent samples t-test, p=0.285).

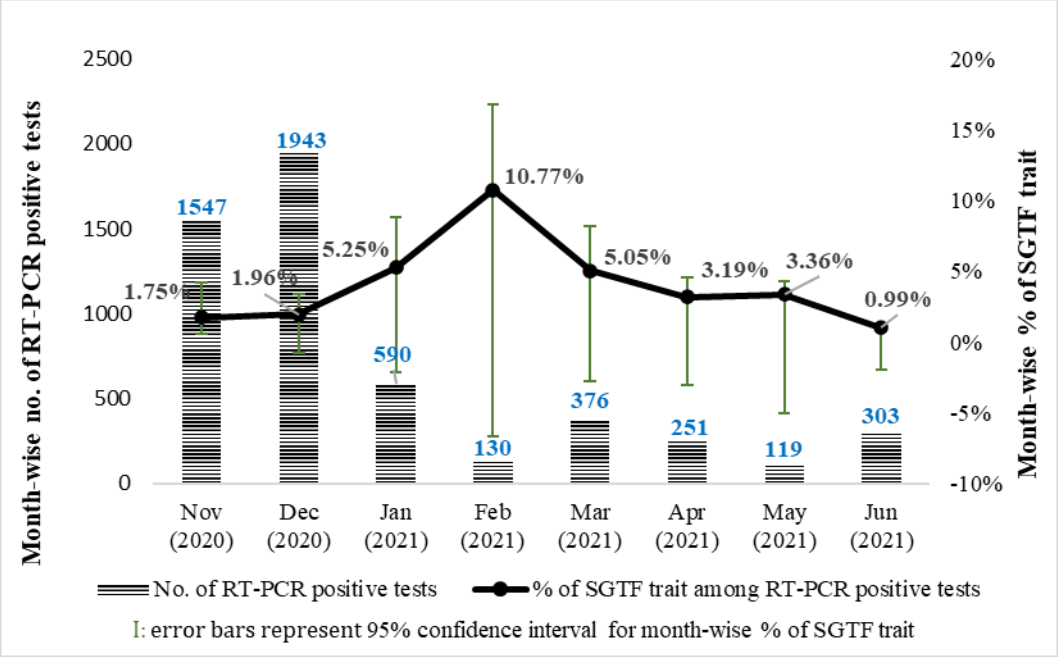

Figure-1: Month-wise proportions of SGTF trait among RT-PCR positive samples (n=144)

Note: SGTF= Spike gene target failure

SGTF trait was first detected at our institute in November 2020, comprising 1.75% of RT-PCR positive tests by the end of the month. The prevalence gradually increased over December 2020 (1.96%) and January 2021 (5.25%), reaching peak in February 2021, during which the marker was present in 10.77% of the positive tests. The rate declined from March (5.05%) onwards, with only 0.99% SGTF-positive samples identified in June 2021. (Figure 1) The month-wise variation in SGTF proportions was statistically significant (Chi-square test of homogeneity, $p<0.001$).

Post-hoc analysis using adjusted standardized residuals revealed that SGTF prevalence was significantly higher than the expected counts in January and February 2021 ($p<0.0031$, after Bonferroni correction).

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DISCUSSION

We identified spike gene target failure trait in 2.74% of RT-PCR–positive SARS-CoV-2 samples tested at NILMRC, Dhaka, between November 2020 and June 2021, with the detection rate peaking in February 2021. This temporal pattern likely reflects the circulation dynamics of the lineages carrying the 69–70 deletion in the spike protein. The overall prevalence of SGTF marker at our institute was higher than the rates reported in France (0.56%)[5], but lower than those reported in Portugal (9.2%)[4], Canada (23.4%)[8] and Pakistan (29.6%)[9]. Such variation in positivity rates may be attributable to differences in the timing of sample collection and the extent of dissemination of strains with the deletion in those regions. Studies conducted during the peak of B.1.1.7 transmission typically reported higher proportions of SGTF.

We were unable to sequence the SGTF-positive samples due to lack of funding, therefore, we could not ascertain their specific lineages, representing a significant limitation of our study. However, we compared our findings with published sequence-based data to provide insight into the potential circulation pattern of 69–70del-carrying lineages in Dhaka city. Analyses conducted by Parvin et al. (2020), Rahman et al. (2021), Saha et al. (2021), and Afrin et al. (2022) indicate the overlapping transmission of three such lineages in Bangladesh during our study period—namely, certain B.1.1.25 sub-lineages, B.1.525 (Eta variant), and B.1.1.7. Among these, B.1.1.25 strains were consistently documented from April 2020 through June 2021, whereas B.1.525 exhibited limited but steady transmission between March 2021 and June 2021.[13-16] The concurrent circulation of these lineages corresponded with the continued detection of SGTF marker at our institute throughout the study period. Notably, we observed a surge in SGTF positivity between January and March 2021 (5.05~10.77%), which aligns with the widely-reported introduction and dissemination of the B.1.1.7 lineage in our population. The peak detection rate demonstrated in February 2021 is consistent with the 16~32% prevalence of this lineage reported by other studies. The subsequent decline in SGTF mirrors the replacement of B.1.1.7 variants with other emerging strains.[15,16]

The 69–70del in the spike protein has independently appeared several times in different SARS-CoV-2 lineages during the course of the COVID-19 pandemic. Its recurrent emergence supports the hypothesis that it may enhance viral fitness and immune evasion capacity, thereby imparting greater epidemic potential and posing a heightened threat to public health globally. To effectively guide public health responses to evolving variants, vigilant tracking and monitoring are essential.[17,18] The World Health Organization (WHO) recommends sequencing at least 1% of a country's confirmed COVID-19 cases for national genomic surveillance, a target that contrasts sharply with Bangladesh's estimated sequencing rate of <0.3% of confirmed cases as of 2023.[19,20] Therefore, a critical gap prevails in the country's capacity to monitor emerging variants.

In such context, the concordance between our findings and published genomic data supports the validity of this PCR-based marker as an indirect surveillance tool. Tracking SGTF trends can serve as an early warning system for the emergence and spread of strains harbouring 69-70del, and complement genome sequencing in resource-limited settings. Such an approach may enable more rapid epidemiological responses, inform public health policy, and ultimately contribute to reducing COVID-19–associated morbidity and mortality.

CONCLUSION

We observed a 2.74% prevalence of spike gene target failure (SGTF) among RT-PCR positive SARS-CoV-2 samples in Dhaka city, with the highest detection rate in February 2021, consistent with the circulation dynamics of Alpha variant (B.1.1.7) in Bangladesh. A sudden rise in SGTF marker at PCR laboratories should warrant careful investigation. Integrating SGTF trend analysis into routine surveillance programs could strengthen early detection and monitoring capacity for any emerging SARS-CoV-2 strain carrying the 69–70 deletion, particularly in resource-limited settings.

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Authors' contributions

AM and AA designed the study protocol. AM, MBBM, TN and AA conducted the RT-PCR testing. TM and AM carried out data recording and analysis.

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AM and TM wrote the original draft. AA edited and reviewed the manuscript.

Conflict of interest

The authors declare no conflict of interest.

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