Epidemiological Characteristics of SARS-CoV-2-Positive Samples in Japan: A Retrospective Study

Kenichi Tadokoro¹*, Katsuhiko Miki¹, Tadashi Yamasaki¹, Toshikazu Yamaguchi¹, Kazuhiro Tateda², Kensuke Kondo¹, Nobuki Arai¹

¹BML, Inc., 1361-1, Matoba, Kawagoe-shi, Saitama, 350-1101, Japan
²Department of Microbiology and Infectious Diseases, Toho University School of Medicine, 5-21-16 Omori-nishi, Ota-ku, 1438540, Tokyo, Japan

*Corresponding author: Kenichi Tadokoro, BML, Inc., 1361-1, Matoba, Kawagoe-shi, Saitama, 350-1101, Japan


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Abstract

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) causes coronavirus disease 2019 (COVID-19). In Japan, infections began in January 2020, and the number of infections has continued to increase. Specimens requested by medical institutions were measured for ORF1a/b, N-gene, and S-gene using TaqPath SARS-CoV-2 real-time polymerase chain reaction detection kits in our biomedical laboratory from January to August 2021. The distribution of threshold cycle (Ct) values of positive samples was analyzed separately every 2 months. The Ct values of saliva samples were normally distributed for all genes, except for the May/June S-gene. By contrast, nasopharyngeal swab samples showed a bimodal distribution from January to June, with a shift toward lower median values in July/August. The results suggest that viruses with different properties may be captured in saliva and nasopharyngeal samples.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), which causes coronavirus disease 2019 (COVID-19), was first described in Wuhan, China, in 2019 [1,2]. COVID-19 has spread globally and was declared a pandemic by the World Health Organization in March 2020. In Japan, the first patient with COVID-19 was reported in January 2020; by October 2022, the cumulative number of infections had reached 22 million. By June 2022, six peaks of COVID-19 had occurred in Japan: in March–April 2020, July–August 2020, October 2020–February 2021, March–May 2021, July–September 2021, and January–June 2022. The number of infected individuals increased significantly, especially during the six peaks. To provide rapid treatment and to understand the dynamics of COVID-19, many public and private laboratories performed clinical tests and reverse transcription-quantitative polymerase chain reaction (RT-qPCR) assays.

A good mechanistic approach to the pathogenesis is essential for the development of effective therapeutics and vaccines against infectious disease. There were also various reports on thrombosis and disease severity in relation to the COVID-19 immunological status. Toll Like Receptors (TLRs) play a considerable role in the host defense against microorganism [3]. Activation of the TLRs in COVID-19 infection could lead to the production of pro-inflammatory cytokines [4]. Serum levels of PD-L1 have a prognostic role in COVID-19 patients and associated to COVID-19 pathogenesis [5]. Also, a higher expression of PDL-1 is associated with poor response to induction therapy in AML patients [6]. TGF-β is a prominent regulator of immune reactions [9], and it causes a comorbidity of severe COVID-19 patients [10]. Mean platelet volume (MPV), and platelet distribution width (PDW) were increased in non-survivors of COVID-19 [11]. Also, MPV and PDW are valuable diagnostic markers for diagnosis of Spontaneous bacterial peritonitis (SBP) [12].

A variety of SARS-CoV-2 variants have been reported worldwide. These variants have been reported to differ in contagiousness and incubation period depending on the mutation acquired [13]. In early 2021, variants with the NS01Y mutation,
such as B.1.1.7 (alpha variant) and B.1.351, spread rapidly around the world. In Japan, the alpha variant, which had been feared to be increasing on April 20, had reached 80% of the total by May 26. On June 6, the spread of the B.1.617 lineage (including the delta variant) was reported. The positive rate of the delta variant in screening tests was approximately 5% on June 30 and had increased to 79% by August 18. The omicron variant was detected in international travelers on December 6, and its rapid replacement with the delta variant was reported on January 26, 2022 [14].

The study of SARS-CoV-2 variants has, thus far, been conducted primarily via next-generation sequencing [15,16]. Next-generation sequencers can provide detailed information on genetic changes, but it is difficult to determine virus load using this method. Herein, the SARS-CoV-2 epidemic was analyzed from different aspects by comparing real-time PCR results according to time and age group.

**Materials and Methods**

**Study setting**

This study used initial values of positive cases (saliva: 167,466 and nasopharyngeal swabs: 127,165) measured at the biomedical laboratory from January to August 2021 and January to February 2022. The Ct values of positive samples were used in this study. The analysis was divided into the following months: January/February 2021, when the prevalent strain was the wild type; March/April 2021, when the wild type was being replaced by the alpha variant; and May/June 2021, July/August 2021, and January/February 2022, when the dominant variants were alpha, delta, and omicron, respectively.

**Laboratory procedures**

Nasopharyngeal swab samples were collected using nylon-flocked swabs and vessels containing virus transport medium, made up of the following: cobas® PCR medium (Roche, Basel, Switzerland), transport medium (Miraclean Technology, Shenzhen, China), or SGVTM-3R (SUGIYAMA-GEN, Tokyo, Japan). Nasopharyngeal swabs and saliva samples were mixed with sample preservative fluid (Hangzhou BIOER, Hangzhou, China) and dithiothreitol. The SARS-CoV-2 nucleic acid tests were performed in amplitude diagnostic testing solution using TaqPath™ COVID-19 HT Kits (Thermo Fisher Scientific, Waltham, MA, USA). The TaqPath SARS-CoV-2 real-time PCR detection kit measures the ORF1a/b, N-gene, and S-gene of SARS-CoV-2, simultaneously.

**Statistical analysis**

Histograms were created for each gene to examine the distribution and trends of the Ct values (from 7 to 37). A box-and-whisker diagram was created to show the distribution of Ct values for the N-gene during each period. The Ct values of the N-gene during each period were compared by age group. The data were divided into age group and examined by t-test to determine whether there was a significant difference.

**Ethics statement**

This study was approved by the Institutional Review Board of the biomedical laboratory (reference number 21-006). Due to the retrospective nature of the study, the need for written informed consent was waived.

**Results**

Figure 1 depicts the histograms showing the distribution of Ct values for the three genes in positive samples during each period. The Ct values were normally distributed for all periods in the saliva samples.

The number of positives was divided into Ct values ranging from 7 to 37. The median is indicated by (▼). (A) Saliva samples. (B) Nasopharyngeal swab samples.
Figure 1: Distribution of Ct values for positive samples. The number of positives was divided into Ct values ranging from 7 to 37. The median is indicated by (▼). (A) Saliva samples. (B) Nasopharyngeal swab samples.

By contrast, the nasopharyngeal swab samples showed a bimodal distribution in January/February 2021 and May/June 2021. In May/June 2021, July/August 2021, and January/February 2022, the distribution skewed toward lower values. Although the ORF1a/b and N genes showed approximate distributions, the number of positive S-genes was halved in March/April 2021 and was markedly decreased in May/June 2021 and January/February 2022. Figure 2 shows the box-and-whisker diagram for the N-gene.
Figure 2: Box-and-whisker diagram of Ct values for samples positive for the N-gene. The mean value is indicated by (×).

The mean value is indicated by (×). The Ct values for saliva and nasopharyngeal swab samples decreased the most in July/August 2021. In all periods, nasopharyngeal swab samples showed a lower median value than the mean. Figure 3 shows the distribution of Ct values by age group.

Figure 3: Box-and-whisker plot for the Ct values of the N-gene in positive samples by age group. The mean value is indicated by (×). The t-test results showed there was a significant difference between the 50–59 and 60–69 age groups in May/June and July/August 2021 for nasopharyngeal swabs. (A) Saliva samples. (B) Nasopharyngeal swab samples.

There were no differences in the distribution of Ct values for the saliva samples, except in the 0–9 age group. In the July/August 2021 nasopharyngeal swab samples, the Ct values were decreased in the lower age groups (under 70–79 age), except for the 0–9 age group.

Discussion

This study had two limitations: the clinical backgrounds of the patients were unknown, and only one type of sample was collected from each patient. Therefore, this study solely focused on statistical analysis in the group comparisons. In this study, the measurement of SARS-CoV-2 was performed under a consistent protocol, from RNA extraction to real-time PCR, using an automated measurement system. Thus, human error and other variable factors were extremely unlikely.

In Japan, there have been six waves of the epidemic up to June 2022. The Ct values of the saliva and nasopharyngeal swab samples were compiled every 2 months and analyzed for distribution. Significant decrease in the number of S gene positive samples in March/April 2021, May/June 2021, and January/February 2022 compared to other genes. Detection of the S-gene using the TaqPath SARS-CoV-2 real-time PCR detection kit has been reported to be affected by the variant of SARS-CoV-2. This suggests that changes in the prevalent strains in Japan are reflected in the number of positive S-genes [17]. The distributions of Ct values for saliva samples all showed a normal distribution; therefore, saliva samples seem to be a suitable material for intergroup comparisons. The distribution of Ct values in nasopharyngeal swabs showed a bimodal pattern (Jan/Feb 2021, May/June 2021) or were lower than saliva (July/Aug 2021, Jan/Feb 2022). Although direct rubbing of the growth site captures a large amount of the virus, differences in collection methods at different medical institutions might be apparent in the distribution of the virus. Box-and-whisker analysis showed different distributions of Ct values for each period. The Ct values decreased the most in July/August 2021, during the peak of the delta variant. The delta variant possesses increased transmissibility and decreased vaccine efficacy compared to other variants of concern, such as alpha and beta [18]. In various countries, the omicron variant spread faster than the delta variant [19,20], but the Ct values in January/February 2022 were greater than the Ct values in July/August 2021. This may be related to vaccination coverage in Japan. In January/February 2022, the all-age vaccination coverage (second dose) reached 80%, whereas in July/August 2021, it was approximately 40% [21]. The association between vaccination coverage and Ct value is also evident in the age group analysis. The age group analysis by t-test showed no differences in saliva samples, but the nasopharyngeal swab samples in July/August 2021 showed significant differences between the 60–69 age group and the younger age groups. During this time, vaccination coverage was approximately 90% among the elderly and approximately 40% among other age groups [21].
Conclusions

This analysis focused on the Ct values of real-time PCR for comparison between groups. The saliva samples showed a clean normal distribution and seemed to be a suitable sample type for a comparative study of each period. By contrast, nasopharyngeal swabs tended to show more pronounced changes than saliva, although there was some disorder in the distribution. Group comparisons of Ct values for positive samples may indicate vaccine effects other than clinical symptoms.

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References


