Jundishapur J Microbiol. 2021 June; 14(6):e117424. doi: 10.5812/jjm.117424.

Published online 2021 August 30. **Research Article**

A Pilot Study on Presence of SARS-CoV-2-RNA in Iranian Blood Donors

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Received 2021 July 07; **Revised** 2021 August 01; **Accepted** 2021 August 01.

Abstract

Background: With the rapidly increasing incidence of the novel coronavirus disease 2019 (COVID-19) and the detection of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) RNA in plasma, blood supply safety has become a main concern.
Objectives: Due to some reports on the detection of RNAemia in SARS-CoV-2-infected blood donors, this study examined the presence of SARS-CoV-2 RNA in asymptomatic blood donors.
Methods: In this cross-sectional study, about 400 blood donors from the Tehran Blood Transfusion Center with negative results for viral serological markers of hepatitis B virus (HBV), hepatitis C virus (HCV), and human immunodeficiency virus (HIV) were included in the study. Moreover, all samples were tested for anti-SARS-CoV-2 ELISA (IgG) to detect antibodies against SARS-CoV-2. The Presence of SARS-CoV-2 RNA in blood donors was identified by targeting RNA-dependent, RNA polymerase (RdRp), and N (nucleocapsid protein) genes using Real-Time PCR. Furthermore, the RNase P gene was used as an internal control.
Results: The SARS-CoV-2 ELISA test showed that 60 (15%) of blood donors had antibodies against SARS-CoV-2 nucleocapsid protein, and 340 (85%) of the participants have not been exposed to the virus. The cycle threshold (Ct) for positive control in the RT-PCR test for nucleocapsid (N) and RdRP SARS-CoV-2 genes was < 40 (CT = 20.37). Moreover, internal control (RNase P gene) in all samples had $Ct < 40$. The presence of SARS-CoV-2 RNA was detected in the blood sample of none of the blood donors. In this regard, there has been no report of SARS-CoV-2 transmission to blood recipients yet.
Conclusions: The blood-borne transmission of SARS-CoV-2 seems to be highly unlikely, and coronavirus RNA screening is unnecessary among blood donors. Preventive measures should be adopted to reduce the theoretical risk of transmitting SARS-CoV-2 by the blood from asymptomatic COVID-19 cases.

Keywords: Real-Time PCR, RNA, SARS-CoV-2, Blood Donors

1. Background

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), as the leading cause of coronavirus disease 2019 (COVID-19), is a positive single-stranded RNA virus consisting of four structural proteins (namely spike (S), membrane (M), envelop (E), and nucleocapsid (N) proteins). SARS-CoV-2 belongs to the family of coronaviruses and the genus betacoronavirus. The disease was first identified in December 2019 in Wuhan, Hubei Province, China, and the World Health Organization (WHO) declared a pandemic on March 11, 2020 (1, 2). Most infected individuals with the SARS-CoV-2 experience mild to moderate respiratory illness and recover with no special treatment. Older persons and those with underlying diseases such as cardiovascular disease, diabetes, chronic respiratory disease, and cancer are more likely to develop severe illness (3).

The SARS-CoV-2 spreads by saliva droplets or nasal discharge with a cough or sneezing. To date, there is no specific treatment for SARS-CoV-2. The laboratory diagnosis of coronaviruses, including SARS- CoV-2, identifies viral RNA genome in the respiratory tract (nasopharyngeal/oropharyngeal swabs, sputum). Moreover, anal/rectal swab and stool tests are used to detect SARS-CoV-2 RNA by RT-PCR. The isolation of coronaviruses requires conditions (biosafety level 3) for CoV-2-SARS (4-9). In Iran, the first case of SARS-CoV-2 diagnosis was reported on February 20, 2019. In the initial stage of the outbreak in all blood transfusion centers, the blood donation with a history of traveling abroad (Southeast Asia) was postponed for 28-days to minimize the risk of transmission of the virus. Furthermore, preventive measures were adopted to reduce the theoretical risk of SARS-CoV-2 by blood transfusions. Moreover, donors were asked to report any symptoms of COVID-19 or any close contact with a confirmed person before donating blood, and their blood components were not consumed if there were such reports.

With the rapidly increasing incidence of COVID-19 and

the detection of SARS-CoV-2 RNA in plasma, blood supply safety had become a main concern (10, 11). On the other hand, due to high mutation in RNA viruses, their transmissibility and virulence may be increased (12). Although there has been no report on the blood-borne transmission of respiratory viruses; it is crucial to detect the status of the asymptomatic SARS-CoV-2 infected blood donors across the country.

2. Objectives

This study aimed to investigate the presence of SARS-CoV-2 RNA in asymptomatic blood donors.

3. Methods

3.1. Sample Collection

In this cross-sectional study, about 400 blood donors were included regarding their medical history, physical examination, and the negative results of blood screening tests. On the other hand, samples with reactive results for viral serological markers, HBsAg (Siemens, Germany), HCV Ab (Monalisa V.3, BIORAD, Germany), and HIVAg & Ab (HIV Ag-Ab, BIORAD, USA) detected by the ELISA method were excluded from the study. Moreover, all samples were tested for anti-SARS-CoV-2 NCP ELISA (IgG) (Euroimmun, Germany) to detect antibodies against SARS-CoV-2 according to the instruction kit. The cut-off values of > 1.1 and < 0.8 were considered as positive and negative, respectively. Blood samples containing anticoagulants were collected and transferred from the Tehran Blood Transfusion Center to the virology laboratory under cold chain conditions. This study was approved by the Ethic Committee of High Institute for Research and Education in Transfusion Medicine (Code: IR.TIM.REC.1399.011). Furthermore, informed consent was obtained from all participants.

3.2. RNA Isolation

The extraction of viral RNA was performed using the DynaBio ™ Viral Nucleic Acid Extraction Mini Kit (DynaBio ™ Viral Nucleic Acid Extraction Mini Kit), and the quality and concentration of the extracted RNA were then checked. To ensure the quality of the extracted RNA concentration, its light absorbance at 260/280 nm was measured by a nanodrop device, and then the light absorption ratio was calculated at 260/280 nm.

3.3. Real-Time PCR Test to Detect SARS-COV2 RNA

A One-Step RT-PCR COVID-19 kit from Pishtaz Teb Company was used in the experiments. The kit simultaneously targets two viral genes (namely RNA-dependent RNA polymerase (RdRp) and (N) Nucleocapsid) and contains a primer and probe for the RNase P gene, which controls the extraction and the amplification steps to prevent false negatives. The amplification of the target genes was measured qualitatively by increasing the fluorescence signal. To analyze the results, the internal control in the channel, ROX, and negative and positive controls in FAM, HEX channels, were examined. The negative control has no fluorescence signal; however, the positive and internal control in all channels has a Ct $\leq$ 35 cycles. If the above conditions are not met, the test must be repeated. The positive threshold of this kit for both genes and the internal control should be Ct $\leq$ 40 with the sigmoid S curve.

4. Results

In this study, 400 samples of blood donors were collected during April to December 2020 from the Tehran Blood Transfusion Center. The participants' mean age was 35.47 ± 10.02 years, and the research sample encompassed 20 (5.00%) females and 380 (95.00 %) males. According to their blood donation records, 97 persons (24.25%) were first-time donors, and 119 and 184 individuals (29.75% and 46%) were repeated and regular donors, respectively. The SARS-CoV-2 ELISA test result showed that 60 cases (15%) had antibodies against SARS-CoV-2 nucleocapsid protein, and 340 persons (85%) have been not exposed to the virus. After the viral RNA extraction and Real-Time PCR test, the first negative control, positive control, and internal control (RNase P gene) were examined to analyze the results. Real-time PCR test on RNA extracted from the samples showed that all samples were negative for amplifying both RdRp and N genes associated with SARS-COV2, and only positive control had Ct = 20.37.

5. Discussion

The severity of the COVID-19 symptoms varies widely in individuals infected with SARS-CoV2, some of whom may be asymptomatic, while others may experience a combination of coughs, fever, and pneumonia. SARS-CoV-2 RNA can be detected one to two days before the onset of the symptoms after being exposed to coronavirus in the upper respiratory tract; however, it may last 7 - 12 days in moderate cases, and up to two weeks in severe cases (13). Moreover, asymptomatic patients have detectable levels of viral RNA in the pharyngeal cavity, indicating the possibility of virus

transmission during the incubation period. The SARS-CoV-2 viral load in the blood is 10^2-10^4 copies /mL, which is much less in the respiratory tract and fecal samples (14, 15). Furthermore, the long-term or asymptomatic presence of the infectious SARS-CoV-2 virus in the blood and its potentials to induce infection is under question.

In this study, considering the non-exposure of 85% of blood donors to the virus, SARS-CoV-2 RNA was not detected in their blood samples. The results of this study were in line with those reported in a study in China (2020) on 98,342 blood donors (including 87,095 whole blood and 11,247 platelet units), indicating that all samples were negative regarding the presence of SARS-CoV-2 RNA (14). In another study in Germany, SARS-CoV-2 RT-PCR was performed on 77 patients, including 18 patients with symptoms (sore throat and fever), 15 patients with different symptom severities, and three asymptomatic patients. In this study, consistent with the results of this study, despite a clear positive case in throat swab, no case of SARS-CoV-2 RNA was observed in the blood or serum of the participants (16). These findings confirm that SARS-CoV-2 infection may be cleared with no significant clinical manifestations, and the risk of SARS-CoV-2 transmission via the blood is highly low.

In Canada, preventive measures were adopted at blood transfusion centers from the beginning of the epidemic to reduce the theoretical risk of SARS-CoV-2 transmission. In this regard, the COVID-19 cases were confirmed in six donors after donating blood, among whom one donor had donated whole blood (which was converted to a single packed red blood cell unit and a plasma unit), and the other five donors only had plasma donation. One of the six donors had mild COVID-19 symptoms at the time of the blood donation, and the other donors had mild to moderate symptoms; however, none of the donors were hospitalized.

The samples were sent to four independent laboratories for SARS-CoV-2 RNA testing, and RT-PCR was performed by targeting N regions of the viral genome. Three of the four laboratories used both regions N and E of the viral genome to enhance specificity. Finally, from the six tested samples, only one sample had a weak positive result for the E gene (Ct = 36.1). Moreover, serological tests were performed on the samples from all six donors to check the presence of antibodies to SARS-CoV-2, and the results were negative, implying that the donors were at the beginning stage of the disease (13). A plasma recipient, who received this component from the same donor, was recalled, and its RT-PCR test was negative for SARS-CoV-2 RNA. Then the infectivity of the SARS-CoV-2 virus in this donor was assessed using Vero E6 cell culture, and the results showed that the virus isolated from the blood was not infectious.

The results of the abovementioned study were consistent with those reported by Chang et al. (10) from China. Only four blood donors (out of above 7,000 donors tested for SARS-CoV-2 RNA) had weak positive SARS-CoV-2 RNA in their blood. According to these studies, like other respiratory viruses such as SARS-CoV and MERS-CoV, SARS-CoV-2 is not present in the blood components of asymptomatic infected individuals, and RNAemia is associated with the severity of clinical symptoms (17, 18). In March 2020 in Korea, infection with SARS-CoV-2 was confirmed in seven blood donations, and all their blood components were discarded. Due to the short shelf life of platelets, all six platelet units were transfused to six patients, and three units of red blood cells were also transferred to three recipients. After receiving blood components, the recipients had no COVID-19-associated symptom and revealed no positive results for SARS-CoV-2 RNA (19). Accordingly, there was noSARS-CoV-2 transmission to the recipients, implying that the risk of COVID-19 transmission by blood transfusion is highly unlikely (19).

5.1. Conclusions

In this study, 400 samples of asymptomatic blood donors were examined for RNAemia by RT-PCR testing, and the results revealed that SARS-CoV-2 RNA was not present in none of the blood donors. Since no case of SARS-CoV-2 transmission by blood transfusion has been reported, and given that patients with symptoms of SARS-CoV-2 infection are not accepted for blood donation at blood transfusion centers across the country, the risk of SARS-CoV-2 transmission by blood transfusions is highly low. However, preventive measures should be adopted to reduce the theoretical risk of transmitting SARS-CoV-2 by blood donation, and donors are asked to report any illness or close contact with confirmed cases to the blood transfusion centers. Moreover, blood donation should be delayed for 28 days in donors with a history of contact or infection to reduce the theoretical transmission of SARS-CoV-2.

Footnotes

Authors' Contribution: Z. S. developed the original idea and the protocol, abstracted and analyzed the data, wrote the manuscript, and was a guarantor. M. Z. and S. S. contributed to the development of the protocol, abstracted the data, and prepared the manuscript.

Conflict of Interests: The authors declare no conflict of interests.

Ethical Approval: This study was confirmed by the Ethic Committee of High Institute for Research and Education in Transfusion Medicine (Code: IR.TIM.REC.1399.011).

Funding/Support: This study was conducted with the financial support of the High Institute for Research and Education in Transfusion Medicine, Blood Transfusion Research Center.

Informed Consent: All participants' informed consent was obtained before the study.

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