Original Article

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Antibody False Positivity Among COVID-19 Convalescent Plasma Donors: A Comparative Study from the Turkish Red Crescent Blood Center

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Abstract

Aim: During our routine work at the Turkish Red Crescent (TRC) laboratories, human immunodeficiency virus (HIV) 1/2 antibody false-positive results were observed among Coronavirus disease-2019 convalescent plasma (CP) donors more frequently than healthy donors. We aimed to determine anti-HIV 1/2 antibody false-positivity rates among the CP donors and healthy blood donors.

Methods: The present study was designed as a cross-sectional study which was a type of observational study. Total 3689 donations from 2593 donors donated CP to the TRC between 11 April-06 July 2020, were screened by electrochemiluminescence immunoassay for the presence of antibody against HIV ½. The confirmation tests were performed with line immunoassay. All of the donors were non-remunerated CP donors between the ages of 18-60. For the control group, 411078 donations from 407363 healthy blood donors were received on the same days.

Results: Repeated reactivity rates (1.87%) were significantly higher than the control group (0.13%, $p<0.05$). However, there was not a statistically significant difference between the confirmed reactivity rates of the study group (0.03%) and the control group (0.01%, $p=0.217$).

Conclusion: In our study, it was determined that the false-positive results obtained from serologic HIV screening tests of CP donors were significantly higher when compared to the healthy blood donors.

Keywords: False HIV, convalescent plasma, serologic tests, immunoassay

Introduction

As a virus from the coronavirus family, the Severe Acute Respiratory syndrome-Coronavirus-2 (SARS-CoV-2), which was firstly defined in Wuhan -a sub-provincial city in China- towards the end of 2019 and assumed to be transmitted to humans from bats, has spread very fast and taken effect on global health, economy and social behavior around the world at short notice. During the writing of this paper, it was denoted that millions of people were infected with the virus and it led to the death of almost four and half million people in 235 countries/regions around the world (1). Naming the disease caused by this virus as Coronavirus disease-2019 (COVID-19) on 11 February 2020, World Health Organization (WHO) declared the outbreak as pandemic on 11 March 2020 (2).

There is no definite cure for the disease yet. The practice of CP, which comes up as a treatment choice and is received from the recovered patients, is an acquired passive immunity treatment. CP was used as post-exposure prophylactic for diseases such as viral hepatitis, measles, epidemic parotitis, and polio while it was used as the medical purpose for diseases such as influenza, bird influenza, SARS-CoV, Middle East Respiratory syndrome

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and Ebola (3-9). First practices related to the CP use in COVID-19 have come from the people's republic of China, the center of the disease. It was reported that the first CP was obtained in Wuhan on February 1st, 2020 and was given to a patient on February 9th, 2020 in this country (10). U.S. Food and Drug Administration (FDA) approved the use of the plasma received from people who were infected with and recovered from COVID-19 in the treatment of existing patients with a declaration of "COVID-19 Convalescence Research-Emergency" on March 24th, 2020 (11). Following all these news, it was decided by the Republic of Turkey Ministry of Health that the CP can be used in the treatment of COVID-19 patients. As part of this, in order to start receiving CP donations, a call was made by the TRC to those, who recovered from the disease and met the requirements of being a donor.

In the serologic tests conducted on the CP donors at the TRC laboratories, it was observed that anti-human immunodeficiency virus (HIV) ½ antibody was found to be false positive more often than other blood donors. In our study, it was aimed to show if there is any significant difference between the CP donors and healthy blood donors regarding the false positive HIV 1/2 test positivity rates.

Methods

Study design

The present study was designed as a cross-sectional study which was a type of observational study. This study was approved by the Turkish Red Crescent Ethical Committee (09.11.2020/2020-01). Total 3689 donations from 2593 donors, who donated CP to the TRC between 11 April-06 July 2020, were screened for the presence of anti-HIV ½ antibody. All of the donors in the study group were between the ages of 18-60 (median age 21.5) and were the voluntary and non-remunerated CP donors. The study group consisted of 2361 males (91.1%) and 232 females (8.9%). The clinical symptoms of CP donors in study group resolved at least 14 days before donation and in 48 hours before they had negative SARS-CoV-2 polymerase chain reaction test results for last consecutive two tests. For the control group, the test results of 411078 donations from 407363 healthy blood donors who donated within the same period were used. The blood donors in control group were between the ages of 18-60 (median age 27). The control group consisted of 350724 male (86.1%) and 56639 female (13.9%). The high male to female ratio in both groups was seen because the TRC does not accept plasma donations of any kind from women with a pregnancy history, including miscarriages or D/C, due to the risk of transfusion-related acute lung injury in the recipient. All the donors in the study and control groups gave the written consent before donation. These demographic data are summarized in Table 1.

Table 1. Demographic data of study and control groups

	Study group (n=2593)		Control group (n=407363)	
	Male	Female	Male	Female
Number (%)	2361 (91.1)	232 (8.9)	350724 (86.1)	56639 (13.9)
Median age (min-max)	21.5 (18-60)		27.0 (18-60)	
Donation number	3689		411078	

As part of the infectious serologic screening tests of blood donors, the anti-HIV 1/2 + p24 antigen tests were conducted on the electrochemiluminescence immunoassay (eCLIA) method and via the Cobas 8000 e801 (Roche, Germany) device and Elecsys HIV Duo (Roche, Germany) kits. In accordance with our test algorithm, the samples determined to be reactive in the first test were studied twice more and the results found to be reactive in at least two of three studies, were considered as "repeatedly reactive".

The confirmation tests were studied with the line immunoassay (LIA) method and via Auto-LIA 48 (Fujirebio, Belgium) device and INNO LIA HIV I/II score kits for the samples which were found to have repeated reactivity

Statistical Analysis

The data that used in our study were received from the digital archives of the TRC. The universe of our study consists of voluntary COVID-19 CP and healthy blood donors. The power of our cross-sectional study was calculated as 100%. For the statistical comparison of the reactivity rates of these two groups, Mid-P Exact test was used through OpenEpi v3.01 program, because it was recommended by the software for the actual distribution of data. The flow chart of the study is demonstrated on Figure 1.

Results

Sixty-nine (1.87%) of 3689 CP donations in the study group were found to have repeatedly reactive for anti-HIV 1/2 in the serologic tests. The confirmation test was negative (false positivity) in 68 donations (1.84%) among CP donors and 9 of them were female (0.35%), 59 of them were male (2.28%). In the study group confirmation test was positive (true HIV infection) in one male donor (0.03%). In the control group, 520 (0.13%) of 411078 blood donations were found to have repeated reactivity for HIV 1/2 antibodies in the serologic tests. The confirmation test was negative in 461 (0.12%), positive in 49 (0.012%) and indeterminate in 10 (0.002%) of them. In the control group, 84 (0.021%) of unconfirmed donors were female

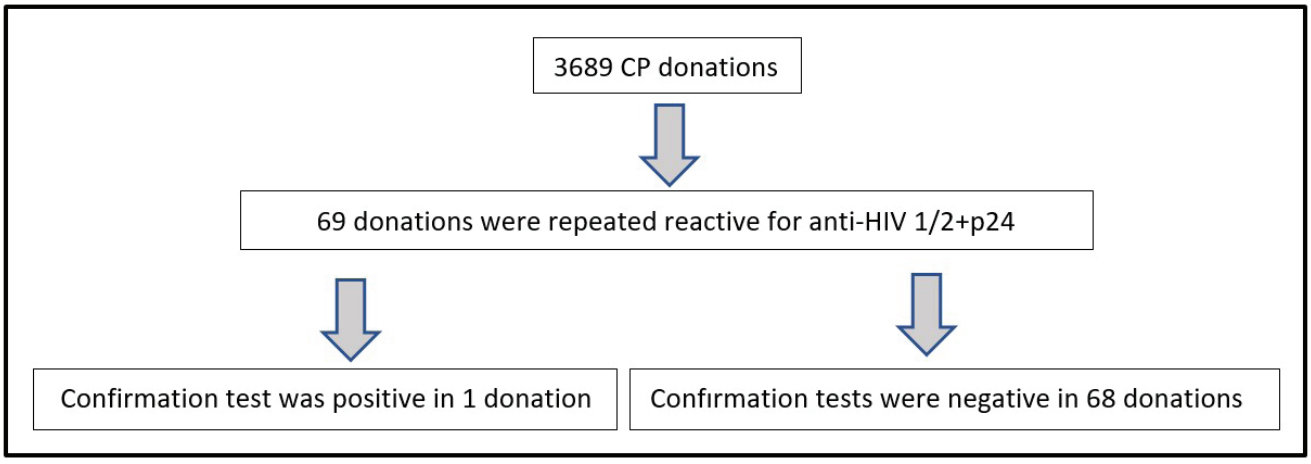

Figure 1. Flow chart of study

and 377 (0.093%) were male.

When the repeated reactivity rates of the study group (1.87%) and control group (0.13%) were compared, the difference was found to be statistically significant (p<0.05).

When the confirmed reactivity rates of the study group (0.039%) and the control group (0.012%) were compared, the difference was not statistically significant (p=0.217). In our study, any confirmed female donor was detected. Because of this reason, statistical comparison between genders was not calculated in the confirmed study group.

When the unconfirmed reactivities found in the study group (1.84%) and in the control group (0.12%) were compared, the difference was found to be statistically significant (p<0.05). In unconfirmed group, difference between female - male donors rates of the study group (0.35% and 2.28%, respectively) and control group (0.021% and 0.093%, respectively) were statistically significant (p<0.05). The findings are summarized in Table 2,3.

Table 2. Anti-HIV 1/2 reactivities of the CP and blood donations

	CP donations (n=3689)	Blood donations (n=411078)	p*
Repetitive reactivity % (n)	1.87 (69)	0.13 (520)	<0.05
Confirmed % (n)	0.03 (1)	0.012 (49)	0.217
Unconfirmed % (n)	1.84 (68)	0.12 (461)	<0.05
Indeterminate % (n)	0	0.002 (10)	-

*Mid-P exact test was used for comparison of two groups, HIV: Human immunodeficiency virus, CP: Convalescent plasma

Table 3. Unconfirmed anti-HIV 1/2 test results related to gender

	Male % (n)	Female % (n)	p*
CP donors (n=2593)	2.28 (59)	0.35 (9)	<0.05
Blood donors (n=407363)	0.093 (377)	0.021 (84)	<0.05

*Mid-P exact test was used for comparison of two groups, HIV: Human immunodeficiency virus, CP: Convalescent plasma

Discussion

The findings of our study support our hypothesis that COVID-19 patients might have a more false positive anti-HIV 1/2 test result than healthy blood donors in the serological methods. In our study, we found that false positivity rate in male donors was significantly higher than female donors. We think that this difference resulted from low number of female donors in study and control groups. Serologic tests for HIV 1/2, hepatitis B, C virus and syphilis are performed by the TRC to the plasma received from the CP donors due to biosafety reasons. As is known, in the tests based on the antigen-antibody interaction principle, cross-reactivity can be seen since the binding domain of each antibody or the molecular association may interact with more than one antigenic determinant or more than one antigen, respectively. In other words, the cross-reactivity can occur because of the antigen that shares single epitope or of the structural similarity of epitopes (12).

In the tests based on the SARS-CoV antigen-antibody interaction, cross-reactions similar to this can also be observed. For example, it was reported that dual antigenic cross-reactivity with N proteins was seen between SARS-CoV and swine group 1 CoVs [TGEVs (M6 and P115 and PRCV-ISU1] in a study conducted (13). Accordingly, there are studies showing that auto-antibodies in some autoimmune diseases can cross-react with the nucleocapsid protein of SARS-CoV and cause false positivity (14,15). Also, false positivities due to cross-reaction have been found between SARS-CoV and HCoV-229E & HCoV-OC43, which are among the other coronaviruses that cause common cold in humans (16). Similar cross-reactions have been observed for Human T-lymphotropic virus (HTLV) I

and II, which rank among the Retroviridae family just like HIV 1/2. It was suggested that these reactions can be associated with rgp46-1 and rgp46-2 antigens of HTLV-I and GD21, p19, p24, gp21 and gp46 antigens of HTLV-II (17). In a study conducted by Pradhan et al. (18), it was stated that the amino acid array of four domains located on the SARS-CoV-2 S glycoprotein shows similarity with HIV-1 gp 120 and gag glycoproteins. Finally Mannar et al. (19) reported that host-derived glycans on spike proteins displayed high levels of cross-reactivity with anti-HIV 1 gp120 antibodies. These findings support the idea that the significantly high false reactivity rate we encountered results from the similarity of antigenic epitopes. The false positive test results for anti-HIV 1/2 were reported with another device system that used eCLIA test method. Tan et al. (20) and Papamanoli and Prevdos (21) reported three acutely ill COVID-19 patients had false positive anti-HIV tests. In these patients negative test results were detected with repeated serologic tests with different devices and and with molecular techniques.

Study Limitations

The main limitation of our study was that only one device and kit system developed by one company was used in our study. The second limitation of our study was low percentage of female donors in study and control groups (8.9% and 13.9%, respectively), so our results are not generalizable to both genders. The third limitation of our study was indeterminate confirmation test results and difficulty of follow-up sample obtaining.

Conclusion

Despite these limitations our test systems are safe and accepted worldwide, due to the national blood-banking algorithm of our country. We think that the results of this study warn us to be careful about the serological HIV 1/2 tests for the COVID-19 patients. Because the number of patients who had experienced COVID-19 and recovered has been increasing day by day; false-positive anti-HIV 1/2 results might increase in hospital settings. HIV 1/2 serological tests are being ordered for many screening purposes so this cross-reactivity might be a real problem. It is needed to be investigated and reported for different device and kit systems. It seems that the manufacturers will need to study on and solve this cross-reactivity problem to avoid false positive results. We think that difference of false positivity rates between genders needs new studies including a higher female population than our study.

False-positive results in anti-HIV 1/2 tests might be observed in the patients recovered from COVID-19. Defined cross-reactivity should be taken into account both in blood banking, CP treatment process and routine clinical practice.

Authorship Contributions

Concept: L.H., C.M.B., A.K., N.H., K.K., F.M.Y., Design: L.H., C.M.B., A.K., N.H., K.K., F.M.Y., Data Collection or Processing: L.H., C.M.B., Analysis or Interpretation: L.H., C.M.B., A.K., Literature Search: L.H., C.M.B., Writing: L.H., C.M.B.

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