HBsAg, anti-HCV, anti-HIV 1/2 and syphilis seroprevalence in healthy volunteer blood donors in southeastern Anatolia

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Abstract
Introduction: This study investigated the seroprevalence of hepatitis B virus surface antigen (HBsAg), antibody against hepatitis C virus (anti-HCV), antibody against human immunodeficiency virus type 1/2 (anti-HIV 1/2), and antibody against Treponema pallidum (anti-Treponemal or syphilis antibody) in healthy volunteer blood donors, and assessed their distribution according to the years and genders.
Methodology: HBsAg, anti-HCV, anti-HIV ½, and syphilis screening results of a total of 266,035 healthy volunteer blood donors who had been admitted for blood donation to the Regional Blood Center of Dicle University Hospital between January 2000 and December 2010 were evaluated, retrospectively. HBsAg, anti-HCV, and anti-HIV 1/2 screening were performed using a fully automated device with the microparticle enzyme immunoassay method (MEIA). Syphilis screening was performed by Rapid Plasma Reagin (RPR) carbon test between January 2000 and December 2009, and by using a fully automated device with the MEIA method between January 2010 and December 2010. Results: Of 266,035 healthy volunteer blood donors, 259,384 (97.5%) were male and 6,651 (2.5%) were female. Statistically, there was not any significant difference between male and female genders for HBsAg, anti-HCV and syphilis seropositive (P = 0.729, P = 0.748, and P = 0.861, respectively). HBsAg was found to be positive in 8,422 (3.17%), anti-HCV in 1,703 (0.64%), anti-HIV 1/2 in one (0.0004%) of 266,035 healthy volunteer blood donors, and syphilis antibody with RPR in 166 (0.07%) of 246,341 healthy volunteer blood donors.
Conclusion: Blood donor forms should be carefully tailored to improve the identification of possible risks of transfusion-transmitted infections.

Key words: blood transfusion; blood donors; hepatitis B surface antigens; hepatitis C antibodies; HIV seropositivity; syphilis


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Introduction
In the past, transmission of infections by blood and blood products transfusion was a major health problem in Turkey as all over the world. Today, in spite of tremendous efforts, safe blood and blood products transfusion remains a serious problem. The most important problem during transfusion is the risk of transmission of infections during the “window period” in which the serological indicators are negative. Therefore, making a decision for blood and blood products transfusion, it is useful to know the epidemiological situation of transfusion-transmitted infections in a region [1].

To minimize the risk of transfusion-transmitted infection, screening tests for HBsAg, syphilis, and malaria in volunteer blood donors were adopted in 1983 in Turkey, an anti-HIV type 1/2 screening test was added in 1987, and an anti-HCV screening test was added in 1996. In 1997, routine malaria screening in volunteer blood donors was discontinued [2-5]. In addition to these tests, antibody against human T-lymphotropic virus type 1/2 (anti-HTLV 1/2) and antibody against hepatitis B virus core antigen (anti-HBc) are also screened in the United States and the European Union countries [4,6]. Although not mandatory in our country, some of the HIV diagnostic tests are still used in some laboratories as a screening test, including the p24 antigen.

Data obtained from the Ministry of Health, the Republic of Turkey, between October 1985 and December 2001, show that 39 (3.1%) of 1,246 acquired immune deficiency syndrome (AIDS) cases resulted from transfusion. In 2007, this 46 (1.5%) of 2,920 AIDS cases resulted from transfusion [6]. These results show that transmission of HIV by transfusion is still a major problem in Turkey. It is observed that
transmission rate of HIV by transfusion is 1.2% in the United States and 10% in the developing countries. Since HBV and HCV infections can become chronic and lead to cirrhosis and hepatocellular carcinoma, they are major threats to public health. In the United States, while HBV is responsible for 24% of post-transfusion hepatitis, HCV is responsible for 43% of post-transfusion hepatitis. Today, it is estimated that approximately 400-500 million people have been infected with HBV, and 100-150 million people with HCV, throughout the world [6-9].

In the present study, the seroprevalence of HBsAg, anti-HCV, anti-HIV 1/2, and syphilis in healthy volunteer blood donors was investigated, and the distribution was assessed in terms of the years and genders.

**Methodology**

HBsAg, anti-HCV, anti-HIV 1/2 and syphilis screening results of a total of 266,035 healthy volunteer blood donors who had been admitted for blood donation at the Regional Blood Center of Dicle University Hospital between January 2000 and December 2010 were included retrospectively in the present study. HBsAg, anti-HCV, and anti-HIV 1/2 screening tests were performed by using a fully automated device (Architect, Abbott, IL, USA) with the microparticle enzyme immunoassay method (MEIA) method. The blood samples of anti-HIV 1/2-positive donors were sent to the Refik Saydam National Public Health Agency for laboratory confirmation by Western-blot analysis. The Rapid Plasma Reagin (RPR) carbon test (Plasmatec, Kent, United Kingdom) was used as a screening test for syphilis infection between January 2000 and December 2009. Syphilis screening tests were performed by using a fully automated device (Architect, Abbott, IL, USA) with the MEIA method between January 2010 and December 2010.

Data of this study were analyzed by SPSS version 11.5 (IBM, SPSS Inc., Chicago, IL, USA) software statistical package program for Windows (Microsoft, Redmond, WA, USA). Statistical evaluation of the differences in seropositivity according to the gender was performed with the Chi-square test and Yates’s continuity correction. Additionally, statistical evaluation of distribution according to the years of seropositivity rates was performed with the Chi-square trend analysis. The P value of < 0.05 was selected for statistical significance.

**Results**

Of a total of 266,035 healthy volunteer blood donors, 259,384 blood donors (97.5%) were male and 6,651 blood donors (2.5%) were female. The distribution according to the gender of HBsAg, anti-HCV, and syphilis seropositivity rates is presented in Table 1. Statistically, there was not any significant difference between male and female gender for HBsAg, anti-HCV, and syphilis seropositivity with RPR (P = 0.729, P = 0.748, and P = 0.861, respectively). A total of 8,422 blood donors (3.17%) for HBsAg and 1,703 blood donors (0.64%) for anti-HCV of 266,035 healthy volunteer blood donors in the present study were found to be favourable. Although a total of 151 blood donors (0.057%) for HIV 1/2 266,035 healthy volunteer blood donors were found to be favourable by the MEIA method, only one blood donor (0.0004%) was found to be positive for HIV by Western-blot analysis, which is a confirmation method. The HBsAg seropositivity rate does not show significant changes over the years; however, the anti-HCV seropositivity rate increased gradually and the syphilis seropositivity rate decreased gradually. The increasing and/or decreasing of seropositivity rates over the years were evaluated by the Chi-square trend analysis. There was no significant relationship between HBsAg seropositivity and seropositivity rates according to the years (P = 0.595). There was a linear relationship between anti-HCV seropositivity and an increase according to the years of seropositivity rates (P < 0.001).

<table>
<thead>
<tr>
<th>Name of test</th>
<th>Male</th>
<th>Female</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>HBsAg</td>
<td>8,217 (n = 259,384)</td>
<td>205 (n = 6,651)</td>
<td>0.729</td>
</tr>
<tr>
<td>Anti-HCV</td>
<td>1,663 (n = 259,384)</td>
<td>40 (n = 6,651)</td>
<td>0.748</td>
</tr>
<tr>
<td>Syphilis*</td>
<td>162 (n = 240,182)</td>
<td>4 (n = 6,159)</td>
<td>0.861</td>
</tr>
</tbody>
</table>

*Syphilis data of 2010 was not evaluation
Table 2. The distribution according to the years of seropositivity rates of HBsAg, anti-HCV, anti-HIV 1/2, and syphilis antibodies

<table>
<thead>
<tr>
<th>Year</th>
<th>n</th>
<th>HBsAg % (n)</th>
<th>anti-HCV % (n)</th>
<th>anti-HIV % (n)</th>
<th>Syphilis % (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>P</td>
<td>P</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>18,753</td>
<td>2.66 (498)</td>
<td>0.40 (75)</td>
<td>0</td>
<td>0.2 (37)</td>
</tr>
<tr>
<td>2001</td>
<td>20,274</td>
<td>4.00 (812)</td>
<td>0.59 (119)</td>
<td>0</td>
<td>0.1 (20)</td>
</tr>
<tr>
<td>2002</td>
<td>25,061</td>
<td>3.66 (916)</td>
<td>0.62 (155)</td>
<td>0</td>
<td>0.1 (25)</td>
</tr>
<tr>
<td>2003</td>
<td>21,218</td>
<td>3.30 (700)</td>
<td>0.69 (147)</td>
<td>0</td>
<td>0.11 (23)</td>
</tr>
<tr>
<td>2004</td>
<td>25,039</td>
<td>2.89 (723)</td>
<td>0.64 (161)</td>
<td>0</td>
<td>0.07 (18)</td>
</tr>
<tr>
<td>2005</td>
<td>26,923</td>
<td>2.98 (803)</td>
<td>0.63 (169)</td>
<td>0</td>
<td>0.04 (11)</td>
</tr>
<tr>
<td>2006</td>
<td>31,320</td>
<td>2.47 (774)</td>
<td>0.42 (130)</td>
<td>0</td>
<td>0.04 (12)</td>
</tr>
<tr>
<td>2007</td>
<td>26,715</td>
<td>2.96 (790)</td>
<td>0.76 (202)</td>
<td>0</td>
<td>0.03 (8)</td>
</tr>
<tr>
<td>2008</td>
<td>29,537</td>
<td>3.24 (957)</td>
<td>0.73 (216)</td>
<td>0</td>
<td>0.02 (5)</td>
</tr>
<tr>
<td>2009</td>
<td>21,501</td>
<td>3.39 (728)</td>
<td>0.79 (169)</td>
<td>0</td>
<td>0.03 (7)</td>
</tr>
<tr>
<td>2010</td>
<td>19,694</td>
<td>3.66 (721)</td>
<td>0.81 (160)</td>
<td>0.005 (1)</td>
<td>0.27 (53)*</td>
</tr>
<tr>
<td>Total</td>
<td>266,035</td>
<td>3.17 (8,422)</td>
<td>0.64 (1,703)</td>
<td>0.0004 (1)</td>
<td>0.07 (166)*</td>
</tr>
</tbody>
</table>

*Syphilis data of 2010 was not evaluation

RPR carbon test was used as a screening test for syphilis antibodies between January 2000 and December 2009. During this period, 166 (0.07%) of a total of 246,341 healthy volunteer blood donors were found to be favourable. After the date of January 2010, the syphilis screening test was performed by the MEIA method and found to be positive in 53 (0.27%) of 19,694 healthy volunteer blood donors. There was a linear relationship between syphilis seropositivity and a decrease according to the years of seropositivity rates obtained with the RPR carbon test, excluding the seropositivity rate obtained with the MEIA method in 2010 (P = 0.022). The results are presented in Table 2.

Discussion

In the present study, the annual distribution of HBsAg, anti-HCV, anti-HIV 1/2 and syphilis seropositivity rates in healthy volunteer blood donors were investigated retrospectively. In addition, distribution of the blood donors according to the years and gender was investigated. It was determined that 97.5% of the blood donors were male. In other studies performed in Turkey, the number of male blood donors was detected to be higher than that of female blood donors [10].

In the current study, a total of 8,422 (3.17%) healthy volunteer blood donors were determined to have HBsAg seropositivity, and when investigated in terms of distribution according to the years of HBsAg seropositivity rate, it was seen that the rates had no significant changes. In two different studies, similar results (3.62% and 2.75%) were reported by Temiz et al., in Turkey [7,8]. When the blood donor screening tests of the blood centers were examined by Mese et al., the HBsAg seropositivity rate was detected as 3.28%, and the rate in this study is consistent with that of the average rate of Turkey [4]. The data obtained from the present study and other studies show that carrying HBsAg is still a serious health problem in our region, as well as all over the Turkey.

The anti-HCV seropositivity rate was determined as 0.64% in the present study. It has been estimated that seroprevalence of HCV is approximately between 2.2% to 3.0% worldwide [11,12]. According to other studies in developing countries, seroprevalence of HCV, which is responsible for post-transfusion hepatitis, has been reported as 0.4% to 6.0% and as 0.40% in the USA [3,13-16].

In Turkey, the anti-HCV seropositivity rate obtained from blood donor screening in different centers was approximately 0.54% between 2000 and 2006. Furthermore, the anti-HCV seropositivity rate was over 1% in the Afyon, Düzce, Erzurum, Manisa and Samsun provinces of Turkey. When the data of the 1990s and 2000s are compared, it is seen that there is no significant difference [17,18]. When the blood donor screening tests of the blood centers in our region were examined by Mese et al., the anti-HCV seropositivity rate was detected as 0.76% [4]. While the anti-HCV seropositivity rate of 0.64% in the present study is similar to the rates obtained in other studies in Turkey, it shows that HCV incidence has been decreasing gradually according to the years.
Worldwide, 3% to 5% of transmission of HIV is through blood transfusion. In the present study, a total of 151 blood donors (0.057%) were found to be favourable for HIV 1/2, and 266,035 healthy volunteer blood donors with the MEIA method. Anti-HIV 1/2 seropositivity that was confirmed by Western-blot analysis at the Refik Saydam National Public Health Agency was determined in only one (0.0004%) of 266,035 healthy volunteer blood donors. A total of 150 blood donors (0.0004%) for HIV were found to be negative by Western-blot analysis. There were 150 false-positive results. When the blood donor screening results of the blood centers in our country were examined by Tore et al., anti-HIV seropositivity was reported as approximately 0.05% [19]. When the blood donor screening tests of the blood centers in our region were examined by Mese et al., the anti-HIV seropositivity rate was determined as 0.14% [4]. In the present study, the HIV seropositivity of 0.0004% is quite below the average of Turkey, and it is a favourable result.

Syphilis was the first infection to be reported as blood-borne. Today, post-transfusion syphilis infection is rarely seen because Treponema pallidum is inactive in routine screening and in blood samples which are kept at +4°C for 48 to 72 hours. Syphilis screening, which causes transfusion-derived infection, is suggested because of the possibility that this factor may exist with sexually transmitted infections [3,5,20].

In studies that were performed abroad between 2004 and 2008, syphilis seropositivity rates were detected as 1% and 0.03%, respectively, with the RPR carbon test, whereas in international studies, these rates were detected as 0.2%, 0.012% and 0.47% from two centers in the provinces of Istanbul and Trabzon in Turkey [21-25]. When the blood donor screening tests of the blood centers in our region were examined by Mese et al., syphilis seropositivity rate was determined as 0.16% [4]. In the present study, the RPR carbon test was used as a syphilis screening test between January 2000 and December 2009. RPR seropositivity was determined in 166 (0.07%) of a total of 246,341 healthy volunteer blood donors. After January 2010, syphilis screening was studied with the MEIA method, and test positivity was detected in 53 (0.27%) of total 19,694 healthy volunteer blood donors. Moreover, quantitative ratios (sample/threshold ratio absorbance; s/co) of microparticle positive blood samples were compared with the results of Treponema pallidum hemagglutination assay (TPHA) in another study. In the MEIA method, it was observed that 100% of the samples whose s/co ratios were greater 12 were confirmed with TPHA [26]. As a result, safe blood can be achieved only from safe blood donors. Otherwise, the unsecured blood transfusion will endanger the life of patients.

**Conclusion**

Transfusion-transmitted infections are major threats. The results obtained in this study show that transfusion-transmitted infections continue to be an important cause of morbidity and mortality in our region. The risk of infection can be reduced by determination of the regional spread of transfusion-transmitted infections, by effectively using questionnaires in blood donation centers, HBV vaccination programs, and awareness-raising activities. Especially, blood donor forms should be carefully tailored to improve the identification of possible risks of transfusion-transmitted infections.

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**Conflict of interests:** No conflict of interests is declared.