

HBV, HCV and HIV seroprevalence among blood donors in Istanbul, Turkey: how effective are the changes in the national blood transfusion policies?

ABSTRACT

The national blood transfusion policies have been changed significantly in recent years in Turkey. The purpose of this study was to determine the prevalence of HBV, HCV, and HIV in blood donors at the Red Crescent Center in Istanbul and to evaluate the effect of changes in the national blood transfusion policies on the prevalence of these infections. The screening results of 72695 blood donations at the Red Crescent Center in Istanbul between January and December 2007 were evaluated retrospectively. HBsAg, anti-HCV, and anti-HIV-1/2 were screened by microparticle enzyme immunoassay (MEIA) method. Samples found to be positive for anti-HIV 1/2 and anti-HCV were confirmed by Inno-Lia HCV Ab III and Inno-Lia HIV I/II Score, respectively. The seropositivity rates for HBsAg, anti-HCV, and anti-HIV-1/2 were determined as 1.76%, 0.07%, and 0.008%, respectively. Compared to the previously published data from Red Crescent Centers in Turkey, it was found that HBV and HCV seroprevalences decreased and HIV seroprevalence increased in recent years. In conclusion, we believe that the drop in HBV and HCV prevalence rates are likely multifactorial and may have resulted from more diligent donor questioning upon screening, a higher level of public awareness on viral hepatitis as well as the expansion of HBV vaccination coverage in Turkey. Another factor to contribute to the decreased prevalence of HCV stems from the use of more sensitive confirmation testing on all reactive results, thereby eliminating a fair amount of false positive cases. Despite similar transmission routes, the increase in HIV prevalence in contrast to HBV and HCV may be linked to the increase in AIDS cases in Turkey in recent years.

Keywords: HBV, HCV, HIV, seroprevalence, blood donors, Turkey.

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INTRODUCTION

A number of viruses, bacteria and parasites can be transmitted through blood or blood products. Amongst these, the hepatitis B virus (HBV), hepatitis C virus (HCV), and the human immunodeficiency virus (HIV) are mandatorily tested on blood donors worldwide due to the potential serious chronic clinical sequelae associated with these readily transmitted agents.^{1,2}

Nevertheless, since donor history questioning is performed more diligently and due to the advances in screening techniques, the risk of transmission has decreased considerably.³ However, small risks of infection transmission persist due to several factors such as genetic variations of infectious agents, presence of an immunologically silent carriage, laboratory errors, and variations in the window period of an infectious agent as well as limitations in screening testing methodology.^{2,4}

As mandated by the Turkish legal system, it is a law to screen all voluntary blood donors for HBV, HCV, HIV-1/2, and *Treponema palladium*. The Blood and Blood Products Act, accepted by the Turkish parliament in 2007, has dropped the rate of transfusion mediated infections by raising the standards of donor screening through improved history questioning and risk factor assessments. Intense campaigns for public awareness against HBV, HCV, and HIV have also been launched. This study is aimed at presenting the effects of the new Turkish legislature and particularly looks at how these policies have brought upon changes in the prevalence rates of transfusion mediated infections in the city of Istanbul where approximately one fourth of the entire Turkish population resides.

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MATERIAL AND METHODS

During the period of January to December 2007, the HBV, HCV, and HIV-1/2 screening test results of blood donors and the confirmatory test results of HCV and HIV-1/2 seropositive donors were retrospectively obtained from laboratory records.

HBsAg, anti-HCV, and anti-HIV-1/2 screening tests were performed on the automatic EIA instrument (Davinci, Biomerieux) with the microparticle enzyme immunoassay (MEIA) method using the micro-Elisa kit (Hepanostika, HBsAg Uni-Form II, bioMerieux, NL) based on the single step "sandwich" principle for HBsAg, the 4th generation EIA kit (Innotest, HCV Ab IV, Innogenetics N.V. Belgium) containing CORE, NS3, NS4, and NS5 antigens of HCV for anti-HCV, and the micro-Elisa kit (Vironostika, HIV Uni-Form II Ag/Ab, NL) based on the single step "sandwich" principle for anti-HIV-1/2.

In the methods used, the signal-to-cutoff (S/CO) ratio ≥ 1 for HBsAg, anti-HCV, and anti-HIV were considered as reactive. Initial S/CO ratios in the range of 0.70-0.99 for HCV and 0.75-0.99 for HIV were considered as being in the grey zone. There was no grey zone establishment for HBV. Reactive samples or samples in grey zone in the screening tests were retested using the same immunoassay. If one or both of the retested specimens reacted then, it was referred as repeatedly reactive and was considered screening tests positive.

For samples that showed repeatedly reactive with HBsAg EIA, no confirmatory tests were carried out and those repeatedly reactive samples were interpreted as positive. Repeatedly reactive samples in the anti-HCV EIA testing were confirmed using the 3rd generation Line Immune Assay (LIA) test method (INNO-LIA, HCV Score, Innogenetics, Belgium), which included the C1, C2, E2, NS3, NS4, NS5 regions of the HCV genome. The anti-HIV EIA repeatedly reactive results were confirmed by the LIA kit (INNO-LIA, HIV I/II Score, Innogenetics, Belgium) containing HIV 1.2 and HIV 1 group O antigens. LIA test results were evaluated according to the recommendations of the assay manufacturer company and results for both anti-HCV and anti-HIV were interpreted as positive, indeterminate or negative.

Statistical analysis

All statistical analyses were carried out with computer software (SPSS; Version 10.0; SPSS, Inc; Chicago, IL). Means, coefficients of variation, standard deviations and confidence intervals were determined. Statistical studies were evaluated using Pearson chi-square and Fisher's exact test. A p-value < 0.05 was considered to be statistically significant.

RESULTS

Screening test results

During the period of January to December 2007, EIA screening testing was performed on 72695 blood donors at the Red Crescent Center in Istanbul, Turkey. At baseline screening testing, 1377 HBsAg, 393 anti-HCV, and 198 anti-HIV positive results were detected (Table 1). Baseline tests revealed that 130 of the anti-HCV positive samples (33.1%) and 79 of the anti-HIV samples (39.9%) were within the grey zone index values (Table 1). For samples that fell within the grey zone index value from the initial testing, we determined the rate of repeated reactivity as 80.8% (105/130) for anti-HCV and 65.8% (52/79) for anti-HIV, whereas those above the positive limit index value were determined as 97% (255/263) and 59.7% (71/119), respectively. When the total sample size was considered, the repeatedly reactive rate for HBsAg, anti-HCV, and anti-HIV was found to be 92.8% (1278/1377), 91.6% (360/393), and 62.1% (123/198) respectively (Table 1). Based on these results, the overall seropositivity rate for the initial test results were 1.89% for HBsAg, 0.54% for anti-HCV, and 0.27% for anti-HIV. Based upon repeated reactive test results, the seropositivity rate dropped to 1.76% for HBsAg, 0.50% for anti-HCV, and 0.17% for anti-HIV. When repeat reactive samples were sorted out and the rate of true positivity was determined, it was found that 99 of the 1377 HBsAg results (7.2%), 33 of the 393 anti-HCV results (8.4%), and 75 of the 198 anti-HIV results (37.9%) were biologically false positive (BFP) (Table 2).

Table 1. HBs Ag, anti-HCV, anti-HIV EIA screening test results and repeating reactivity rate

	Number tested	Number of initially reactive samples			Number of repeatedly reactive samples		
		Grey zone limit index	Positive	Total	Grey zone	Positive limit index	Total
		N	N	N	N (%)	N (%)	N (%)
HBsAg	72,695	-	1,377	1,377	-	1,278 (92.8)	1,278 (92.8)
Anti-HCV	72,695	130	263	393	105 (80.8)	255 (97)	360 (91.6)
Anti-HIV	72,695	79	119	198	52 (65.8)	71 (59.7)	123 (62.1)

Table 2. The biologically false positive rate in the first tests, after results with repeating reactivity in screening tests were accepted as true positive

	Initially reactive samples N (%)	Repeatedly reactive samples N (%)	Biologically false positive N (%)
HBsAg*	1,377 (1.89)	1,278 (1.76)	99 (% 7.2)
Anti-HCV	393 (0.54)	360 (0.50)	33 (% 8.4)
Anti-HIV	198 (0.27)	123 (0.17)	75 (% 37.9)

Confirmatory test results**HBsAg**

For the evaluation of the HBsAg results, samples showing repeating reactivity with EIA were accepted as positive results and no further confirmatory tests were carried out. As a result, our study revealed the HBsAg seroprevalence to be 1.76% among blood donors.

HCV

In 360 serum samples with positive anti-HCV screening test results (29.2% $s/co = 0.70-1$; 70.8% $s/co \geq 1$), 54 (15%) positive, 217 (60.3%) negative, 89 (24.7%) indeterminate results were obtained by LIA. When indeterminate results were excluded, the anti-HCV seroprevalence was 0.07% (54/72695).

Out of the 54 LIA positive samples, 2 were from the of (grey zone) 0.70-0.99 and 52 were from samples that had a S/CO value of ≥ 1 . In other words, for initial EIA test samples that were considered positive but within the grey zone (S/CO value between 0.70 and 1.0), the rate of confirmation upon LIA testing was only 2%, whereas samples with an initial S/CO index value >1 had a confirmation test positive rate of 20.4%. The difference in their rates of LIA confir-

mation positivity was statistically significant ($p < 0.0001$). Thus, samples within grey zone index values had significantly higher negative outcome rates as determined by LIA confirmation, compared to samples with s/co rates above the positive threshold value ($p < 0.0001$). However, among indeterminate results, no difference was found between the s/co rates in the grey zone and those with positive threshold values ($p > 0.05$) upon LIA testing (Table 3). When indeterminate results were excluded, 60.3% of the anti-HCV EIA positive results were concluded to be BFP.

HIV

In 123 serum samples with positive anti-HIV screening test results (42.3% $s/co = 0.75-1$; 57.7% $s/co \geq 1$), 8 (6.5%) positive and 115 (93.5%) negative results were obtained using LIA. According to results verified by LIA, the HIV seroprevalence among our blood donors was 0.008% (6/72695).

All samples with grey zone s/co rates had negative results with LIA, compared to 8 positive and 63 negative results in samples with $s/co \geq 1$ ($p = 0.020$), (Table 4). When indeterminate results were excluded, 95.1% of the anti-HIV EIA positive results were concluded to be BFP.

Table 3. Confirmatory results of anti-HCV reactive results with LIA

	Anti-HCV confirmatory test (LIA) results		
	Positive N %	Negative N %	Indeterminate N %
Grey zone (105)	2 (1.9)	80 (76.2)	23 (21.9)
Positive index (255)	52 (20.4)	137 (53.7)	66 (25.9)
Total (360)	54 (15.0)	217 (60.3)	89 (24.7)
p	< 0.0001	< 0.0001	> 0.05

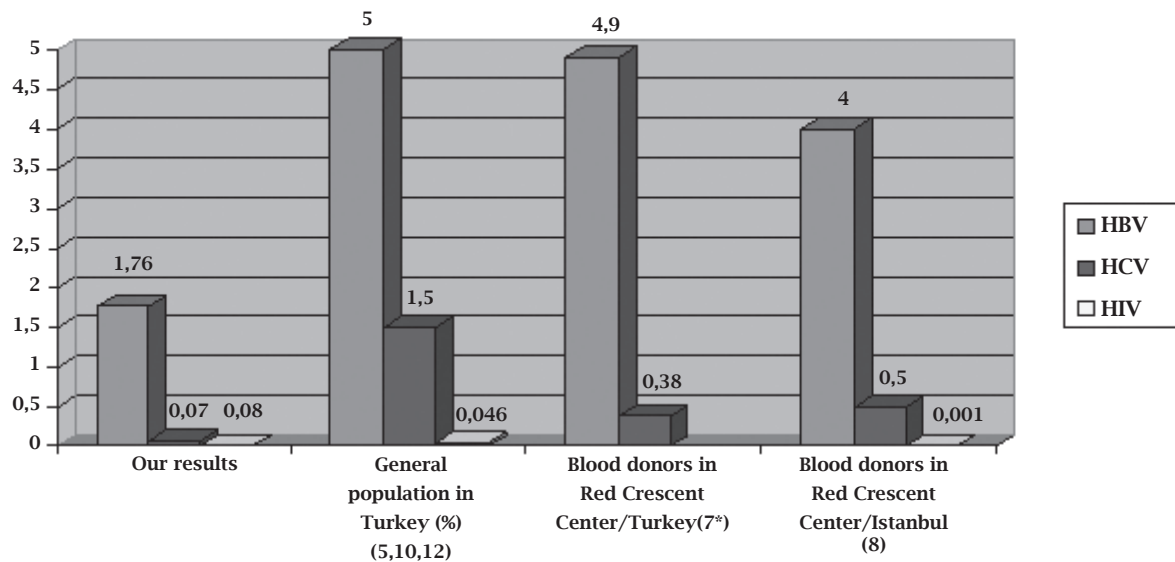
Table 4. Confirmatory results of anti-HIV reactive results with LIA

	Anti-HIV confirmatory test (LIA) results		
	Positive N %	Negative N %	Indeterminate N %
Grey zone (105)	0	52 (100)	0
Positive index (255)	6 (8.5)	64 (91.5)	1
Total (360)	6 (4.9)	117 (95.1)	1
p	0.038	0.038	

Table 5. Biologically false positive rate in anti-HCV and anti-HIV EIA screening results according to confirmatory test results

	Prevalence in accordance with the screening test N (%)	Prevalence in accordance with the confirmatory test N (%)	Biologically false positive N (%)
HCV	360 (0.50)	54 (0.07)	306 (% 85)
HIV1,2	123 (0.17)	6 (0.008)	117 (% 95)

Figure 1: Comparison of HBV, HCV and HIV seroprevalance among our blood donors with seroprevalance reported in the most extensively ranging studies conducted in the general Turkish population and the Red Crescent Centres. (*No HIV prevalence was reported at the Red Crescent Centre in Turkey results).



DISCUSSION

This study contains important results showing that, compared to previous years, the infection rates have decreased among blood donors and that transfusions are being carried out more safely as a result of vaccination and training campaigns launched in Turkey's largest metropolitan city of Istanbul, all of which have been the results of the private sector efforts as well as governmental agencies under the directive of the Turkish Ministry of Health.

In screening tests performed on the general public in Turkey, the incidence of HBsAg positivity has ranged between 1.7-21%.⁵ According to studies conducted by various blood bank centers, HBsAg positivity amongst blood donors was 5.2% during the periods between 1985 and 1999, which then decreased to 2.97% between 2000 and 2003.⁶ HBsAg positivity among blood donors and the general population has been gradually declining over the years. Reasons for this drop have been attributed to heightened public knowledge about HBV as well as the expansion and implementation of public vaccination policies.⁶⁻⁸ The most extensive data pertaining to HBV seroprevalence in blood donors is derived from the Red Crescent Centres. HBV seroprevalence

of 6,240,130 candidates giving blood at the Red Crescent Centres throughout Turkey in 1989-2004 was on average 4.9%.⁷ In the same study, HBsAg positivity was found to be 4.92% in 1989, 5.23% in 1991, and 2.10% in 2004. The decrease in Hepatitis B seroprevalence was attributed to the decrease in the number of military blood donors (positive correlation) and to the increase in voluntary donors (negative correlation).⁷ Based on the results of the Red Crescent Centers from Istanbul between 1987 and 2003, the mean HBsAg positivity was 4%, with a decrease in seroprevalence from 5% to 2.07%.⁸ This decrease in HBV seroprevalence was emphasized as being attributed to greater care taken in donor questioning and to the use of more sensitive screening tests from 1997 onwards.⁸ Our study determined HBsAg positivity to be 1.7%, which is 2.6 times ($p < 0.05$) lower than the HBV seroprevalence detected by the Red Crescent Centre's throughout Turkey⁷ and 2.3 times ($p < 0.05$) lower than the results given for the city of Istanbul.⁸ Based on our results, a general trend towards a decline in HBsAg positivity in donor population was observed over the past several years. In addition to improved public awareness and expan-

sion of vaccination policies, another reason for the observed decline seen in our results likely stems from the use of highly sensitive test kits by our group upon which samples were accepted as "True Positive" only if clear cut repeat reactivity was demonstrated, thereby excluding false positives that would have otherwise been accepted as positive.

In Turkey, HCV incidence in the general population ranges between 1-2.4%. In contrast, the ratio among blood donors is 0.3-1.8%.^{7,10} Unlike HBV, the incidence of HCV infection does not show significant difference between regions.^{9,10} Anti-HCV positivity in blood donations reported from various centres was 0.6% between 1991 and 1999 and 0.54% between 2000 and 2006, showing no significant change over the years.⁶ Based on the Red Crescent Center's results reflecting the general population across Turkey, anti-HCV seroprevalence between 1996 and 2004 was 0.38%.⁷ In comparison, anti-HCV positivity for the period of 1996-2003 was 0.5%, based on the Istanbul's Red Crescent Center results.⁸ In our study, anti-HCV positivity among blood donors was found to be 0.07%. This result is 7 times lower than the overall Red Crescent Centre's results throughout Turkey ($p < 0.05$) and 5 times lower than the results in Istanbul ($p < 0.05$). If the incidence in the overall population is accepted as 1%, it can be said that, compared to the general public, the HCV seroprevalence of our blood donors is approximately 14 times lower. We believe that the reason for the results to vary to such a degree stems from the fact that most Turkish conducted studies report reactive screening results as positive without verifying anti-HCV positivity by confirmation testing. In comparison, in our study repeat reactivity was confirmed with the use of a sensitive confirmatory test. Our view is further supported by the fact that 85% of the samples determined as positive during the screening test were actually BFP according to the confirmatory test results.

It is estimated that 33.2 million people throughout the world carry the HIV virus.¹¹ Based on the data obtained from the Ministry of Health, as of 2006, 2544 cases of HIV/AIDS have been reported in Turkey.¹² In 3% of these cases, the route of transmission was through blood and blood product transfusions.¹² Based upon data obtained from the Red Crescent Center between the periods of 1996 and 2003, Anti-HIV positivity on 1,737,943 screened donors was 0.001% and no significant changes were observed in this rate over the years.⁸ Based upon data obtained from the Turkish Ministry of Health, HIV/AIDS incidence rates between 2000 and 2005 were 0.023%, 0.027%, 0.027%, 0.028%, 0.030%, and 0.046%, respectively.¹² Data from the Red Crescent Center revealed that over the past seven years, the incidence of HIV amongst donors remained low without an increase, while the incidence in the general population gradually increased over the years. The rate of anti-HIV positivity amongst blood donors from our study was 0.008%, based upon our con-

firmatory test results. From the Red Crescent Center's data, it was noted that the rate of anti-HIV positivity in Istanbul had increased eightfold amongst screened blood donors ($p < 0.05$). This might be attributable to the fact that donor candidates with risk factors for HIV tend to conceal their condition during donor questioning out of fear of associated social labelling.

There are however some shortcomings in our study. One of these is the lack of confirmation testing of our HBsAg results. Although the specificity of the EIA tests used during HBsAg screening is nearly 100%, there was a need to confirm the positive results with a neutralization test, particularly in communities with a low prevalence of Hepatitis B, such as voluntary blood donors.^{13,14} In a study recently conducted in Turkey, nearly 70% of the weak positive HBsAg results (with a S/CO ratio between 1 and 2.5) were determined to be negative (false positive) in the confirmatory neutralization tests.¹⁵ Another study found the false positivity rate in weak positive samples to be significantly higher than those of the strong positive samples and recommended that confirmatory tests should only be performed on weakly positive samples.¹⁶ Based on these results, if confirmatory testing were carried out in our HBsAg positive results, a part of the weak positive samples in particular would have had a negative result and the subsequent HBV prevalence among our blood donors would have been even lower. A further shortcoming was that indeterminate results for anti-HCV and anti-HIV results were not finalized with further testing. When anti-HCV EIA positive results are shown to be indeterminate upon immunoblot confirmatory testing, it is recommended to either repeat the anti-HCV EIA test or perform a HCV RNA quantification for the same donor.¹⁷ Amongst blood donors, most indeterminate anti-HCV immunoblot results test negative upon repeat testing,¹⁷ however, occasionally serum samples that had been in the pre-seroconversion window period can later seroconvert to positive.¹⁸ Similarly, during the window period for individuals recently infected, HIV confirmatory testing may have an indeterminate outcome. For these cases, repeat HIV testing (screen with confirmation) are recommended after 1-2 months.¹⁹ In low risk HIV/HCV populations, it has been hypothesized that repeat indeterminate results are largely due to the development of auto antibodies or from the cross-reaction of antibodies with certain irrelevant antigens.²⁰ Donors with indeterminate or positive anti-HIV/anti-HCV confirmatory results are directed to a healthcare facility for further evaluation and management, but unfortunately no feedback has been received about the definite clinical status of these cases.

Although not recommended by the assay manufacturer company, another important point we want to emphasize from our study is the value of using a grey zone with positive threshold determination as a means of increasing the sensitivity of anti-HCV and anti-HIV screening tests. Posi-

tive results were obtained in only 2 out of the 105 (1.9%) anti-HCV reactive samples that had fallen within the grey zone index (s/co value 0.70-0.99). For both samples, HCV RNA testing was performed simultaneously, however in both samples the results were found to be negative. In comparison, all confirmatory test results of the anti-HIV positive samples that were within the grey zone index value range were determined to be negative. In anti-HIV and anti-HCV EIA positive samples whose values were within the grey zone index (s/co 0.70-0.99), significantly more samples tested negative upon confirmation than those samples above the positive threshold value ($s/co > 1.0$). This suggests a contradiction with regards to 2 issues, the first is the negative zone application requirement, the other is how to deal with donors whose test result is positive, but HCV RNA tests negative. In this case, it can be considered that the grey zone application is at least not required in anti-HIV screening tests. Nevertheless, further research is required to establish whether the grey zone application leads to unnecessary donor loss and increase in costs.

In conclusion, we believe that the decrease in HBV and HCV prevalence over time has been multifactorial and has been attributed to a higher public awareness compared to previous years as a result of intensive training and campaigns, an increase in the volunteer donor numbers, more diligent donor questioning and examination, expansion of HBV vaccination coverage as well as confirmation of our screening results with sensitive confirmatory testing. While a decrease in HBV and HCV prevalence has been observed, the increase in HIV prevalence rate, despite similar transmission routes, may be linked to a recent increased trend of HIV/AIDS cases observed in Turkey over the past few years. Another factor to consider with regards to HIV donor questioning is the fact that many donors still have reservations in answering questionnaires to the full extent out of fear that risk behaviour questions may somehow be used against them. We believe that the healthcare system would be better served by developing strategies with improved donor questioning that address the psychosocial aspects of HIV screening. We hope that the creation of these changes can minimize the fear of social stigmata associated with HIV donor questioning while preserving donor confidentiality.

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