SEROPREVALENCE OF HEPATITIS C VIRUS INFECTION AMONG BLOOD DONORS IN A TEACHING HOSPITAL IN NORTHEASTERN MALAYSIA

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Abstract. The aim of this study was to determine the prevalence of HCV infection and the signal/cutoff (S/CO) value for false reactive, false positive, indeterminate and true positive HCV infection among apparently healthy blood donors in our area. This retrospective study was conducted at the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia from June 2008 to June 2009. Blood samples were screened for anti-HCV using enzyme immunoassay (EIA). Reactive cases were confirmed by recombinant immunoblot assay (RIBA). Sixty-one blood donors were found to be reactive after the first screening test. Twenty-nine blood donors had reactive repeat screening, with only 9 samples being true positives. The S/CO for false reactive, false positive, indeterminate and true positive anti-HCV samples were 1.02 to 1.45, 1.01 to 2.09, 1.07 to 2.43 and 35.95 to 119.89, respectively. The analysis showed the low incidence of HCV infections among blood donors in our area, however, thorough donor screening and stringent selection criteria are still recommended to eliminate high risk donors to improve our blood transfusion service.

Keywords: hepatitis C infection, anti-HCV seropositive, anti-HCV screening, blood donor, Malaysia

INTRODUCTION

Hepatitis C infection is a worldwide problem in public health and has become a significant cause of morbidity and mortality, especially in developing countries (Andrade *et al*, 2006). Blood transfusions save millions of lives, but transfusion transmitted infections (TTIs) still put millions of people at risk and pose a serious problem, especially in multitransfused patients (Diro *et al*, 2008).

Blood donors in low hepatitis C virus infection prevalence settings have a greater possibility of false positive results. All positive anti-HCV EIA results in blood donors should have additional confirmatory testing (Dufour *et al*, 2003). Laboratories need to report the signal/cut-off (S/CO) ratio whenever a positive anti-HCV result is found. Laboratories

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need to determine an appropriate cutoff point based on the incidence of hepatitis C infection in the area (Dufour *et al*, 2003). Low S/CO levels (<4.5) results in more anti-HCV false positives and can result in exclusion of up to half of blood donors (Contreras *et al*, 2009).

The recombinant immunoblot assays (RIBA) should be performed first as a confirmatory test, because a negative RIBA prevents false labeling of an individual as HCV exposed and reduces the frequency of false positives in those with a weakly positive anti-HCV EIA (Dufour *et al*, 2003). The risk for TTIs has reduced significantly with improvement in screening for anti-HCV and implementation of nucleic acid testing (NAT) to confirm HCV infection among blood donors (Qiu *et al*, 2008).

Mandatory screening of blood for infections is one of several strategies to ensure safe blood transfusions and prevent HCV transmission (Thakral *et al*, 2006). Public awareness should target identified risk factors to prevent HCV infection among blood donors (Khattak *et al*, 2008). It is important to study the prevalence, age distribution and risk factors for HCV infection in order to make appropriate changes in blood donor selection criteria and recruit more low risk voluntary blood donors (Thakral *et al*, 2006).

The aim of this study was to determine the prevalence of HCV infection and the S/CO value for false positives, indeterminates and true positives among apparently healthy blood donors in our area.

MATERIALS AND METHODS

This retrospective study was carried out from June 2008 to June 2009 in the Transfusion Medicine Unit, Hospital Universiti Sains Malaysia. This center serves approximately 800 users and recruits 12,000 donors annually.

All blood donors were screened for anti-HCV using an enzyme immunoassay, ABBOT HCV EIA version 3.0 (third generation). Reactive cases were repeat tested. A S/CO value of >1 was considered as reactive. Repeat reactive cases were confirmed by third generation RIBA (Chiron Corp, Emeryville, CA).

Definitions

False reactive is when the samples are negative after repeat screening with an immunoassay test. A positive anti-HCV is when the tested specimens are repeatedly reactive and describes the final interpretation of screening the immunoassay test results. A false positive anti-HCV is when samples test negative or have an indeterminate result with third generation RIBA. A true positive anti-HCV is when samples are positive after testing with third generation RIBA.

RESULTS

Six thousand four hundred ninety-five blood donors were included in the study. Sixty-one donors (0.94%) were reactive for hepatitis C virus infection with the first screening test. Twenty-nine donors were anti -HCV positive with repeat testing and 32 were negative on repeat testing. When a confirmation test was performed on those with repeatedly positive test results, 24.13% of samples (7/29) had an indeterminate result, 31.0% of samples (9/29) had a positive result (true positive) and 44.8% of samples (13/29) had a negative results (false positive initial testing). The S/CO values for false reactive and false positive ranged from 1.02 to 1.45 and 1.01 to 2.09, respectively. The S/CO ratios for donors with indeterminate results and true positive results ranged from 1.07 to

2.43 and 35.95 to 119.89, respectively.

DISCUSSION

The seroprevalence of HCV infection among blood donors worldwide ranges from 0.4% to 19.2 % (Memon, 2002). The risk factors contributing to the transmission of HCV infection explain the variability in HCV seroprevalence (Thakral *et al*, 2006). The overall seroprevalence of anti-HCV positive status was 0.45% (29/6495) and of true positive HCV infection was 0.14% (9/6495) among blood donors in our area. A study in Singapore also reported a low prevalence of hepatitis C infection among blood donors of 0.37% (Wang, 1995). Similar prevalence were reported from India (0.44%) and Brazil (1.37%) (Akhtar *et al*, 2005; Andrade *et al*, 2006); Karachi was also reported to have an incidence of 1.8% among healthy blood donors (Thakral *et al*, 2006). A retrospective study conducted by the Nepal Central Blood Transfusion Service reported a seroprevalences of anti-HCV among the general population and blood donors of 0.3% to 1.7% (Karki *et al*, 2008). Slightly higher prevalences of anti-HCV were observed in the Philippines (2.2%), Indonesia (2.3%) and Thailand (2.9%) among blood donors (Arguillas *et al*, 1991; Darmadi *et al*, 1996; Luksamijarulkul *et al*, 2004; Karki *et al*, 2008). Seroprevalences of HCV infection have been reported among blood donors in North West Pakistan (4.1%), Nigeria (5.0%), Ethiopia (5.8%) and Georgia (7.8%) (Zaller *et al*, 2004; Diro *et al*, 2008; Jeremiah *et al*, 2008; Khattak *et al*, 2008). The prevalence of confirmed HCV infection among bloods donor in Beijing was 31.3% (Qiu *et al*, 2008).

Out of the 61 initial screening positive samples in our study, 32 were confirmed negative (false reactive) when tested with a screening immunoassay with S/CO values of 1.02 to 1.45. Thirteen samples were negative on RIBA (false positive) but reactive with EIA with S/CO values of 1.01 to 2.09. Our findings support those of Contreras *et al* (2009) who determined the optimal S/CO point to be 4.5 to identify a major proportion of anti-HCV false positive results. Very low levels of anti-HCV have been reported to be associated with negative supplemental testing and may reflect false or nonspecific reactivity (Contreras *et al*, 2009). Similar findings were reported by Sayan *et al* (2006) who observed that HCV RNA was not detected in samples with S/CO ratios less than 3.8. A previous study (Dufour *et al*, 2003) reported 86% of samples with low levels of anti-HCV on EIA were RIBA negative (Dufour *et al*, 2003). It was also reported(Contreras *et al*, 2009) that donors with low levels of anti-HCV, positive on RIBA and negative for HCV RNA were found to remain negative for HCV RNA after five 3 monthly follow-ups, therefore supplemental testing or samples with very low levels (<4.5) of S/CO (<4.5) can be avoided (Contreras *et al*, 2009).

False positive antibody results may be due to cross reaction with other antibodies, however the exact cause is not clear (Contreras *et al*, 2009). Huang *et al* (2005) reported levels ≥10 are more likely to be cases of previous infection. Half of individuals with low positive anti-HCV results have no recognized risk factors for HCV(Huang *et al*, 2005). However, donors who had vaccination for influenza may have false positive results for anti-HCV and other serologic tests (Dufour *et al*, 2003).

Seven of our subjects had indeterminate (false positive) results with a S/CO value of <2.5. One previous study (Dufour

et al, 2003) reported that patients with low positive anti-HCV EIA results had indeterminate results on RIBA in 12% of samples. It is appropriate and more economical to test patients with borderline anti-HCV levels with another enzyme immunoassay prior to use of the HCV RNA test (Sayan *et al*, 2006). There may be various causes of indeterminate results, such as recovering from a self limited acute HCV infection, partial seroconversion, early seroconversion or nonspecific false reactivity on a RIBA test (Contreras *et al*, 2009).

In our study 9 of our donors had a true positive HCV result with levels ranging from 35.95 to 119.89. This supports previous studies who found a value >40 represents a current or persistent infection (Huang *et al*, 2005). Dufour *et al* (2003) reported that 90% of specimens with high anti-HCV levels had positive HCV RNA results. One study (Contreras *et al*, 2009) found that antibody levels >20 were associated with viremia, and no viral replication was seen in subject with low antibody levels.

The majority of HCV RNA positive results were found in samples with levels >11. Antibody levels have been reported to be directly correlated with the presence of HCV RNA (Bossi and Galli, 2004). A direct relationship has been observed between high levels of antibodies and viral replication (Contreras *et al*, 2009).

We do not have a figure for blood donors during the window period because nucleic acid amplification testing (NAT) has not yet been implemented in our hospital. This screening test can identify donations made during the immunological window period before HCV seroconversion. Lei *et al* (2008) found the introduction of NAT would add an extra layer of safety to the blood supply in regard to transmission of HCV during the window period of infection. The majority of HCV NAT positive subjects were found to be in the preseroconversion phase of infection (Hyland *et al*, 2003). In a multi-Chinese blood center study, it was found that incorporating NAT technology into blood donor screening would reduce the risk of HCV infections eightfold over current EIA screening (Shan *et al*, 2007).

Lower education level, being a laborer/agriculture worker, residence in a rural area, history of a blood product transfusion, tattooing, intravenous drug use and sexual promiscuity were among the significant risk factors for HCV infection among donors (Luksamijarulkul *et al*, 2004). Other factors that contribute to the increased rate of HCV infection were increasing demand for blood supply, emergency need for blood, asymptomatic HCV carrier status in donors and increase in test seeking behavior. HCV infection was reported to be more common in commercial donors (Jeremiah *et al* 2008). O'Brien *et al* (2008) reported that most HCV positive donations occurred most in donors born between 1945 and 1964; the reasons for this are unclear. A declining rate in HCV infection is noted in first time donors born after 1964.

The prevalence of anti-HCV confirmed positive donations in our blood donor population was low (0.14%) and comparable to other countries in Southeast Asia. However detection of HCV infection among donors is still of major importance to prevent TTIs because it is a virus of major concern in multiply transfused patients (Rezvan *et al*, 2007) .

Health care professionals needs to understand to use the S/CO level in determining the next step in hepatitis C diagnosis (Contreras *et al*, 2009). When a low

S/CO value is used, confirmatory testing is necessary to exclude false positive results. High antibody levels are associated with HCV RNA positivity and confirmatory testing may not be needed except in those with a normal ALT. Understanding risks for TTIs will help improve pretesting donor screening (Qiu *et al*, 2008). More information about HCV transmission in the general population, the risk factors and its prevention is needed.

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