ARTICLE IN PRESS

Transfusion and Apheresis Science ■■ (2014) ■■–■■

Contents lists available at ScienceDirect

Transfusion and Apheresis Science

journal homepage: www.elsevier.com/locate/transci

Serological characterization of occult hepatitis B virus infection among blood donors in India

Veena Doda *, Satyam Arora, Tapannidhi Kirtania

Blood Bank, Post Graduate Institute of Medical Education and Research (PGIMER), Dr Ram Manohar Lohia Hospital, New Delhi, India

ARTICLE INFO

Article history:
Received 4 February 2014
Received in revised form 4 April 2014
Accepted 25 July 2014

Keywords:
OBI
Hepatitis B
NAT
TTI
Blood bank

ABSTRACT

Introduction: Discovery of hepatitis B infections characterized by the presence of viral genome without detectable HBsAg (Occult Hepatitis; OBI) has initiated a considerable amount of research in this regard. Our study is a serological and molecular characterization of OBI among the donors who donated at our blood bank during the study period.
Material and Method: During the study period HBsAg ELISA non reactive ID-NAT reactive donors samples were screened for presence of antibody against HBc, HBs and HBe. Molecular analysis of these NAT yield samples was undertaken for detection of the viral load and genotyping.
Result: We studied 28,134 HBsAg ELISA non reactive donor samples. On testing them with ID-NAT, HBV DNA was detected in 25 samples. Eighteen samples out of these 25 NAT yield were further worked up. The 66.6% of the NAT yield samples (12 out of 18) were reactive for antibody against HBc. The 25% (3 out of 12) of these NAT yield samples having antibody against core antigen also had antibody against HBs. The 27.7% (5 out of 18) of NAT yield detected by ID-NAT did not have any detectable serological marker in blood. Four out of 12 core antibody positive NAT yield samples had genotype A HBV infection.
Conclusion: As per our study molecular detection of HBV DNA by ID-NAT, we were able to analyze 18 HBV NAT yield cases among 28,134 HBsAg ELISA non reactive donors. Out of 18, 12 donors were OBI whereas the rest (6) were in window period (WP yield) of HBV infection. One out of every 3.6 NAT yield detected by ID-NAT was non reactive for all serological markers.

1. Introduction

Hepatitis B virus (HBV) infection is one of the major threats to human health worldwide, especially to the population in developing countries. Detection of HBV is primarily based on screening for HBsAg (surface antigen) as a marker of infection for the virus. However, the discovery of infections characterized by the presence of viral genome without detectable HBsAg (occult hepatitis infections; OBI) has initiated a considerable amount of research. OBI cases have been identified in patients with clinical complications of chronic infection as well as co-infections with other viruses such as hepatitis C virus (HCV) or human immunodeficiency virus-1 (HIV).

Occult hepatitis B infection is defined as the presence of circulating hepatitis B virus (HBV) DNA detected by HBV nucleic acid test (NAT), with or without detectable antibody against the core antigen. NAT yields are detection of HBV DNA as a marker of HBV infection when HBsAg is absent in blood. These NAT yields for HBV can be of two types based on presence of antibody against core antigen. NAT yield without antibody to core is considered as window period phase of the infection (WP yields) whereas yields with antibody to core can be considered as an occult hepatitis B (OBI). Detection of a NAT yield depends upon the sensitivity of the assay used for detection of both HBsAg and HBV

* Corresponding author. Tel.: +91 9810626226; fax: 0091-11-23348033.
E-mail address: veenadoda@gmail.com (V. Doda).

http://dx.doi.org/10.1016/j.transci.2014.07.008
1473-0502/

Please cite this article in press as: Veena Doda, Satyam Arora, Tapannidhi Kirtania, Serological characterization of occult hepatitis B virus infection among blood donors in India, Transfusion and Apheresis Science (2014), doi: 10.1016/j.transci.2014.07.008

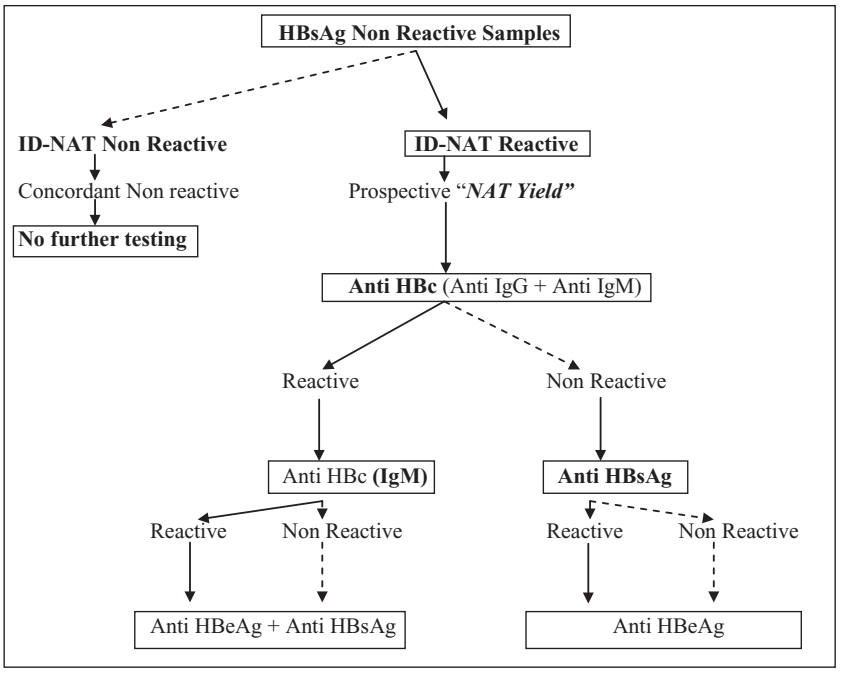

Fig. 1. Algorithm for further studying a prospective HBV NAT yield.

DNA. Levels of both of them vary in the blood depending on the phase of the infection as well as the immune status of the individual.

With recent addition of ID-NAT for screening of donated blood, detection of OBI among the blood donors has been possible at the molecular level. This study provides prevalence of OBI among whole blood donors at our blood bank in India. Further we studied the serological characterization and molecular analysis of the hepatitis B virus NAT yield samples of those asymptomatic whole blood donors.

2. Material and methods

This study was done on the 28,465 donors who donated at our blood bank, in a Central Government hospital from January 2012 to December 2013. In addition to mandatory screening by ELISA (for anti HIV, anti HCV and HBsAg) all the samples were subjected to ID-NAT. Voluntary: replacement donors were 14,927 (52.5%): 13,538 (47.3%). Blood units non reactive for HBsAg and non reactive with ID-NAT were not further tested considering them to be concordant HBV non reactive donor samples. Blood units which were HBsAg non reactive but ID-NAT reactive (NAT Yield) were further worked up with anti HBc (anti IgG and IgM), anti HBsAg, anti HBe, viral DNA load and viral genotyping according to the algorithm in Fig. 1.

2.1. Sample collection and testing

Serology and ID-NAT were done on the samples from the pilot tubes initially (serum, plasma) followed by repeat testing from the blood bag. Remaining tests were done on NAT yield samples stored at –70 °C. The testing kits used for the study are summarized in Table 1. Tests were done as per the manufacturer's instructions.

A sample was termed NAT yield when found reactive by ID-NAT and non reactive by serology. NAT co-yield was termed when sample was found reactive for two markers by ID-NAT and both the marker were non-reactive by serology. A sample was termed as ID-NAT co infection yield when two markers were reactive by ID-NAT, out of which one was detected by serology; hence the other viral marker detected only by NAT was considered as the ID-NAT co infection yield.

2.2. Serological testing

Screening for HBsAg (hepatitis surface antigen) was done for each donor by ELISA. The assay was based on one step enzyme immunoassay technique of the sandwich type for

Table 1
Details of principles and kits of the tests performed.

Test	Principle	Kit
HBsAg	ELISA	BioRad "MONOLISA HBsAg Ultra"
ID-NAT	TMA	Procleix Ultrio
Anti HBc (anti IgG&IgM)	CMIA	Abbott, ARCHITECT
Anti HBc (anti IgM)	ECi	Ortho Diagnostics, Vitros 3600
Anti HBsAg	CMIA	Abbott, ARCHITECT
Anti HBeAg	CMIA	Abbott, ARCHITECT
Quantitative HBV DNA	Real Time PCR	Abbott, m2000
Quantitative HCV RNA	Real Time PCR	Taqman technology

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