Incidence of Hepatitis C Virus RNA in Anti HCV Negative Blood Donors

A thesis Submitted by

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بسم الله الرحمن الرحيم

(قالوا سبحانك لا علم لنا إلا ما علمتنا إنك أنت العليم الحكيم)

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Abstract

Hepatitis C has been recognized as a global health problem. The detection of hepatitis C virus (HCV) infection is of major importance for the prevention of transfusion-transmitted hepatitis.

The present study was carried out to evaluate the commercially available antibody tests in the diagnosis of hepatitis C virus (HCV) infection in comparing with the results of RT-PCR test and Evaluation of the seroprevelance of hepatitis C among Egyptian blood donors.

90,886 blood donors were submitted to antibody screening tests for HCV. All anti- HCV –ve cases were submitted to screening for HCV - RNA by RT-PCR assay using a pooling method.

The total number of HCV antibody reactive cases was 6,940 cases out of 90,886 blood donors with a seroprevalence of 7.64%.The total number of cases found RNA positive and HCV by EIA –ve were 5 cases among 83,946 donors giving a rate of 1: 16,789.

In conclusion the prevalence of HCV in Egypt is very high (7.64%). The routine use of EIA for detection of anti-HCV antibody is a highly accurate method and does not show a significant difference with the NAT technique.

Key words: hepatitis C virus, EIA, RT-PCR, NAT and Pooling.

CONTENTS

ABBREVIATIONS

AB	Applied Bio-Systems.
ALT	Alanine Amino Transferase.
bDNA	branched DNA.
cDNA	complementary DNA.
DNA	Deoxyribonucleic Acid.
EIA	Enzyme Immuno Assay.
ELISA	Enzyme Linked Immuno Sorbent Assay.
FDA	Food and Drug Administration.
HBV	Hepatitis B Virus.
HCC	Hepatocellular Carcinoma.
HCV	Hepatitis C Virus.
HCV-LPs	HCV like Particles.
HENCORE	Hepatitis C European Network for C-operative Research.
HIV	Human Immunodeficiency Virus.
IFN-α	Interferon-α.
IgG	Immunoglobulin G.
IVDU	Intravenous Drug Use.
NANBH	Non-A Non-B Hepatitis.
NAT	Nucleic Acid Amplification Testing.
NCI	National Cancer Institute.
NHANESIII	Third National Health and Nutrition Examination Survey.
NK	Natural Killer Cells.
NS	Nonstructural.
OPD	0-Phenyl Enediamine.
PCR	Polymerase Chain Reaction.
PKR	Dependent Protein Kinase RNA.
PWP	Preseroconversion Window Period.
rHCV	recombinant Hepatitis C Virus.
RNA	Ribonucleic Acid.
RT-PCR	Reverse Transcriptase-Polymerase Chain Reaction.
SVR	Sustained Viral Response.
TMA	Transcription-Mediated Amplification.
WHO	World Health Organization.

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INTRODUCTION &
AIM OF THE WORK

Introduction

Hepatitis C virus (HCV) is a major cause of chronic liver disease and hepatocellular carcinoma worldwide. The most recent World Health Organization (WHO) estimate of the prevalence of HCV infection is 2%, ranging from 0.6% to 2.3% in North America, Northern and Western Europe and Australia **(*Roman et al., 2008*).**

The lowest prevalence (0.01%-0.1%) has been reported from countries as the United Kingdom and Scandinavia. The highest prevalence (15%-20%) has been reported from Egypt ***(Frank et al., 2000 & Shepard et al., 2005 b)***.

The most efficient transmission of HCV is through large or repeated direct percutaneous exposures to blood (e.g., transfusion or transplantation from infectious donors, injecting drug use) ***(Alter, 2007)***.

Routine screening of blood donors for anti-HCV is important to prevent HCV infection via blood transfusion **(*Dow et al., 1994*)**. The major complication of chronic **HCV** infection is progressive hepatic fibrosis leading to cirrhosis, which develops in about 20% of those with chronic **HCV** patients ***(Alter et al., 1999 & Conry-Cantilena et al., 1996)***. Several factors have been identified to influence the rate of progression to cirrhosis in the HCV chronically infected population. Unfavourable factors include male age, (age >40 years at infection), significant alcohol consumption (>30 g/day) and co-infection with human immunodeficiency virus (HIV) or hepatitis B virus (HBV) (***Zarski et al., 1998 and Benhamou et al., 1999***).

However, HCV antibody detection depends on many factors such as:

(1) The time of infection where in acute infection anti-HCV could not be detected although the HCV-RNA exists in the blood ***(Abdel-Hamid et al., 1997)***.

(2) The specificity and sensitivity of the available screening assays are very important, that is why several generations of the assays have been developed ***(Colin et al., 2001)***.

(3) Increased false negativity for antibody tests compared with PCR-RNA detection in patients who have an impaired immune system e.g. patients with human immuno-deficiency virus (HIV) infection, those on hemodialysis and patients on chemotherapy drugs ***(Fabrizi et al., 2002)***.

(4) Technical or cleric errors could be the reason of false negative results.

To avoid such false negative results we can depend on a Nucleic Acid Amplification Testing (NAT) which may be used to confirm the negative results (***Saldanha et al., 1998)***. Because NAT technology is a highly sensitive and specific technique, it is possible to identify viremic samples in which antibodies are not yet present and therefore reduce the window period to 15 to 20 days ***(Seme and Poljak, 1996 & Muller-Breitkreutz et al., 1999)***.To reduce the cost, pooling of blood sample are requested.

Aim of the work

- The aim of this work was to screen blood donor samples which are tested to be negative of anti-HCV by Enzyme Immuno Assay (EIA) third generation and to find out the incidence of HCV RNA

in the plasma apparently of healthy blood donors, with negative EIA third generation.

- To reduce the costs of NAT technique through pooling of the samples.
- Estimation the true up to data incidence of hepatitis C in Egypt.

REVIEW OF LITERATURE

Hepatitis C virus

Infection with the hepatitis C virus (**HCV**) is a leading cause of liver disease worldwide ***(Kim, 2002)***. This disorder was first recognized in the mid-1970s and was categorized as non-A, non-B hepatitis (NANBH) ***(Feinstone et al., 1975)***. HCV was subsequently identified in 1988 as a ribonucleic acid (RNA) virus ***(Alter et al., 1989)***. Progression to chronic hepatitis C occurs in most people acutely infected with HCV, and persistent infection is an important cause of cirrhosis, end stage liver disease, and hepatocellular carcinoma ***(Alter et al., 1999 and Kim, 2002)***.

Hepatitis C Virus Structure

HCV is a member of the Flaviviridae family, genus Hepacivirus ***(Simmonds et al., 2005)*** which is an enveloped virus with a positive-strand RNA genome, approximately 9.6 kilobases in length. It encodes a single large polyprotein of about 3,000 amino acids (***Liang et al., 2000***).

HCV virion is made of a single-stranded positive RNA genome, contained into an icosahedral capsid, itself is enveloped by a lipid bilayer into which two different glycoproteins are anchored ***(Penin et al., 2004)***. See figure (1).