Hepatitis C Virus-Antibodies In Egyptian Volunteer Blood Donors, Comparison Of 5 Screening Assays (ELISA) Confirmed By Immuno-Blot Assay

Thesis Submitted For Partial Fulfillment Of Master Degree
In Clinical Pathology

By

Hatem Ali Saber Kamel Sarg

M.B.B.Ch

Supervised By

Prof. Dr / TARIF HAMZA SALLAM

Prof. Of Clinical Pathology

Dr. / MANAL HASHIM AHMED FAYEK

Lecturer Of Clinical Pathology

Dr. / HODA MOHAMED EL GENDI

Lecturer Of Clinical Pathology

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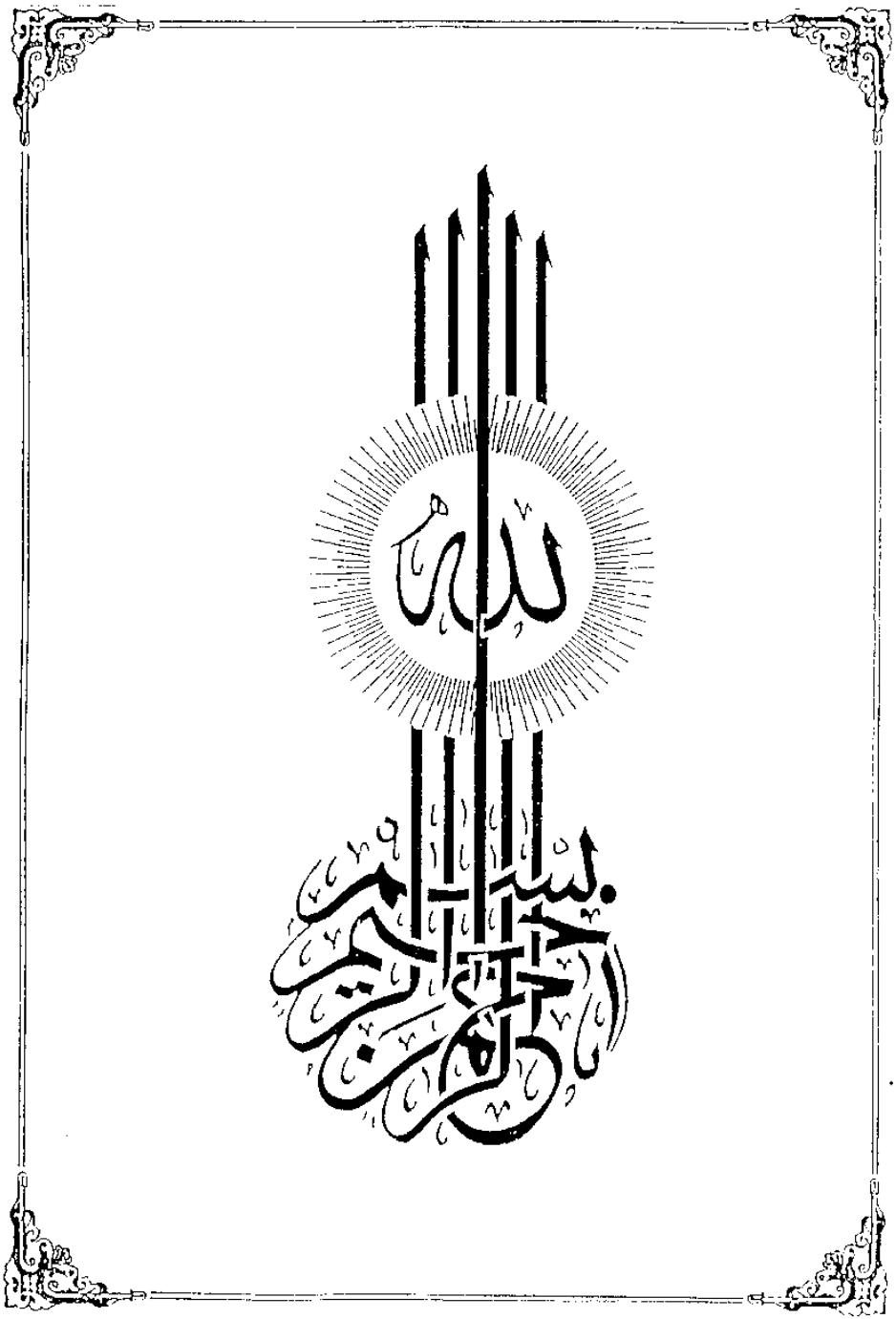

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List Of Abbreviations

- AA	Amino acids.
- Ab	Antibodies.
- ALT	Alanine aminotransferase enzyme.
- AST	Aspartate aminotransferase enzyme.
- cDNA	Complementary deoxyribonucleic acid.
- CMV	Cytomegalovirus.
- C	Core.
- EBV	Ebestein Bar virus.
- E.Coli	Escherichia- coli.
- ELISA	Enzyme Linked Immuno-Sorbant Assay.
- E	Envelop.
- GP	Glycoprotein.
- HAV	Hepatitis A virus.
- HBV	Hepatitis B virus.
- HBsAg	Heppatitis B surface antigen.
- HCV	Hepatitis C virus.
- HIV	Human Immunodefeciency viruses.
- IFN	Interferon.
- Ig	Immunoglobulin.
- KD	Kilo dalton.
- NANB	Non-A, Non-B.
- nm	Nano meter(10^{-6} meter)
- NS	Non structural protein.
- OD	Optic density.
- ORF	Open Reading Frame.
- P	Peptides.
- PCR	Polymerase Chain Reaction.
- RIBA	Recombinant Immuno-Blot Assay.
- RNA	Ribonucleic acid.
- SOD	Super oxide dismutase.
- UTR	Untranslated region.

Introduction and Aim of The Work

Introduction

HEPATITIS C VIRUS (HCV) is now known to be the major cause of more than 90% of post-transfusion hepatitis (Choo et al., 1990). HCV infection is difficult to recognize because it has a mild subclinical course, clinical signs are often absent and the transmission from acute to chronic stage is silent (Bruno et al., 1994).

Currently it is estimated that 40-60% of these infected persons may become chronic HCV carriers and those carriers may be infectious for many years (Houghton et al., 1991). Chronic HCV infection is commonly associated with cirrhosis and hepatocellular carcinoma (Simonetti et al., 1992).

With cloning of HCV, it is found to be a positive single stranded RNA molecule, The diagnosis of HCV infection became more reliable (Choo et al., 1990).

Because HCV is parenterally transmitted virus, the agent is of particular interest to Hematologists.

In Egypt, Hepatitis is a major public health problem; viral hepatitis competes with schistosomiasis as a leading cause of chronic liver disease. In preliminary serosurveys, remarkably high rates of HCV-Ab seropositivity have been reported in presumably healthy volunteer Egyptian blood donors (Darwish et al., 1993).

Therefore, screening of blood units for antibodies to HCV (HCV-Ab) is becoming an important part of the routine analytical work of blood banks. This practice leads to several problems; not only with the interpretation of results and counselling of donors. but also with the selection of the appropriate screening test.

Nowadays, several enzyme immunoassays (EIAs) for detecting anti-HCV in human serum using recombinant antigens or synthetic peptides have been developed and commercialized and many of them are available in Egypt. However, these tests can yield false positive reactions (Leon et al., 1993). To discriminate between false and true anti HCV reactivity, first, second and third generations Recombinant Immuno-Blot Assays (RIBA-I, RIBA-II and RIBA-III) have been developed. However, the interpretation of their results remains a problem (Allain et al., 1992 and Tong & Codd, 1992).

The Polymerase Chain Reaction (PCR) assay for the cDNA is still the only test that directly detects HCV-RNA and thus discriminates between viremic and non viremic (or low viremic) individuals (Bresters et al., 1993).

Recently, HCV genotyping takes marked importance as different HCV genotypes infections resulted in different serological reactivities and responses to interferon therapy (Yoshioka et al., 1992).

Aim Of The Work

This study aims at determining the prevalence of Hepatitis C Virus infection among blood donors attending Blood Transfusion Center of Ain Shams University Hospitals.

Also to evaluate the specificity of the most commonly used, ELISA tests for HCV-Ab detection, in Egypt.to decide which is more suitable for Blood Banks screening work to minimize the un-necessary loss of donations.

Review of Literature

History Of The Disease

The first reference to epidemic infective jaundice has been ascribed to Hippocrates, The earliest record was in a letter in 751_{AD}. by Pope Zacharias as there have been numerous accounts of epidemics particularly during wars (Zukerman, 1977). During the early 1960s, up to 33% of patients who received blood transfusion got hepatitis. By the 1970s the organism causing hepatitis type A and type B were detected and identified but hepatitis still developed as a post transfusion complication in recipients, 90% of this cases were diagnosed as non-A ,non-B (NANB) hepatitis (Aach et al., 1978).

The laboratory diagnosis of NANB hepatitis until recently has been only possible by exclusion of known hepatotropic viruses e.g. HAV, HBV, CMV, EBV,... and other etiologic factors (Bradley, 1985).

Transfusion associated hepatitis has been reported to occur in about 2.5-15% of patients who received blood transfusion after exclusion of HAV and HBV positive blood, the majority of these cases (75-90%) diagnosed as NANB hepatitis (Feinstone et al., 1975).

In 1986, screening of blood donors for surrogate markers (elevated ALT activity or/and presence of HBc-Ab decreased the incidence of post-transfusion hepatitis (Koziol et al., 1986).

However in 1989, a unique cDNA clones were isolated from a chimpanzee chronically infected with NANB hepatitis which was found to belong to a new hepatitis virus termed HCV.

Filtration experiments showed that the agent size ranges between 40-80 nm, its low density and sensitivity to organic solvents such as chloroform suggest that the virus has a lipid containing envelop similar to some RNA viruses as Flaviviruses or Togaviruses (Choo et al., 1989).

Virology Of HCV

Hepatitis C Virus is now believed to be a lipid enveloped virus, 55 to 65 nm. in diameter and is known to possess a single stranded RNA genome. Both structural and non-structural proteins are also recognized (Dusheiko et al., 1993).

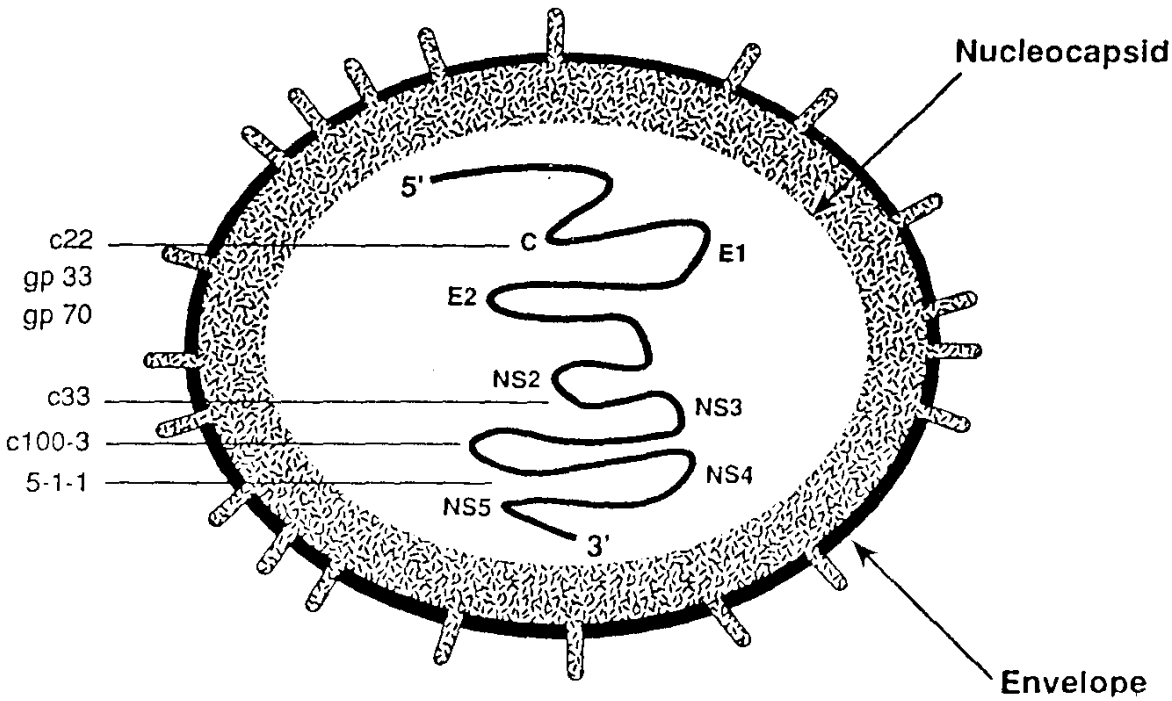

Fig. 1 . A stylized depiction of the hepatitis C virus, showing an artistic representation of the viral particle, RNA genome, non-coding 5′ region, coding region, and proteins expressed from the viral genome and used in serological assays. C, nucleocapsid protein-coding region; E, envelope protein-coding region, NS, non-structural region. (Dusheiko et al., 1993).