STUDY OF THE HIGH PREVALENCE OF HEPATITIS C VIRUS IN EGYPT

A Thesis

Submitted for partial fulfillment of M.D. Degree
Microbiology and Immunology
(Bacteriology)

By

Nebal Medhat Darwish
M.Sc. (Bact.)

Supervisors

Prof. Dr. Tahani Abdel-Hamid Mohamed
Professor of Microbiology and Immunology
Faculty of Medicine – Ain Shams University

Dr. Samir I. Abdel-hady
Assistant Professor of Microbiology and Immunology
Faculty of Medicine – Ain Shams University

Dr. Mona Omar Abass
Assistant Professor of Microbiology and Immunology
Faculty of Medicine – Ain Shams University

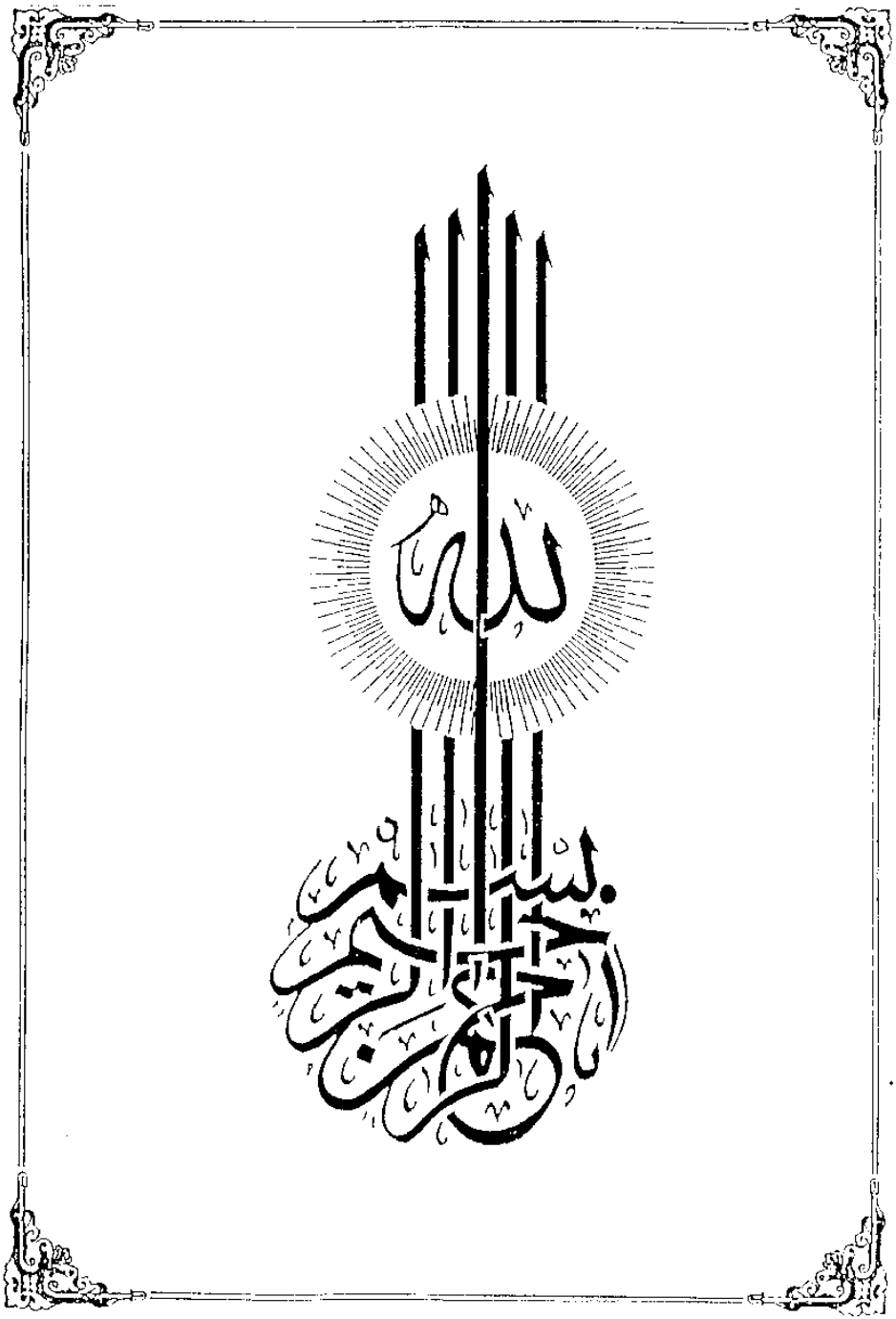
1995

بسم الله الرحمن الرحيم

وقل رب زدني علما

صدق الله العظيم

سورة طه آيه ١١٤



Acknowledgement

Thanks to God for helping me to accomplish this work.

The author would like to express grateful acknowledgment to her major advisor Professor Dr. Tahani Abdel-Hamid Mohamed, Professor of Microbiology and Immunology, Faculty of Medicine, Ain Shams University, for her helpful guidance and direction throughout this work.

I would like to thank Dr. Samir I. Abdel-Hady, Assistant Professor of Microbiology and Immunology, Faculty of Medicine, Ain Shams University, for his advice and generous help.

Special thanks are due to Dr. Mona O. Abbas, Assistant Professor of Microbiology and Immunology, Faculty of Medicine, Ain Shams University, for her kind instructive supervision and valuable guidance.

Acknowledgement is also due to Professor Dr. Mostafa El Awady, National Research Institute, Doki, and to Dr. Maged El-Sherbiny Assistant professor, Cairo University for their help.

I would like to pay a special tribute to all staff members in the department of Microbiology and Immunology, Faculty of Medicine, Ain Shams University.

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

ALT	: Alanine amino transferase
Anti-HBc	: antibody to hepatitis B core antigen
Anti-HCV	: Antibody to HCV
AST	: Aspartate amino transferase
bp	: Base-pair
CAH	: Chronic active hepatitis
CDC	: Centers for disease control
cDNA	: Complementary DNA
CPH	: Chronic persistent hepatitis
EIA	: Enzyme immunoassay
ELISA	: Enzyme linked immunosorbent assay
EM	: Electron microscope
GGT	: Gamma–glutamyl transferase
HA	: Hepatitis A
HAV	: Hepatitis A virus
HB	: Hepatitis B
HBsAg	: Hepatitis B surface antigen
HBV	: Hepatitis B virus
HC	: Hepatitis C
HCC	: Hepatocellular carcinoma

HCV	:	Hepatitis C virus.
HDV	:	Hepatitis D virus
HEV	:	Hepatitis E virus
HFV	:	Hepatitis F virus
HGV	:	Hepatitis G virus
HIV	:	Human immunodefficiency virus
HLA	:	Human leucocyte antigen
PCR	:	Polymerase chain reaction
PTH	:	Posttransfusion hepatitis
PT-NANBH	:	Posttransfusion non-A, non-B hepatitis
PTH NANB	:	Posttransfusion hepatitis non-A,non-B
NANB	:	Non-A, Non-B
NANBH	:	Non-A, Non-B hepatitis
RIBA	:	Recombinant immunoblot assay
RT-PCR	:	Reverse transcription polymerase chain reaction
SIA	:	Strip immunoblot assay
TAH	:	Transfusion associated hepatitis

INTRODUCTION

disease, and hepatocellular carcinoma (HCC) are recognized public health problems in Egypt (Hibbs et al., 1993). Egypt has remarkably high rates of HCV–seropositivity, highest rates yet reported in the world among blood donors (Ghaffar et al., 1991).

A high endemicity of schistosomiasis (40%) is also present in Egypt. In a recent study on blood donors from Cairo, it was found that a history of schistosomiasis is a risk factor significantly associated with HCV infection. In Egyptians, uncomplicated Schistosoma mansoni appears to potentiate acute viral hepatitis by hepatitis B virus (HBV) and hepatitis D virus (HDV) superinfection, increasing and prolonging transaminase levels and the hepatitis B surface antigen (HBsAg) carrier rate. Such evidence may suggest that Schistosoma mansoni may similarly potentiate HCV infection in humans (Ghaffar et al., 1991).

Schistosomiasis, hepatitis B (HB), and hepatitis C (HC) virus infections which are endemic in Egypt, represent 3 important risk factors for HCC. The co–existence of these factors may have an interacting role in the development of HCC (Simonetti et al., 1992).

Hepatitis F virus (HFV)

In liver samples from patients who died of fulminant hepatitis, examination by electron microscopy has revealed 60 nm ogavirus–like particles. These has provisionally been referred to as "HFV". This agent appears to cause a malignant form of hepatitis, including rejection and necrosis of transplanted livers. HFV is currently being investigated by molecular biological techniques, but little else is known about this virus at present (Fagan, 1992).

Hepatitis G virus (HGV)

More of a rumor than a virus, HGV is reported to be a paramyxovirus–like agent transmissible to chimpanzees, causing syncytial giant cell hepatitis. Nothing else is known about HGV at present (Fagan, 1992).

NON–A, NON–B HEPTITIS

The term NANBH was adopted 15 years ago to designate those cases of transfusion associated hepatitis (TAH) that were serologically uncleared to any known hepatotropic virus (Prince et al., 1974 and Feinstone et al., 1975). An enormous amount of information has been accumulated over the years regarding the epidemiology and natural history of this infection, the recognition of other potential sources of infection, the preventive measures directed to diminish blood–borne transmission, and the treatment of the chronic sequelae of this disease.

Despite this intensive investigation, and until very recently, the identification of the causative agent of NANBH had remained elusive (Genesca et al., 1991).

The presumption that this form of NANBH was caused by a collection of serologically unrelated hepatotropic agents is derived primarily from the results of cross–challenge studies in chimpanzees experimentally infected with blood or blood components. Two distinct agents (type I and type II) have been

postulated on the basis of sequential episodes of disease in chimpanzees after they were inoculated with plasma treated by different physiochemical procedures (Alter, 1989).

HEPTITIS C VIRUS

Characterisation of HCV

Recently in 1988, workers at Chiron corporation reported the cloning of the major blood–borne NANB virus (type I) which is designated by utilization of the chimpanzee model and by application of recombinant DNA technology (Choo et al., 1989). They isolated all nucleic acid from known infectious serum and, from this pool, formed complementary DNA (cDNA) fragments using reverse transcriptase with random primers. This process yielded approximately 6 million sequences complementary to random segments of nucleic acid found in the infectious serum. These sequences were then individually inserted into phage vectors and expressed in Escherichia coli. Each resulting polypeptide was tested with serum from patients with chronic NANBH to detect reactivity with serum antibodies. Each polypeptide was also screened with control specimens of noninfected serum. After about a million such specimens were screened, one was found to react with antibodies in infected serum but not with control serum.

The cDNA fragment used to generate this polypeptide was then used as a hybridization probe to extract the original nucleic acid from which the fragment was generated. In this way, the entire genome of the suspected agent was identified. It was found to be composed of a single positively stranded RNA about 10,000 nucleotides long. From homologies found within the genomic structure, it appeared that this agent, now termed HCV, was related to the family of flaviviruses. This identification was consistent with the 30 to 60 nm size of the virus as determined by infiltration studies, and with the presence of a viral envelope, as recognized by the agent's sensitivity to chloroform (Choo et al., 1990 and Tang, 1991). It is associated with formation of cytoplasmic tubules seen by electron microscopy (EM), in the liver of experimentally infected chimpanzee, but not human, hepatocytes (Yoshizawa et al., 1981; Bradley et al., 1983 and Alter, 1989).

The minor blood–borne NANBV (type II) has been reported but it has not been clearly established that it is epidemiologically important. It is thought to be smaller than type I (25 to 30 nm versus 30 to 60 nm), naked versus enveloped type I, it is resistant to chloroform and not associated with