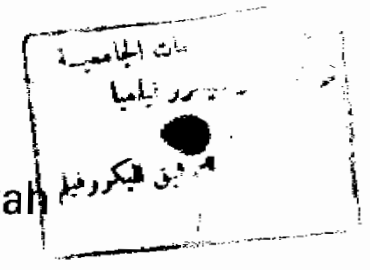
THE FREQUENCY OF AUTOANTIBODIES [ANTISMOOTH MUSCLE] AMONG HCV-POSITIVE CHRONIC LIVER DISEASE EGYPTIAN POPULATION

THESIS
Submitted for Partial Fulfillment
of The Master Degree in
Tropical Medicine



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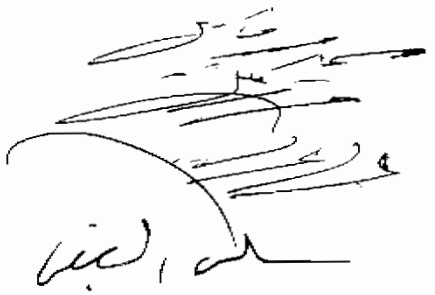

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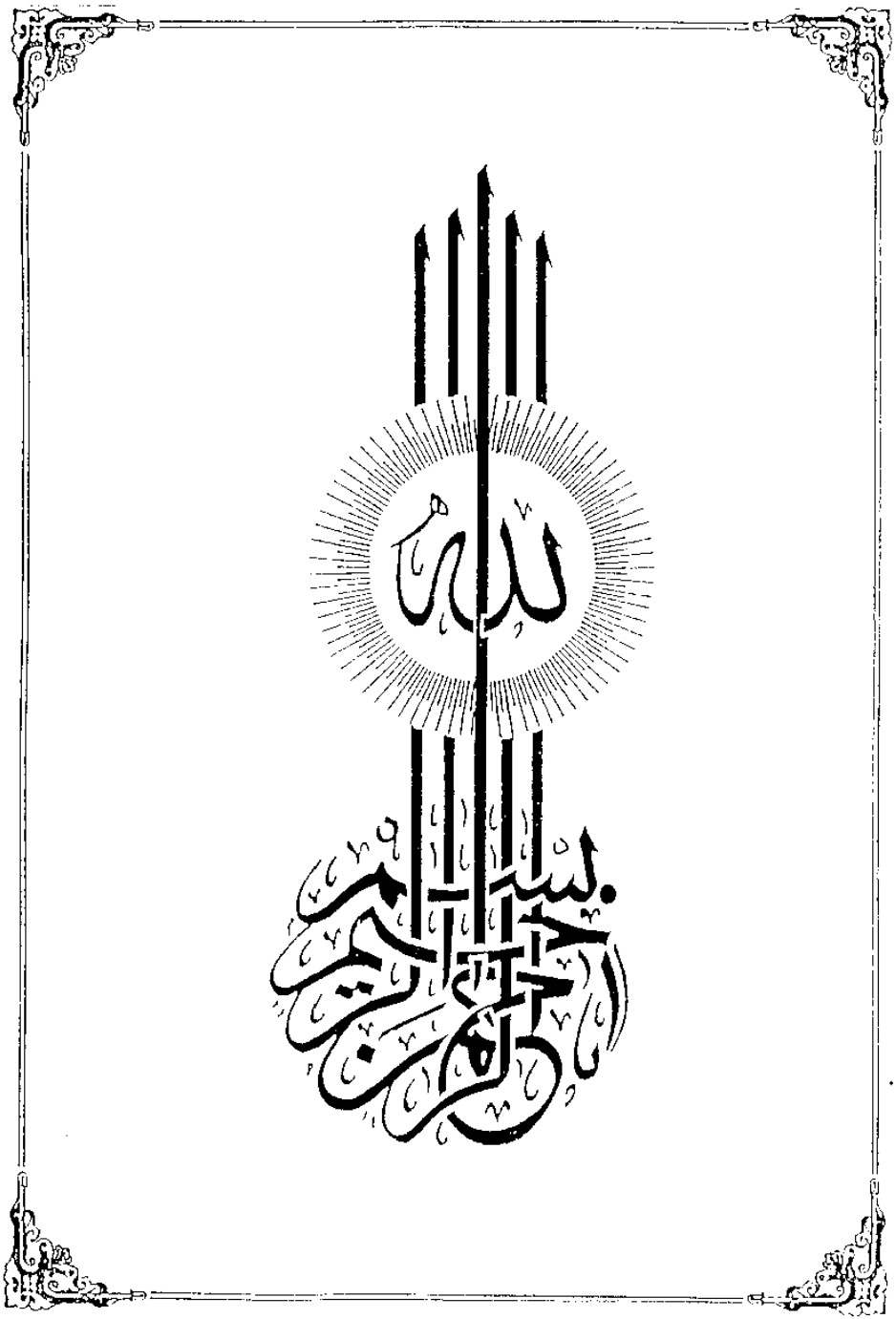

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LIST OF ABBREVIATION

AI-CAH:	Autoimmune chronic active hepatitis.
ALT:	Alanine aminotransferase.
ANA:	Anti-nuclear antibody.
AST:	Aspartate aminotransferase.
C.A.H.:	Chronic active hepatitis.
CHC:	Chronic hepatitis C.
CLD:	Chronic liver disease.
ELISA:	Enzyme linked immunosorbant assay.
HCC:	Hepatocellular carcinoma.
HCV:	Hepatitis C virus.
HIV:	Human immunodeficiency virus.
IFL:	Indirect immunofluorescence.
IgG:	Immunoglobulin G.
IgM:	Immunoglobulin M.
LKM1:	Liver and kidney microsomes.
NANBH:	None-A, none B hepatitis.
PCR:	Polymerase chain reaction.
RIBA:	Recombinant immunobolt assay.
SGOT:	Serum glutamic oxaloacetic transaminase.
SGPT:	Serum glutamic pyruvic transaminase.
SMA:	Smooth muscle antibody.

INTRODUCTION AND AIM OF THE WORK

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The autoimmune chronic active hepatitis (AI-CAH) was originally described as affecting young individuals, mainly, females and there is considerable evidence that the condition is related to aberrant autoreactivity to hepato-cellular antigens **(McFarlance and Eddlestone, 1989)**. However, it is now known that the disease is biphasic with respect to age of onset with many cases presenting later in life (above 40 years). This raises the question of whether some factors, possibly environmental, is required to trigger the disorder is susceptible subjects, the measles virus may be such a trigger **(Roberston et al., 1987)**.

The question of whether HCV has a role in the pathogenesis of AI-CAH was first raised when anti-HCV was identified in the sera of some spanish patients with AI-CAH **(Esteban et al., 1989)**.

Antibodies to HCV are present in high proportion of AI-CAH (44%) cases in Spain, and in 86% of patients in Italy with anti-LKM1 sera **(Lenzi et al., 1990)**.

Previous studies have described a high frequency of antibodies to hepatitis C virus in patients with type

2 autoimmune hepatitis expressing anti-LKM1 in their sera. Resolution of this question has major implications for therapy, because corticosteroids are of proven benefit in AI-CAH, whereas interferon can have adverse effect in patients with underlying autoimmune liver disease **(Burman et al., 1986)**.

Thus, it was appealing to study the pattern of autoantibody prevalence among HCV-CLD Egyptian patients and therefore possible to evaluate its therapeutic and diagnostic implications.

To fulfil the aim of this study, 200 HCV-positive-CLD cases (group A) [112 males and 88 females with a mean age of 38 years] will be matched to 35 HCV-negative-CLD patients as a control group (group B) [22 males and 13 females with a mean age of 41 years]. Both groups will be evaluated clinically, biochemically, pathologically, radiologically and serologically. HCV-antibody was screened by second generation ELISA, ortho and antibodies [SMA, ANA, AMA, LKM1 and SMA-anti-actin] were screened by immuno-fluorescence on cryostat murine section using 1:40 serum dilution.

The study will be carried out in Cairo Liver Center.

REVIEW OF LITERATURE

HCV: Morphology

The recent identification of the major non-A, non-B virus, which has been termed HCV, was the result of the major advances in molecular cloning techniques **(Choo et al., 1989)**. At around the same time, HCV was also cloned in Japan **(Arima et al., 1989)**.

Two more HCV genomes from Japanese patients were recently reported and was found to have 70-80% homology with the American HCV nucleotide sequence **(Kato et al., 1990a and Takamizawa et al., 1991)**.

The current understanding is that HCV is an approximately 1000 nucleotide linear, single stranded, positive polarity RNA virus and shares nucleotide and amino acid sequence homology with pesti viruses and flavi viruses as well as two plant virus super groups [alpha virus like and picornavirus-like] indicating that HCV may be evolutionary related to plant and animal viruses **(Miller et al., 1990)**.

As demonstrated in flaviviruses **(Chambers et al., 1990)** and supported by in vitro translations of cloned HCV

sequences **(Hijikata et al., 1991)**, the HCV polyprotein is processed into the N-terminal putative nucleocapsid protein termed C (19 to 22 KD) followed by two envelope glycoprotein, E1 and E2 (approximately 35 and 72 KD, respectively), and the non structural proteins.

The second envelope glycoprotein, E2, maps to a region of the genome that codes for the NS1 protein of flaviviruses, in HCV this protein is thought to be a second envelope glycoprotein; therefore it has been called E2/NS1 **(Choo et al., 1991)**.

The cleavages between C and E1 and between E1 and E2 are thought to result from the action of host signal peptidases, whereas processing of nonstructural proteins is thought to be accomplished by a viral-coded protease, among the HCV structural proteins, C is highly basic and relatively well conserved among different HCV isolated **(Takeuchi et al., 1990a)**, whereas the envelope glycoproteins E1 (glycoprotein 35) and E2 (glycoprotein 72) show substantial amino acid sequence variation including

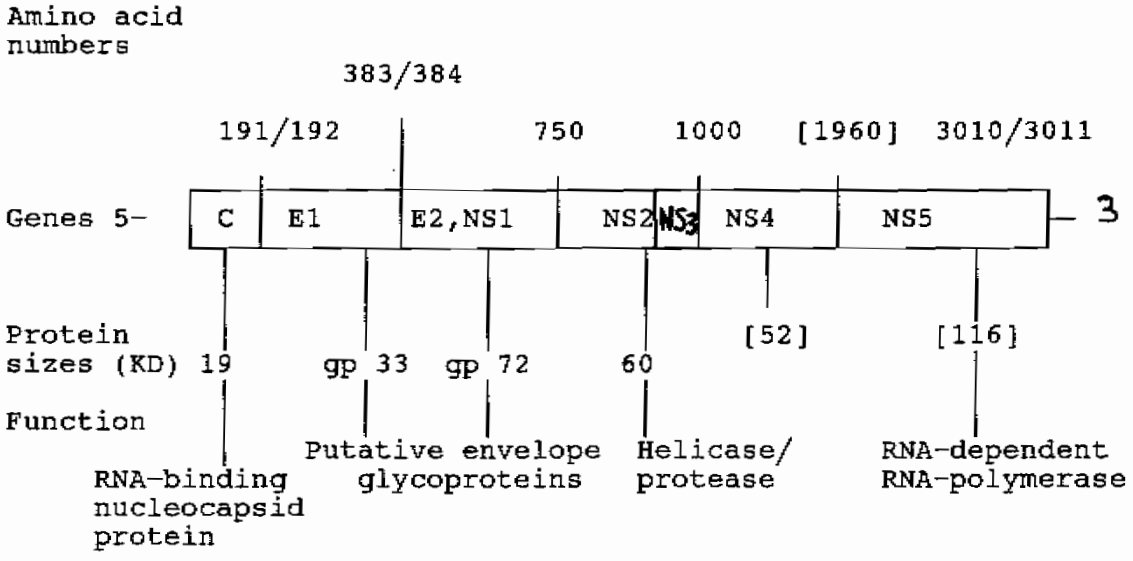
hypervariation in the N-terminal protein of E2, which may reflect selective pressure of host's immune responses **(Weiner et al., 1991 and Weiner et al., 1992).**

Further study showed that the putative nucleocapsid protein gene sequence are high conserved, suggesting that the core protein may play an important regulatory role in the life cycle of HCV **(Takeuchi et al., 1990b).**

HCV is a single-stranded, enveloped RNA virus with one reading frame, and appears to be distantly related to flavivirus, although hepatitis C virus is not transmitted by arthropod vectors **(Lau et al., 1991 and Miller et al., 1990).** HCV is 50-60 nm in size and contains 3011 amino acids and 9033 nucleotides, virus particles have not be visualized by immune-electron microscopy **(Sherlock and Dooly, 1992a).** A scheme of the genetic organization of HCV is shown in figure (1):

Fig (1) organization of the HCV genome. The observed and potential (brackets) sizes of viral proteins are shown in kilo-daltons, along with their putative functions. The

observed predicated location of polyprotein cleavage sites are indicated (C. nucleocapsid protein gene; E1-envelop gene; E2/NSI-envelope gene or NS gene I; NS2-NS genes 2-5) **(Houghton et al., 1991).**



Virion structural proteins are processed from approximately the N-terminal quarter of the polyprotein with a variety of monostructural (NS) proteins being processed from the remainder of the polyprotein. Viral proteins and particles have still not yet proved possible to infect cells in vitro. However, it has been possible to identify viral

proteins by transcribing RNA from cloned HCV, cDNA in vitro and then translating the RNA in vitro translation system such as the rabbit reticulocyte lysate.

Cloned HCVc RNA DNA has also been transfected into mammalian cells in different expression vectors, and the protein products have been identified. In these various ways, **Houghton et al. (1991)** had been able to identify a nucleocapsid protein of about 19 KD (kilo-dalton) and two glycoproteins of approximately 33 KD and 72 KD that were processed from the N-terminal region of the HCV polyprotein.

Like its pestivirus and flavivirus, HCV does not appear to produce DNA replication intermediates, and integrated forms of the viral genome in the host genome have not been detected, **Choo et al.(1989)**. Recently,evidence of subgenomic HCV RNA species has been obtained using polymerase chain reaction (PCR) analysis **(Han et al., 1991)**. This is in contrast to the absence of subgenomic RNA species during replication of the flaviviruses and pestiviruses **(Collett et al., 1989)**. However, these subgenomic HCV RNA species may