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The prevalence of HCV antibody among haemodialysis patients in Cairo governorate sector C and its relation to their glucose homeostasis

A thesis protocol submitted for partial fulfillment of MD degree in internal medicine

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Abbreviation list

ALT	Alanine transaminase
AST	Aspartate Aminotransferase
CDC	Center of disease control
CKD	Chronic kidney disease
Cr	Creatinine
DOPPS	Dialysis Outcomes and Practice Patterns Study
EHM	Extrahepatic manifestations
ELISA	Enzyme-linked immunosorbent assay
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HGB	Haemoglobin
HIV	Human immunodeficiency virus
HOMA	Homeostatic model assessment
HVR	Hypervariable region
MC	Mixed cryoglobulinemia
NHANES	National Health and Nutrition Examination Survey
NIDDK	The National Institutes of Diabetes and Digestive and Kidney Disease
PBMCs	Peripheral blood mononuclear cells
Plt	Platelets
PTDM	Post-transplantation diabetes mellitus
RIBA	recombinant immunoblot assay
SIGN	Safe Injection Global Network
SOCS-3	suppressor of cytokine signaling 3
SVR	Sustained virologic response
TLC	Total leucocytic count
UA	Uric acid
Ur	Urea
WHO	World Health Organization

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Hepatitis C virus

Nature:

HCV, a member of the Flaviviridae family, has a single-stranded positive RNA genome with high genetic diversity. The diversity is due to defective repair activity of the RNA dependent RNA polymerase, which results in nucleotide substitution, as well as the absence of 5′ to 3′ exonuclease activity causing a lack of editing **(Guillemette et al., 2007)**

At least six HCV genotypes and a large number of subtypes have been identified, some with different clinical outcomes. More recently, chimeric viruses generated by intergenotypic homologous recombination events in the NS2 gene have been described. **(Shetty et al., 2009)**

One of the most remarkable features of hepatitis C virus is the frequency with which a chronic infection can be established, occurring in 55–85% of patients **(Liang et al., 2000)**.

During the course of an infection, *HCV* mutations accrue at the rate of 1.4×10^{3} to 1.9×10^{3} substitutions per nucleotide per year. Variants reflecting slight modifications to the coding sequence, so-called "quasispecies", are commonly found to coexist simultaneously within a patient, and any given serum-derived HCV inoculum contains a population of closely related viruses. The regions of the genome corresponding to essential viral functions such as those involved in translation or replication, as well as the noncoding 5`- and 3` -ends, are highly conserved. The most variable part of the genome is the region encoding the envelope glycoproteins E1 and E2, and certain strains have shown more than 50% variability **(Guillemette et al., 2007)**.

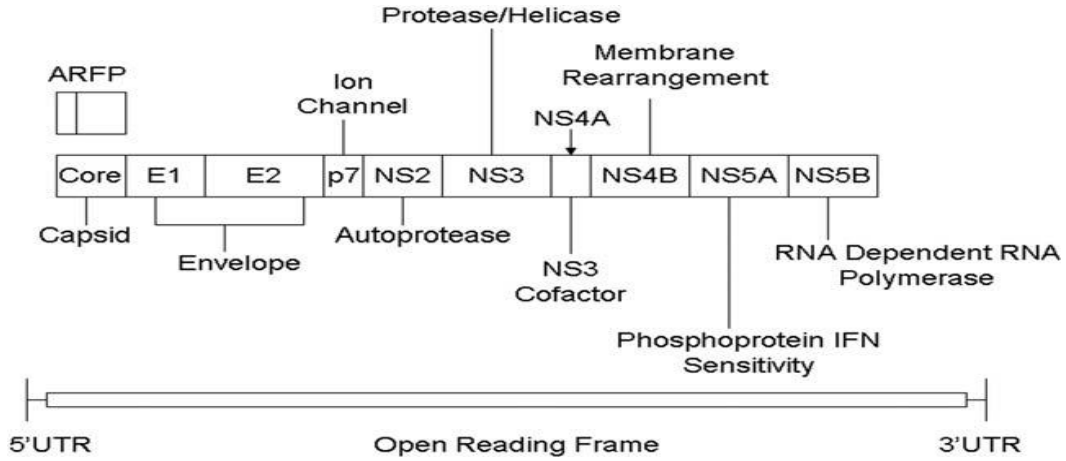

Figure 1. Structure of the *HCV* gene. A long single open-reading frame encodes a polyprotein of ~3010 amino acids. The structural proteins are core, E1, and E2, the envelope proteins. The rest of the genome contains nonstructural proteins. Another protein known as ARFP (alternative reading frame protein) may be expressed from the core region of the genome as a result of translational frame shift. **(Qureshi, 2007)**

The observed "hypervariability" in *HCV*-envelope proteins is one explanation for the exceptional ability of *HCV* inocula to reinfect the same host following seroconversion and resolution of acute infection. The absence of protective immunity against *HCV* continues to thwart vaccine development, and the emergence of drug-resistant strains will likely pose a future obstacle to long-term efficacy of inhibitors designed to target the enzymes responsible for viral replication. **(Shetty et al., 2009)**

The nomenclature system

The scale of genetic heterogeneity of HCV became clear in the early 1990s, and a number of different, often incompatible, methods were used to classify variants, resulting in differences in the letters or numbers assigned to each recognized genetic group **(Simmonds et al., 1993)**

Progress toward resolving these uncertainties were made by publication of a consensus paper in 1994, which proposed the classification of HCV by phylogenetic methods into six genotypes. **(Simmond et al., 2005)**

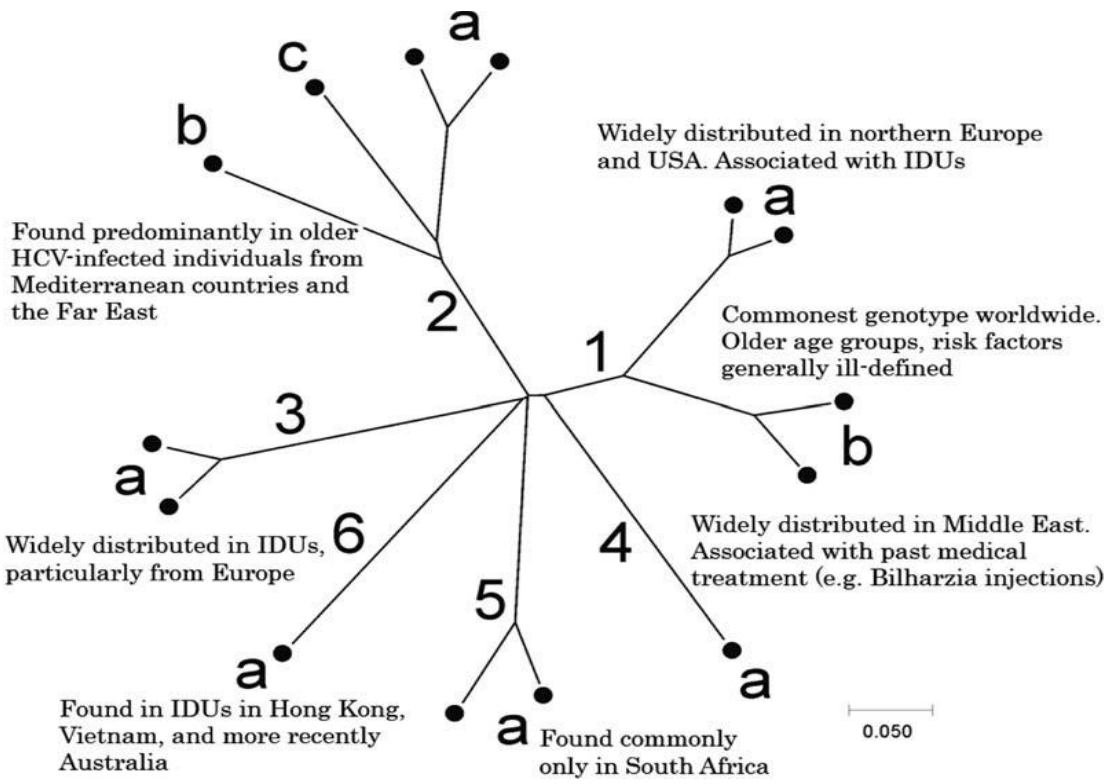

Figure 2. Evolutionary tree of the principal genotypes of HCV found in industrialized countries. Phylogenetic analysis was carried out on complete genome sequences of genotypes of HCV found in the main identified risk groups for HCV infection: injection drug users (IDUs), recipients of unscreened blood or blood products, and those experiencing other parenteral exposures. These represent the main variants believed to have become prevalent over the course of the 20th century. The tree was constructed with the phylogeny program Neighbor-Joining implemented in the MEGA package, with Jukes-Cantor corrected distances (**Hengli, 2009)**

Each of the approximately equidistant genetic groups contains a variable number of more closely related, genetically (and epidemiologically) distinct "subtypes." Genotypes differ from each other by 31–33% at the nucleotide level, subtypes by 20–25%. (**Carla et al., 2009)**

Despite the sequence diversity of HCV, all genotypes share an identical complement of colinear genes of similar size in a large open reading frame, and the genetic inter-relationships of HCV variants are remarkably consistent throughout the genome **(Robertson et al., 1998)**.

This consistency has allowed provisional classification of many of the currently recognized HCV variants, on the basis of partial sequences from subgenomic regions such as core/E1 and NS5B **(Simmonds et al., 1994)**.

Subsequent molecular epidemiology studies have revealed great HCV diversity in certain regions of sub-Saharan Africa and in south and south-east Asia (**Fig. 3**). Most newly described variants originate from specific geographical regions. For example, infections in western Africa are predominantly genotype 2 **(Candotti et al., 2003)**, whereas those in central Africa, such as the Democratic Republic of Congo and Gabon, are genotypes 1 and 4 **(Ndjomou et al., 2002)**.

Genotypes 3 and 6 show similar genetic diversity in south and eastern Asia **(Tokitaet et al., 1995)**. This "endemic" pattern of sequence variability suggests that HCV has probably circulated in human populations in these parts of Africa and Asia for centuries, millennia, or longer (**Carla et al., 2009)**

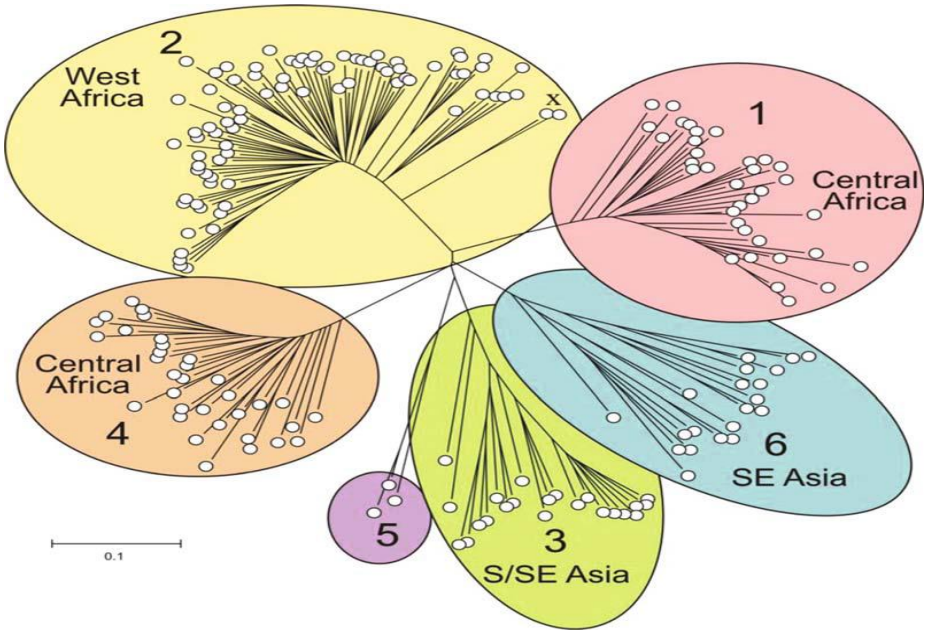

Figure 3. .Evolutionary tree of all known subtypes and genotypes HCV. This phylogenetic analysis of the NS5B region of all published sequences demonstrates that HCV variants still fall into six distinct genotypes, but each genotype includes numerous novel variants discovered in high-diversity areas in sub-Saharan Africa and southeast Asia. The tree was constructed with the phylogeny program Neighbor-Joining implemented in the MEGA package, with Jukes-Cantor corrected distances. More divergent members of genotype 2 are marked with an "x." **(Hengli, 2009)**

In contrast, the most common variants found in Western countries (1a and 1b in genotype 1; 2a, 2b, and 2c in genotype 2) have become widely distributed over the past 50–70 years as a result of transmission through blood transfusion and other invasive medical procedures and of needle sharing by injection drug users (IDUs) **(Cochrane et al., 2002)**

They now represent the vast majority of infections encountered clinically in Western countries. Subtypes 1a, 1b, 2a, 2b, 3a, and 4a are likely to be the descendants of HCV variants from endemic areas that "seeded" these new, rapidly expanding transmission networks. As discussed below, HCV classification should recognize the epidemiological associations of these "founder" viruses and incorporate their subtype names into the genotype nomenclature, while acknowledging that such labels are of little or no value in the description of HCV variants in high-diversity areas in sub-Saharan Africa and Southeast Asia (**Carla et al., 2009)**

A recent discovery with implications for HCV classification is recombination between genotypes of HCV. Homologous recombination in HCV could clearly be facilitated by the overlap in genotype distributions in many parts of the world. Because of the nature of HCV risk behavior, frequent exposures may surround the time of primary infection (e.g., repeated needle sharing by several IDUs), when protective immunity from reinfection has not yet been established. The true frequency of recombination may be underestimated because of the difficulty of detecting within-subtype recombination. In regions where HCV is highly diverse, such as West Africa, even intersubtype recombination will be difficult to detect. Finally, although a comparatively great number of complete genome sequences are available for common HCV genotypes, such as 1b, most studies of HCV variability in high-diversity areas are based on analysis of single subgenomic regions, such as NS5B or core/E1, making detection of potential recombinants unlikely **(Legrand et al., 2007)**

The observed "hypervariability" in HCV-envelope proteins is one explanation for the exceptional ability of HCV inocula to reinfect the same host following seroconversion and resolution of acute infection. The absence of protective immunity against HCV continues to thwart vaccine development, and the emergence of drug-resistant strains will likely pose a future obstacle to long-term efficacy of inhibitors designed to target the enzymes responsible for viral replication **(Shetty et al., 2009)**

Replication Cycle:

Recent studies have identified viral and host cell components involved in HCV cell entry. Hepatocytes are the main target cells for HCV infection. However, HCV has also been found in B cells, dendritic cells, and other cell types. The HCV envelope integral membrane proteins are glycosylated noncovalent heterodimers of two polypeptides, E1 and E2. The latter of these is apparently responsible for viral attachment, as E2-specific antisera can prevent HCV binding to cultured cells **(Mardapour al., 2007)**

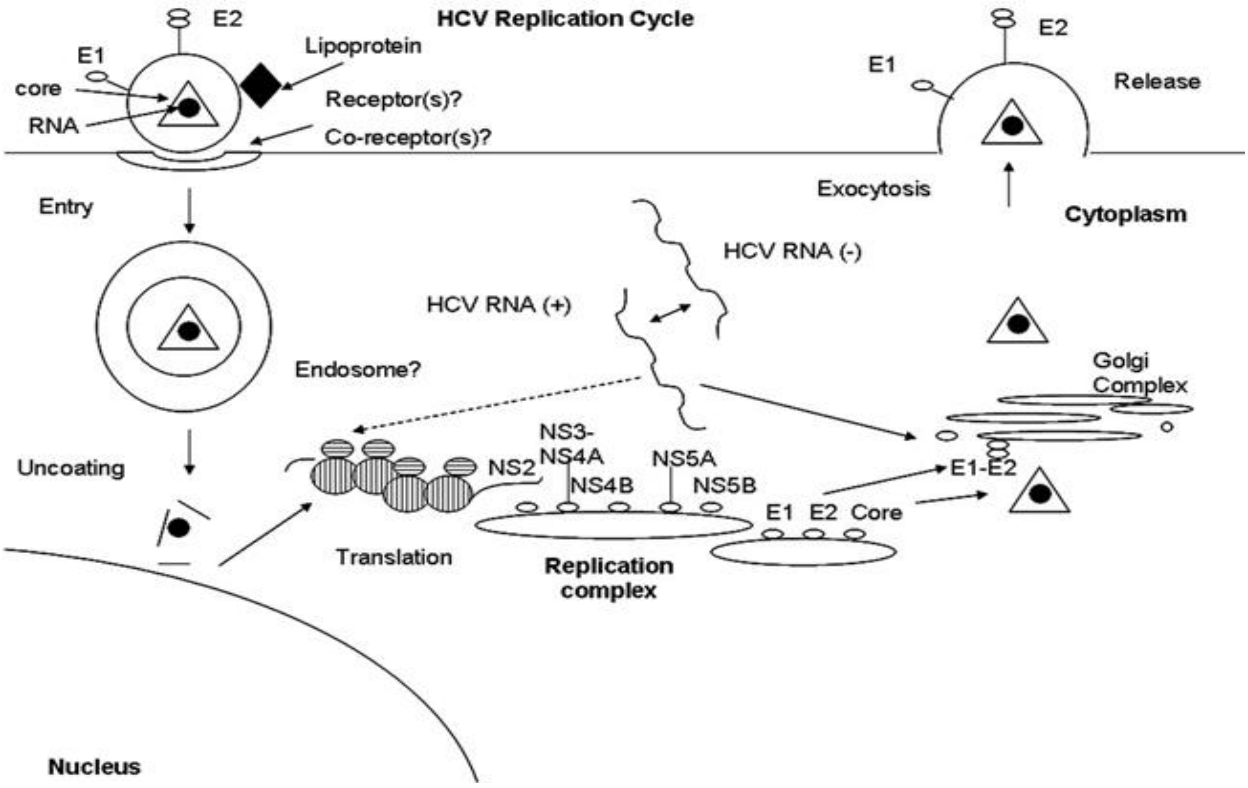

Figure 4. *HCV* life cycle. After *HCV* entry into the cell, the nucleocapsids are delivered to the cytoplasm, where the viral RNA acts as an mRNA for translation of a long polyprotein. RNA-dependent RNA replication converts the (+) into (−), which serves as a template for further (+) synthesis. Cytoplasmic replication occurs via membrane-associated replication complexes in a perinuclear membranous web. Genomic RNA-containing plasmids bud into cytoplasmic vesicles through intracellular membranes, which fuse with the plasma membrane. **(Alter et al., 1989)**

Circulating HCV virions have been shown to associate with β-lipoproteins. The capsid protein, core, colocalizes with apolipoprotein AII and intracellular lipid droplets; this interaction may contribute to hepatic steatosis observed in core

transgenic mice. Notably, the expression of LDL receptor in some cell types was shown to induce HCV binding, which does not otherwise occur. The endocytosis of HCV particles appears to be mediated by LDL receptor, but may be assisted by other interactions. E2 was shown to bind a human cell surface membrane protein, CD81. This interaction may contribute to entry of HCV virions into hepatocytes, although expression of CD81 is a protein that has been found on the surface of many cell types. Other proposed HCV receptors in addition to LDL and CD81 include scavenger receptor class B type I (SR-BI), dendritic cell-specific intercellular adhesion molecule-3-grabbing nonintegrin (DC-SIGN), liver/lymph node-specific intercellular adhesion molecule-3-grabbing integrin (L-SIGN), and more recently, claudin-1 which has been found to be essential for HCV entry into hepatic cells and has been termed a coreceptor **(Mardapour et al., 2007)**

HCV replication has been reported to occur in extrahepatic sites, most notably peripheral blood mononuclear cells (PBMCs) from chronically infected patients. High HCV RNA titers are observed in lymph nodes and approx. 50% of patients develop circulating HCV-associated cryoglobulins. An association with lymphoproliferative disorders such as non-Hodgkin's lymphoma has been reported. Lymphotropic variants of HCV have been isolated from passage in cell culture and chimpanzee PBMCs. Comparison of HCV RNA sequences isolated from viruses propagated in lymphocytes vs. hepatocyte cell lines revealed that the majority of amino acid positions which putatively influence cell tropism are located in E2 **(Shetty et al., 2009)**

Events following HCV cell attachment remain to be elucidated. It is known that HCV enters by clathrin-mediated endocytosis via transit through an endosomal low pH compartment and presumably by endosomal membrane fusion. The actual mechanisms involved in activating the low pH-induced fusion as well as the fusion peptides still remain unknown **.**The E2 glycoprotein is insufficient for membrane fusion, and a hydrophobic region of the E1 glycoprotein has been proposed to mediate fusion of the viral envelope to the cell membrane. Upon cell entry, core associates with 60S ribosomal subunits, which may contribute to uncoating of RNA and initiation of (internal ribosome entry site) IRES-mediated HCV translation and RNA replication **(Mardapour et al., 2007)**

Once synthesized, the various HCV proteins are subject to host-mediated proteolytic degradation and presentation on the cell surface as foreign antigens. Epitopes from all HCV proteins can be presented by multiple MHC Class I and II haplotypes and are susceptible to recognition by immunoglobulins and T-cell receptors **(Cerny et al., 1999)**

In acute and IFN-resolved cases, humoral, CD4+ T cell, and CD8+ CTL responses are directed against all viral antigens and are especially vigorous against epitopes on core and NS3. In comparison to HBV, viral antigen expression is relatively low in hepatitis C; but, unlike some other viruses, HCV does not appear to actively subvert antigen processing and/or presentation. To the contrary, the expression of molecules which mediate antigen recognition, such as MHC, intracellular adhesion molecules, TNF-α, and Fas antigen, is upregulated in HCV-infected cells. In chronic infection, the HCV-specific CTL response is effective enough to maintain some control over viral load; but, the majority of patients develop only a modest humoral response and are unable to establish a robust CTL activity sufficient to achieve clearance**.** How HCV is able to persist in the face of an active, concerted immune response is unclear. The lack of a virus-associated reverse transcriptase activity precludes integration into the host genome. Given the quasispecies nature of HCV infection, it is likely that "escape mutation" arises from nucleotide misincorporation by NS5B RNA-dependent RNA polymerase activity. It has long been presumed that sequence heterogeneity in immunodominant epitopes allows immune-mediated selection of resistant quasispecies. There is some evidence that selection of humoral escape variants can occur during the course of HCV infection in humans and chimpanzees. The principal neutralization epitope, hypervariable region-1 (HVR1, a 27–30–residue amino acid sequence located in the E2 N-terminus), exhibits extreme variation among known isolates. Quasispecies with mutated HVR1 have been shown to arise in response to antibody selection against the parent sequence. It is also possible that mutations modify a dominant CTL epitope, as has been observed in chimpanzee acute infection. Mutation may convert viral polypeptides to potent antagonists of CTL directed against the wild-type sequence. This scenario has been observed with an NS3 epitope as well as an NS5B epitope, resulting in persistent infection **(Dustin et al., 2007)**