An-Najah National University
Faculty of Graduate Studies

Virological Features of Hepatitis C Virus In Hemodialysis Patients: Northern Districts of Palestine

By

Fekri Helmi Samarah

Under the Supervision

of

Dr. Nael Abu-Hasan **Dr. Kamel Adwan**

This Thesis was submitted in partial fulfillments of the requirements for the degree of masters of Science in Biology

Nablus, Palestine
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This Thesis was defended successfully on the 23rd of May 2001 and approved by

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3. Dr. Tamer Essawi - External Examiner	
4. Dr. Jumanah Odeh - Internal Examiner	

This Work is Fully Dedicated

To My
Parents, Brother, Sisters

And Beloved
Wife and Daughter

Acknowledgment

I would like to express my deep thanks and gratitude to my academic supervisors Dr. Nael Abu-Hasan and Dr. Kamel Adwan for their kind and unlimited professional and personal support. Special thanks to Dr. Hasan Hijaz (Nephrologist, Al-Watani Hospital) for his kind encouragement and guidance. My greatest appreciation is also due to the staff nurse in the dialysis unit and my colleges in the lab. at AL-Watani Hospital for their kind help and support in data collection.

Last but not least, all the love to my wife and family for their patience, support and encouragement during the course of the work.

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List of Abbreviations

Abbreviation	Meaning
NANA	Non-A, non-B
RdRp	RNA-dependent RNA polymerase
ORF	Open readingframe
UTR	Untranslated region
IRES	Internal ribosome entery site
PKR	RNA-dependent protein kinase
NAT	Nucleic acid testing
ELISA	Enzyme-linked immunosorbent assay
RIBA	Recombinant immunoblot assay
HCC	Hepatocellular carcinoma
IFN	Interferon
ESRD	End stage renal disease
IVDA	Intravenous drug abuse
HD	Hemodialysis
NS	Non-structural
MEIA	Micro-particle enzyne immunoassay
EIA	Enzyme immunoassay
RRT	Renal replacement therapy
CVA	Cerebro-vascular accident
LFT	Liver function test
ALP	Alkaline phosphatase
AST	Aspartate transaminase
ALT	Alanine transaminase
OD	Optical density

Abstract

A total of 71 HD patients were followed up with respect to HCV markers for 18 months. Initial findings showed that 37 (52.11%) were HCV positive and 12 (16.9%) were HBV positive. By the end of the first period (6 months), the prevalence of HCV increased to 65.15 % as 6 new cases became HCV positive. With respect to HBV the prevalence dropped to 10.61 %. ALT levels were monitored on a weekly basis as an indicator for episodes of HCV infection. This marker strongly correlates with HCV infection as all seroconverted HCV new cases showed clear ALT elevation in the acute phase of infection. Fluctuation in ALT levels among HBV positive cases were not as clear as for those with HCV infection, however, HBV positive cases seem to exhibit constantly high levels. Such fluctuations were also observed among some of the initially HCV positive cases, indicating possible HCV re-infection among such cases. Preliminary evidence suggests that a nosocomial infection was behind the observed increase in the prevalence rate of HCV. By the end of this period 5 HCV negative and 1 HCV positive subject died due to various complications.

During the second phase of the study, non-of the HCV negative cases (41) were seroconverted to HCV positive and only 1 case was seroconverted to HBV positive. ALT elevation was observed among 2 HCV negative cases, however, non-of these 2 cases entered the acute phase of infection to either HCV or HBV infections. Strict hygienic conditions based on the recommendations of this study is more likely behind the limited number of newly infected cases. By the end of this phase 2 of the HCV positive cases and 7 of the HCV negative died.

During the third phase HCV seroconversion was observed in 1 case out of initially 40 HCV negative cases involved. Non of the 80 cases involved at the beginning of this phase was seroconverted to HBV positive. Out of the 40 HCV negative cases, 5 died, 1 of which was HBV positive. Out of HCV positive cases involved in this phase (40), 5 patients died and 2 of them were with dual infection (HCV/HBV).

All HCV seroconverted cases (7 cases) clearly showed a strong association between high ALT levels and the acute phase of infection. Incubation period ranged between 21-109 days, based upon the findings of high ALT levels in two successive samples and the appearance of either anti-core or anti-NS antibodies. Anti-core antibodies seem to appear in an earlier time compared to anti-NS antibodies as 4 of the seroconverted cases expressed anti-core antibodies as early as 21 days and up to 30 days. The production of an antibody response to the NS antigen occurs relatively late (56-109) days. ALT elevation was found to be associated with both antibodies in the acute phase of infection. Subjects expressing anti-NS seem to show much higher ALT levels, that last for longer time.

Infection rates increased dramatically with duration of dialysis, thus confirming of the role of dialysis as a risk factor for HCV infection. With increased duration of infection a shift from NS or core to both markers was also observed.

CHAPTER I

Introduction

1.1 General introduction

The hepatitis C virus (HCV) spreads by parenteral transmission of body fluids, primarily blood or blood products. Hepatitis C is a ubiquitous disease, affecting many individuals worldwide. The prevalence rate ranges from 0.15% (Scandinavia) to as high as 44% (northwest Egypt and southern Cameroon) in the general population.[1] On average, 230,000 new infections occurred in the United States each year during the 1980s.[2] With the advances in serologic assays in 1989, including tests for detection of genotype-specific antibodies and screening tests based on enzyme immunoassays, the incidence of new HCV infections dropped to 80% of the pre-1989 level.[2,3] Although many individuals are chronically infected, only a small percentage of those with newly acquired infection actually show symptoms. Therefore, many remain unaware of their disease. Hepatitis C-associated chronic liver disease is a leading cause of death among adults in many countiries and a leading cause for liver transplantation. It is anticipated that the number of deaths attributed to HCV-related liver disease will increase substantially over the next 10-20 years.[4]

1.2 Physical characteristics, molecular genetic structure and diversity of HCV

HCV was first isolated and characterized in 1989. The viral genome is a positive-sense, single-stranded RNA molecule of approximately 9.4kb in length, which encodes a polyprotein of about 3,100 amino acids. This polyprotein is cleaved into functional proteins by cellular and viral proteases. At present, there

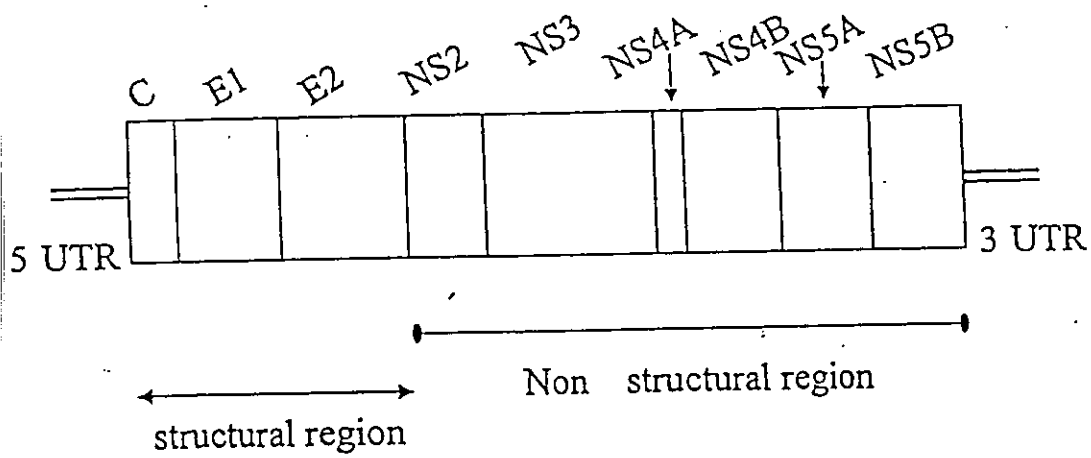
are 6 main genotypes of HCV, each further stratified by subtype as discussed later [5].

Hepatitis C virus exists as a heterogeneous mixture of closely related viruses called quasispecies. Analysis of individual isolates demonstrates that there are 2 hyper-variable regions located within the virion structural proteins (see figures 1 and 2) [6]. The continuous evolution of new variant glycoproteins is a major mechanism of viral evasion. Substantial evidence indicates that HCV genotype is clinically important with respect to efficacy of interferon therapy; the likelihood of response to antiviral therapy appears to be associated with the degree of quasispecies diversity. Additionally, mutations in the viral population likely contribute to the emergence of interferon "resistance" during interferon therapy. The rate of disease progression in hepatitis C infection is influenced by both virus and host-related factors. However, the phenomenon of quasispecies is likely responsible for the ineffectiveness of isolate-specific vaccines and will challenge the development of pan-genotype antiviral therapies. New strategies based on anti-sense and ribozyme technologies may hold future promise as therapeutic modalities. [7,8]

In 1989, several reports detailing the association of this newly identified virus with post-transfusion non-A, non-B hepatitis (NANB).[9] Kubo and colleagues[10] subsequently confirmed the global distribution of the virus through isolation of a partial HCV clone from an implicated donor of NANB.

The negative strand replicative intermediate is synthesized by the virally encoded RNA-dependent RNA polymerase (RdRp);[11] the lack of a 3'-5' proofreading activity ensures the generation of a population of similar but distinct quasispecies. The HCV genome

consists of a single open readingframe (ORF) flanked by 5' and 3' untranslated regions (UTR). The 5' UTR contains a series of stem-loop structures that interact with host factors to initiate synthesis of the polyprotein through an internal ribosome entry site (IRES). [12,13] The 3' UTR can be divided into 3 domains by virtue of the sequence composition.[14,15] The first domain constitutes the most heterogeneous sequence and is followed by a domain composed of a poly-A or poly-UC nucleotides . The third domain consists of a highly conserved 3' tail of 98 nucleotides, which is important for initiation of viral RNA replication. [16]



Boxed area represents the single open reading frame (ORF)

UTR : untranslated region

Figure 1. HCV Genome

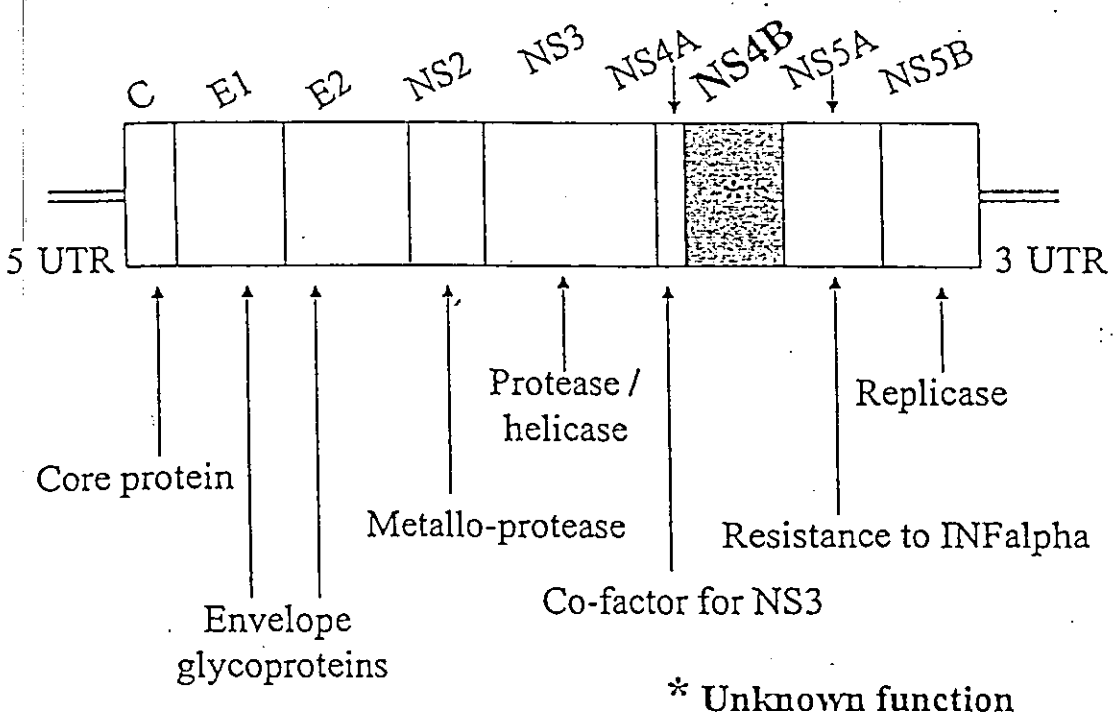


Figure 2. The RNA genome and the encoded polyprotein

There are 6 main genotypes of HCV, which are classified numerically from 1 to 6. Each genotype is further stratified by subtype, which is alphabetically assigned as "a," "b," and so on. [17] Molecular characterization of the virus, which is approximately 45-60nm in diameter, [18] has revealed heterogeneity between isolates from different genotypes and individual subtypes. The prevalence of individual genotypes varies by geographic region, with genotype 1 showing a worldwide distribution. Other HCV isolates are region specific as shown in table 1. [5,19,20]

Table 1: HCV genotypes and their distribution

Genotype	Geographical predominance
1a*	USA and developed western countries
1b*	USA, Japan, Europe
2	Most developed countries, but not very common
3	Rising in prevalence among injecting-drug users
4*	Confined to the middle east and north Africa
5	South Africa
6	Asia

*Genotypes 1 and 4 do not respond as well to interferon therapy as other genotypes (Adopted from, Adrian, 1998)

1.3 Variants of HCV

Hepatitis C virus exists as a heterogeneous mixture of closely related viruses called quasispecies. The heterogeneity of sequence composition among these different genotypes and subtypes is not evenly distributed across the RNA genome. [21-23] The spectrum of nucleotide changes within these hyper-mutable regions is influenced by the strength/effectiveness of both endogenous and exogenous applied antiviral modalities. However, it is important to emphasize that individual quasispecies have been synthesized prior to exposure to either immune-mediated or exogenous antiviral selection pressures. Individual quasispecies sensitive to these selection pressures are "culled" from the viral population.

Analysis of individual isolates demonstrates that there are 2 hyper-variable regions located within the E1 and E2 virion structural proteins at the 5' end of the ORF and a third one located within the NS5A domain. The continuous evolution of new variant

E1 and E2 glycoproteins, as permitted by these hypervariable domains, poses a considerable challenge to host immunity. The effectiveness of the viral evasion strategy relies upon the ability to remain ahead of the host's immune system and thus maintain viral persistence. This very effective strategy of continuous alteration of antigenic motifs is not unique to HCV and can be generated by a different maneuvers in organisms.

Mutations found within the NS5A domain, for example, are associated with resistance to interferon.[24,25] This "survival" protein blocks activation of the double-stranded RNA-dependent protein kinase (PKR) which is a primary mediator of the interferon-induced antiviral response. Failure to prevent activation of PKR would result in downregulation of protein translation through EIF-2alpha phosphorylation, with a concomitant reduction in viral replication.[26]

The quasispecies nature of HCV has several potentially important biological consequences. The phenomenon is likely to be a significant factor in the inability of acutely infected individuals to clear infection. Mutations in the viral population will likely contribute to the emergence of interferon "resistance" during interferon therapy. The occurrence of quasispecies is likely then to be responsible for the ineffectiveness of isolate-specific vaccines and presents considerable difficulties for the design of pan-genotype antiviral therapies.[27]

1.4 Implications of Quasispecies Diversity

The primary target organ of HCV is the liver. However, recent evidence suggests that diversity of cellular tropism exists. The virus has been detected in bone marrow, kidney, monocytes/macrophages (CD14), B lymphocytes (CD19), and

granulocytes (CD15) as well.[28-30] The clinical importance of these extrahepatic reservoirs as pools of replication-competent virus remains to be defined. Interesting to note, however, is that the quasispecies diversity exhibited in isolates from the liver, peripheral blood monocytes, and serum has been found to differ. Thus suggesting that altered cellular tropism and viral partitioning in extrahepatic reservoirs is in part a consequence of quasispecies-related divergence.[31,32]

Substantial evidence that HCV genotype is clinically important with respect to efficacy of interferon antiviral therapy is clear.[33] In addition, likelihood of response to antiviral therapy appears to be associated with the degree of quasispecies diversity.[34]

1.5 Hepatitis C Viruses and Host

Hepatitis C infection occurs in individuals of all ages. Transmission is mainly through direct percutaneous exposure to blood or blood products. The rate of infection through sexual and household contact is rare.[35,36] The incidence of transfusion-associated and blood product-acquired iatrogenic HCV infection has been reduced considerably since the introduction of serologic screening and again more recently, with the introduction of nucleic acid testing (NAT) of donated blood.[37,38] Currently, the predominant route of community-acquired HCV is through intravenous drug use.

1.6 Primary Infection

The mechanisms employed by HCV to penetrate the primary cellular target, the hepatocyte, are not well known. However, recent evidence suggests that the molecule CD81, which is expressed on various cell types, may be involved.[39,40] The region of CD81 that

interacts with the HCV E2 envelope glycoprotein is highly conserved in humans, and this may in part explain the host restriction exhibited by the virus. Other molecules purportedly involved in mediating viral entry include low-density lipoproteins and immunoglobulins.[41,42] Only about 5%-20% of individuals infected with HCV spontaneously clear the virus [43]; the remaining stay viremic and develop chronic infection (see Figure 3). The disease may remain undiagnosed in as many as 30%-40% of individuals who have normal serum alanine-transaminase (ALT) levels. [44-46]

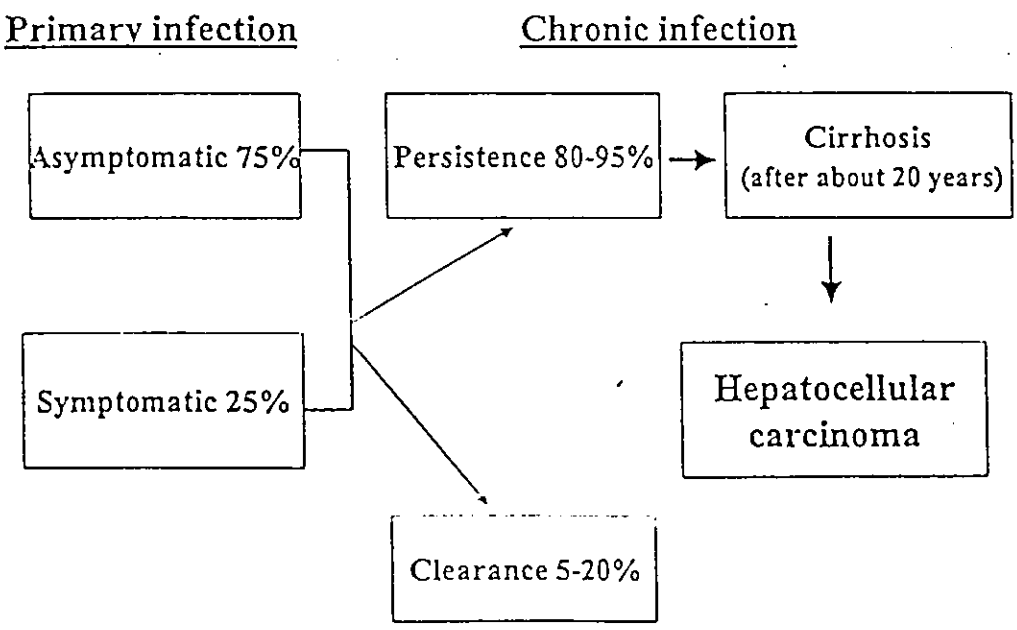


Figure 3. Natural history of hepatitis C infection.

1.7 Infection markers of HCV

Hepatitis can occur at about 2-26 weeks post-exposure to HCV. A frequent indication of infection is raised ALT levels. [47] HCV RNA can be detected in some individuals as early as 1 week post-infection depending on initial viral burden and relative viral fitness of the inoculum. [48] Seroconversion is evident approximately 2 weeks to 6 months following infection, although studies with primates have reported delayed seroconversion up to 5 years after exposure. [49,50] Thus, the number of unidentified carriers may be underestimated in the general population. Therefore, testing blood with NAT should clarify this situation and identify potentially infectious carriers.

Jaundice occurs in only 20%-30% of cases. [2] ALT level fluctuates during HCV infection, and this likely reflects perturbation of the homeostasis within the liver. Therefore, a single ALT measurement should not be considered to exclude hepatitis C infection if a discernible risk factor exists. Subsequent to the identification of elevated ALT levels, identification of seroconversion to anti-HCV antibody positivity can be achieved with enzyme-linked immunosorbent assays (ELISA) and recombinant immunoblot assays (RIBA).

There is no specific antibody associated with active infection; the presence of anti-HCV antibodies can be evidence of either a past, resolved infection or current infection. Hemodialysis or immunocompromised patients may have anti-HCV antibody titers below the threshold for detection by ELISA or RIBA, resulting in false-negative serology. Using NAT methodologies (branched-chain DNA assays, reverse transcription-polymerase chain reaction, transcription-mediated amplification, and the ligase chain reaction)

can provide a definitive diagnosis of current HCV infection by detection of specific viral nucleic acid sequences. [51]

The natural course and progression of this infection is difficult to determine and several reports indicated the absence or presence of associations between histology, biochemical, and virologic markers of disease activity. [43,52-54]

The study groups are often of mixed genotype, of undefined age at infection, have unknown duration of infection, have possible variation in viral burden at time of exposure, and have competing risk factors for infectious and other liver diseases. The investigation of disease progression in a cohort of female patients exposed to genotype HCV 1b through contaminated anti-D immunoglobulin represents one of the few globally homogeneous study groups within which the natural course of HCV infection can be studied.[53,55,56] Despite this seeming dichotomy in the literature, it has been possible to delineate the likely sequence of biochemical, virologic, and histologic events during both primary and chronic hepatitis C infection.

1.8 Chronic Infection and Disease Progression

HCV infection is characterized by a slow indolent progression to liver failure. Alterations of the liver architecture, i.e, inflammation and hepatocellular necrosis, are not pathognomonic for HCV infection. The period of infection prior to liver failure can range from 10-40 years.[57,58] The gold standard for staging liver damage associated with viral hepatitis is liver biopsy. Disease progression is defined according to grade and stage, with grade referring to the degree of inflammation and stage referring to the degree of fibrosis/cirrhosis. At present, an international system

(Histological Activity Index) is recommended as a scoring system for appraisal of hepatic damage. In this system, inflammation is scored on an 18-point scale and fibrosis is scored on a scale of 0-6. [59-61]

Mechanisms of induced liver damage are not clearly defined; however, hepatic injury is predominantly immune mediated, because HCV is not obviously cytopathic. Evidence of hepatic injury is primarily indirect. This is due to the following factors: (1) treatment with corticosteroids and other immunosuppressive agents can reduce ALT levels even though viral load increases [62,63]; (2) HCV-infected liver transplant patients, when immunosuppressed, have very high viral titers but can remain asymptomatic[63]; (3) activated CD8+ T cells can migrate from the periphery to the liver and initiate tissue damage[64]; and (4) viral replication can occur in the absence of evidence of liver cell damage.[65]

1.9 Manifestations of HCV Infection

Chronic hepatitis C infection is often accompanied by complications of autoimmune hepatitis and cryoglobulinemia. Mixed cryoglobulinemia does not appear to influence response to interferon therapy. However, treatment of chronic HCV infection with interferon may lead to the exacerbation of autoimmune hepatitis.[66-69]

Finally, hepatocellular carcinoma (HCC) may develop after about 20-40 years of infection.[66] Studies with transgenic mice have demonstrated that the hepatitis C core protein may have a significant role in the development of this malignancy.[70] Immune-mediated liver damage evident in chronic hepatitis C infection triggers the cyclic events of cellular replication and repair. Concomitantly, inflammation, necrosis, and cirrhosis appear to

contribute to the pathogenetic mechanisms that result in HCC. Progression to HCC can take several decades and can occur in the absence of cirrhosis.[71] The development of HCC has been associated with HCV genotype.[72] In particular, HCV 1b appears to be associated with a greater prevalence of HCC than is 2a/c. However, this assessment is not uniformly accepted.[73]

1.10 Factors Influencing Disease Course

The rate of disease progression in hepatitis C infection is influenced by both virus- and host-related factors. Virus-related factors include size of initial inoculum at infection, quasispecies diversity, and genotype. Transfusion-associated infection has a more rapid progression to active liver disease than needlestick-associated infection.[43,74] This is presumably related to the smaller viral burden at exposure in the case of the latter. In addition, a low quasispecies burden is associated with a greater likelihood of response to antiviral therapy.[34] There is disagreement about the role of hepatitis C genotype as a prognostic tool for disease progression. While genotype 1b, for example, has been reported to be associated with a more rapid progression to severe liver disease, this finding is not uniform throughout the literature.[53,75-77] It was also reported that genotype 2-infected individuals were almost twice as likely to develop cirrhosis than other types.[78, 79]

Viremia can be detected in individuals chronically infected with HCV up to several decades after initial infection. The extent of viremia can range from 2000 to 50 million viral copies/ml serum [54]. While spontaneous viral clearance is an unlikely event in those with chronic HCV infection, several studies on the natural fluctuations in serum viral load during chronic hepatitis C have shown conflicting results.[48,54,80-85] This diversity in opinion is

undoubtedly influenced by the heterogeneity of patient populations with respect to age at infection, gender, duration of disease, viral type/subtype, probable mode of viral transmission, duration of study period, and means of quantifying viral load.

Individuals > 50 years of age who are infected with HCV have a more severe and rapid disease course, as well as higher mortality, than do younger people.[86,87] Lifetime consumption of alcohol plays an additive role in progression to cirrhosis and Hepatocellular Carcinoma (HCC).[88] In addition, host-dependent genetic factors, as assessed by HLA typing, have been shown to be related to viral clearance.[89-91] Extraneous risk factors for infectious and other liver diseases include superinfection with hepatitis A virus (HAV), hepatitis B virus (HBV), or HIV. Coinfection with these viruses is likely to augment the rate at which HCV disease progresses.[92,93] Coinfection with HAV or HBV is also associated with both increased severity of disease as well as increased risk of developing HCC.[94]

1.11 Antiviral Therapy

At present, 2 strategies are approved for the treatment of HCV infection: (1) interferon monotherapy, and (2) interferon and ribavirin (a guanosine nucleoside analogue) combination therapy. The goal of antiviral therapy is achievement of sustained virologic clearance. Sustained response is defined as the absence of detectable HCV RNA 6 months after the completion of therapy. Many of the commercially available assays have clinically relevant detection thresholds of approximately 100 viral genomes/ml of serum. Patients can achieve virologic clearance if there is no detectable HCV RNA as determined by NAT. Although

interferon/ribavirin combination therapy seems to be effective , it is associated with significant side effects, primarily hemolytic anaemia.[83,84]

1.12 Implication of Genetic Diversity

Many of the clinical trials that have investigated interferon treatment of chronic hepatitis C infection have demonstrated the effect HCV genotype has on response to therapy. Genotype 1b is not effectively treated with interferon monotherapy, with only 10%-15% of treated patients exhibiting long-term response.[83,84] By contrast, individuals infected with HCV of genotypes 1a, 2a, 2b, and 3a have significantly higher long-term response rates.[95-97] Additionally, the identification of subtypes is of clinical importance, because it has been demonstrated that subtype 1a-infected individuals respond better to interferon monotherapy than their 1b-infected counterparts.[98] This effect was observed to be independent of age, gender, and duration of disease. Large clinical trials demonstrated an efficacy rate of 65% for combination therapy in non-1b infected individuals. [83, 84]

1.13 Viral interference

Viral interference is a well-known phenomenon in hepatitis virus infection. Hepatitis B virus (HBV) infection in humans may be suppressed by hepatitis A virus or hepatitis delta virus (HDV) super-infection with subsequent clearance of hepatitis B surface antigen (HBsAg) in serum [99,100].

Simultaneous acute infections with NANB and HBV were first described in an intensive care unit nurse who needle-sticked her finger during venipuncture of an HBsAg-positive uremic patient on chronic hemodialysis. Her clinical and serological changes after the needle stick were characterized by late onset of an attenuated B

hepatitis, that was preceded by an earlier onset of NANB hepatitis, suggesting viral interference.[101]

Patients with acute HBV and HCV co-infection generally have a delay in the appearance of HBsAg with shortened duration of HBsAg antigenemia, and a lower level of HBsAg and ALT as compared with patients with acute HBV infection alone.[102] These observations suggest that HCV coinfection interferes with HBV and attenuates its clinical presentation. Coinfection with HCV and HBV can significantly increase the risk of fulminant hepatitis. Whether this is because of additive effect of the hepatocyte damage by two concurrent viral infections or other unknown mechanisms requir further studies. Interestingly, the interaction between HCV and HBV is similar to the obligatory interaction between HDV and HBV in that dual infection may cause a much more severe disease and may also result in suppression of HBV replication.[103] Earlier studies in chimpanzees showed that acute NANB (HCV) infection resulted in acute ALT elevation with precipitous decline in serum HBs-Ag titer.[104] A 4 to 6 times reduction in HBV DNA polymerase activity in HBV carrier chimpanzees was also observed after the NANB (HCV) infection.[105] All these studies illustrate that NANB (HCV) super-infection exerts a suppressive or inhibitory effect on the replication of the preexisting HBV. Clinical studies in-patients with chronic HBV infection have also shown that anti-HCV positive patients generally have a low HBV DNA polymerase activity [106,107] or weak HBV DNA positivity.[108,109] In fact, gradual loss of HBV DNA, with subsequent HBeAg seroconversion and even HBsAg clearance, has been observed in acute HCV superinfection [109,110]. A recent study on patients with HCV and HBV dual infection has shown that HCV RNA-positive patients

rarely had detectable IgA anti- HBcore, suggesting a decrease of active immune response against HBV.[106] Furthermore, an immunological study assessing prolifeative response of peripheral blood mononuclear cells to viral antigens showed that such patients responded primarly to HCV antigens.

In most of the above mentioned cases of dual infection, the cellular response had increased toward multiple HCV antigenic components, increased liver damage, and suppression effect on HBV in humans.[111]

1.13.1 Aggravation of disease by HCV

HCV superinfection can cause a much more severe liver disease in-patients with chronic HBV infection. In terms of conventional liver biochemical tests and the clinical presentation, an acute HCV superinfection in previously unrecognised asymptomatic HBs-Ag carriers is not more severe than when it occurs in non-HBV patients. However, the former group of patients tends to have higher serum alphafetoprotein level, which may reflect more extensive hepatic necrosis [112] than the later. In fact, acute HCV superinfection in HBs-Ag carriers may be the major cause of fulminant/subfulminant hepatitis as reported in India.[113] Two independent studies from Taiwan have also shown that a significant proportion (10% to 20%) of fulminant/subfulminant hepatitis in chronic HBs-Ag carriers could be attributed to HCV superinfection.[114,115] These studies confirm the suggestion that HCV superinfection on HBV carriers may enhance the risk of fulminant hepatitis.[116] Besides such catastrophic effect of acute HCV superinfection, liver disease appears to be more sever in terms of histology and clinical decompensation in patients seropositive for both HBsAg and anti-HCV than in patients seropositive for HBsAg

alone [107,116] or HCV alone.[117] Furthermore, case control studies have indicated that dual infection with HCV and HBV has a much higher relative risk for the development of HCC.[116,118] A prospective follow-up study in Italy suggested that dual HBsAg and anti-HCV positivity in patients with cirrhosis is an independent and significant determinant for the development of HCC.[119] Several studies on HBsAg-negative patients with HCC have indeed shown that a significant number of patients seropositive for anti-HCV have both HBV and HCV genomic sequences in tumorous and non-tumorous liver tissue.[120] These findings provide the evidence implicating the importance of persistent dual infection with HCV and HBV in hepatocarcinogenesis. Without the molecular studies, such cases in the case-controlled studies could have been considered as HCV-related, and the relative role of dual infection with HCV and HBV in the development of HCC might have been consequently underestimated.

In conclusion, dual infection with HCV and HBV, or triple infection with HCV, HBV, and HDV tends to aggravate the severity and progression of the liver disease that appears to be resistant to antiviral therapy [121] . On the other hand, the more recent virus infection tends to suppress the preexisting virus(es). In terms of suppressive effect, HCV seems to be the strongest virus, because it has been shown to influence the rate of HBsAg clearance, to suppress HDV, and even to usurp the role of HBV to cause continuing chronic hepatitis. It seems clear that serum HCV markers, at least anti-HCV, should be tested in all patients with chronic HBV and HDV infection, particularly those who develop acute hepatitis-like episode, or those undergo spontaneous HBsAg clearance. For patients having dual or triple infection with HCV,

HBsAg clearance can be expected if follow-up HBsAg assays show a decreasing serum to negative control count ratio. To the contrary, more active and progressive liver disease will follow if coreplication is evident, as indicated by simultaneous presence of nucleic acids of these viruses.

At present, antiviral therapy outside of clinical trial is not recommended for such patients with concurrent infections until more information is available to suggest otherwise. The understanding of the mechanisms by which hepatotropic viruses interact is also very much incomplete. Possible alterations in the mechanism(s) responsible for virus absorption, penetration and/or replication have been suggested.[104] Induction of interferon (IFN), IFN-like substance, or other soluble mediator(s) were also considered.[103,104] A recent cotransfection study in a human hepatoma cell line showed that HCV suppresses the HBV replication involving the process of transcription and encapsidation of HBV pregenomic RNA. This suppressive effect may be mediated by HCV core protein which seems to function as a gene-regulatory protein.[122]

Although, the above mentioned mechanisms shed some light on the issue, several important issues remain to be answered:

(1) what are the viral factors, such as genotype/strain and concentration of each virus, that may determine the impact of HCV on the outcome of dual or triple hepatitis virus infection?

(2) What are the molecular mechanism(s) regulating these changes? And

(3) what are the immune responses of the host and other host factor(s) that may be involved in dual or triple virus infection?

We hope that future research, and in particular, the molecular mechanism(s) of suppressive effect of HCV on HBV or HDV will clarify these issues. Such knowledge may help in designing new therapeutic approach in the treatment of chronic HBV and HDV infection .

1.14 HCV in renal disease and dialysis

Estimates of the prevalence of HCV antibodies in patients undergoing hemodialysis range from 15% to 48% in North America. However, prevalence rates are somewhat higher in other parts of the world, such as Saudi Arabia and Egypt.[123] Incidence rates among such groups are declining probably due to blood screening and the titer infection control measures in hemodialysis units. The presence of anti-HCV antibodies in patients with end stage renal disease (ESRD) correlates with multiple transfusion, time on hemodialysis, history of intravenous drug abuse (IVDA), and previous renal transplant.[124] Although the natural history of HCV infection in the hemodialysis population is largely unknown, liver failure is the cause of death in 8% to 28% of long-term renal transplant survivors.[125]

The prevalence of HCV in patients with ESRD on hemodialysis is 30%, compared with less than 5% for those on peritoneal dialysis and this can be attributed mailnly to nosocomial infections.[126, 127] Steps to reduce nosocomial infection in hemodialysis units include single-use heparin vials; glove changes between patients; and cleaning and disinfection of all instruments and environmental surfaces.

1.15 The Future of HCV Therapy

Advances in our understanding of the dynamics of viral replication, the evolution of treatment-resistant quasispecies will aid

in the development of novel therapeutic approaches. Multidrug approaches similar to those currently used to treat HIV infection may also have utility for the treatment of HCV infection. These drugs may include inhibitors of HCV polymerases (NS5B), proteases, and/or helicases (NS3).[128] Antiviral strategies based on antisense oligonucleotide and ribozyme methodologies also hold therapeutic promise.[129,130]

Aims of the study

In Palestine, no previous studies were carried out in search for the viral features among dialysis patients. The present study aims to achieve the following objectives:

1. Determination of the prevalence rate of HCV among renal failure patients.
2. To asses the influence of virological and host factors on serum ALT levels.
3. To study the acute phase and co-infection with HBsAg.
4. To asses the significance of antibody patterns agianst HCV antigens in terms of biochemical liver abnormalities.
5. To evaluate the sensitivity and specificity of the used ELISA kits.

CHAPTER II

Materials and Methods

2.1. Study population

The study was conducted during the period of June 1999 to November 2000. The subjects of the study were grouped into two categories.

I. Chronic Renal Failure Patients.

A total of 95 HD patients (64 M, 31 F; mean age 44.6 years [range, 14-70 years]) enrolled at AL-Watani dialysis unit, the only governmental center in the City of Nablus in the northern part of the West Bank (Palestinian area). The mean duration of dialysis was 43 months (range 13-124 months).

The patients were distributed on three daily shifts and each shift serve 10 patients. The causes of their renal failure were chronic glomerulonephritis (n=41), diabetic nephropathy (n=28), polycystic kidney disease (n=2) and obstructive nephropathy (n=24).

Except for 31 patients who needed HD twice a week, the majority of the patients were on a 3-days/week schedule. Routine blood examination included total blood count, blood sugar, total protein, BUN, creatinine, Na^{+}, K^{+}, Cl^{-}, Ca^{++}, P^{+++}, and liver function test (LFT) including (ALT, AST, ALP, TSB) were done monthly. Sera samples were also tested for anti-HCV, HBs-Ag, and HIV at enrollment. Anti-HCV positive sera were confirmed using immunocomb II (Organics-Israel). Information regarding sex, age, and duration of dialysis for all subjects involved was collected from patient's charts.

On follow up, patient's sera were tested on weekly basis for ALT, and aliquots of the sera samples were stored at –80°C for further analysis. ALT test was used as an indicator for the episode of hepatitis. The episode was defined as elevation of serum ALT (normal < 32 IU/L) in more than two sequential weekly tests, with a peak value of greater than 64 IU/L. Patients with previous history of elevated ALT levels due to other hepatic diseases were excluded. Hepatitis events were calculated to clarify its relationship with seroconversion to either HBsAg or anti-HCV. By the end of 18 months all stored sera samples from all patients were tested for HCV and HBV. For conformation and comparison reasons, all follow-up patients were tested for anti-HCV using three different third-generation ELISA kits.

II. Kidney Transplant Patient.

A total of 112 (74 M, 38 F; mean age 38.9 year [range 9 to 72 years]) kidney transplant recipients referred to AL-Watani Hospital, were included and tested for ALT, HIV and HCV/ HBV markers.

2.2. Dialysis Procedure

The unit (11 machines- Fresenious AG, Germany) serves hemodialysis patients that are distributed on three daily shifts of 10 patients each. All machines are located in a single room. No dedicated areas or machines were used for either HCV and/or HBV infected subjects. Anti-HCV positive patients were not isolated, as they constitute a high proportion of the patients and because of undesirable psychological effects. It is reasonable to assume that the same staff treated all involved patients.

Both acidic and alkaline bicarbonate dialysis fluids were used at a flow rate of 500 ml/min. Water quality standards were fulfilled by a reverse osmosis system. Hemodialysis patients have been dialyzed

with Cuprophan (E3, Fresenious AG, Germany or Presto and Allegro, Organ Teknika, Boxtel, Holland) or polysulfone (F6, F8, F40, and F60, Fresenious AG, Germany) dialyzers. No dialyzers were reused. Heparin was given as a bolus dose followed by an individually determined continuous infusion. All dialysis machines were disinfected by citrosteril and heat between treatments.

2.3. Serologic assays

All assays were carried out according to manufacturer's instructions.

2.3.1. Anti- HCV Testing

2.3.1.1. HCV EIA 3.0

Anti-HCV status, for all sera samples, was determined using Abbot third-generation HCV ELISA (Abbot Diagnostic Division, USA).

The assay employed polystyrene beads coated with recombinant (*Escherichi coli* and yeast) HCV antigens from structural and non-structural regions of the genome (core, NS3, NS4 and NS5regions).

Beads were washed on quick wash instruments, optical densities (O.D) were measured, and cut off values were determined on a Quantum spectrophotometer.

Initially reactive samples were repeated in duplicate as recommended by the manufacturer and were considered repeat reactive when at least one of the repeated tests was again reactive.

2.3.1.2. Murex anti-HCV (version III)

The Murex anti-HCV (version III) enzyme immunoassay utilizes antigens from the putative core (C, structural), protease/helicase (NS3, non structural), NS4 (non-structural) and

replicase (NS5, non-structural) regions of the virus to provide a sensitive diagnostic test.

2.3.1.3. IMx HCV Version 3.0

Abbot IMx HCV version 3.0 is a micro-particle enzyme immunoassay (MEIA) for the detection of antibodies to hepatitis C virus. MEIAs are a variation of the enzyme immunoassay (EIA) principle. Solid phase EIAs, first described in the early 1970s, use antigens and/or antibodies coated on a surface to bind complementary analytes. The bound analyte is detected by a series of antigen-antibody reactions. EIAs are available to identify many antigens and antibodies related to viral hepatitis infection. In the IMx final reaction, an antibody coupled to an enzyme acts upon a substrate to produce a fluorescent end product. The fluorescence produced by the enzyme reaction is measured and is proportional to the amount of bound antibody.

2.3.1.4. Immunocomb II

Immunocomb II (organics, Israel) is an indirect solid-phase EIA composed of a 12 teethed plastic comb; each tooth is sensitized at three spots:

1- an upper spot: human immunoglobulin (internal control).

2- a middle spot: HCV structural antigen (synthetic peptide of HCV core antigen).

3- a lower spot: HCV non-structural antigens (recombinant protein NS3 and synthetic peptide NS4).

Results are visible within minutes, appearing as gray-blue spots on the surface of the comb's teeth (see figure 4).

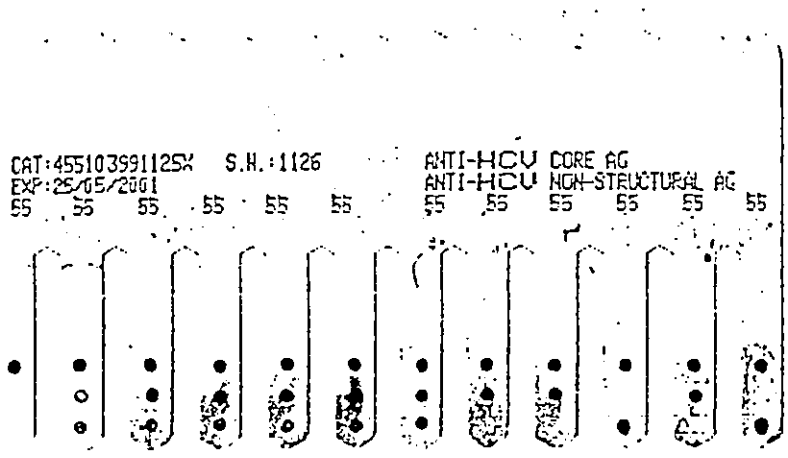


Figure 4. HCV markers incorporated in immuno Comb II (lanes 1 and 2 negative and positive markers, lanes 3-7 patients showing both markers, lanes 8, 9 and 11 patients with anti-core alone, lanes 10 and 12 patients with anti non-structural proteins.

2.3.2. HBV Testing

HBsAg was detected using Auszyme Monoclonal EIA (Abbot laboratories Diagnostic Division, USA).

2.3.3. Anti-HBV Testing

Anti-HBV was detected using bioelisa anti-HBsAg kit, which is a direct immunoenzymatic method of the "sandwich" type in which the wells of a microtiter plate are covered with highly purified HBsAg (ad and ay subtypes) and the conjugate is HBsAg marked with horseradish peroxidase. The sample to be evaluated is incubated in one microplate well. HBsAg bound to the plate well is able to specifically capture anti-HBs-Ag when it is present in the sample. After washing to remove any unbound material the conjugate is added and will bind to the antigen-antibody complex formed during the first incubation. After this second incubation and washing, an enzyme substrate containing a chromogen is added.

This solution will develop a blue color if the sample contains anti-HBs. The blue color changes to yellow after blocking the reaction with sulpharic acid. The intensity of the color is proportional to the amount of anti-HBs present in the test specimens.

2.3.4. Human Immunodeficiency Virus (HIV) Testing

Anti- HIV status, for all sera samples, was determined using Abbott HIV-1/HIV-2 3RD Generation Plus EIA

The assay employed polystyrene beads coated with recombinant HIV-1 *env* and *gag* and HIV-2 *env* proteins.

Beads were washed on quick wash instruments, optical densities (O.D) were measured, and cut off values was determined on a Quantum spectrophotometer.

Specimens, which produce absorbance values less than the cutoff value are considered negative for antibody and need not be tested further.

Specimens, which are repeatedly reactive, by Abbott HIV-1/HIV-2 3RD Generation Plus EIA are considered positive by the criteria established by this test and should be further tested using a supplemental test.

2.4. Biochemical Assays

2.4.1. ALT

ALT values were determined by a kinetic method using the KoneLab (Kone instruments, Finland) and Cobas Mira (Roche, Germany) analyzers. Reading less than 32 IU/L were considered normal.

HCV, HBV and HIV status among dialysis patients are presented in table 2. Patients were studied in three constitutive periods, each period lasted for 6 months. This strategy was adopted due to the complications generated by the high mortality rate and the introduction of new cases to the dialysis unit at various stages of the study. It is important to note that due to the limited number of cases we were obliged to include newly admitted cases.

Data presented in table 2/A shows that 6 new cases were infected with HCV within the first period of the follow up. On the other hand, 5 out of 12 HBV positive cases (dual infection) showed HBsAg clearance. Out of the 71 studied patients, 5 HCV negative and 1 HCV positive cases died during this period.

Data presented in table 2/B shows that none of the 41 HCV negative (18 newly admitted) cases were seroconverted to HCV positive during the second period of the follow up study; however, ALT elevation was observed in 2 cases. Seroconversion into HBV was observed in one case. By the end of this period 7 HCV negative and 2 HCV positive cases died.

During the third period of the study that included 40 HCV positive and 40 HCV negative (6 newly admitted cases), seroconversion to HCV positive was observed in 1 case only. Out of the 40 HCV negative cases 5 died of which one case was HBV positive. On the other hand 5 of the HCV positive cases died and 2 of these cases were with dual infection (HCV/HBV).

Table 2. HCV, HBV and HIV status among HD patients (A-C).

A- Period-I (June 99-Nov 99).

	INITIAL READINGS			FINAL READINGS				
	HCV	HBV	HIV	HCV	HBV	HIV	DEATHS HCV	DEATHS HBV
-VE	34 (47.89%)	59 (83.1%)	71 (100%)	23 (34.85%)	59 (89.39%)	66 (100%)	5 (83.33%)	0 (0%)
+VE	37 (52.11%)	12 (16.9%)	0 (0%)	43 (65.15%)	7 (10.61%)	0 (0%)	1 (16.67%)	0 (0%)
Total	71 (100%)	71 (100%)	71 (100%)	66 (100%)	66 (100%)	66 (100%)	6 (100%)	0 (0%)

B- Period-II (Dec 99-May 2000).

	INITIAL READINGS			FINAL READINGS				
	HCV	HBV	HIV	HCV	HBV	HIV	DEATHS HCV	DEATHS HBV
-VE	41 (49.4%)	76 (91.57%)	83 (100%)	34 (45.95%)	66 (89.19%)	74 (100%)	7 (77.78%)	0 (0%)
+VE	42 (50.6%)	7 (8.43%)	0 (0%)	40 (54.05%)	8 (10.81%)	0 (0%)	2 (22.22%)	0 (0%)
Total	83 (100%)	83 (100%)	83 (100%)	74 (100%)	74 (100%)	74 (100%)	9 (100%)	0 (0%)

C- Period- III (June 2000-Nov 2000).

	INITIAL READINGS			FINAL READINGS				
	HCV	HBV	HIV	HCV	HBV	HIV	DEATHS HCV	DEATHS HBV
-VE	40 (50%)	72 (90%)	80 (100%)	34 (48.57%)	65 (92.86%)	70 (100%)	5 (50%)	0 (0%)
+VE	40 (50%)	8 (10%)	0 (0%)	36 (51.43%)	5 (7.14%)	0 (0%)	5 (50%%)	3* (100%)
Total	80 (100%)	80 (100%	80 (100%)	70 (100%)	70 (100%)	70 (100%)	10 (100%)	0 (100%)

*** 2 patients were with dual infection (HCV/HBV)**

Data presented in table 3 shows hepatitis onset based on ALT peak level. Out of 10 patients with clear elevated ALT levels (ranged 213-1200 IU/ml), 7 were seroconverted into anti-HCV positive. The conversion required time in days ranged 21–109. Based on Immunocomb II assay, 4 of the seroconverted subjects expressed anti-core in an earlier date ranging between 21-30 days, while the rest 3 expressed anti-NS on a later date ranging between 56-109 days. It is worth noting that of the 7 seroconverted subjects, 4 showed hepatitis episodes within the same month (Aug. 99), 2 were seroconverted in Nov.99, and 1 case in August 2000. The data also shows that most (6 out of 7) of the seroconversion was observed within the first 6 months of the study. Seroconversion into positive HBsAg was observed in one case.

Table 3. Patients with acute hepatitis and peak ALT levels.

No.	Sex	Age	Onset of hepatitis	Peak ALT IU/L	Seroconversion /days	Antibody Type
1.	F	53	Aug.1999	580	56	NS*
2.	M	53	Aug.1999	488	21	Core
3.	F	39	Aug.1999	213	70	NS
4.	M	70	Aug.1999	332	28	Core
5.	M	35	Nov.1999	313	109	NS
6.	M	14	Nov.1999	599	30	Core
7.	M	50	Feb.2000	437	No serocon.	-----
8.	M	14	Feb.2000	246	No serocon. /D*	-----
9.	M	30	Aug.2000	600	23	Core
10.	F	65	Feb.2000	1200	HBV infection (one case)	

***D = dead**
***NS= Non structural**

The data presented in table 4 shows the correlation between anti-HCV markers patterns and ALT levels among HD patients. The subjects were categorized into 4 distinct patterns:

I-Anti-core negative/ anti-NS negative

II-Anti-core positive/ anti-NS negative

III-Anti-core negative/ anti-NS positive

IV-Anti-core positive/ anti-NS positive

ALT elevation seems to be associated with both markers as they appear in the acute phase of the infection. It is worth noting that subjects expressing anti-NS at early stages of infection (before the appearance of anti-core) seems to show a much higher elevated ALT levels and such levels persist for a much longer time than that observed for anti-core (data not shown).

Table 4. Correlation between anti-HCV pattern and ALT levels among HD patients.

	Anti-HCV patterns				
ALT level	**Core-ve/ NS-ve**	**Core+ve/ NS-ve**	**Core-ve/ NS+ve**	**Core+ve/ NS+ve**	**Total**
Normal	**47**(92.16%)	**0**(0%)	**0**(0%)	**27**(84.38%)	**74**(77.89)
Elevated	**4**(7.84%)	**4**(100%)	**8**(100%)	**5**(15.6%)	**21**(22.11%)
Total	**51**(100%)	**4**(100%)	**8**(100%)	**32**(100%)	**95**(100%)

The data presented in table 5 shows the association between dialysis duration and HCV infection and its markers. Infection rates of 14.29%, 71.61%, 83.33% and 100% for those on dialysis for 0-20, 21-40, 41-60 and above 60months, respectively. Table 5 also shows that with increased duration of dialysis, a shift from anti-core or anti-NS to both markers and with a clear longer persistence for anti-NS marker compared to anti-core.

Table 5. Association between HCV markers, infection and duration of dialysis.

Anti-HCV markers	Duration of dialysis / months				
	0-20	21-40	41-60	>60	Total
Core-ve/NS-ve	42 (85.71%)	8 (27. 58%)	1 (16. 67%)	0 (0%)	51 (53. 68%)
Core+ve/NS-ve	3 (6. 12%)	1 (3. 45%)	0 (0%)	0 (0%)	4 (4. 21%)
Core-ve/NS+ve	3 (6. 12%)	2 (6. 9%)	1 (16.67%)	2 (18.18%)	8 (8.42%)
Core+ve/NS+ve	1 (2. 05%)	18 (62. 07%)	4 (66.66%)	9 (81.82%)	32 (33.69%)
Total	49 (100%)	29 (100%)	6 (100%)	11 (100%)	95 (100%)

Table 6 represents the association between ALT levels and HCV/HBV patterns. Based on HCV/HBV status the patients were clustered in four basic patterns; I. **HBsAg/HCV negative** (49), II. **HBsAg positive/HCV negative** (2), III. **HBsAg negative/HCV positive** (38) and IV. **HBsAg/HCV positive** (6). Elevated ALT levels were observed in 4.08%, 100%, 31.58% and 66,67% of subjects in patterns I, II, III and IV, respectively. These data show a clearly high association between HBV infection and ALT elevation compared to HCV positive cases.

Table 6. Correlation between Anti-HCV pattern/ HBs-Ag patterns and ALT levels among HD patient.

ALT level	HBV/HCV Patterns				
	I. HBsAg-ve HCV-ve	II.HBsAg+ve HCV-ve	III. HBsAg-ve HCV+ve	IV. HBsAg+ve HCV+ve	Total
Normal	47(95.92%)	0(0%0)	26(68. 42%)	2(33. 33%)	76(80%)
Elevated	2(4. 08%)	2(100%)	12(31. 58%)	4(66. 67%)	19(20%)
Total	49(100%)	2(100%)	38(100%)	6(100%)	95(100%)

Data presented in table 7 shows the clinical picture of all cases (95) by the end of the follow up study of hemodialysis patients. 46.64% and 53.68% were observed for HCV positive and HCV

negative cases, respectively. Compared to the initial findings a clear decrease in HCV seropositivity was observed (52.11%) as shown in table 2/A. With respect to gender, males showed a much higher percentage of HCV seropositivity compared to females (63.64%nd 36.36% for males and female, respectively). Regarding HBV status among the studied group, 13.63% were with dual infection (HBV/HCV) and HBV seropositivity among HCV negative cases was 3.92%. Association between HCV infection and ALT levels showed 5-fold increase in HCV positive cases compared to HCV negative subjects (38.64% to 7.84%).

Table 7. Clinical data for HCV positive/negative HD patients by the end of follow up period.

Patient characteristic	**Patients**	
	Anti-HCV +ve	**Anti-HCV -ve**
No. of patients	44(46.32%)	51(53.68%)
Male	28(63.64%)	36(70.59%)
Female	16(36.36%)	15(29.41%)
Median age (yr)	43.07	45.92
Age range (yr)	14-70	14-70
Median duration of dialysis (mo)	43	13.92
Range of duration of dialysis (mo)	13-124	4-57
No. HBsAg positive	6(13.63%)	2(3.92%)
ALT patterns		
Normal	27(61.36%)	47(92.16%)
Elevated	17(38.64%)	4(7.84%)
Causes of renal failure		
- Diabetic nephropathy.	8	20
- Obstructive nephro.	10	14
- Glomerulonephritis	25	16
- Polycystic kidney disease	1	1

Data presented in table 8 shows the clinical picture of the kidney transplant subjects (112) by the end of the follow up study.

The percentages of 33.93% and 66.07% were observed for HCV positive and negative cases, respectively. Compared to the initial findings none of the cases were seroconverted to either HCV or HBV. With respect to gender, males showed a much higher percentage of HCV seropositivity compared to females (63.16% and 36.84% for males and female, respectively). With respect to HBV status among the studied group, 10.53% were with dual infection (HCV/HBV) and the percentage of HBV seropositivity among HCV negative cases was 18.92%. Association between HCV infection and ALT levels showed a slight increase in HCV positive cases compared to negative subjects (21.05% to 13.51%).

Table 8. Clinical findings among HCV positive/negative kidney transplant patients by the end of follow up period.

Patient characteristic	**Patients**	
	Anti-HCV +ve	**Anti-HCV -ve**
No. of patients	38(33.93%)	74(66.07%)
Male	24(63.16%)	50(67.57%)
Female	14(36.84%)	24(32.43%)
Median age (yr)	43.5	36.5
Age range (yr)	28-68	9-72
No. HBsAg positive	4(10.53%)	14(18.92%)
ALT patterns		
Normal	30(78.95%)	64(86.49%)
Elevated	8(21.05%)	10(13.51%)

Data presented in table 9 represents HCV, HBV and HIV status among hemodialysis and kidney transplant patients. The percentages of 46.32% and 33.93% were observed among hemodialysis and kidney transplant patients for HCV infection, respectively. This clearly indicates that dialysis is a major risk factor for HCV infection. HBV seropositivity rates of 8.42% and 16.07% were observed among both subjects. None of the hemodialysis patients was HIV positive and only one subject (0.89%) was HIV positive among kidney transplant cases.

Table 9. Serological status of HIV (anti-HIV), HBsAg, and HCV (anti-HCV) among hemodialysis and kidney recipients by the end of the follow up.

Subjects	Anti-HCV		HBsAg		HIV	
	+ve	-ve	+ve	-ve	+ve	-ve
Haemodialysis	44 (46. 32%)	51 (53. 68%)	8 (8. 42%)	87 (91. 58%)	0 (0%)	95 (100%)
Kidney Recipient	38 (33. 93%)	74 (66. 07%)	18 (16. 07%)	94 (83. 93%)	1 (0.89%)	111 (99.11%)

Data presented in table 10 shows clinical diagnosis of death in association with HCV, HBV and duration of dialysis. Out of 25 cases, 8 were HCV positive of which 2 were with dual infection with HBV. Out of the 8 HCV positive cases 2 (25%) died due to liver failure.

Table 10. Association between HCV, HBV and duration of dialysis with respect to cause of death.

No.	Duration of dialysis/months	HCV	HBV	Cause of death
1	21	-VE	-VE	CVA
2	15	-VE	-VE	CVA
3	22	+VE	-VE	Pulmonary Edema
4	20	-VE	-VE	CVA
5	21	-VE	-VE	CVA
6	78	+VE	-VE	Septicaemia
7	67	+VE	-VE	Septicaemia
8	20	-VE	-VE	CVA
9	16	+VE	-VE	Septicaemia
10	40	+VE	+VE	Liver Failure
11	28	-VE	-VE	CVA
12	83	+VE	+VE	Liver Failure
13	33	+VE	-VE	Pulmonary Edema
14	20	+VE	-VE	CVA
15	4	-VE	-VE	Septicaemia
16	17	-VE	+VE	CVA
17	5	-VE	-VE	CVA
18	20	-VE	-VE	CVA
19	5	-VE	-VE	CVA
20	6	-VE	-VE	CVA
21	6	-VE	-VE	CVA
22	7	-VE	-VE	CVA
23	7	-VE	-VE	CVA
24	6	-VE	-VE	Septicaemia
25	6	-VE	-VE	Pulmonary Edema

For confirmation, all HCV positive cases were tested for anti-HCV antibodies using three different kits (Murex, Abbott and IMx). All sera were positive in the three kits except for two patients who were only positive in Murex kit.

Prevalence of HCV infection among hemodialysis patients differs in various countries, ranging from 2 to 6 % in North Western Europe to more than 20% in Japan and over 60% in Saudi Arabia [131]. In the northern Palestine, Al-Kurdi 1998 [132] reported the prevalence of 0.24%, 34.4%, 10.8%, and 39.6% for blood donors, kidney transplant, transfusion dependent and hemodialysis patients, respectively. The findings of Alkurdi, 1998 encouraged us to evaluate HCV status among HD patients. Due to the limited number of cases and the complications generated by the high mortality rate, this study was conducted in three constitutive periods; each period lasted for 6 months. During the first phase of the study a clear increase (13%) in HCV rate was observed (see table 2/A). This sudden increase took place within three months as 4 subjects were seroconverted during August 99 and the other 2 in November of the same year. Such a finding clearly indicates a nosocomial infection within the unit. To confirm this proposed hypothesis it was essential to carry out genotyping analysis for the involved subjects. Unfortunately, such techniques are not available in the area. Further evidence in support for the proposed hypothesis came from the findings in the second and third phases through which a limited increase in the prevalence of HCV was observed as shown in tables 2/B and 2/C. During the first phase we have also observed a striking interaction between HCV and HBV, in patients co-infected by these agents, suggestive of the phenomenon of viral interference as 5 out of 12 co-infected cases showed HBsAg clearance. Our data is in agreement with many reports [133]; were HCV super-infection was reported to suppress HBV and leads to the clearance of HBsAg. In vitro studies on HCV/HBV co-infection showed that prolonged expression of the HCV core protein in human hepatoma (HuH-7)

cells resulted in a shift from cytoplasmic to nuclear localization of this protein. This shift is correlated with a suppression of HBV transcription, assembly, and release. The core protein was suggested to act as a gene regulatory protein and phosphorylation of some of its serine residues seems to be essential for the suppressive activity of HBV genes [134]. It is worth noting that it was difficult to determine the onset of HCV among those cases, thus, whether further HCV infection has any thing to do with HBsAg clearance is not clear. We should point out that those individuals were initially with elevated ALT levels that dramatically dropped in association with HBsAg clearance. The lack of routine ALT tests and good filing system limits speculations in this respect.

During the second phase of the study none of the 41 HCV negative cases were seroconverted to Anti-HCV positive as shown in table 2/B. Thus, providing evidence in support of the nosocomial mode of transmission within the unit during the first phase of the study. The observed reduction in HCV prevalence rate compared to that observed during the first phase is most likely due to the strict recommendations of this study based on the initial findings in phase one. We would like to add that the recommendations included the use of separate heparin vials and the frequent change of gloves.

Although, HBsAg positive cases were not on a separate machines, the prevalence rate is within the international range [135] and this could be due to the fact that all HBV negative cases are subjected to a vaccination program that is adopted within the last two years. The single HBsAg positive case found during this phase is an old female that was not vaccinated.

Data presented in table 2/C clearly indicates the drop in the prevalence of both HBV and HCV infections, although the majority of the study cases were on maintained hemodialysis for longer than 12 months within the same unit. Thus, confirming that the adopted hygiene policy for the control of HCV is effective.

ALT status could be of a limited diagnostic value in chronic patients, however it seems to be a valuable indicator for those in the acute phase of infection. Monitoring of ALT levels, among follow up patients, seems to provide more support to the significant association between elevated ALT levels and HCV seropositivity. Thus, it seems reasonable and essential to take in account that one ALT measuring is not a good enough indicator for the association between HCV infection and ALT.

In our study, monitoring of ALT levels during the first phase (weekly basis) showed a significant increase in ALT levels among HCV negative cases (10 out of 34) as shown in tables 2/A and 3. Out of those subjects, 7 entered the acute phase of HCV, 1 case seroconverted into HBsAg positive and 2 showed elevated levels without any signs of seroconversion to either HCV or HBV. Such data is in agreement with previous reports on the association between HCV/HBV and ALT elevation. It also confirms that ALT monitoring is a valuable tool in HCV/HBV diagnosis. Reason(s) for the observed fluctuation and abnormality in ALT levels in the other 2 cases is not known, however, other viral infections and or drug interactions may account for such elevation.

The finding of periodic elevation of ALT levels among HCV positive cases (11 out of 44/ data not shown) could be attributed to virus diversity, rapid mutation rates, episodic immune

responsiveness by the host, and/or simultaneous infection with various subspecies [136].

With respect to HCV related antigens (core and NS antigens), our findings are in agreement with previous observations [137] in which antibodies to the core appeared earlier (range 21-30 days) than NS antigens (range 56-109 days) as shown in table 3. Anti-core antibody seems to be closely associated with HCV RNA and considered as a reliable marker for viral replication [138]. On the other hand, anti-NS antigens may appear first [139], as observed among 3 out of 7 HCV seroconverted subjects in our study.

Our data also show that subjects expressing anti-NS during the acute phase and before the onset of anti-core seems to express a much higher ALT levels as shown in table 4. Such levels persist for a much longer time compared to that observed when anti-core expressed first (see table 5).

A shift from anti-core or anti-NS to both markers was observed in association with increased dialysis duration as shown in table 5. These findings suggest that immune response changes are not influenced by dialysis duration and could be a consequence of HCV infection duration. Such finding is in agreement with previous studies [140]. The findings of 14.29%, 71.61%, 83.33% and 100% HCV seropositivity rates among those with dialysis duration of 0-20, 21-40, 41-60 and above 60 months, respectively, strongly indicate that dialysis is a major risk factor for HCV infection (see table 5).

More pronounced ALT levels were found among HBsAg carrier patients irrespective of their anti-HCV status (Table 6). This is in agreement with previous reports [141], as the risk for the development of chronic hepatitis and liver damage among HD patients is more with HBV than HCV infection.

By the end of the study period, a decrease in HCV infection rate was observed (46.32% final/ 52.11% initial). This could be attributed to: first, the observed nosocomial infection at the first phase of the study and second, to the fact that 18 and 6 newly admitted cases were enrolled during the second and third phases of the study. The adoption of new hygiene strategies based on our recommendations by the end of phase 1 might be another factor that account for such decrease in HCV infection rate (see table 7). The finding of 38.64% and 7.84% with elevated ALT levels among HCV positive and HCV negative cases, strongly link ALT elevation with HCV seropositivity. This finding is in agreement with previous reports on the behavior of ALT marker [142].

With respect to gender, males showed a much higher rate (63.64%) of HCV infection compared to females (36.36%) among dialysis patients. Reasons behind such variations are not clear, however, findings of much higher rate of diabetic males compared to females, which seems to lead to further complications including renal failure may account for this. On the other hand, renal obstruction due to prostate cancer, congenital reflux and gouty may contribute to such variations. One should point out in this respect that such complications are usually the result of lack of good follow up regimes.

A prevalence rate of 33.93% was observed for HCV among renal transplant recipients (see table 8); this rate is higher than the rates of 13.1% and 10.3% reported in Germany and USA, respectively [143]. However, such rates are comparable with that observed in Spain (46.5%), a country with 10 folds higher HCV seropositivity rate among blood donors compared to our status [144]. Risk factors for developing HCV among this group includes; the

duration of renal replacement therapy (RRT), past history of hemodialysis treatment, and blood transfusion before transplantation [145]. Data regarding multiple transfused patients, in our country, indicate that blood transfusion is relatively not a high risk factor for HCV infectivity among renal transplant recipients [132]. However, hemodialysis seems to be accused as the major risk factor among this group as most if not all were on hemodialysis prior to transplantation. It is interesting to note that gender difference with respect to HCV seropositivity is in favor of males (63.16%) compared to (36,84%) among females among kidney transplant patients (see table 8). Such situation is similar to that observed among HD patients. HBV status among this group was more pronounced (18.98%) compared to that of HD (3.92%) patients (tables 7 and 8). Vaccination prior to transplantation, contaminated blood and equipments during surgery may account for the observed differences. Compared to HD patients a weak association between HCV seopositivity and ALT levels was observed (see table 8). Such finding confirms the previous speculation of increased risk of recurrent HCV infection during dialysis.

A comparison between HCV, HBV and HIV status between HD and kidney transplant patients is shown in table 9. The finding of only one HIV positive case among kidney transplant patients, strongly indicates that HIV is not an endemic disease in our region and medical history of the affected subject indicates that the infection was acquired during the transplantation process.

The finding of high death rate among dialysis patient (see table 10) is expected among this group. Septicemia is a clear indication of neglect as such patients are most likely immuno-compromised, thus are at high risk of infection [146]. Hemorrhagic cerebrovascular

accident (CVA) or stroke is also common as a result of persistent hypertension [147]. Pulmonary edema is also another expected complication among this group that usually results from fluid retention in the body tissues especially the chest region and leads to death [148]. Liver failure was also another cause of death among this group, which is expected as a result of hepatitis infection [125]. Further studies are required to further elucidate death causatives and the associated risk factors.

Data regarding the confirmation of HCV positive cases probably indicates that Murex test Kit was with the highest specificity. It is worth noting that the two patients were HBV positive. Over more, noticeable low anti-HCV titers were also found among the majority of HBV positive patients. Such findings were in agreement with previous reports in this respect [133] where viral interactions seem to take place. ALT levels were also more pronounced between these two subjects compared to the other four cases of dual infection. The findings of ALT levels might reflect high HBsAg, low anti-HCV and could explain the findings on specificity between the used kits.

Recommendation and suggestion for further studies

1. Patients should have specific dialysis stations assigned to them, and chairs and beds should be cleaned after each use.
2. Sharing among patients of ancillary supplies such as trays, blood pressure cuffs, clamps, scissors, and other nondisposaple items should be avoided.
3. Nondisposaple items should be cleaned or disinfected appropriately between uses.
4. Medications and supplies should not be shared among patients, and medication carts should not be used.
5. Medications should be prepared and distributed from a centralized area.
6. Clean and contaminated areas should be separated (e.g., handling and storage of medications and hand washing should not be done in the same or an adjacent area to that where used equipment or blood samples are handled).
7. Intensive efforts must be made to educate new staff and reeducate existing staff regarding hemodialysis-specific infection- control practices that prevent transmission of HCV and other bloodborne pathogens.
8. Anti-core antibody is a reliable marker of acute HCV infection specially when combined with elevated ALT levels among HD patients and should be carried out routinely.
9. Monitoring of ALT levels seems to be a more sensitive indicator of acute HCV infection compared to anti-HCV and should be adopted as a routine test.
10. Our data indicates a nosocomial infection in the dialysis unit, however, mode of transmission is not clear and further

investigation at the molecular level (genotyping) seems to be essential at this stage.

11. Affected patients should be always dealt with as at high risk of HCV transmission to their family members and should be informed of such consequences.

12. Specially designed follow up system should be implemented in order to reduce further complications that may lead to death among this group.

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ملخص

لقد تمت دراسة و متابعة مدى تطور و انتشار مرض ا لتهاب الكبد الوبائي الفيروسي C على 71 مريضا ممن يتابعون عملية الغسيل الكلوي في المستشفى الوطني- نابلس. لقد بينت الدراسة الأولية أن37 مريضا أي ما يعادل حوالي52 % من المرضى في هذه الوحدة من المصابين بهذا المرض في حين كان 12 مريضا أي ما يعادل 17 % من المصابين بالتهاب الكبد الفيروسي B.

لقد أجريت الدراسة على ثلاث فترات زمنية متلاحقة كل فترة منها ستة شهور, نظرا لمتغيرات عدة أهمها التحاق مرضى جدد و موت آخرين مما تسبب في عدم ثبات أعداد عينة الدراسة و مما استدعى لزيادة فترة المتابعة و مع نهاية الفترة الأولى لوحظ زيادة في نسبة الإصابة بالتهاب الكبد الفيروسي C, حيث وصلت النسبة إلى 65.15% حيث انضم ستة مرضى جدد إلى قائمة المصابين, في حين لوحظ انخفاض في نسبة الإصابة بالتهاب الكبد الفيروسي B, حيث انخفضت النسبة من حوالي17% إلى 10.61% وكانت العدوى المزدوجة و أصابه هؤلاء المرضى بالفيروس C هي السبب في هذا الانخفاض.

وقد تم استخدام الإنزيم ALT المفرز من الكبد كمقياس ومؤشر للإصابة حيث تمت دراسة مستويات الأنزيم بشكل أسبوعي, وقد دلت الدراسات على أن هذا المؤشر مرتبط و بشكل كبير مع الإصابة بالالتهاب الفيروسي C. وقد بينت الدراسة مثل هذا التوافق حيث لوحظ ارتفاع حاد في مستوى الإنزيم لدى جميع المرضى الذين أصيبوا خلال الفترة الأولى من الدراسة, كما لوحظت ذبذبات في مستوى الإنزيم لدى المرضى الذين كانوا مصابين أصلا بهذا الفيروس مما يؤكد على إمكانية تكرار حدوث العدوى. وتشير النتائج الأولية إلى أن العدوى من داخل الوحدة هي السبب الرئيسي وراء هذه الإصابات و المسؤولة عن الزيادة في نسبة الإصابة بالالتهاب الفيروسي C, ومع نهاية هذه الفترة لوحظ وفاة ستة مرضى لأسباب وتعقيدات فسيولوجية مختلفة, كان من بينهم أحد المصابين بالتهاب الكبد الفيروسي C.

خلال فترة الدراسة الثانية لم تسجل أية أصابه جديدة بالتهاب الكبد الفيروسي C من بين 41 حالة سالبة, في حين سجلت أصابه واحدة بالتهاب الكبد الفيروسي B, كما لوحظت ارتفاعات حادة في مستوى الأنزيم ALT لدى

مريضين ولم يلاحظ دخول أي من هذين المريضين في الأعراض الحادة للألتهاب الفيروسي B أو C, ويبدو أن ظروف الرقابة الصحية المشددة و التي استخدمت في الوحدة بناءا على نتائج وتوصيات المرحلة الأولى من هذه الدارسة كانت وراء الحد من انتشار المرض وعدم تسجيل أية أصابه جديدة, وقد توفي في نهاية هذه الفترة تسعة مرضى كان من بينهم اثنين من المصابين بالتهاب الكبد الفيروسي.

أمّا في المرحلة الثالثة فقد لوحظ حالة عدوى جديدة بالتهاب الكبد الفيروسي C من بين 40 حالة سالبة تضمنتها هذه المرحلة, ولم تسجّل أية إصابة بالتهاب الكبد الفيروسي B. ومع نهاية الفترة الثالثة توفي 10 مرضى من اصل 80 مريضا. (40 حالة مصابة HCV, و 40 حالة غير مصابة) كان من بينهم ثلاثة مرضى من المصابين بالتهاب الكبد الفيروسي.

و تشير النتائج السريرية بأنّ الحالات السبع التي أصيبت خلال فترات الدراسة الثلاث إلى وجود علاقة قوية تربط بين الإنزيم ALT مع الطور المرضي للعدوى, حيث لوحظ ارتفاع في مستوى الأنزيم ALT لدى جميع الحالات المصابة حديثا. أمّا فيما يتعلق بفترة حضانة فيروس HCV فقد تراوحت ما بين (21-109) أيام اعتمادا على ارتفاع مستوى الأنزيم ALT في قراءتين متتاليتين متزامنا مع ظهور الأجسام المضادة لكل من أنتجن NS و أنتجن Core في العينات المصابة. وقد لوحظ أن الأجسام المضادة لأنتجن Core تظهر مبكرا مقارنة مع الأجسام المضادة لأنتجن NS, و لوحظ كذلك بأنّ الارتفاع في مستوى الأنزيم ALT كان مرتبطا بكلا النوعين من المضادات الحيوية في الطور الحاد من المرض, مع ارتفاع أعلى في مستوى الأنزيم لدى المرضى الذين ظهرت لديهم المضادات الحيوية لأنتجن NS.

أمّا فيما يتعلق بنسبة الإصابة, فقد لوحظ زيادة مطردة مع ازدياد فترة الغسيل الكلوي مما يؤكد أن مثل هذه العملية تعتبر من العوامل الخطرة و المسببة لانتقال المرض (HCV), كذلك و مع ازدياد فترة الغسيل هنالك انتقال من نمط المضادات الحيوية للأنتيجن NS أو Core إلى كلا المؤشرين.