HEPATITIS C VIRUS ANTIBODIES IN PERITONEAL DIALYSIS PATIENTS COMPARED TO HEMODIALYSIS PATIENTS

Thesis

SUBMITTED FOR PARTIAL FULFILMENT OF THE

MASTER DEGREE IN INTERNAL MEDICINE

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1994

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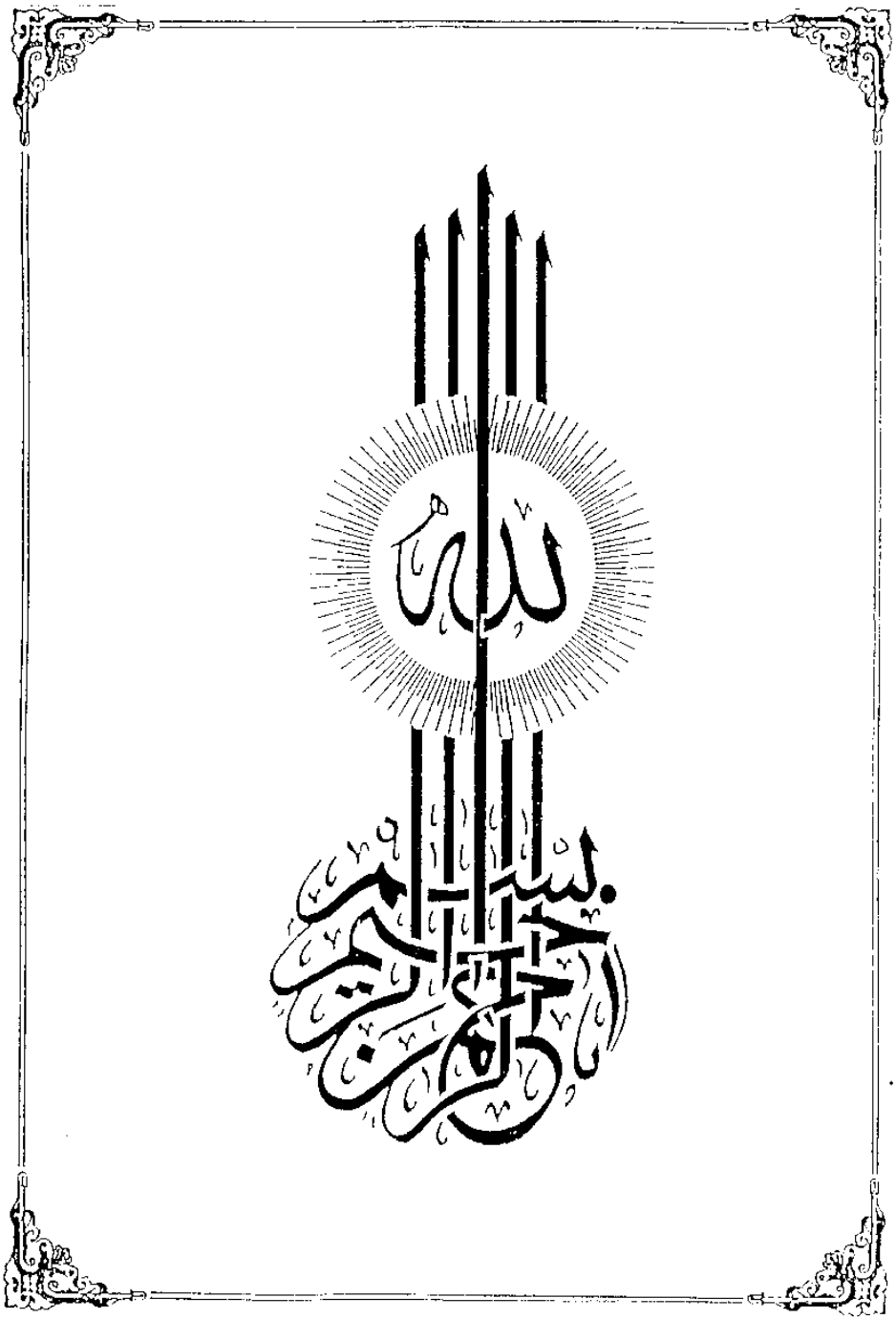

ACKNOWLEDGEMENT

A word of thanks giving to **ALLAH,** the source of all knowledge, by whose abundant aid this work has come to fruition.

I would like to express my deep thanks to **Professor Dr. SABRY GOHAR,** Professor of Internal Medicine and Nephrology , for his general supervision , creative guidance , continuous encouragement and general help.

I ' m deeply indebted to **Dr . ADEL AFIFI** , Professor of Internal Medicine and Nephrology , for his unlimited help through out this work , wise directions and constructive suggestions.

I am grateful to **Dr . KHALED HUSSEIN ABOU SEIF ,** Lecturer of Internal Medicine and Nephrology , for all the help he offered me.

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TO MY WIFE

INTRODUCTION AND AIM OF THE WORK

INTRODUCTION

Chronic non-A, non-B hepatitis has been recognized for about two decades; 90% or so of these cases have been found to be due to Hepatitis C virus (HCV), while 9 - 10% are due to, still, an unknown factor. HCV itself, though better understood, is not fully "explored "; and everyday new knowledge appears. It is a single stranded open frame RNA virus of the Flavivirus group. Its full structure is not fully understood, but some sequences of aminoacids in some areas of its structure have been recognized (Abd El-Fattah , 1993).

In 1989 Choo Q L et al developed the first generation anti-HCV test which tested for the nonstructural protein of the virus, C100-3 (first generation ELISA test), but this test had some specificity problems and was found to be falsely positive in some immunologically determined liver diseases.Further tests were refined to detect anti-bodies to C100-3, C33, C22 (second generation ELISA test) and then the confirmatory test that added a fourth parameter, 5-1-1, using recombinant immunoblot assay (RIBA2) (Van der Poel et al., 1991). These newer tests increased the sensitivity of detection of hepatitis C infection and allowed its earlier diagnosis (Cristiano et al., 1991). The presence of these antibodies indicates that the individual (donor or patient) has been infected with HCV, may harbour infectious HCV, and may be capable of transmitting HCV(Weiner et al, 1990 and Tedder et al,1991). Polymerase Chain Reaction (PCR) is a new technique that allows us to direct assay HCV-RNA (Cristiano et al.,1991), even quantitatively (Brillanti et al., 1991). PCR has shown that the HCV has many sequence variables. Also PCR has become the gold standard of infection detection, because it detects HCV-RNA when all other tests for HCV are negative; and can detect it in the acute phase also (Lazizi et al., 1992).

HCV-RNA is present mainly in the serum and in the liver tissue, and is also detectable in the saliva, sweat and semen; but in very low concentrations, which makes

them very unlikely sources of infectivity (Nakano et al.,1992). About 54% of cases are community acquired. Many patients may have been infected through repeated IV injections e.g. for schistosomiasis, ritual circumcision, tattoo, dental therapy, barber blades, manicure or sharing personal utensils (Abd El Fattah et al.,1993). Direct mother to neonate does not seem to pause a problem except when the mother harbors HIV infection (Novati et al., 1992). This also applies for sexual transmission (Tedder et al., 1991).

Chronic uraemia causes a profound impairment of the immune system (Kallinowski et al.,1991).Infections are therefore one of the major causes of morbidity and mortality in patients with end-stage renal disease accounting for 20% of the deaths in this population (Keane et al., 1983 - Ruiz et al., 1990 and Tolkoff-Rubin et al., 1990). 19% of all deaths among hemodialysed patients are regarded as sequelae of viral hepatitis (Jakobs et al., 1977).

Hemodialysis patients run the high risk of parenterally transmitted viral hepatitis including hepatitis B and HCV (Kallinowski et al.,1991). However, the incidence of viral B hepatitis in hemodialysis units has declined over the past 20 years with improved infection control strategies, including patient surveillance and segregation, plus improved prophylaxis with immune globulin, hepatitis B immune globulin, hepatitis B vaccines, and disinfectant procedures (Alter et al., 1986).

Hepatitis C virus infection has long been a concern for dialysis patients, who may require repeated transfusions and have defective immune defence mechanism. Patients on haemodialysis have the additional risk of acquiring hepatitis C virus infection through sharing of dialysis machines (Chan et al.,1991).In contrast to hemodialysis patients, patients on peritoneal dialysis have reduced transfusion requirements and are not exposed to the potential risks of cross-infection through sharing of dialysis machines (Korbet.,1989).

So theoretically, patients on peritoneal dialysis are expected to have a lower prevalence of HCV infection than patients on hemodialysis and according to our knowledge only few studies were done regarding this subgect.

AIM OF THE WORK

The aim of this study is to determine the seroprevalence of anti-HCV among uraemic patients and to compare the prevalence of anti-HCV in this group of patients on different modalities of treatment i.e hemodialysis, peritoneal dialysis and conse- rvative treatment. The association of these antibodies to blood transfusion, duration of dialysis, sex, age, liver functions and HBsAg status as well as which factors predispose patients to have anti-HCV are investigated.

REVIEW OF LITERATURE

HEPATITIS C VIRUS

The term non-A, non-B hepatitis (NANBH) has been used to describe hepatitis in patients who do not develop antibodies to hepatitis A virus, HBV, cytomegalovirus or Epstein Barr virus and do not have a clinical history of other potential causes of hepatitis (Wick et al ., 1985).

Two forms of NANBH have been identified :-

1) An epidemic type usually transmitted enterically and

2) A parenterally transmitted form caused by hepatitis C virus (HCV) (Polesky; Hanson., 1989).

After 15 years of unsuccessful attempts, the most frequent of NANBH viruses has recently been identified and designated HCV. It is by an original and direct molecular biology approach, leading to the cloning of nucleic acids presumed to be present in an infectious plasma, that this virus could be partially characterized. The viral genome was sequenced before the agent could be detected by serology or electron microscopy (Trepo et al.,1990).

The discovery of HCV and its serological markers represents a new milestone in the history of viral hepatitis and allows a specific diagnosis of viral hepatitis in more than 90 % of cases. In addition, testing for anti-HCV plays a crucial role in prevention of post-transfusion (PT)- NANBH (Bonino et al.,1991).

Physicochemical and molecular characterization of HCV, strongly suggest that it is a pesti-/ Flavivirus like virus. Additional studies show that the buoyant density of plasma derived HCV in sucrose is significantly lower than of most tissue culture derived flaviviruses (1.20 g/cm3). This finding suggest, but does not prove, that at least one physicochemical property of HCV is more similar to that of the pestiviruses, bovine viral

diarrhea virus(BVDV) and hog cholera virus (Hog CV), than that of the flavivirus (Bradley et al.,1991).

HCV is a small (10-Kb) single stranded RNA virus. The virus is global in distribution with a prevalence between 0 . 3 % and 1 . 5 % (Alter, 1991). It is composed of at least 3 structural proteins (Roggendorf et al., 1991). The viral genome encodes a polyprotein with the putative structural and non-structural (NS) proteins located at the N- and at the C-terminus, respectively (Houghton et al., 1990; Kato et al, 1990;Takeuchi et al .,1990 and Takeuchi et al.,1990).

TYPES OF HEPATITIS C VIRUS

HCV was classified genetically into 2 types, HCV-K1 and HCV-K2, which show 67 % and 71 % identity at the nucleotide and amino acid sequence levels in a 340 bp region which encodes the NS5 gene Gly-Asp-Asp motif. To develop a rapid method to classify the genomes of HCV isolates, restriction fragment length polymorphism (RFLPS) in reverse transcriptase-polymerase chain reaction products encoding a portion of the NS5 gene was identified. ALu I and Acc II enabled HCV to be classified into the K1 and K2 types, & Sau 961 enabled classification into the K1 type and the K2a and K2b subtypes. These RFLPs also generally allow Japanese isolates to be distinguished from the prototype (PT, an isolate from the U.S.A.), which is a K1 type. Sequence analysis of the 5' - untranslated regions of Japanese isolates revealed near identity between the K1 type and PT types, and 93 to 94 % identity between the K1 type and K2 types indicating that there are type K1-and K2-specific RFLPs in this region. These results suggest that the nucleotide sequences of the K1 and K2 types are different throughout the HCV genome. The incidence of HCV types K1, K2a and K2b, and PT in 50 samples was 74 %, 16%, 8 % and 2%, respectively (Nakao et al.,1991).

Based on variation in nucleotide sequence within restricted regions in the putative C (core) gene of HCV, 4 groups of HCV have been postulated in a panel of 44 isolates. They were provisionally designated types I, II, III and IV. A method for typing HCV was developed, depending on the amplification of a C gene sequence by polymerase chain reaction (PCR) using a universal primer (sense) and mixture of 4 type-specific primers (antisense). HCV were determined by the size of the products specific to each of them.Type II was found in HCV samples from 131 (82 %) of 159 blood donors, more often than in those from 48 (60 %) of 80 patients with NANB liver disease in Japan. In 11 hemophiliacs who had received imported coagulation factor concentrates, type I was found in 5, as against type II in 4. Double infection with 2 different HCV types was found in 2 patients with chronic NANB liver disease (types I and II; II and III) and 2 hemophiliacs (types I and II; I and III). HCV types were identical in mother and baby in

each of 2 examples of perinatal transmission, and were also identical in donor and recipient in a case of accidental needle exposure(Okamoto et al., 1992).

Partial nucleotide sequences in the tentative NS5 region of the hepatitis C viral genome obtained from patients with chronic hepatitis in Thailand were analyzed by reverse transcription followed by the PCR. Of ten samples studied, 4 showed low homologies to any known type of HCV : the homologies of the nucleotide sequences of these clones with HCV-J,-US, K2a and-K2b, were 66.5-69.1 %, 66.5-68.2 %, 61.2-64.1 % and 64.4 - 66.2 %, respectively, and the homologies of their deduced amino-acids sequences were 71.7 - 75.2 %, 71.7 - 75.2, 69.0 - 72.6 % and 69.9- 73.5 %, respectively. These 4 clones were classified a new distinct type of HCV, named HCV-T. Moreover, the nucleotide and amino-acid sequence homologies of the 4 HCV-T clones showed that the HCV-T type could be classified into 2 genotypes, HCV-Ta and HCV Tb (Mori et al.,1992).

TRANSMISSION OF HCV

Although hepatitis C infection has been clearly demonstrated to be transmitted through blood products or blood contamination, most cases of sporadic hepatitis C infection are unassociated with parenteral risk factors, and it is unclear how infection might be acquired by nonparenteral means (Hsu et al.,1991). Human intravenous immunoglobulins prepared by the cold ethanol fractionation technique of Cohn are considered safe with respect to infectivity. However, there have been several instances of transmission of both hepatitis B and NANB viruses after administration of intravenous immunoglobulins. The high prevalence rate of HCV antibody (anti-HCV) in intravenous immunoglobulins has important implications for follow up of recipients, selection of serum donors, and implementation of anti-HCV testing (Dodd et al.,1992).

Some HCV positive subjects are not infectious and some asymptomatic blood donors carry a serologically undetected HCV; the liver of at least 10 % of patiens with chronic NANBH without anti-HCV antibodies contains the RNA of HCV detectable by molecular amplification. All this leads to the concept of HCV negative viral hepatitis (Trepo et al.,1990).

Percutaneous transmission of HCV can occur among medical employees who stuck themselves accidentally by the needles used by the patients. However it was concluded that the risk of HCV infection by the needle stick accident is not so high (Okamoto et al.,1991). Studies also showed the high percutaneous transmission of hepatitis C in contrast with the low sexual transmission (Maisonneuve et al.,1991). The seroprevalence of HCV was investigated in 16 tattooed men with chronic liver disease.In 11 of the 16 men, the result was positive, which was significantly higher as compared with that of presumable healthy blood donors in the Tokyo area, i.e., 1.14 % of 2470 donors tested. These results suggest that HCV infection occurred at the time of tattooing in these 11 patients (Iwamura et al .,1992).