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Original Article

Anti-ENA Antibody Profile in Hepatitis C Patients Undergoing Hemodialysis

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ABSTRACT. Infection with hepatitis C virus (HCV) is increasing all over the world, especially among hemodialysis patients. HCV is one of the major autoantibody inducing viruses, where anti-nuclear antibodies (ANA), anti-smooth muscle antibodies (ASMA), anti-liver kidney microsome antibodies (LKM-1), and rheumatoid factor (RF) have been related to HCV. Few studies have investigated the presence of anti-extractable nuclear antigens (ENA) antibodies in chronic liver diseases, especially in chronic hepatitis C cases, but none investigated its immunostimulation role in hemodialysis units. The aim of the study was to assess the prevalence of HCV among chronic kidney disease- Stage 5 (CKD5) patients undergoing hemodialysis and the prevalenceof ENA antibodies among them. Sera of 134 patients with chronic kidney disease undergoing hemodialysis, were screened for HCV antibodies and ENA antibodies profile, using ELISA and Immunoblot technique. 41 HCV-positive blood bank donors were used as controls. Sixty-four (47.7%) of 134 patients undergoing hemodialysis were infected with HCV. Thirty-three (51.6%) of 64 patients with HCV infection undergoing hemodialysis had anti-ENA antibodies: 9 (27.3%) showed anti-SSA antibodies and 22 (66.7%) had anti-SSB antibodies. The prevalence of anti-ENA antibodies was significantly higher in the patients with HCV infection, undergoing hemodialysis, compared with both control groups (hepatitis C-positive blood bank donors and hepatitis C-negative patients undergoing hemodialysis). Seventeen of 33 HCV antibodies-positive males undergoing hemodialysis had anti-ENA antibodies, compared with 16 of 31 females, indicating no sex related difference. This study emphasizes the high prevalence of HCV infection in our hemodialysis patients, comparable to that of other middle eastern countries, but higher than Western ones. A strong association was observed between anti-HCV positivity and hemodialysis duration, as well as anti-ENA antibody profile. However, these antibodies were gender independent.

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Introduction

Hepatitis C virus (HCV) is becoming a major threat in hemodialysis units all over the world, and predominantly in the Mediterranean countries, Middle East, and Far East.[1-3,] Risk factors aggravating HCV infection in hemodialysis units include multiple blood transfusion, dialysis age, dialyzer reuse, and environment of hemodialysis which may contribute to in noso-

comial HCV transmission.[4-8]

Viral infections often result in the appearance of autoantibodies that may precede organ damage and development of autoimmune disease in susceptible individuals.[9,10]

HCV is becoming one of the major autoantibody inducing viruses. Antinuclear antibodies (ANA), anti smooth muscle antibodies (ASMA), anti-liver kidney microsome antibodies (LKM-1), rheumatoid factor (RF), and antithyroid antibodies have been found in association with HCV.[11-14]

Few studies have investigated the presence of anti-extractable nuclear antigens (ENA) antibodies in chronic liver diseases, especially in chronic hepatitis C cases.[15,16] Garcia-Carrasco et al[17] detected a high prevalence of anti-ENA antibodies viz., anti SSA and SSB, both in primary Sjogren's syndrome and HCV infection. D'amico et al[16] reported that 46.3% of 96 patients with chronic hepatitis C had anti-ENA antibodies, specifically anti-SSA, -SSB, and -RNP at a prevalence of 23.1, 20.2 and 11.2%, respectively.

So far, no reports are available about the prevalence of anti-ENA antibody profiles among HCV-infected hemodialysis patients. The purpose of this study was to assess the prevalence of HCV infection among patients undergoing maintenance hemodialysis, their anti-ENA antibody profiles, and to assess the correlation of HCV infection, hemodialysis, and anti-ENA antibody profile.

Materials and Methods

Patients

A total of 134 patients (67 males and 67 females) with chronic kidney disease, undergoing regular renal dialysis in one of the three major hospitals in Jordan (Al-Basheer Hospital, Amman; Al-Hussain Hospital, Alssalt; and Jordan University Hospital, Amman), were recruited into the study. These dialysis patients were selected as they are possible victims of hepatitis C infections. Blood bank donors who were found to be HCV positive were used as controls. All patients and controls had signed consent to participate in this study.

Blood specimens

Ten milliliters of blood was collected from the chronic kidney failure patients, prior to hemodialysis in a plain tube; serum was separated and frozen in 0.5 mL aliquots at −20°C until use. Similar preparations were done for samples of HCV-positive blood donors' samples.

Screening for hepatitis C and B viruses

Sera of patients and controls were tested for HCV antibodies using enzyme-linked immunosorbent assay (ELISA) technique according to the recommendations of the kit manufacturers (Diasorin, S.A., USA), for HbsAg using chemiluminescent sandwich method (Roche, Belgium), and for HBV-core antibodies using DPC-kits, USA.

ENA screening

Sera of patients and controls were qualitatively screened for IgG antibodies to eight cellular and nuclear antigens (Sn RNP, SSB, SSA, Scl 70, CENP B, Jo 1, Sn RNP/sm and Sm) using ELISA technique (AESKU Diagnostics, Germany).

ENA profile

Sera of patients and controls were assayed for human autoantibodies of IgG class to nine lines of highly purified ENA (nRNP, Sm, SSA, SSB, Scl 70, Jo 1, nucleosomes, CENP B, and dsDNA) using immunoblot technique and the manufacturer's instructions (EUROIMMUN, Euroline system, Germany).

Statistical Analysis

Data analysis was done using χ^2 test and SPSS computer software.

Results

Patients' classification and test groups

A total of 134 hepatitis B-negative renal hemodialysis patients (67 males and 67 females) with a mean of 8.6 years of continuous hemodialysis were included in this study. Forty-one hepatitis C positive blood bank donors (31 males

Table 1. Classification of patients.

Group	Group size	Males		Females		Status
		No.	%	No.	%	
1	64	33	51.6	31	48.4	Hepatitis C-positive patients undergoing hemodialysis
Mean age distribution in years	47.6 ± 13.5	50.3 ± 12.8		46 ± 15.6		
2	41	31	75.6	10	24.4	Hepatitis C-positive patients blood bank donors
Mean age distribution in years	42.0 ± 8.8	41.0 ± 7.0		46.0 ± 1.5		
3	70	34	48.6	36	51.4	Hepatitis C-negative patients undergoing hemodialysis
Mean age distribution in years	41.0 ± 11.2	43.0 ± 11.5		40 ± 10.7		
Total	175	98		77		

and 10 females) were included in the study as control group. All patients who were hepatitis B-positive (HbsAg or HBV-core antibodies) were excluded from the study.

Based on the confirmed hepatitis C results, the total 175 hemodialysis patients and blood bank donors were divided into three groups: group 1: hepatitis C-positive patients undergoing hemodialysis; group 2: hepatitis C-positive blood bank donors; group 3: hepatitis C-negative patients undergoing hemodialysis (Table 1). Sixty-four patients (33 males and 31 females) were hepatitis C-positive patients, constituting 51.6% and 48.4% respectively, with a prevalence rate of HCV infection of 47.7%.

ENA screening

Thirty-three patients (51.6%) of group 1 had antibodies to at least one ENA. The mean age of patients in the group was 47.6 ± 13.5 years, and there were 17 males and 16 females, with a mean age of 50.3 ± 12.8 and 46.0 ± 15.6 years, respectively. Similarly, 14 patients (34.1%) of group 2 were also positive to at least one ENA antibody, with a mean age of 35 ± 8.8 years and a male to female ratio of 11:3. On the other hand, only three patients (two males and one female) of group 3 were positive to at least one ENA antibody (4.2%) (Table 2).

ENA profile

Autoantibodies to nRNP/Sm, CENP B, nucleosomes, and Sm were detected in hepatitis C-positive patients undergoing dialysis, with almost equivalent ratio among both males and females (Figure 1). However, four patients (12.1%) had antibodies to dsDNA with male to female ratio of 1:3, whereas the ratio was reversed for autoantibodies to Scl 70. Further more, autoantibodies to Jo 1 were rare among the group. On the other hand, autoantibodies to SSA and SSB were prevalent among the group (66.7%) with almost equivalent ratios in both hepatitis C-positive males and females undergoing hemodialysis.

Both male and female genders of hepatitis C-

Table 2. ENA screening results of the three groups and age distribution

Group number	Mean age ± SD	Number of positive ENA patients (%)
Group 1	47.6 ± 13.5	33 (51.6)
Male	50.3 ± 12.8	17 (51.5)
Female	46.0 ± 15.6	16 (48.5)
Group 2	35.0 ± 8.8	14 (34.1)
Male	41.0 ± 7.0	11 (78.6)
Female	46.0 ± 1.5	3 (21.4)
Group 3	41.0 ± 11.2	3 (4.2)
Male	43.0 ± 11.5	2 (66.7)
Female	40.0 ± 10.7	1 (33.3)

Group 1: hepatitis C-positive patients undergoing HD, Group 2: hepatitis C-positive blood donors, Group 3: hepatitis C-negative patients undergoing HD

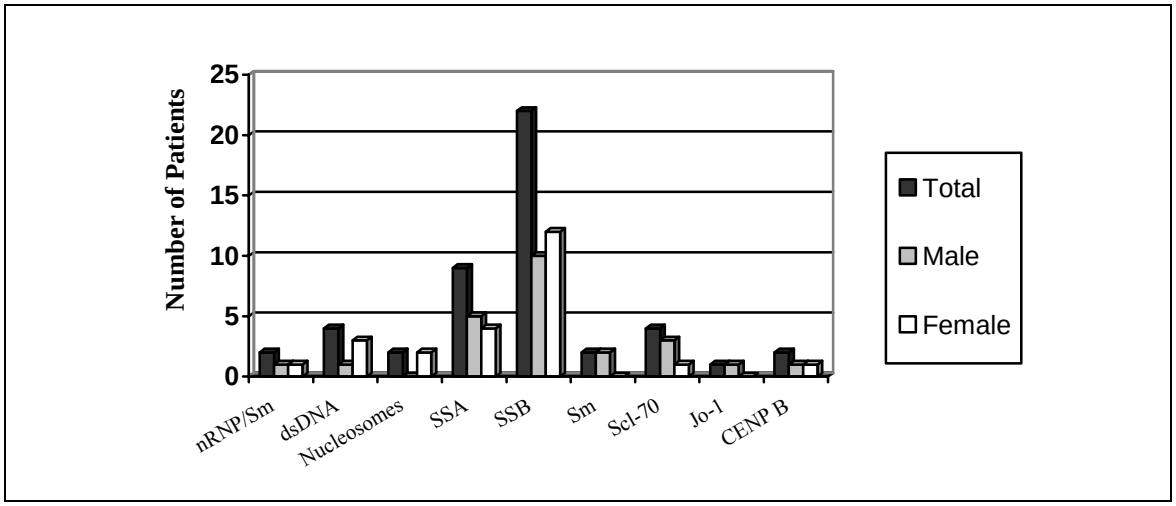

Figure 1. Hepatitis C-positive patients undergoing HD: ENA profile results (group 1).

positive blood donors (group 2) produced autoantibodies to dsDNA and Sm antigens in equal proportion, but at low frequencies. However, 64.3% of the group 2 (eight males and one female) produced antibodies to SSA antigen, and 78.6% (nine males and two females) produced antibodies to SSB antigens. Furthermore, only one male of the group produced autoantibodies to Scl 70. Contrary to group 1, none of the hepatitis C-positive blood donors produced autoantibodies to nRNP/Sm, nucleosomes, Jo 1, and CENP B (Figures 2 and 3).

Out of the hepatitis C-negative patients undergoing hemodialysis (group 3), only two (a male and a female) had detectable autoantibodies to SSB antigen. Similarly, two males were positive to nucleosomes' autoantibodies, but only one male patient had autoantibodies to dsDNA. None of this group produced autoantibodies to nRNP, Sm, SSA, Jo 1, Scl 70, and CENP B.

Discussion

Prevalence of anti-HCV in hemodialysis patients

The prevalence of anti-HCV in the hemodialysis patients studied was 47.7%, which is comparable to that reported for Kuwait and Turkey,[18,19] but higher than that reported for USA[20] and Sudan,[8] and lower than that reported for Saudi Arabia, Syria, and Egypt.[3,21,22] There was a strong correlation between anti-HCV positivity and duration of hemodialysis

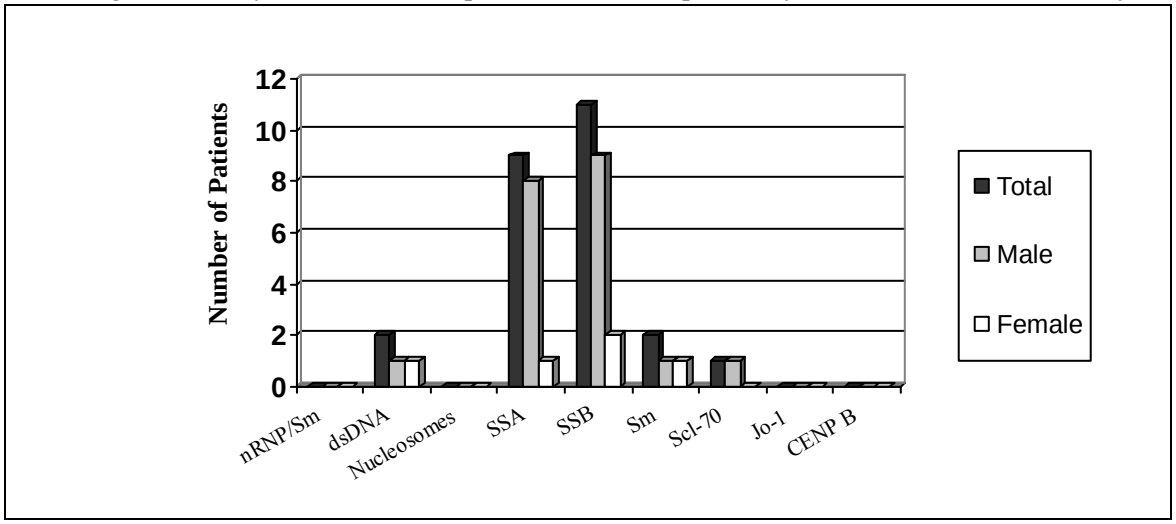

Figure 2. Hepatitis C positive blood bank donor: ENA profile (group 2).

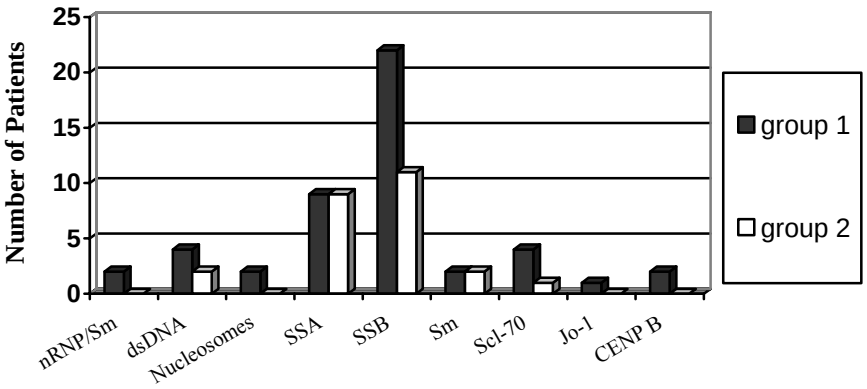

Figure 3. Comparison of hepatitis C-positive ENA profile results between group 1 and group 2.

(8.3 years for HCV-positive patients and 4.9 years for HCV-negative ones) which may be attributed to the continuous exposure to the HCV acquisition risk factors and a possible nosocomial source.[3,5,6] However, there was no correlation between anti-HCV positivity and age, gender, or exposure to HBV.

Correlation between anti-ENA antibodies and HCV positivity in patients undergoing hemodialysis

Thirty-three patients (51.6%) of group 1 and 14 patients (34.1%) of group 2 had anti-ENA antibodies, with no significant difference ($P > 0.05$) between the two groups. SSB autoantibodies were present in 78.6% of group 2 patients and 66.79% of group 1 patients, with no significant difference between the two groups ($P > 0.42$). On the other hand, SSA autoantibodies were significantly more prevalent in group 2 (64.3%) than in group 1 (27.3%) patients ($P < 0.05$). This observation suggests that maintenance hemodialysis may reduce the effect of HCV by decreasing the HCV-RNA in these patients.[23]

Contrary to what was reported by D'Amico et al,[16] the frequency of SSA and SSB in our patients was higher than that reported earlier for patients with chronic hepatitis C infection.[16] However, such autoantibodies are known to be associated with Sjogren's syndrome.[17] Furthermore, such reports may suggest that HCV may have a probable role in the pathogenesis of Sjogren's syndrome through a possible reactivation of an endogenous retroviral protein or via molecular mimicry between viral proteins and native autoantigens.[24] The other anti-ENA autoantibodies (nRNP/Sm, Sm, and Jo 1) were detected in very low frequencies among group 1 patients, but were higher than that reported by D'Amico et al,[16] which may be related to the difference in the patients' group. On the other hand, antibodies to dsDNA, nucleosomes, Scl 70, and CENP-B ranged between 6.1 and 12.1% of the group.

Although autoantibodies to Sm (14.3%), dsDNA (14.3%) and Scl 70 (7.1%) were detected in group 2, none of them had antibodies to nRNP/sm, nucleosomes, Jo 1, or CENP-B, which differed from that of group 1. Such a finding may be caused by disturbances in the immune system in hemodialysis patients, resulting from hemodialysis procedures. These results are in agreement with those reported by Garcia-Carrasco et al[17] and Lobreglio et al[15] for Mediterranean groups. Such discrepancies in the types and prevalence of autoantibodies could be attributed to the possible variation in the infective viral genotypes, or to the genetic and environmental diversities among the populations, as documented in oral lichen planus cases, which occurs frequently in association with HCV infection in Mediterranean populations.[25]

Correlation between anti-ENA antibodies and anti–HCV-negative patients undergoing hemodialysis (group 3)

Our results indicate that only three patients (4.2%) had positive anti-ENA antibodies; two of them had antibodies to nucleosomes, two to SSB, one to Sm, and one to dsDNA, while none of them had autoantibodies to the other ENA tested. These results indicate that HCV infection in hemodialysis patients probably had a triggering effect on autoantibodies' formation, and moreover, the hemodialysis procedure itself may affect the immunological status of patients undergoing prolonged hemodialysis as in the three ENA-positive patients who had hemodialysis for 8.5 years.

Correlation between anti-ENA antibodies and gender

Our results show lack of significant correlation ($P > 0.2$) between genders among hepatitis C-positive patients undergoing hemodialysis and hepatitis C-positive blood donors, and their total ENA antibodies and ENA-profiles. Such results contradict those reported by D'Amico et al.[16] Furthermore, hepatitis C-positive male blood donors showed a highly significant correlation with prevalence of SSA antibodies ($P < 0.05$), whereas females of the same group showed a correlation for Sm antibodies.

The data obtained from this study emphasize the fact that anti-HCV antibodies in our hemodialysis patients had a high prevalence which was comparable to that reported in Middle Eastern countries, but higher than Western ones. There was a strong correlation between anti-HCV positivity and hemodialysis duration and anti-ENA antibody production. Furthermore, there was a strong correlation between HCV positivity and anti-ENA profile antibodies among hemodialysis patients. However, these were gender independent. Further studies are required to investigate the role of these anti-ENA antibodies' profile in possible complication of hemodialysis patients.

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