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Hepatitis C Genotype 4 with Normal Transaminases: Histological changes, Schistosomiasis, and Response to Treatment

Moutaz F. Derbala¹, Aliaa M. Amer², Muneera Almohanadi³, Anil John³, Ashraf Amin³, Anjum John⁴, Manik Sharma³, Saad R. Alkaabi³, Nazeeh Z. Al Dweik³, Fuad Pasic³, Rafie Yaqoob³, Mohammed T. Butt³, and Fatma M. Shebi^{5,6}

¹ Department of Gastroenterology and Hepatology, Hamad Hospital and Weill Cornell Medical College in Qatar, Doha, Qatar

² Department of Laboratory Medicine and Pathology, Hematology Section, Hamad Hospital, Doha, Qatar

³ Department of Gastroenterology and Hepatology, Hamad Hospital, Doha, Qatar

⁴ Medical Research Centre, Hamad Hospital, Doha, Qatar

⁵ Infections and Immunoepidemiology Branch, Division of Cancer Epidemiology and Genetics, National Cancer Institute, Rockville, MD, United States

⁶ National Liver Institute, Public Health Branch, Shibin Elkom, Menoufia, Egypt

Abstract

Introduction and aims—Among individuals with chronic HCV infection, approximately 30% of patients show persistently normal alanine aminotransferase (PNALT). Individuals with PNALT have been historically excluded from antiviral treatment. However, some studies have reported sudden worsening of disease in patients with PNALT, suggesting the need to treat individuals with PNALT. To evaluate this further, we compared fibrosis severity and response to treatment in patients with PNALT to patients with abnormal ALT. In addition, we investigated whether liver histology and schistosomiasis affect response to treatment differently in those with PNALT and abnormal ALT.

Methods—A retrospective cohort study of 176 HCV-Genotype 4 (HCV-G4) patients treated with pegylated interferon and ribavirin.

Results—Of 176 cases studied, 53 (30.1%) had normal ALT. Prevalence of pretreatment severe fibrosis, sustained virological response (SVR) and relapse were not significantly different in patients with PNALT (26%, 66% and 5.7% respectively) compared to those with abnormal ALT (32.5%, 60.7%, and 6.6% respectively). Multivariable logistic regression revealed that pretreatment ALT, pretreatment viral load, inflammation, and schistosomiasis were not significantly associated with SVR [OR (95% CI), 0.75 (0.34-1.65); 0.92 (0.61-1.37); 1.64 (0.64-4.18); 0.90 (0.44-1.84) respectively]. Severe fibrosis was the only significant predictor of SVR [OR (95%CI), 0.38 (0.14-0.99)].

Conclusion—PNALT does not reflect the degree of fibrotic changes or predict SVR.

Furthermore, schistosomiasis is a predictor of neither fibrosis nor poor response in patients with PNALT. Severe fibrosis is a strong and independent predictor of response to treatment. Therefore, it is important to treat individuals with PNALT levels regardless of schistosomiasis.

Keywords

HCV; persistently normal alanine aminotransferase; response to treatment

Background

The hepatitis C virus Genotype 4 (HCV-G4) is responsible for 20% of chronic hepatitis (1). It is the most common variant of HCV in the Middle East and it has been reported that its prevalence is increasing in some southern European countries in the Mediterranean basin (2). The exact causes of the consistent the high rates of HCV-G4 transmission in Middle East are not yet clear. Approximately 30% of patients with chronic HCV infection show persistently normal ALT (PNALT) (3). Although formerly referred to as 'healthy' or 'asymptomatic' HCV carriers, and thus historically excluded from antiviral treatment, it has now become clear that they are at risk for developing significant liver disease; up to 20% might progress toward a more severe degree of liver fibrosis (4). However, the virological characteristics, histological features, and optimal response rate of chronic HCV-G4 patients with PNALT are poorly studied.

HCV patients coinfecting with schistosomiasis are expected to have accelerated fibrosis, increased haematological adverse effects, and poor response as schistosomiasis leads to down regulation of immune response to HCV in the form of reduced IFN γ , IL-4, and IL-10 secreted by HCV-specific T cells (5,6).

The objective of the current study was to assess the effect of pretreatment ALT status on the extent of liver fibrosis, treatment response and haematological adverse effect in HCV-G4 patients, and the impact of coinfection with schistosomiasis in this group of patients.

Patients and methods

Study design

We conducted a retrospective cohort study of 176 HCV-G4 patients treated with pegylated interferon and ribavirin, to compare patients with PNALT to patients with ALT and to examine the impact of schistosomiasis coinfection on fibrosis progression, response to treatment, and haematological adverse effects.

Study population

In this Retrospective study, 176 HCV-G4 consecutive, eligible patients who attended the Hepatitis clinic in Hamad Hospital and fulfilling the inclusion/exclusion criteria were recruited in the period between January 2004 and 2006. All individuals with persistent normal ALT or abnormal ALT levels were offered treatment if they did not meet any of the exclusion criteria. In addition, all subjects had liver biopsy regardless of their ALT levels. The study was performed according to the applicable regulations and ethical principles defined in the Declaration of Helsinki 1997, and approved by the ethics research committee of the Hamad Medical Corporation.

Chronic HCV infection was evidenced by positive serology for anti-HCV, active virus replication by detection of HCV-RNA in serum and histological findings of chronic active hepatitis according to the Scheuer Score. The necro-inflammatory and fibrosis scores were assigned based on the Scheuer scoring system from 0 to 4. Patients were further subdivided into mild fibrosis (stages I&II) and severe fibrosis (stages III & IV). We classified subjects into two transaminases groups: Group A) HCV-G4 with normal pretreatment hepatic transaminases and Group B) including HCV-G4 with high (> normal limit) pretreatment

hepatic transaminases. We considered subjects to have persistent normal ALT values if they had three or more determinations identified to be within the normal range over a 6-month period or longer. We further divided individuals with normal pretreatment hepatic transaminases into “low-normal” (< 50% upper normal limit) and “high-normal” (>50% upper normal limit).

None of the patients participating in our study had serologic evidence suggestive of autoimmune liver disease or inheritable disorders such as hemochromatosis or Wilson's disease, or a history of alcoholism or drug abuse. Hepatocellular carcinoma was excluded by testing of α -fetoprotein and by ultrasound scanning. None of the female patients was pregnant as evidenced by negative serum pregnancy test. Breast feeders were excluded. We excluded patients if they had severe cardiac or pulmonary disease, concurrent hepatitis B or human immunodeficiency virus infections, history of seizure disorder, psychiatric disease, and organ transplantation.

Diagnosis of schistosomiasis was based on presence of one or more of the following: history of treatment for schistosomiasis, detection of ova in urine or stool analysis, positive serology or histological evidence of schistosomiasis.

Testing for anti-HCV was carried out using a commercial ELISA kit (AxSYM 3.0; Abbott Laboratories, Chicago, IL, USA). All patients were HCV-G4 as detected by the Inno-LiPA HCV II assay (Innogenetics Inc., Alphen aan de Rijn, The Netherlands). Monitoring serum HCV RNA levels was by Amplicor (version 2.0, Hoffmann-La Roche) with a minimum detection limit of 50 IU/mL.

All patients included in this study were previously naive to HCV treatment. They were treated subcutaneously, with 180 μ g of peginterferon α -2a once weekly and ribavirin given at an oral daily dose of 1000 mg (body weight >75 kg) or 1200 mg (body weight <75 kg) for 48 weeks.

Definition of virological responses

End-of-treatment response (ETR) was defined as loss of detectable serum HCV RNA at the end-of-treatment, while SVR was defined as the absence of detectable HCV RNA levels at end of the follow-up (24 weeks after the end of the treatment). Relapse was defined as the reappearance of HCV RNA during the follow-up in subjects with previous ETR

Statistical analysis

Analyses were conducted using SAS version 9.1.3 (SAS Institute Inc., Cary, NC). We explored the data for outliers and checked for normality. When necessary, variables were log transformed if normality assumption was violated.

We evaluated the unadjusted association between pretreatment ALT and response to treatment, fibrosis, inflammation, anaemia, viral load, and schistosomiasis. For categorical variables, the unadjusted association was tested using chi-square statistics (Fisher exact statistics if there was limited sample size thus violating the chi-square statistics assumptions), while for continuous variables, ANOVA (or Kruskal-Wallis test when the assumption of normality is violated) were used.

Multivariable logistic regression (for categorical variables) and multivariable linear regression (for continuous variables) were employed to test for the null hypothesis of no significant association between pretreatment ALT and important variables (response to treatment, fibrosis, and grade) controlling for possible confounders and covariates, such as age, sex, schistosomiasis, and viral load.

Results

One hundred-seventy-six HCV-G4 cases were enrolled in the study (table 1), of whom 150 (85.2%) were men. The median age of study participants was 46 years (inter-quartile range 40-52). In the current study, we observed no response, relapse, and SVR in 31.8%, 6.3%, and 61.9% respectively. Out of 176 cases, 53 (30.1%) had normal ALT.

In this study, severe fibrosis was not significantly different in patients with schistosomiasis (30.6%) compared to those without schistosomiasis (30.5%), $p = 0.99$.

In the unadjusted analysis individuals with and without normal ALT were significantly different by pretreatment viral load but did not significantly differ by age, pretreatment hemoglobin, or pretreatment neutrophils (table 2).

Most importantly, there was 1) lack of association of PNALT with fibrosis [severe fibrosis was not different in patients with PNALT (26%) compared to those with abnormal ALT (32.5%), $p = 0.41$]; 2) lack of association of PNALT with inflammation [severe inflammation was observed in 13 (25%) PNALT compared to 46 (39%) of those with abnormal ALT, $p = 0.08$]; 3) lack of association of PNALT with schistosomiasis [schistosomiasis was detected in 60.4% of patients with PNALT compared to 63.6% in those with abnormal ALT, $p = 0.08$]; 4) lack of association of PNALT with response to treatment [among individuals with PNALT 35 (66%) had SVR compared to 74 (60.7%) among individuals with abnormal ALT, $p = 0.50$, and 5.7% of individuals with PNALT had relapse compared to 6.6% of those with abnormal ALT, $p = 0.80$]. (Table 2) Schistosomiasis effect on SVR did not differ by ALT status (p for interaction 0.15). Similarly, the effect of severe fibrosis on SVR did not differ by ALT status (p for interaction 0.54). In addition, among individuals with PNALT, there was no significant difference in fibrosis between those with low normal and upper normal ALT ($X^2 = 2.40$, $p = 0.12$).

Pretreatment anaemia was significantly different in patients with PNALT (15.9%) compared to those with abnormal ALT (3.3%), $p < 0.01$. Week 4 and 12 anaemia was not significantly different in patients with PNALT (32.7%, and 50.9% respectively) compared to those with abnormal ALT (23.1%, and 43.6% respectively). Pretreatment, week 4 and 12 neutrophils was not significantly different in patients with PNALT (39.6%, 73.6%), and 69.6% respectively) compared to those with abnormal ALT (43.0%, 78.2%, and 87.1% respectively).

In the multivariable analysis, adjusting for age and sex, we examined the interplay of ALT status, fibrosis, inflammation, pretreatment viral load, and schistosomiasis on response to treatment.

Severe fibrosis was the only significant predictor of the lower likelihood of SVR (OR 0.38, 95%CI 0.14-0.99), but not of the lower likelihood of ETR (OR 0.43, 95%CI 0.16-1.79). Elevated pretreatment ALT, pretreatment viral load, and inflammation were not significantly associated with SVR [OR (95% CI) 0.75 (0.34-1.65); 0.92 (0.61-1.37); 1.64 (0.64-4.18) respectively] or ETR [OR (95% CI) 0.70 (0.30-1.63); 0.99 (0.65-1.50); 1.78 (0.66-4.79) respectively]. Schistosomiasis was not significantly associated with SVR or ETR [OR (95% CI) 0.90 (0.44-1.84), and 0.85 (0.40-1.79) respectively] i.e. SVR and ETR are not different in those with coinfection compared to those who has only HCV infection.

Discussion

Patients exhibiting PNALT present a unique challenge for hepatologists. In spite of our increasing understanding of the epidemiology, natural history and treatment of patients with

chronic HCV infection, debate still exists concerning patients with PNALT and the role of cofactors in the evaluation of the disease and the decision for treatment. In this study we focused specifically on HCV genotype 4, not only because it has not received intensive study, but also for its prevalence among Egyptians who are also subjected to schistosomiasis. Schistosomiasis is a factor that has long been incriminated as a cofactor in the increased incidence of HCV infection in Egypt, before implantation of oral therapy owing to widespread use of parenteral antimony salts therapy using unsterile syringes in 1960 to 1982 (2,7,8). The notion that treatment of schistosomiasis led to transmission of HCV infection is no longer accepted per se since, at least 20% of our HCV Egyptian patients either never had schistosomiasis or were born in the era of oral therapy. Other ways of transmission were sought, including sexual or vertical modes of transmission, but these routes are now known to account for less than 5% of cases (9-12).

In our study, none of our patients with PNALT had normal histology, hence as reported with other genotypes the persistently normal transaminases in HCV-G4, does not reflect the fibrotic changes (13). Some authors attributed this to the ultimate increase in ALT during the natural course of the disease (14). While others suggested that ALT levels are released by direct virus-related cytopathic activity and/or by an immune-mediated process which is less activated in patients with PNALT (15). Less likely, clinical outcome of HCV infection can be influenced by the HCV genotype and/or to a lower degree of viral replication (16). Our observation of the significant high SVR rate of all PNALT group (64%) compared to the high ALT one is in accordance with Yagura et al (17), Puoti et al (18), Snoeck et al (19), and Roulot et al (2). They attributed this to that the upper limits of normal range for serum transaminases in HCV patients with SVR were far lower than the current accepted value and that the upper limit of normal for serum ALT is 25 U/L is useful to consider as an indication of the interferon therapy. Our study confirms safety of treatment with PEG-IFN α -2a plus RBV in HCV carriers with PNALT, and similar adverse effect to that in patients with elevated ALT levels.

Recent reports suggested that HCV may induce auto-immune haemolytic anaemia. The increased anaemia noticed in our patients with normal ALT may reflect extrahepatic replication, as bone marrow cells of patients displaying haematological disorders represent a putative site of extrahepatic HCV replication (20).

All our chronic HCV with PNALT, whether with or without schistosomiasis, were comparable in terms of fibrosis and response to treatment rate, suggesting that, in this context, schistosomiasis coinfection is not a surrogate of poor response. Our results reinforce Roulot et al (2) findings of high response among Egyptian patients with schistosomiasis patients despite the presence of a more severe fibrosis. Increased susceptibility to HCV in schistosomiasis patient due to increased production of IL-10, is no longer accepted (21). The reported normal thrombopoietin (22) and elevated von Willebrand factor antigen (23) levels in schistosomiasis, may explain absence of thrombocytopenia as an adverse effect in our patient with coinfection.

The correlational relationship between pre-treatment HCV-RNA titre and the response to treatment is ambivalent.

This result has been affirmed by our observation in the current study and many recent studies, emphasizing that the baseline HCV RNA is trivial for SVR [13,23,24]. As serum HCV viral load fluctuates and HCV replicates in extrahepatic sites, so the viral load becomes unstable failing to show the degree of liver damage [13]. Thus, there is no direct proportion between the high amount of circulating HCV and the active state of viral reproduction in liver or the severity of liver disease, and as previous reports suggested,

treatment of HCV infection might be directed by the early kinetics independently of the baseline HCV-RNA level [24,25]. We can conclude that, in HCV-G4 the pretreatment ALT status does not reflect the fibrotic changes. In addition, severity of fibrosis predicted the response to treatment independent of ALT levels. The higher SVR in individuals with PNALT emphasizes the importance of offering treatment to individuals with PNALT levels independent of schistosomal coinfection, as schistosomal coinfection did not enhance liver pathology and did not affect response rate.

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Table1
Study subjects characteristics

Variable	Median (inter-quartile range)
Age	46 (40,52)
Male, N (%)	150 (85.2)
Pretreatment ALT [#] (U/L)	61 (37.0,105.0)
Pretreatment haemoglobin	15.2 (14.1,16.1)
Pretreatment log viral load	5.7 (5.1,5.8)
Pretreatment neutrophils	2.8 (1.9,4.1)
No response, N (%)	56 (31.8)
Relapse, N (%)	11 (6.3)
End of treatment response, N (%)	120 (68.2)
Sustained viral response, N (%)	109 (61.9)

Table 1 notes:

[#] ALT: alanine aminotransferase

Table 2
Unadjusted association of selected study subjects characteristics by alanine aminotransferase status[@]

	ALT [#] status	
	Normal N=53	Elevated N=123
Variable	Median (range)	Median (range)
Age	47 (40-55)	45 (40-51)
Male, N (%)	43 (81.1)	106 (86.1)
Pretreatment haemoglobin [*]	14.9 (13.3-15.8)	15.3 (14.3-16.2)
Pretreatment viral load [*]	5.5 (2.8-5.8)	5.8 (5.3-5.8)
Pretreatment neutrophil	3.2 (1.9-4.4)	2.8 (1.9-4.0)
Schistosomiasis, N (%)	32 (60.4)	77 (63.6)
Sustained viral response, N (%)	35 (66.0)	74 (60.7)
Relapse, N (%)	3 (5.7)	8 (6.6)
Grade (severe inflammation), N (%) ^{\$}	13 (25.0)	46 (39.0)
Stage (severe fibrosis), N (%) [^]	13 (26.0)	38 (32.5)

Table 2 notes:

[@] P value were calculated by Fisher exact statistics for categorical variables, and Kruskal-Wallis test for continuous variables

[#] ALT: alanine aminotransferase

^{*} P value <0.05

^{\$} severe inflammation: grades III & IV

[^] severe fibrosis : stages III & IV