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What is Vitamin D doing in the Management of Chronic Hepatitis C?**Complementary role of vitamin D deficiency and the interleukin-28B rs12979860 C/T polymorphism in predicting antiviral response in chronic hepatitis C***Bitetto D, Fattovich G, Fabris C, Ceriani E, Falletti E, Fornasiere E, Pasino M, Ieluzzi D, Cussigh A, Cmet S, Pirisi M, Toniutto P*

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Hepatology 2011;53:1118–26**Abstract**

The widely accepted interleukin-28B (IL-28B) rs12979860 C/T polymorphism and the more recently proposed vitamin D serum concentration are two novel predictors of the response to antiviral treatment in chronic hepatitis C virus (HCV) infection. This study aimed to verify whether the IL-28B rs12979860 C/T polymorphism and pre-treatment serum vitamin D levels have independent or complementary roles in predicting the rates of sustained viral response (SVR). The present study included 211 consecutive, treatment-naïve chronic HCV patients who had their pre-treatment serum 25-OH vitamin D level and IL-28B rs12979860 C/T genotype determined. Overall, SVR was achieved by 134/211 (63.5%) patients and by 47/110 (42.7%) patients infected with difficult-to-treat HCV genotypes. On multivariate analysis, SVR was predicted by the HCV genotype, the IL-28B rs12979860 C/T polymorphism, and γ -glutamyl transpeptidase, HCV RNA, cholesterol, and 25-OH vitamin D serum levels, with an area under the receiver operating characteristic (ROC) curve of 0.827. When difficult-to-treat HCV genotypes were analyzed separately, the SVR was predicted by the IL-28B rs12979860 C/T polymorphism, viral load, and serum vitamin D level, with an area under the ROC curve of 0.836. Moreover, by categorizing these latter patients into 4 groups—C/C homozygotes with vitamin D levels >20 ng/mL (group A) or $\leq$ 20 ng/mL (group B) and C/T heterozygotes or T/T homozygotes with vitamin D levels >20 ng/mL (group C) or $\leq$ 20 ng/mL (group D)—a significant linear trend was observed, with SVR rates in the following descending order: group A, 18/21 (85.7%); group B, 6/11 (54.5%); group C, 14/38 (36.8%); and group D, 9/40 (22.5%) ($p < 0.0001$).

Conclusion: Vitamin D serum levels are complementary to the IL-28B rs12979860 C/T polymorphism in enhancing the correct prediction of the SVR in treatment-naïve chronic hepatitis C.

Comments

Ability to predict response to current antiviral regimens remains an important goal in patients with chronic hepatitis C (CHC). In last few years, several factors have been identified to help physicians predict the probability of achieving a sustained viral response (SVR) prior to treatment with combination therapy of pegylated interferon (PEG-IFN) alpha and ribavirin in patients with CHC. Two such new predictors of response to antiviral treatment include the interleukin-28B (IL-28B) rs12979860 C/T polymorphism and vitamin D serum concentration. The IL-28B rs12979860 C/T polymorphism, located on chromosome 19 upstream of the gene encoding IFN- γ 3, represents a host-related, nonmodifiable variable that strongly predicts the response to antiviral treatment. Among hepatitis C virus (HCV)-1-infected patients,

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SVR rates higher than 60–80% were achieved by C/C homozygotes compared with the 15–30% achieved by carriers of the T/T or T/C alleles.¹ Similar data favoring the predictive role of IL-28B polymorphism is available for even in non-genotype 1 patients (genotype 2 and 3).² The second novel predictor, serum vitamin D concentration, is also of great interest because it is easily modifiable by dietary supplementation and appears to possess important immunomodulated and antiproliferative effects. It has also been shown that cholecalciferol supplementation added to combination therapy with PEG-IFN plus ribavirin could enhance the rates of SVR in patients with genotype 1 chronic hepatitis C.³

Since no study had evaluated earlier the role of the serum vitamin D concentration as a predictor of SVR in conjunction with the IL-28B rs12979860 C/T polymorphism, the present study evaluating the role of both these factors in combination is a welcome step.

Vitamin D deficiency is common not only among patients with chronic cholestatic liver disease or advanced fibrosis/cirrhosis, where it can be expected, but also in patients with mild CHC. The reasons for vitamin D deficiency in patients with CHC are still not clear. The likely explanation includes interaction between vitamin D, the immune response and inflammatory status and an impaired liver synthesis of 25-OH vitamin D in patients with chronic liver disease. In the present study, the occurrence of vitamin D deficiency (≤ 20 ng/mL) was observed in approximately one-half of the patients and severe vitamin D deficiency (≤ 10 ng/mL) in approximately 16% of them. On multivariate analysis, only age, time of the year of blood sampling, and grading score were found to be independent predictors of low serum vitamin D levels. The histology staging score, which had a strong inverse association with serum vitamin D level on univariate analysis, did not appear to be an independent predictor of vitamin D deficiency. This is in contrast to earlier reports which reported an inverse association not only between histology grading and vitamin D but also between the staging of liver fibrosis and the vitamin D level.⁴ More data is required before definite conclusions are drawn for the possible reasons for vitamin D deficiency in patients with CHC.

Vitamin D has emerged as a key regulator of the innate immunity response in humans and the relationship between the rapid decline of the HCV RNA level after therapy initiation and vitamin D suggests that the latter could amplify the immunological effect of IFN.⁵ In the present study, vitamin D levels were found to influence the achievement of viral clearance after antiviral therapy in patients with CHC. In particular, there was a highly significant association between progressively lower baseline serum vitamin D levels and the rates of viral clearance. This outcome was evident in all HCV genotypes but was particularly important in patients infected by difficult-to-treat genotypes. Baseline vitamin D affected not only SVR but also the rapid virological response (RVR) and complete early virological response (cEVR) and therefore suggested the possible predictive role of baseline serum vitamin D on the attainment of SVR through achieving RVR. The data raises several questions particularly for the nutritionally challenged populations. Whether vitamin D levels should be assessed before starting antiviral therapy and treatment initiated only in the presence of normal serum vitamin D values? In the presence of vitamin D deficiency, should we first correct the deficiency before starting antiviral therapy? We probably need more data before vitamin D level estimation and its supplementation becomes a standard of care in the treatment of patients with CHC.

The study demonstrated that the vitamin D level and the IL-28B rs12979860 C/T polymorphism are two independent predictors of SVR achievement in difficult-to-treat HCV genotypes and combined together these factors enhance the ability to identify patients who will respond to treatment. Compared with patients carrying the IL-28B rs12979860 C/C genotype and who have a normal vitamin D level, vitamin D deficiency identifies patients with a lower probability of SVR attainment. Furthermore, carrying at least one T allele along with vitamin D deficiency was associated with the lowest probability of attaining the same viral endpoint.

Even though the data is convincing, we will have to wait for similar prospective data with dynamic determination of vitamin D levels during the course of treatment of patients with CHC before we conclude that pre-treatment serum vitamin D determination is a useful adjunct to IL-28B rs12979860 C/T polymorphism evaluation in the treatment of patients with CHC.

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Apolipoprotein C3 Gene Polymorphism in Nonalcoholic Fatty Liver Disease—To Be or Not To Be

Apolipoprotein C3 gene variants in nonalcoholic fatty liver disease

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Abstract

Background: Nonalcoholic fatty liver disease is associated with hepatic insulin resistance and type 2 diabetes mellitus. Whether this association has a genetic basis is unknown.

Method: In 95 healthy Asian Indian men, a group known to have a high prevalence of nonalcoholic fatty liver disease, we genotyped two single-nucleotide polymorphisms (SNPs) in the gene encoding apolipoprotein C3 (APOC3) that are known to be associated with hypertriglyceridemia (rs2854116 [T-455C] and rs2854117 [C-482T]). Plasma APOC3 concentrations, insulin sensitivity, and hepatic triglyceride content were measured. We also measured plasma triglyceride concentrations and retinyl fatty acid ester absorption as well as plasma triglyceride clearance after oral and intravenous fat-tolerance tests. Liver triglyceride content and APOC3 genotypes were also assessed in a group of 163 healthy non-Asian Indian men.

Results: Carriers of the APOC3 variant alleles (C-482T, T-455C, or both) had a 30% increase in the fasting plasma APOC3 concentration, as compared with the wild-type homozygotes. They also had a 60% increase in the fasting plasma triglyceride concentration, an increase by a factor of approximately 2 in the plasma triglyceride and retinyl fatty acid ester concentrations after an oral fat-tolerance test, and a 46% reduction in plasma triglyceride clearance. The prevalence of nonalcoholic fatty liver disease was 38% among variant-allele carriers and 0% among wild-type homozygotes ($p < 0.001$). The subjects with nonalcoholic fatty liver disease had marked insulin resistance. A validation study involving non-Asian Indian men confirmed the association between APOC3 variant alleles and nonalcoholic fatty liver disease.

Conclusion: The polymorphisms C-482T and T-455C in APOC3 are associated with nonalcoholic fatty liver disease and insulin resistance.

Comments

In the present study, authors found that the APOC3 variants (C-482T and T-455C) were associated with approximately a 30% increase in the fasting plasma apolipoprotein C3 concentration, approximately a 60% increase in the fasting plasma triglyceride concentration, and an increase by a factor of approximately 2 in postprandial plasma triglyceride and retinyl fatty acid ester concentrations after an oral fat tolerance test. Authors had recruited all healthy subjects with no risk factors (non-obese) for nonalcoholic fatty liver disease (NAFLD) and found that 38% of the Asian Indian men with APOC3 variants had NAFLD and marked insulin resistance, whereas no APOC3 wild-type homozygotes had NAFLD. In the non-Asian Indian cohort the difference in NAFLD between those with and without variant alleles was 9% and 0%. APOC3 variants C-482T and T-455C lead to increased plasma concentrations of APOC3, which in turn inhibit lipoprotein lipase and triglyceride clearance, thus conferring a predisposition to both fasting and postprandial hypertriglyceridemia due to an increase in chylomicron-remnant particles. Increased concentrations of circulating chylomicron-remnant particles are then preferentially taken up by the liver by means of a receptor-mediated process, resulting in NAFLD and hepatic insulin resistance.¹

Authors in the present study also found that the prevalence of variant APOC3 alleles was similar among Asian Indian men and non-Asian Indian men, suggesting that other factors are responsible for the increased prevalence of NAFLD (38% vs 9%) among the Asian Indian men. In this regard they also genotyped the Asian Indian men for the SNP rs738409 in PNPLA3, which has recently been shown to be associated with NAFLD by means of an unknown mechanism. They found that the CG and GG genotypes of this SNP were also associated with increased liver triglyceride content, as compared with the CC genotype in this population. They also examined the fraction of variance in the risk of nonalcoholic fatty liver disease attributable to APOC3, PNPLA3, or both and found that they accounted for 11%, 6.5%, and 13.1%, respectively, of the variance, suggesting that these gene variants have an interactive effect.

Ethnic differences in hepatic steatosis have been shown earlier and are thought to have the genetic basis.² An association of a PNPLA3 variant with NAFLD in Hispanic Americans and African Americans has been shown earlier, suggesting that PNPLA3 contributes to the variation in NAFLD across multiple ethnicities.^{3,4} Though Petersen et al suggested a strong association of APOC3 variants with NAFLD amongst Asian Indian and non-Asian Indian men, a recent study has shown that APOC3 variants probably had no effect in causing NAFLD amongst African Americans,

European Americans and Hispanics.⁵ Two APOC3 variants were genotyped in 1228 African Americans, 843 European Americans and 426 Hispanics from a multiethnic population based study—the Dallas Heart Study. The same study also examined the relationship between these two variants and HOMA-IR in the Atherosclerosis Risk in Communities (ARIC) study. No significant difference in hepatic fat content was found between carriers and noncarriers in the Dallas Heart Study. Neither APOC3 variant was associated with HOMA-IR in the Dallas Heart study; this lack of association was confirmed in the ARIC study, even after the analysis was restricted to lean (BMI < 25 Kg/m²) individuals (n = 4399).⁵

Thus, more data would be required before any firm conclusions about the role of APOC3 gene variants in causing NAFLD amongst various races are drawn.

Another interesting aspect of the influence of APOC3 promoter variants on insulin resistance, type 2 diabetes mellitus, and possibly NAFLD is that it may vary with the level of adiposity. In a recent study, lean carriers of the -482T allele had increased type 2 diabetes risk, while such an effect was not observed in overweight participants. Conversely, in overweight participants the -455C allele seemed protective against type 2 diabetes.⁶ These findings of a divergent role of Apo C variants in lean and overweight patients may be particularly relevant in Indian setting and requires further exploration in a subset of patients with lean NAFLD.

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