

## Correspondence

### **Chemokine receptor 5 (CCR5) polymorphism in chronic hepatitis B patients treated with three different nucleos(t)ide analogues**

Sir,

Most adults infected with hepatitis B virus (HBV) recover and develop protective antibodies. However, approximately 5 per cent of adults remain chronically infected with HBV and are at risk for developing end-stage liver disease and hepatocellular carcinoma<sup>1</sup>. Chemokine receptor 5 (*CCR5*) is a gene located on short (p) arm at position 21 on chromosome<sup>2</sup>. *CCR5* is critical in regulating T cell functions by mediating recruitment, polarization, activation and differentiation of type 1 cytokine secreting T helper and cytotoxic T cells. A 32 bp deletion in the coding region of *CCR5* gene leads to complete loss of the functional *CCR5* protein synthesis. This mutation was found to be associated with reduced risk of HIV susceptibility<sup>3</sup>.

*CCR5* deficiency is particularly intriguing because it offers a potential means to enhance the T-cell response to HBV by blocking *CCR5*. *CCR5Δ32* homozygous mutation is known to provide genetic protection against HBV persistence<sup>4</sup>. So, we hypothesize that *CCR5Δ32* heterozygous mutation may have an additive effect in nucleoside analogue treatment response in chronic HBV patients so that simultaneous administration of a *CCR5* blocking agent, along with an anti-HBV agent may increase HBV clearance through augmentation of the T-cell response to HBV antigens. This pilot study was designed to analyze the *CCR5* polymorphism in chronic HBV patients with respect to HBV DNA clearance following treatment using different types of nucleos(t)ide analogues (lamivudine/adefovir/telbivudine). The study was conducted in the Department of Medicine, Maulana Azad Medical College (MAMC), New Delhi, India during 2008-10. The study protocol was approved by the ethics committee of MAMC and prior written informed consent was taken from each patient. The study group consisted of 45 consecutive chronic hepatitis

B patients; 15 new patients and 30 patients from our previous study<sup>5</sup>. Test for liver functions, serological tests for hepatitis B surface antigen (HBsAg), anti-HBe (antibody to hepatitis B e antigen), HBeAg, anti-Hbc IgG (IgG antibody against HBV core antigen), anti-HCV, HBV DNA and HBV viral load were assessed at baseline for inclusion of patients and after treatment. Liver biopsy was done in all patients to assess severity of disease before the start of therapy and at the end of 6 months and assessed as described earlier<sup>5</sup>. The biopsy specimens were scored according to the original criteria of Knodell histological activity index (HAI)<sup>6</sup>. HBV DNA was extracted from serum by using the standard phenol chloroform method. Detection of HBV DNA was carried out using primers as described earlier<sup>7</sup> to amplify part of the surface gene. Hepatitis B viral load estimations were performed by real time PCR (RT-PCR) method<sup>5</sup>. A 204 bp fragment denotes the *CCR5* wild type allele while a 172 bp fragment denotes the *CCR5Δ32* mutant allele. The patients included were conveniently selected to receive either lamivudine or adefovir or telbivudine (100, 10 and 600 mg, respectively) daily for a period of six months (15 patients in each group). Qualitative values were correlated with Chi-square or Fisher exact tests.  $P < 0.05$  was considered statistically significant. Quantitative values are expressed as means and ranges, and were compared using the Student's *t*-test or the Mann-Whitney non-parametric *U* test.

There was a significant reduction in the serum alanine aminotransferase (ALT in IU/l) levels after treatment for six months ( $P < 0.0001$ ) as compared to baseline values, and results were similar in all drug groups. Compared to baseline, there was a significant ( $P < 0.05$ ) reduction in total bilirubin (mg/dl) levels which was also similar in all drug groups. All the patients remained HBsAg positive at the end of 6 months in all the groups. Four (8.89%) patients seroconverted from

HBeAg positive to negative state. Ten of 45 (22.22%) patients became HBV DNA negative, which included four patients each in the adefovir and telbivudine group while two patients in the lamivudine group. There was significant ( $P < 0.001$ ) reduction in serum viral load after six months of therapy; the mean reduction was 1.92, 2.06 and 2.65 log copies per ml with respect to serum HBVDNA adefovir, lamivudine and telbivudine groups, respectively. There was a non significant reduction with respect to HAI score and fibrosis score after treatment for six months and the results were similar in all the drug groups. Of the 45 patients, five (11.11%) were found to have CCR5 $\Delta$ 32 heterozygous (+/-) genotype and the remaining 40 patients (88.8%) were of CCR5 (+/+) genotype. Of the four patients who seroconverted from HBeAg positive to negative state, CCR5 $\Delta$ 32 (+/-) mutation was found in two patients. The frequency of CCR5 $\Delta$ 32 mutations did not relate with biochemical, virological and histological parameters. There was no difference in response to therapy when patients were categorized on the basis of the response pattern with respect to different drug groups, and the distribution of alleles with respect to CCR5 polymorphism.

The overall allele frequency of CCR5 $\Delta$ 32 for all study subjects was 11.11 per cent, which is consistent with an earlier study<sup>4</sup>. Woitas and group<sup>8</sup> have shown that homozygosity for 32 bp deletions is also more common in HCV infected patients than in healthy controls; they have shown that patients with 32 bp mutation have elevated viral loads. Current therapeutic options [nucleoside (or nucleotide) analogues] to treat chronic hepatitis B led to a sustained therapeutic response in only a minority of treated individuals, thus, simultaneous administration of a CCR5 blocking agent, which is already being developed for HIV infection, along with an anti-HBV agent may increase HBV clearance through augmentation of the T-cell response to HBV antigens.

The study findings suggest that the treatment with antiviral drugs adefovir, lamivudine and telbivudine brings about biochemical and serological improvement when administered for about six months. The treatment also brings about a significant reduction in the serum HBV DNA levels. There was not enough evidence

to show a significant association of CCR5 $\Delta$ 32 gene polymorphism with the treatment outcome (biochemical, virological and histological response) of patients of chronic hepatitis B, and the limitations of this study were small sample size and limited duration of therapy (6 months).

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