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RESEARCH LETTER

Exogenous growth factor based regenerative therapy in alcoholic cirrhosis patients renders limited effect on hemostatic elements

To the editor,

Exogenous growth factors stimulate hepatic regeneration by mobilizing bone marrow precursors to chronically diseased liver [1,2]. Hemostatic factors are synthesized and cleared by liver. Although the coagulation system remains delicately balanced in advanced liver diseases but, it can become grossly deranged with slight perturbations [3]. Whether the growth factor based regenerative therapy can induce hepatocyte functions and improve the hemostatic capacity is not known. The present study was undertaken to investigate the changes in the coagulation profile of patients with alcoholic cirrhosis after growth factor(s) therapy using conventional as well as global tests of coagulation.

Patients with decompensated alcoholic cirrhosis, those were enrolled in the clinical trial — NCT01902511, from October 2013 to June 2015, were prospectively evaluated. After receiving the ethical approval from the institutional ethics committee, patients were enrolled and written informed consent was obtained. Patients on anticoagulant therapy, with known coagulation disorder other than due to liver disease, presence of any other chronic medical condition, hepatocellular carcinoma, sepsis, grade IV hepatic encephalopathy, pregnancy, patients allergic to granulocyte colony stimulating factor (G-CSF) or erythropoietin (EPO) and refusal to participate in the study were excluded. In group 1, G-CSF (Neupogen) was given as 5 ug/kg body weight subcutaneously, on day 1, 2, 3, 4, 5 and then every 3rd day till day 60. In group 2, erythropoietin (darbopoietin) was given subcutaneously at a dose of 500 IU/kg twice a week for 2 months (total 16 doses) along with G-CSF. The EPO was given on a separate site than the G-CSF. Patients were monitored for any local site complications and also for any other adverse effects, especially fever, body rashes, joint pains, abdominal pain, bleeding. Blood samples of every patient were collected before the start of the therapy and again on day 60 of therapy and labeled as pre- and ''post-therapy samples''. Blood samples were collected in two vacutainers containing buffered sodium citrate (0.109 M, 3.2%) in the ratio of blood: anticoagulant 9:1 for coagulation studies. The samples were processed within half an hour of collection. Citrated tube was centrifuged at 3000 rpm for 10 minutes and plasma was obtained. Part of the plasma was run on fully automated coagulometer (CA-1500, Sysmex, Siemens Healthcare Diagnostics, NY, USA) and values for prothrombin time (PT), international normalized ratio (INR), activated partial thromboplastin time (APTT), protein C, protein S, antithrombin and Factor VIII were recorded. Activity of protein C, S and antithrombin III, were estimated by chromogenic method and factor VIII was assessed by clotting method. Remaining plasma was used for the ELISA for von Willebrand factor (VWF) antigen (Assay Pro, EV2030-1, Missouri, USA) and human tissue factor assays (Assay Pro, ET1002-1, Missouri, USA). Global coagulation analysis was done by Sonoclot coagulation and platelet function analyzer (Sienco Inc., Arvada, CO, USA). For this 340 μL of citrated whole blood was added to gb ACT+ (glass bead activated ACT) cuvette pre-warmed to 37 °C along with 20 μL of $CaCl_2$. Sonoclot signatures were recorded for a period of 30 minutes and activated clotting time (ACT), clot rate (CR), time to peak (TP), the peak amplitude (PA) and platelet function (PF) were registered. Clinical details and platelet counts of the respective patients were compiled from the hospital information system. Statistical analysis was done using SPSS version 22.0 (SPSS, Chicago IL). Mean with standard deviation or standard errors and median with inter-quartile range were used to describe the data. Chi-square test or Fischer's exact test was used to compare the results, where required. Student *t*-test was used to compare delta changes in 2 independent groups. Paired *t*-test was used to compare pre with post means of continuous variables with normal distribution. Wilcoxon's signed rank test was used for continuous variables with skewed distribution. A P-value ≤ 0.05 was considered significant.

A total of 60 alcoholic cirrhosis patients were screened at Institute of Liver and Biliary sciences, New Delhi, India, over a period of twenty months. Of these, 18 patients were excluded from the basis of above mentioned exclusion criteria and 42 patients were enrolled in the study. The post-therapy samples were collected from 36 patients

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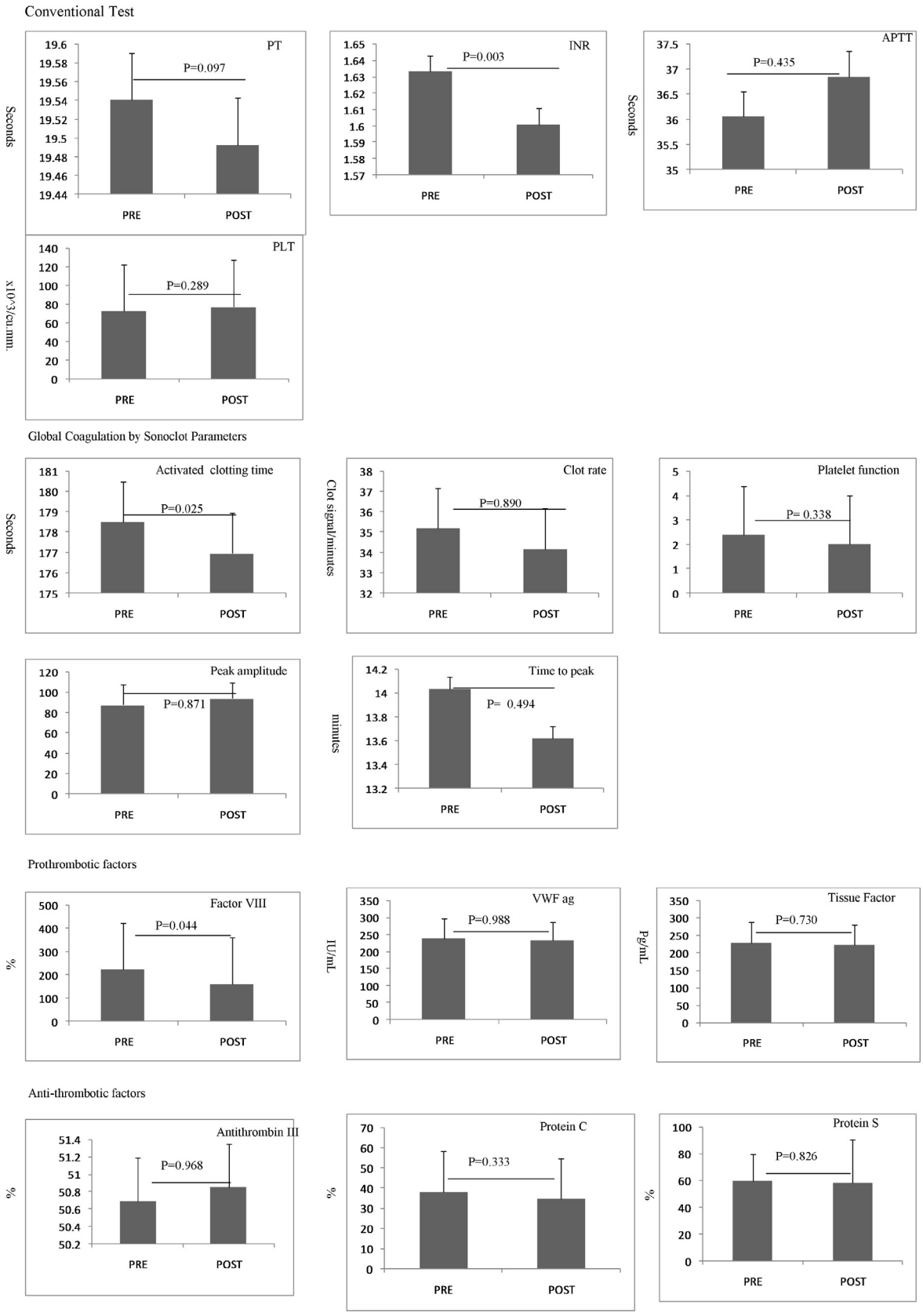

Figure 1 Coagulation profile change, post growth factor therapy represented in mean ± SD. PT: prothrombin time; INR: international normalized ratio; APTT: activated partial thromboplastin time; PLT: platelet count; ACT: activated clotting time; CR: clot rate; PF: platelet function; PR C: protein C; AT: antithrombin; FVIII: factor VIII; VWF: von Willebrand factor; TF: tissue factor; PRE: pre-therapy mean value; POST: post-therapy mean value.

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