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ORIGINAL ARTICLE

PROTEIN C IN CHRONIC LIVER DISEASE

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ABSTRACT

Introduction: In chronic liver disease due to chronic hepatitis and underlying cirrhosis, haemostatic imbalance occurs that leads to hypercoagulability, which favors thrombosis. The cause of Hypercoagulability in chronic liver disease is the reduced level of protein C and increased level of factor VIIIa. This study aimed to measure the levels of protein C in chronic liver disease patients.

Methodology and Result: It was a cross sectional comparative study and included fifty patients of chronic liver disease. They were further divided into two groups on the basis of their histological stages of fibrosis. Group I included stages 0 – 3 of fibrosis whereas group II included stages 4 – 6 of fibrosis. Each group comprised of 25 patients of CLD. Protein C level was done on all patients by synthetic chromogenic substrate method. Results: Mean ± SD of protein C in group I and II patients was 87.8 ± 15.6 and 35.9 ± 17.2% respectively. These results showed level of PC was reduced in advanced stage of fibrosis when compared with early stage of fibrosis in patients of chronic liver disease. A statistically significant difference ($p < 0.001$) was observed when mean levels of PC were compared among two groups; higher in group I as compared to group II.

Conclusions: In chronic liver disease protein C may be useful for assessment of hepatocellular damage in cirrhosis.

Key words: Protein C (PC), Chronic liver disease (CLD).

INTRODUCTION

Liver has the major role in synthesizing all clotting factors (except von Willebrand factor) and coagulation inhibitors. Protein C (PC) is one of the plasma glycoprotein precursors of serine proteases. This vitamin K dependent protein is synthesized by the liver. Vitamin K is required as a cofactor for the post ribosomal modification of this factor to make it physiologically active.[1] It is converted to activated protein C by the action of thrombin in the presence of thrombomodulin. Activated protein C inhibits factors V and VIII in the presence of phospholipid and calcium using protein S as cofactor. In addition to its anticoagulant activity, protein C enhances fibrinolysis as well. Protein C deficiency is either inherited or acquired. Inherited protein C deficiency is classified as type I or type II. In type I PC deficiency, the antigen level and function are reduced whereas in type II, PC is dysfunctional with normal antigen levels. Acquired PC deficiency is usually consumptive and occurs in patients with extensive venous thrombosis, infection, disseminated intravascular coagulation, adult respiratory distress syndrome, chronic liver disease and haemolytic uremic syndrome.[2]

In chronic liver disease the haemostatic failure occurs, that leads to thrombosis and bleeding. The causes of impaired haemostasis due to chronic liver disease consist of several haemostatic defects such as impaired synthesis of coagulation factors,[3] deficiencies of natural anticoagulants, thrombocytopenia and platelet dysfunctions.[4,5] Once the cirrhosis develops, the symptoms may vary from fatigue, myalgias, loss of appetite, bleeding from gastrointestinal tract, fluid retention and abdominal swelling. Signs of liver cirrhosis are spider nevi, palmer erythema, clubbing, gynecomastia, hypogonadism, enlarged, normal or shrunken liver, splenomegaly and ascites.[6]

Under the physiological conditions the balanced levels of procoagulant and anticoagulants determine the risk of hemorrhage and thrombosis. In chronic liver disease due to chronic hepatitis and underlying cirrhosis, this haemostatic imbalance leads to hypercoagulability which favors thrombosis. The end stage cirrhosis is however predominately associated with bleeding tendency.[7] The cause of hypercoagulability in chronic liver disease is the reduced level of protein C and increased level of factor VIIIa. As a consequence of hypercoagulability, the deep vein thrombosis, pulmonary embolism, hepatic and portal vein thrombosis may occur.[8] The conventional laboratory screening of the patients with PT and APTT are based on conversion of fibrinogen to fibrin that starts with 5% of whole thrombin generated thus leaving the remaining 95% unde-

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tected. As these tests are performed in the absence of thrombomodulin so the extent of reduction of anti-coagulants in liver cirrhosis remains undetermined.[9]

The clinical significance of decrease in anticoagulant protein C in chronic liver disease is currently unclear.

MATERIALS AND METHODS

This study was conducted at Department of Haematology, University of Health Sciences Lahore, in collaboration with Holy Family Hospital Rawalpindi. This work was done within time period of six month after obtaining permission from ethical committees of both institutes. It was a cross sectional comparative study. It included fifty patients of chronic liver disease. Liver biopsy of each patient was done to assess the stage of fibrosis. Patients on anticoagulants, oral contraceptives and congenital deficiency of protein C were excluded.

Subjects were divided into two groups according to early and advanced histological stages of fibrosis. Group I included patients of stage 0 – 3 and group II of stage 4 – 6. Each group comprised of 25 patients of CLD. All 50 patients had a percutaneous liver biopsy. All liver biopsies were evaluated blindly by a single liver histopathologist and histological changes of chronic hepatitis were classified according to the modified histologic activity index (HAI). The chronic hepatitis grading score (0 - 18), which represents necro-inflammatory activity was the sum of the piecemeal necrosis score (0 - 4), confluent necrosis score (0 - 6), focal lytic necrosis, apoptosis and focal inflammation score (0 - 4), and portal inflammation score (0 - 4). The chronic hepatitis staging score (0 - 6), which is referred to as the fibrosis score was based on the degree and extent of fibrosis, architectural alterations, and development of cirrhosis. Consecutive sampling technique was used for blood sampling. Consent was taken and an inclusion criterion was fulfilled by each patient selected. Protein C level was done on all patients. The quantitative assay PC is based on determination of protein C (PC) activity level in plasma by synthetic chromogenic substrate method by using STA Compact® auto analyzer (Diagnostica Stago France). The detection of chromogenic assay is based on the absorbance (optical density O.D) of monochromatic (405 nm) light.

Statistical Analysis

The data was analyzed using SPSS 16.0. Mean ± S.D were given for quantitative variables. Two independent sample t test was applied to observe mean differences in different groups. Two - tailed probability values of < 0.01 were considered of statistical significance.

RESULTS

Fifty patients of chronic liver disease were included in this study with mean age of 46.9 ± 6.9 (range 31 - 61) years. Out of 50 patients, 27 were male and 23 were female. They were divided into two groups of early and advanced fibrosis according to histological stages of fibrosis. The higher level of protein C was observed in group I. Mean value was 87.8 ± 15.6% and its range was 68 - 126%. Mean concentration of protein C (35.9 ± 17.2%) was decreased in advanced stage group II patients. Range of protein C in group II was 10 - 66%. A statistically significant difference ($p < 0.001$) was observed when mean levels of protein C were compared within groups.

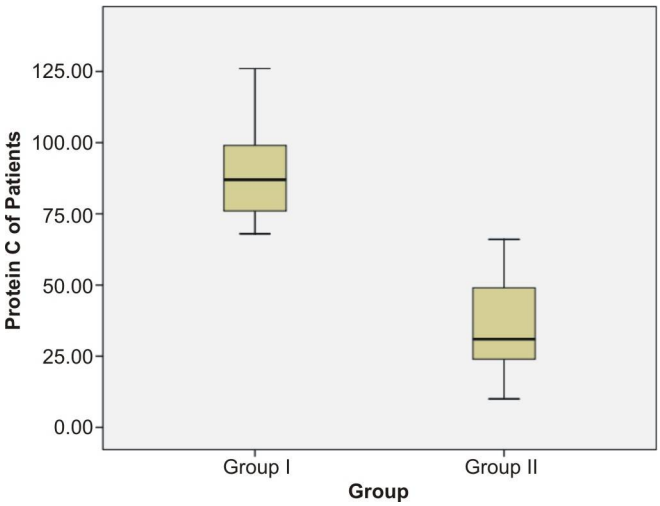

Fig. 1: *Box - plots for mean concentration of protein C in group I and group II. Boxes show range of protein C and horizontal line inside the boxes indicates the mean value. The decreased mean concentration of protein C was observed in advanced stages of fibrosis.*

DISCUSSION

Liver fibrosis and its end stage cirrhosis, is the common outcome of almost all patients of chronic liver disease. The common cause of cirrhosis is hepatitis B, hepatitis C and alcohol. Cirrhosis is a serious and irreversible disease characterized by replacement of liver tissue by fibrotic scar tissue as well as regenerative nodules, the portal flow of blood is blocked through the organ leading to progressive loss of liver function.[10] Complications of cirrhosis are the major cause of mortality and morbidity worldwide. It contributes to the overall deregulation of blood coagulation because of damaged hepatic cells which leads to decreased level of all the vitamin K dependent coagulation factors and anticoagulants especially antithrombin III and protein C.[11]

Our results demonstrated that the mean plasma protein C levels were significantly higher in group I of stage 0 - 3 as compared to the stage 4 - 6 of group II. In the present study we observed decrease in protein C level with advancement of fibrosis. The higher level of protein C was observed in group I with mean value of

87.8 ± 15.6% (range 68 - 126%). Mean concentration of protein C (35.9 ± 17.2%) was decreased in advanced stage group II patients. Range of protein C in group II was 10 - 66%. A statistically significant difference ($p < 0.001$) was observed when mean levels of protein C were compared within groups.

These low levels of protein C in patients with chronic liver disease can be used for assessment of fibrosis. Several studies have inspected the reduction of protein C in chronic liver disease as plasma concentration of protein C is significantly reduced in cirrhosis. In a study protein C levels were observed in advanced and early stages of fibrosis. They observed the decreased level of protein C (3%) in advanced stage as compared to early stage (24%) with statistically significant p value of 0.007.[12]

Another study conducted on 50 cirrhotic patients has shown that in comparison with controls the cirrhotic patients had significantly decreased protein C levels.[13] In another study, the coagulation inhibitor protein C was measured in patients with various liver diseases. The protein C level was significantly decreesed in patients with cirrhosis as compared to patients with steatosis with statistically significant p value of 0.001.[14] The endothelial receptors involved in the protein C pathway are thrombomodulin (TM) and endothelial protein C receptor (EPCR) as they regulate coagulation and inflammation processes. A study conducted on levels of soluble thrombomodulin and EPCR has postulated that they are the markers of hepatocellular carcinoma (HCC) and prognostic indicators of cirrhosis in chronic liver disease. EPCR levels were higher in patients (239 ± 1.8 ng/mL) than in controls (127 ± 1.5 ng/mL) with significant *p value of* 0.0001.[15]

It is ***concluded*** that the levels of protein C is reduced with advancing cirrhosis in patients of chronic liver disease.

Conflict of Interest

The authors declare no financial or non-financial competing interest.

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