



MEASUREMENT OF PLASMA PROTEIN C LEVEL AMONG SUDANESE PATIENTS WITH CHRONIC RENAL FAILURE

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ABSTRACT

Background: Protein C is a vitamin K-dependent plasma glycoprotein produce in liver, it is a key component of the anticoagulant system. Deficiency of this protein may lead to serious thrombotic complications. **Objective:** This study was conducted to investigate the effect of heamodialysis on protein C level among chronic renal failure patients in Sudan. **Material and Methods:** A total of 45 patients with chronic renal failure and 45 control subjects were involved in this study. Blood samples were collected from all patients in 1:9 volumes of sodium citrate solution 3.8%. plasma protein C level was determined by Enzyme Linked Immunosorbent Assay (ELIZA) method. **Results:** The present study reported that there was no significant change in Protein C level in chronic renal failure patients when compared to control group. The level of Protein C:Ag were 92.6+16.3 in CRF patients and 84.8+12.2 in controls ($p > 0.005$). **Conclusions:** there was no significant change in Protein C level in chronic renal failure patients when compared to control group.

KEYWORDS: protein C, chronic renal failure (CRF), Sudan.

INTRODUCTION

Chronic kidney disease CKD is a condition which there is a deterioration in function or structural of kidney with glomerular filtration rate (GFR) <60 ml/min /1.73m.^[1] According to the latest WHO data published in May 2014 Kidney disease death in Sudan reach 1.46% of total death population. Renal failure has been associated with bleeding or thrombotic events and these complications are common among patients with stage5 CRF, end stage renal disease ESRD.^[2] Up to 60% of patients with control venous catheter develop thrombosis disturbance in heamostasis are common complication of kidney disease, thrombotic complications have become the predominant cause of mortality and morbidity in this population.^[3] Hypercoagulable state in patients on chronic heamodialysis can be caused due to decreased in the endogenous levels of natural inhibitors of coagulation like protein C, S and antithrombin III.

Protein C is a single chain precursor synthesized in the liver as a 461 amino acid long PC is vitamin K. Dependent in activated precursor of a serine protease (APC), which is the key component in a physiologically important natural anticoagulant system. That is activated by binding of thrombin to endothelial transmembrane receptor thrombomodulin.^[4] The complex of thrombin and thrombomodulin activates Protein C and the

activated protein C in turn forms a complex with its cofactor protein S that has a high affinity to phospholipid membranes.^[5] That is of physiological importance since aPC inactivates preferentially the membrane-bound coagulation factors Va and VIIIa. Additionally, activated protein C processes profibrinolytic activity by inhibiting plasmin activator inhibitor-1 (PAI-1).^[6] Protein C deficiency may be inherited or acquired and associated with a variably increased risk of thrombosis; the prevalence of PC deficiency is 2-5% in patients with thromboembolic diseases. Several studies have confirmed this initial report and it has been shown that individuals with an isolated PC deficiency may run a 6 to 9 fold increased risk for venous thrombosis.^[7-8] Two types of PC deficiency states are recognized, in type I deficiency the plasma concentration of PC is reduced both in functional and immunological assay, reflecting a genetic defect causing a reduced biosynthesis of PC. Type II deficiency is characterized by normal protein C antigen levels, but with decreased functional activity. This type of defect reflect the synthesis of abnormal molecules with reduced function, the mutation in the PC gene have been characterized in a recently published data base.^[9] Because of the critical role that protein C play as an anticoagulant those deficiencies in PC or APC resistance lead to significant risk of forming blood clots. Previous studies have given somewhat conflicting

evidence on the effects of hemodialysis on protein C. Some studies found hemodialysis was found to induce an increase in protein C.^[17] But other studies found HD seem to favor the reduction of coagulation inhibitor, also HD consumption for such factors; probably the dialysis membrane itself could be a target.^[16] The aim of the present study was therefore to examine possible changes in the protein C system during hemodialysis treatment by studying the effects of hemodialysis on levels of protein C.

MATERIALS AND METHODS

This case-control study was carried out at Dr. Salma dialysis centre, Sudan. Between March 2017 to Aug 2017. Data were collected by structured interview questionnaire and from patients medical files A total of 45 patients with chronic renal failure and 45 control subjects were involved in this study. Patients were dialyzed 4 hours, three times a week. The study has been approved by the local ethics committee of ALneelain University.

Blood samples were collected from all patients in 1:9 volumes of sodium citrate solution 3.8%.

Laboratory methods: Plasma total PC (immunological activities) were measured by the sandwich technique of enzyme immunoassay (ELISA) using commercial assay kits from Aesku Diagnostica (Germany) and following the manufacturer's recommendations. The micro ELISA plate was coated with capture antibody specific for human protein C and the enzymatic activity was obtained by using TMB as a substrate and 1mol/L HCl to block the reaction.

Estimation of urea and creatinine: The method involved colorimetric determination of creatinine Assay Kit from RANDOX Reagents following manufacturer's instructions. Level of urea were also determined using Urea Assay Kit from RANDOX Reagents. The absorbance was measured at 510 nm and 580 nm for creatinine and urea respectively using spectrophotometer.

Data analysis was performed using statistical package for social sciences. SPSS V 21. The result expressed as the mean $\pm$ SD, program by the method of chi-square, student T test and personal correlation. The difference between mean of patients and control group with P-value ≤ 0.05 was considered statistically significant.

RESULTS

A total of 45 sample collected from CRF patients and 45 sample collected as control from healthy (non CRF individual) subject were participated in the study. The result of the study showed that the mean age of chronic renal failure patients was 61.6 $\pm$ 6.9 years; 28 (31.1 %) were males and 17(18.9%) were females. There was no significant change in Protein C level in chronic renal failure patients when compared to control group. Serum

creatinine and urea showed statistically significant increase when compared with control group (table 1). While no correlation existed between blood level of urea, creatinine and the observed protein C values ($r = 0.154$), P value 0.148 and ($r = 0.151$), P value 0.155 respectively as shown in figure (1, 2).

Table. 1: Mean value of protein C, S. Creatinine and urea among chronic renal failure (CRF) patients and control subjects of the.

Variable	CRF patient	Control	P.value
	mean $\pm$ SD	mean $\pm$ SD	
Protein C%	90.6 $\pm$ 14.2	85.41 $\pm$ 11.9	0.065
S.creatinin mg/dl	0.87 $\pm$ 0.29	8.26 $\pm$ 2.81	0.00
Urea	104.5 $\pm$ 28.39	29.0 $\pm$ 6.64	0.00

DISCUSSION

The effect of hemodialysis on Protein C levels remains controversial. The results of the present study showed that patient with renal disease on regular hemodialysis have normal protein C antigen level with no significant change on protein C from healthy person. Which is agreed with other studies done by Sorensen PJ, et al. showed no difference in predialysis plasma PC antigen level was demonstrated between the dialysis patients and normal control^[10], and the same finding found in study done by Yashitaka I et al, showed the predialysis plasma levels of protein C and protein S were not significantly different from normal control value.^[14] On the contrary study by Brandt P et al, in postdialysis reduction of AT III and PC has been suggested to result from increased consumption of AT III by infusing heparin or by thrombin generated through platelet activation, which could then lead to PC consumption.^[13] Also, Alegre et al. observed a decrease in immunoreactive protein C during hemodialysis.^[15]

The dialyzer initiates an inflammatory reaction with a release of cytokines during dialysis.^[12] However, there are no reports on the effects of an acute phase reaction on protein C synthesis, also anticoagulant in routine hemodialysis consists of a standard dose of heparin given as a bolus at the start of the dialysis treatment and this anticoagulant has not effect on immunoreactive protein C, for this reason protein C antigen level within normal range among chronic renal failure patients. Also our results showed that the mean age of CRF patients is 61.6 $\pm$ 6.9 years. This indicated end stage renal disease (ESRD) affect like study done by Stel VS et al, showed mean age of ESRD patients is generally over 60 years.^[11]

CONCLUSION

In conclusion there was no significant change in Protein C level in chronic renal failure patients when compared to control group.

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