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Blood Levels of Protein C Among Intensive Care Unit (ICU) Patients in Gaza, Palestine.

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Abstract*: Intensive care unit (ICU) is one of the largest, most expensive, and complex components of health care worldwide. However, assessment of protein C (PC) levels in ICU patients may help in identifying high risk groups and relevant therapy. The present work aims to estimate the PC levels among ICU patients and follow up their outcome. The present work is a cross sectional comparative study which was performed as at Al-Shifa hospital ICU and included 85 patients as well as 85 apparently healthy controls. Plasma PC levels, Kidney and liver functions were assessed biochemically for all subjects. PC levels were below the lower limit of normal range in 65.9% of patients (n=56) at admission, irrespective of the primary diagnosis and sex. PC levels were lower in non survivors (n=17; 20%) than in survivors. There were statistically significant differences in all hematological and biochemical characteristics of the patients as compared to the control group. however, for all liver and kidney function tests performed in the present study, no significant differences were reported for any of these tests between ICU patients with PC ≤ 70 % and those with PC > 70 %. There was a statistically significant correlation between PC levels and ICU outcomes. It was concluded that organ dysfunction was associated with a higher risk of ICU mortality. PC levels may be considered as early marker of organ dysfunction and hence the expected mortality at ICU.*

Key Words: Protein C, intensive care unit (ICU), Gaza, Palestine

Introduction:

Protein C (PC), an essential constituent of anticoagulation process in the human body, is a vitamin K-dependent zymogen glycoprotein circulating in blood plasma and the Activated protein C (APC) is regulating blood coagulation primarily by proteolytically inactivating proteins factors Va and VIIIa **[Stenflo, 1976; Spek et al., 1998]**.

Therefore, PC deficiency people are susceptible for different forms of blood vessel thrombosis **[Broekmans et al., 1983; Spek et al., 1998].** PC deficiency has been phenotypically classified into two types. In the commonest one, type I deficiency, there is a parallel reduction of PC activity and antigen levels due to the decreased production or the instability of normally functioning molecules. In type II deficiency, which is due mainly to the production of an abnormal PC molecule, PC activity is diminished to a greater extent than the antigen level **[Reitsma, 1997].** PC is activated in vivo by a thrombin–thrombomodulin–dependent reaction **[Esmon, 2004]** that boosted by the endothelial cell protein C receptor (EPCR) **[Taylor et al., 2001]**. Besides its well-defined anticoagulant activity, APC also has other vital functions and cellular activities that are mediated by APC-EPCR binding concomitant to subsequent splitting of proteolytically activated receptor 1 **[Riewald et al., 2002; Cheng et al., 2003].**

The PC system, the activated from APC, plays an essential regulatory role in hemostasis and the systemic response to acute inflammation. The anticoagulant activity of APC arises from activation of its inactive PC through catalysis with thrombin and most effectively when PC is bound to its EPCR and thrombin is complexes with thrombomodulin **[Esmon & Fukudome, 1995; Esmon, 2006].**

Abnormalities of APC or its activation process may be pathologically associated with other clinical conditions like disseminated intravascular coagulation, and purpura fulminans. Murine models of stroke and other coagulopathies were used to further investigate the other activities of APC system **[Griffin et al., 2004; Zlokovic et al., 2005; Fernández et al., 2006].** Counteracting and inhibiting the APC activity have been attributed to different plasma components **[Heeb et al., 1991; Fernández et al., 2006]**. Apart from the mild and moderate clinical conditions, patients managed in intensive care unit (ICU) are susceptible for the activated inflammatory pathways associated to their critical clinical condition. Published data revealed significant alterations in the hemostatic network in patients admitted to the ICU, with laboratory indication of diminished PC concentrations in sepsis patients compared to patients of severe trauma and neurosurgery **[Boldt et al., 2000; Brunkhorst et al., 2007].**

The determination of PC concentrations and their possible relationship to morbidity and mortality may help in identifying high-risk groups and potential therapeutic targets. Therefore, we set up the present work to estimate the PC levels among ICU patients and follow up their outcome.

Materials and Methods

All patients admitted to the ICU at AL-Shifa hospital, between March and July 2008, were screened for the level of protein C (PC), C- reactive protein (CRP) concentrations, as well as, complete blood count (CBC), liver and kidney function tests within 24 hours of admission. We excluded all patients younger than 15 years and patients suffering from malignant disorders. All patients involved in the present study were followed up until their discharge from the ICU. Apparently healthy subjects, matched for age and sex, served as normal controls and were recruited from a health care center to which they had been referred for a routine checkup at Al-Remal clinic. None of the controls had a history of arterial disease (stroke, myocardial infarction, angina, or peripheral vascular disease), venous thrombosis (pulmonary embolism or DVT), or known malignancy. The present study was approved by the Helsinki Ethical Committee at the Palestinian Ministry of Health.

Statistical Analysis

The data were tabulated, encoded and statistically analyzed using the IBM SPSS Statistics (version 17, IBM Corporation, Somers, NY).The Chi square test and the independent-samples t-test, were performed aiming at the description, identification of significant relationship, correlations and differences between the study items, variables and parameters. A p-value < 0.05 was considered statistically significant.

Results

Characteristics of the Study Group

The sample size of this study was 170 subjects with case-control ratio of 1:1 that matched by age and sex. Out of 150 patients admitted to ICU during the study period, 85 patients (57 male and 28 female) met the inclusion criteria and were enrolled in the study. The general characteristics of the study group are presented in table1**.** Forty patients (47.1%) were less than 30 years, 14 patients (16.5%) were 30 to 40 years, and 31 patients (36.5%) were more than 40 years. Twenty four patients (28.2%) were admitted after explosive injury (E.I), 16 patients (18.8%) were admitted after operative procedures, 5 patients

(5.9%) were admitted as acute renal failure (ARF), 4 patients (4.7%) were admitted as road traffic accident (RTA), 5 patients (5.9%) as diabetic ketoacetosis (DKA), 3 patients (3.5%) as chronic obstructive pulmonary disease (COPD), 6 patients (7.1%) as head trauma and head falling, and 22 patients (25.9%) had different primary diagnosis. The mean ICU length of stay (3.8± 4.0 days) in patients with PC concentration ≤70% is higher than that for patients (3.1± 4.2 days) with PC concentration >70%. The mean length of stay in ARF patients (1.6±0.9 days) was the lowest compared to other patients. PC concentrations decreased significantly in both survivors (68.7±23.6%), non survivors (50.5±14.3%), and critically ill (53.5±26.7%) as compared to the control group (95.1±7.9%), but were lower in non survivors than survivors.

The overall ICU outcome rate was 20% (n = 17) non survivors, 67.1% (n = 57) survivors, and 12.9% (n =11) critically ill.

Table 1 Characteristics of the patient group upon admission to the ICU (n = 85)

Variable	Categories	N	%	Length of stay Mean± SD	Protein C % Mean ±SD
	< than 30	40	47.1	4.5 ±5.0	63.9±24.4
	30 to 40	14	16.5	2.4 ±1.8	68.5±22.8
	> than 40	31	36.5	2.9±3.2	59.6±23.3
	Total	**85**	**100**	**3.6±4.1**	**63.1±23.7**
Gender	Male	57	67.1	4.0±4.4	61.8±24.2
	Female	28	32.9	2.6±3.3	65.6±22.9
	Total	**85**	**100**	**3.6±4.1**	**63.1±23.7**
Primary diagnosis	E.I	24	28.2	4.4±5.3	63.5±26.2
	Post operative	16	18.8	2.7±2.4	61.7±20.5
	ARF	5	5.9	1.6±0.9	55.2±19.9
	RTA	4	4.7	8.8±7.7	53.0±22.5
	DKA	5	5.9	1.8±0.8	62.6±18.8
	COPD	3	3.5	7.0±8.7	53.7±2.3
	head trauma and head falling	6	7.1	4.3±2.2	66.0±28.9
	Others*	22	25.9	2.6±1.8	67.9±26.5
	Total	**85**	**100**	**3.6±4.1**	**63.1±23.7**

Blood Levels of Protein C Among Intensive Care Unit (ICU).

ICU outcome	survivors	57	67.1	3.8±4.4	68.7±23.6
	non survivors	17	20	3.3±4.0	50.5±14.3
	critically ill	11	12.9	2.8±2.4	53.5±26.7
	Total	**85**	**100**	**3.6±4.1**	**63.1±23.7**
PC categories	≤70%	56	65.9	3.8±4.0	49.4±13.8
	>70%	29	34.1	3.1±4.2	89.5±14.5
	Total	**85**	**100**	**3.6±4.1**	**63.1±23.7**

EI = Explosive injury, ARF= acute renal failure, RTA= Road traffic accident, DKA = diabetic ketoacetosis, COPD = chronic obstructive pulmonary disease.
*Others=gastrointestinal, urogenital, epilepsy, hyperthyroidism, pre-eclampsia, liver cirrhosis, drug poisoning, burning, septicemia, meningitis, bronchial asthma and respiratory failure.

Hematological and biochemical characteristics of the study groups

There are statistically significant differences in all hematological and biochemical characteristics of the patients as compared to the control group. The mean ± SD as well as the p-values are presented in a comparable manner in table 2.

Table 2. Mean levels of biochemical parameters of patients and controls

Parameter	**Patient N=85** Mean ± SD	**Control N= 85** Mean ± SD	**P-Value**
Blood glucose (mg/dL)	178.5±147.3	93.7±18.4	0.000
Urea (mg/dL)	54.2±41.5	23.9±6.9	0.000
Creatinine (mg/dL)	1.3±1.4	0.7±0.2	0.000
ALT(U/L)	45.9±36.8	22.7±12.7	0.000
AST(U/L)	113.0±431.1	18.5±5.6	0.047
TP (g/dL)	5.4±1.2	7.2±0.2	0.000
Alb (g/dL)	3.0±0.8	3.3±0.2	0.001
TB (mg/dL)	0.9±0.6	0.7±0.1	0.000
DB(mg/dL)	0.4±0.3	0.3±0.04	0.018
Ca^{+2} (mg/dL)	8.7±0.9	10.4±0.4	0.000
WBC (10^9/L)	11.8±6.6	7.1±1.6	0.000
RBC (10^{12}/L)	3.9±0.9	4.8±0.5	0.000
Hb (gm/dl)	11.0±2.2	13.8±1.4	0.000
PLT (10^9/L)	184.2±110.2	265.5±59.8	0.000
PC (%)	63.1±23.7	95.1±7.9	0.000
CRP (mg/dL)	1.6±0.8	2±0	0.000
Neutrophil (%)	83.1±11.6	58.4±8.4	0.000

ALT=Alanine aminoransferase, AST=Aspartate aminotransferase, TP=Total Protein, Alb= Albumin, TB=Total Bilirubin, DB=Direct Bilirubin, Ca+2=calcium, WBC= White blood cells, RBC= Red blood cells, Hb= Hemoglobin, PLT= Platelets, PC= Protein C,CRP= C-reactive protein.

Evaluation of protein C concentration upon admission into ICU.
The initial PC concentration was below the lower limit of normal in 65.9% of patients (n = 56). The evaluation of PC levels upon admission to the ICU, stratified by the primary diagnosis is presented in figure 1. PC concentration decreased significantly in all patients irrespective of sex and diagnosis. Initial PC concentrations were lower in patients with RTA (n=4), compared with those who had COPD (n=3), ARF (n=5), post operative (n=16), DKA (n=5), EI (n=24), head trauma and head falling (n=6) and others (n=22) (Table 1). The minimal value reached was more pronounced in patient with RTA and EI compared with the other diagnosis and control group (Fig. 1). All control group had PC values more than 70%.

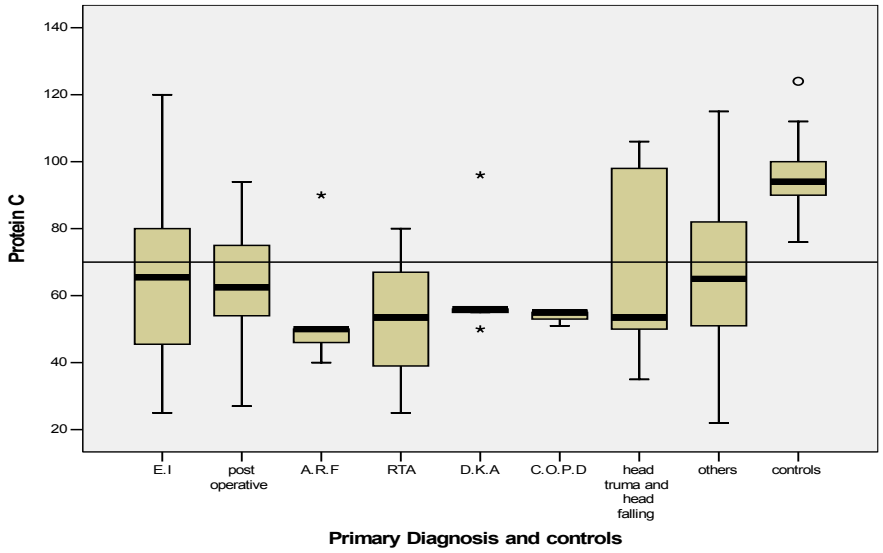

Figure 1. Box plots representing the protein C concentration (%) according to the primary diagnosis upon admission to ICU and controls. The dashed line represents the lower limit of normal for protein C (70%).

Evaluation of liver and kidney function tests among patients in relation to protein C level.

For all liver and kidney function tests performed in the present study, no significant differences were reported for any of these tests between ICU patients with PC ≤ 70 % and those with PC > 70 %. The mean ± SD, and the p-value of these parameters are mentioned in table 3

Table 3. Liver and kidney function tests among patients in relation to protein C.

Variable	Protein C ≤ 70 % N=56 Mean±SD	Protein C >70% N=29 Mean±SD	P-Value
ALT (u/L)	110.6±449.6	46.3±31.1	0.445
AST (u/L)	133.7±527.6	73.1±91.2	0.542
TB (mg/dL)	0.8±0.04	1.0±1.0	0.067
DB (mg/dL)	0.4±0.03	0.5±0.5	0.068
Urea (mg/dL)	59.2±46.1	44.6±29.0	0.124
creatinine(mg/dL)	1.4±1.2	1.2±1.8	0.417
TP (g/dl)	5.2±1.2	5.7±1.3	0.129
ALb (g/dl)	2.9±0.8	3.1±0.8	0.249
Na+(mmol/L)	143.1±8.6	142.3±7.8	0.718
K+ (mmol/L)	4.3±1.0	4.4±1.1	0.601
Ca^{+2} (mg/dL)	8.7±0.9	8.8±1.3	0.649

TP=Total Protein, Alb= Albumin, Na+=Sodium, K+=Potassium, Ca^{+2}=calcium ion. ALT= Alanin Aminotransferase, AST= Aspartate Aminotransferase, TB=Total Billirubin,DB= Direct Billirubin

Relationship between protein C levels and length of stay among survivors patients admitted to ICU.

It was found that there were no statistically significant correlations between PC level and length of stay among survivors patients (r= -0.149 , p= 0.269) as shown in Fig.2.

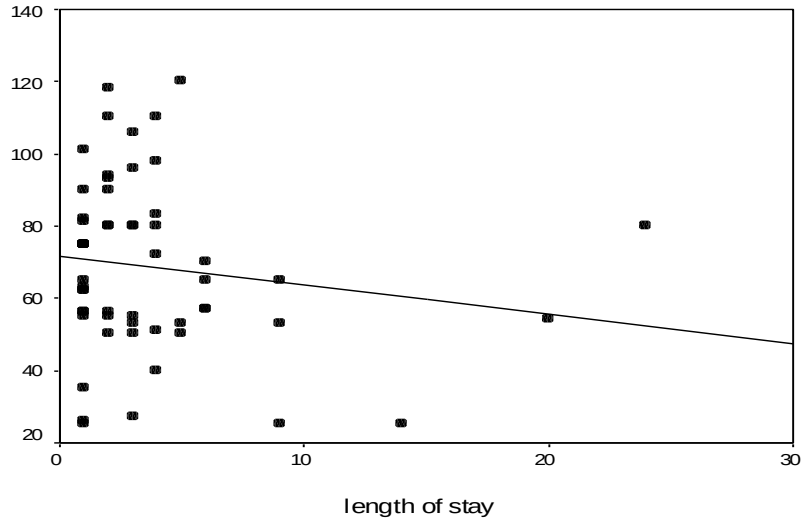

Fig.2. Correlation between protein C and length of stay at admission ICU among survivors patients

Relation between protein C levels and ICU outcome

Table 4. shows that out of 85 patients admitted to the ICU, 56 patients (65.9%) had PC levels below the lower limit of normal. Thirty patients (35.3%) who had PC levels below the lower limit of normal were survivors, and 16 patients (18.8%) who had PC levels below lower limit of normal were non survivors, 10 patients (11.8%) who had PC levels below the lower limit of normal were critically ill. On the other hand, 29 patients (34.1%) had PC levels more than the lower limit. Twenty seven patients (31.8%) had PC levels more than the lower limit were survivors, only one patient (1.2%) had PC level more than the lower limit was non survivor, another one (1.2%) had PC level more than the lower limit was critically ill. There was statistically significant relationship between PC and ICU outcome ($p= 0.001$), Pearson Chi-Square were (13.547).

Table 4. Correlation of protein C level with ICU outcome.

Variables	Categories	Protein C level			Total
		Statistics	≤70 %	>70 %	
ICU outcome	survivors	Count	30	27	57
		% of Total	35.3%	31.8%	67.1%
	non survivors	Count	16	1	17
		% of Total	18.8%	1.2%	20.0%
	critically ill	Count	10	1	11
		% of Total	11.8%	1.2%	12.9%
Total		Count	56	29	85
		% of Total	65.9%	34.1%	100.0%
Pearson Chi-Square = 13.547				P-Value=0.001	

Discussion

In this study, we measured PC levels and assessed both liver and renal functions in all admitted patients in the study period who met the inclusion criteria (n = 85) from the ICU at Al- Shifa hospital (57 males, 28 females). Age and sex matched healthy control group was also included in the study. All healthy control group members had normal PC levels as well as normal liver and kidney functions.

Relationship between protein C with gender among patients and controls.

Data showed that there were no statistically significant differences between males and females with regard to PC levels in patients and controls groups, which means that gender has no effect on the concentration of PC. These results are congruent with others who did

not find any statistically significant differences in PC levels, between males and females [**Brunkhorst et al., 2007**]. This result may be explained by the fact that PC is required for both sexes as they have the same mechanisms for maintaining homeostasis.

Values of protein C upon admission to ICU.

The main finding of our study is that PC concentrations were generally low in patients admitted to the ICU, irrespective of sex, and primary diagnosis. PC concentrations correlated to the organ dysfunction and were associated with a higher risk of ICU mortality. This result is in agreement with the study conducted by Frank Brunkhorst and his colleagues [**Brunkhorst et al., 2007**].

Low PC concentrations have been reported frequently in ICU patients. Previous observations [**Hesselvik et al., 1991; Kollef & Sherman, 1999; Mesters et al., 2000; Fourrier et al., 1992; Macias & Nelson, 2004**], however, have focused mainly on patients with sepsis syndromes, especially those with severe sepsis. The reason for the early decrease in PC concentrations is probably multifactorial [**Levi et al., 1997; Esmon et al., 1999; Osterud & Bjorklid, 2001; Levi et al., 2003**]. Therefore, the subsequent consumption of anticoagulation factors, including PC, is one possible reason for the decreased PC levels seen in ICU patients [**Levi et al., 2003**]. Impairment of hepatic protein synthesis may also be a contributing factor, due to associated hepatic dysfunction or substrate deficiency.

In our study, PC concentrations decreased significantly regardless of the primary diagnosis. However, the minimum PC concentration was observed in RTA patients, ARF and COPD than other patients groups. This may not be surprising, because the high degree of inflammatory reaction in those patients is expected to be associated with the magnitude of tissue damage. Our finding is in agreement with other related studies [**Brunkhorst et al., 2007**].

Intensive care unit length of stay (LOS), and protein C concentrations according to primary diagnosis, gender, age and ICU outcome.

The study showed that the highest number of patients was those who had explosive injury that makes our study different from others. The lower the PC levels, the longer was the LOS which required more

extracorporeal support. Median of LOS in male was higher than in female (Table 3), and the mean of PC levels was lower in non survivors than in survivors. The mean of PC levels in males was lower than in females. This result is supported by another study. On the other hand our study showed that the median of LOS in non survivors was lower than that in survivors a result which is not in agreement with others [**Brunkhorst et al., 2007**].

The ongoing conditions in our area are completely different from other areas in the world. We are subjected to continuous aggression and many people suffer injuries due to Jet-bombardments. In many situations ICU is faced with large numbers of injuries beyond its capacity to absorb them. ICU is forced to rearrange its priorities in dealing with such injuries. Sometimes injured patients are not able to get access to the hospital in the proper time which may be reflected on their outcome. In our study the mean of PC was (68.68±23.6%) in survivors patients, and was (50.47±14.3%) in non survivors patients. But in another study the mean of PC was (76.3±28.8%) for survivors patients and (66.5±36.8%) in non survivors patients [**Brunkhorst et al., 2007**]. This may be explained by the fact that our patients reach the ICU after a relatively long time of injury.

Our observations showed an increased mortality if the baseline of PC level is ≤70%. These results are consistent with previous studies that showed decreases in PC levels which precede overt clinical symptoms [**Bone, 1992; Fourrier et al., 1992; Mesters et al., 1996**], and may be predictive of potential mortality [**Brandtzaeg et al., 1989; Bone, 1992; Fourrier et al., 1992; Levi et al., 1993; Mesters et al., 1996**]. Hence, future investigations should focus on measuring PC levels as soon as possible after sepsis is suspected and it should be evaluated subsequently. This could potentially provide a more rapid and accurate assessment of the patient's status. If such studies confirm that rapid declines in PC levels can be readily detected, and preceding clinical deterioration, this information could be used to guide therapy.

ICU mortality

Our study showed that the overall ICU mortality rate was 20%. It is higher than the findings of another study from Germany that showed overall ICU mortality of 14.7% [**Brunkhorst et al., 2007**]. This finding could be explained on the basis of the conditions that our

society is faced with, especially the ongoing conflict and war on Gaza strip.

Protein C levels and ICU outcome

In our study we found that there was a significant correlation between the survivors and non survivors patients regarding PC level. However, another study showed no correlation at admission, but at 44-hours there was significant correlation [**Yan et al., 2001**]. This difference may be related to the difference between our patients and theirs as well as the time of admission to ICU which may be late in our hospital. Among the homeostatic markers investigated in this study, PC levels correlated best with the outcome of ICU patients (*ie*, survivors, non survivors and critically ill).

In conclusion, our study demonstrates that PC concentrations are generally low in ICU patients at Al-Shifa hospital. PC levels were associated with organ dysfunction/failure and were independently associated with a higher risk of ICU mortality. These findings suggest that targeting the PC pathway may improve outcomes in patients with multi-organ failure of non septic origin. The study confirmed that baseline PC levels are dependent predictor of outcome in ICU patients. Lower PC levels were common in ICU patients and were associated with several severely negative clinical outcomes, including increased mortality. These findings suggest that PC levels can be used prognostically and that such agents as PC, or preferably, activated PC, may reverse the acquired PC deficiency and improve outcome. Further research is needed dealing with large number of cases with emphasis on PC levels, and introduction of PC testing as a routine in ICU at admission and discharge.

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