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Tissue Factor Pathway Inhibitor and P-selectin as Markers of Sepsis Induced Non Overt Disseminated Intravascular Coagulopathy

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ABSTRACT

Background: Inflammation and coagulation occur concomitantly in sepsis and are intimately linked. Proinflammatory mediators can stimulate tissue factor (TF) expression on endothelial cells and circulating monocytes. Expression of TF is a primary mechanism of inflammation induced coagulation activation. It is the most important initiator of thrombin formation. Thrombin activates platelet which leads to P-selectin translocation and glycoprotein (GP) IIb-IIIa activation. P-selectin was shown to up regulate TF generation in monocytes and also to initiate signaling pathways in leucocytes and activate the elaboration of cytokines. Tissue factor pathway inhibitor (TFPI) is an endogenous anticoagulant that modulates initiation of coagulation induced by TF. Disseminated intravascular coagulopathy (DIC) is a major factor influencing mortality in sepsis. The term non overt DIC refers to a state of affairs prevalent before occurrence of overt DIC. In these cases the DIC score is less than 5. It was suggested that initiation of treatment in pre DIC state (non overt DIC) has better outcome than overt DIC.

Objective: This study investigated the role of TFPI level, P-selectin and thrombin activation markers in non overt and overt DIC induced by sepsis and its relationship to outcome and organ dysfunction as measured by the Sequential Organ Failure Assessment (SOFA) score.

Study design: The study included 176 patients with mean age 3±2.5 years. They admitted to the intensive care unit (ICU) of the Assiut University Pediatric Hospital. They included 144 cases of non overt DIC [group I; GIT infection with dehydration (n =66) and group II; respiratory tract infection (n=78)] and 32 children with overt DIC [group I; GIT infection with dehydration (n=15) and group II; respiratory tract infection (n=17)]. Twenty three healthy children of matchable age were included as control.

Results: There was a significant difference in haemostatic markers; platelet count, partial thromboplastin time (PTT), fibrinogen, D dimer levels, platelet activation marker (P-selectin), thrombin activation markers (thrombin antithrombin complex and prothrombin fragment 1+2), TFPI and DIC score between overt and non overt DIC in both groups. Statistically significant differences in plasma levels of haemostatic parameters between the non overt DIC and the control in both groups were found. The highest significant level of TFPI was present in GIT infection. However, in respiratory infection the level of TFPI did not differ significantly from the control. It was noticed that P-selectin was positively correlated with DIC score, fibrinogen consumption, fibrinolysis (D. dimer), thrombin activation markers and TFPI. TFPI was significantly correlated with fibrinolysis, DIC score and prothrombin fragment 1+2. There was a significant difference between overt and non overt DIC in SOFA score in both types of infection. SOFA score was positively correlated with DIC score, thrombin activation markers, TFPI and P-selectin and negatively correlated with platelet count and fibrinogen in overt DIC Patients. In contrast SOFA score was correlated only with prothrombin fragment 1+2 and partial thromboplastin time in non overt DIC.

In conclusion, the plasma TFPI concentration and P-selectin increases in the majority of the patients with non overt DIC with GIT and respiratory tract infection and they are useful for predicting outcome in DIC patients in the ICU. To improve the outcome of DIC patients, there is a need to establish more diagnostic criteria for non-overt-DIC. Plasma levels of TFPI and P-selectin may be helpful in this respect.

Key Words: *TEPI – P-selectin – DIC – Overt.*

INTRODUCTION

Pediatric sepsis remains a leading cause of death in children. Eighty percent of deaths in children can be classified as sepsis deaths **[1]**. Sepsis is defined as systemic inflammatory response syndrome (SIRS) in the presence of documented or suspected infection. When sepsis is associated with acute organ dysfunction, the sepsis is considered severe **[2,3]**. It has been reported that inflammation and coagulation occur concomitantly in sepsis and are intimately linked. Acute inflammation as seen in association with sepsis lead to systemic activation of

coagulation system [4]. Pro inflammatory mediators such as endotoxin, tumor necrosis factor α (TNFα), lipoproteins and growth factors can all stimulate tissue factor (TF) expression on endothelial cells and circulating monocytes [5]. Intravascular expression of TF is a primary mechanism of inflammation induced coagulation activation. It is the most important initiator of thrombin formation [6]. Thrombin activates platelet [7]. This in turn leads to P-selectin translocation and to glycoprotein (GP) IIb-IIIa activation, rendering them able to accomplish their functions in inflammation and hemostasis [8] P-selectin was not only shown to up regulate TF generation in monocytes [9] but also to initiate signaling pathways in leucocytes and activate the elaboration of cytokines in a mechanism that involved P-selectin interaction with its receptors. This process contributes to microthrombi formation. If this process goes unchecked by natural anticoagulants such as tissue factor pathway inhibitor, thrombin will propagate uncontrolled coagulation leading to organ dysfunction as seen in severe sepsis [5].

Tissue factor pathway inhibitor (TFPI) is an endogenous anticoagulant that modulates initiation of coagulation induced by TF [10,11]. It inactivates both factors Xa and TF-VIIa complex [12]. High dose of exogenous recombinant TFPI may increase this threshold and protect against disseminated intravascular coagulation (DIC) and venous thrombosis [13]. DIC is a major factor influencing mortality in sepsis. Previous studies suggest that initiation of treatment in pre DIC state (non overt DIC) gives better outcome than overt DIC [14]. An evolving score based on prothrombin time (PT), platelet count, fibrinogen, partial thromboplastin time (PTT) and D-dimer in the first 48 hours of intensive care reflected clinical severity [15]. A score of 5 or greater could diagnostically define patients with a poor prognosis from haemostatic dysfunction. If the score ≥5 it is compatible with overt DIC. If the score <5 it is suggestive (not affirmative) for non-overt DIC [16]. The non-overt DIC has a prognostic relevance [17,18,19].

Our study aims to investigate the role of TFPI, P-selectin and thrombin activation markers in overt and non overt DIC in gastrointestinal and respiratory infections with severe sepsis in relation to organ dysfunction measured by SOFA score. We also aim to detect their usefulness as markers for diagnosis of non overt DIC.

PATIENT AND METHOD

The study protocol was approved by the Human Ethics Review Committee of Assiut University, and signed consent from patient's parents was obtained. The study included 176 patients with mean age 3±2.5 years. They admitted to the ICU of the Assiut University Pediatric Hospital. They included 144 cases of non overt DIC [group I; GIT infection with dehydration (n=66) and group II; respiratory tract infection (n=78)] and 32 cases with overt DIC [group I; GIT infection with dehydration (n=15) and group II; respiratory tract infection (n=17)]. Twenty three healthy children of matchable age were included as control.

The groups of patients fulfilled the diagnostic criteria of non overt and overt DIC of International Society on Thrombosis and Hemostasis (ISTH) [20-22]. The patients also had manifestation of systemic inflammatory response syndrome (SIRS), which manifests itself as age dependant changes in temperature, abnormal heart rate, elevated respiratory rate and changes in white blood cell count in peripheral blood. However, at least one of them must be either temperature or white blood count [1]. The control group is healthy volunteers of the same age from the healthy pediatric and circumcision office; they were not receiving any medication at the time of blood sampling.

DIC score is a score equal the sum of 5 variables. A score 5 or more means at least 2 elements are affected. The scoring system for DIC proposed by ISTH, suggested that if the score ≥5, compatible with overt DIC. If <5, suggestive of non-overt DIC [16].

DIC score:

Score	P.T sec	PTT sec	Platelet $x10^9$/L	Fibrinogen g/L	D.dimer n/ml
0	≤13.5	28-41	>150	>1.8	≤1000
1	>13.5	<28->41	≤150	≤1.8	≤2000
2	≥15	<24->46	≤100	≤1.5	≤4000
3	≥18	≥61	≤80	≤1	>4000

The degree of organ dysfunction in patients with DIC was assessed by the sequential Organ Failure Assessment (SOFA) score system [18].

Sequential Organ Failure Assessment (SOFA) score system:

	SOFA score				
	0	1	2	3	4
Respiratory PAO2/FO2 mm/Hg	>400	≤400	≤300	≤200	≤100
Coagulation Platelet Count x 10^9/L	>150	≤150	≤100	≤50	≤20
Liver Billirubin mg/dl	<1.2	1.2-1.9	2-5.9	6-11.9	>12
Cardiovascular hypotension	No hypotension	Mean bl.p <70	Dop ≤5	Dop >5, epi ≤0.1	Dop >5, epi >0.1
Central nervous system Glascow coma scale	15	13-14	10-12	6-9	<6
Creatinine mg/dl Or urine output ml/dl	<1.2	1.2-1.9	2-3.4	3.5-4.9 or <500	>5 or <200

Dop, dopamineEpi, epinephrine, and FO2, fraction of inspirited oxygen.
Adenergic agents administrated for at least 1 hour.

Method:

Prothrombin time (PT) was determined by the one-stage method of Quick **[19]** using thromborel S (Behringwerke, Marburg, Germany). Plasma fibrinogen was measured by clotting methods using (fibri-prest Diagnostica Stago). Plasma levels of TAT by Enzygnst TAT micro enzyme immunoassay (Dade Behring) Marburg/Germany, Prothrombin fragment 1+2 by Enzygnst enzyme immunoassay (Dade Behring) Marburg/Germany. D-dimer was the fibrin-related marker utilized and D-dimer levels. By an ELISA kit from Biopool International (Umeå, Sweden; Tintelize. Human soluble P-selectin was detected by ELISA kit from R&D system NE, USA. Total TFPI enzyme immunoassay (Asserachrom Total TFPI, Diagnostica Stago, Asnieres, France).

The non-overt DIC and overt scoring template was applied and the score derived for each patient assessed against the outcome parameters of SOfA score.

Statistical methods:

All data were analyzed using SPSS (Statistical Program for Social Sciences version 11 for windows, 2001, SPSS Inc., Chicago, IL, USA). Comparisons between means for continuous variables were done using independent sample *t*-test and simple ANOVA. (Between groups). Values are represented as mean ± SD. Relationships in variables were assessed by correlation test A p value <0.05 is considered to be significant. All p values were two-tailed.

RESULTS

Our results are shown in Tables (1-4) and Figs. (1-8).

This study comprised 176 (101 males and 75 females with a mean age (3±2.5) patients admitted to the ICU of the Assuit University Pediatric Hospital Underlying conditions included non overt DIC groups [GIT infection (n=66) and respiratory tract infection (n=78)], DIC groups [GIT infection (n=15), respiratory tract infection (n=17)] fulfilled the DIC diagnostic criteria of overt DIC criteria and non overt DIC of ISTH **[13-15]**. Hemostatic data for GIT infection patients with overt and non overt DIC are described in Table (1). There was a significant difference in GIT infection group Between overt and non overt DIC in platelet count, PTT, fibrinogen, D dimer levels, platelet activation marker (P-selectin), thrombin activation markers (Thrombin antithrombin complex and Prothrombin fragment 1+2), Tissue factor pathway inhibitor and DIC score (Table 1) (Figs. 1,2).

Similar differences in haemostatic markers Between overt and non overt DIC in respiratory infection group Table (2) (Figs. 3,4). We demonstrated statistically significant differences of plasma levels of haemostatic parameters between the non overt DIC groups compared to the control in prothrombin time, fibrinogen level, thrombin activation markers and DIC score in the two groups compared to the control (Table 3).

Fibrinolysis diagnosed by qauantitative D-dimer was manifested in GIT infection compared to the other two groups. Platelet activation marker (P-selectin) was significantly increased in GIT infection compared to respiratory tract infection and the control (Fig. 5). The highest significant level of TFPI was achieved GIT infection, however respiratory infection was not differ significantly from the control (Table 3) (Fig. 6).

DIC score is positively correlated with its variables (D. dimer, and PT) also, it was correlated with thrombin activation markers (Thrombin antithrombin complex and Prothrombin fragment 1+2) *p* values were (0,001) for both. It was noticed that P-selectin was positively correlated with DIC score, fibrinogen consumption, fibrinolysis (D. dimer), thrombin activation markers (Thrombin antithrombin complex and Prothrombin fragment 1+2) and TFPI. *p* values were (0.003, 0.03, 0.004, 0.02, 0.001, 0.04) respectively. TFPI was significantly correlated with fibrinolysis, DIC score and prothrombin fragment 1+2 *p* values were (0.002, 0.01, 0.01) respectively. (data not shown in the tables).

SOFA score:

There was a significant difference between overt and non overt DIC in SOFA score *p*= (0.001) Tables (1,2) in both types of infection. The correlation coefficients (r) of SOFA was positively correlated with DIC score, thrombin activation markers, TFPI and P-selectin and negatively correlated with platelet count and fibrinogen in overt DIC Patients. In contrast SOFA score was correlated only with prothrombin fragment 1+2 and partial thromboplastin time in non overt DIC. (Table 4) (Figs. 7,8) (50%) died in overt DIC group versus 9% in non overt DIC *p*=0.01.

Figures: Box plots indicate 25th percentile, median value, and 75th percentile (horizontal lines of rectangle from the bottom, respectively) and 10th and 90th percentiles (horizontal lines outside rectangle).

Table (1): The studied parameters in overt and non overt DIC in patients with GIT infection.

Haemostatic parameters	Overt DIC Mean±SD N=15	Non overt DIC Mean±SD N=66	*p* value
Platelet count x 10^9/L	96±28	320±165	0.001
Prothrombin time (seconds)	17±2.7	17±3	NS
Partial thromboplastin time (seconds)	47±4	39±14	0.001
Fibrinogen (g/L)	0.7±0.4	1.2±0.5	0.01
Ddimer (ng/ml)	2300±1240	691±311	0.005
DIC score	5.8±0.8	2.9±1	0.001
Thrombin antithrombin complex (ug/L)	35±9	21.9±18	0.001
Prothrombin fragment 1+2 (pmol/L)	563±179	284±185	0.001
P-selectin (ng/ml)	63±9.0	51±17	0.004
Tissue factor pathway inhibitor (ng/ml)	159±42	92±39	0.01
Organ dysfunction (SOFA score)	6.6±1.2	2±1.8	0.001

Table (2): The studied parameters in overt and non overt DIC in patients with respiratory tract infection.

Haemostatic parameters	Overt DIC Mean±SD N=17	Non overt DIC Mean±SD N=78	*p* value
Platelet count x 10^9/L	91±28	316±19	0.001
Prothrombin time (seconds)	16.7±1.9	17±2	NS
Partial thromboplastin time (seconds)	46±2	39±12	0.001
Fibrinogen (g/L)	0.7±0.4	1.4±0.8	0.01
Ddimer (ng/ml)	1908±1000	476±297	0.005
DIC score	6±0.9	2.2±1	0.001
Thrombin antithrombin complex (ug/L)	35±9	13±10	0.001
Prothrombin fragment 1+2 (pmol/L)	524±190	215±144	0.001
P-selectin (ng/ml)	64±5.0	36±19	0.001
Tissue factor pathway inhibitor (ng/ml)	151±41	85±24	0.01
Organ dysfunction (SOFA score)	4.8±1.2	1.8±1.8	0.001

Table (3): The studied parameters in non overt DIC in patients compared to the control.

Haemostatic parameters	Group I GIT infection Mean±SD N=66	GroupII Respiratory infection Mean±SD N=78	Control Mean±SD N=23
Platelet count x 10^9/L	320±165	316±19	269±91
Prothrombin time (seconds)	17±3*†	17±2*	13.5±0.6
Partial thromboplastin time (seconds)	39±14	39±12	41±1
Fibrinogen (g/L)	1.2±0.5*	1.4±0.8*†	3.0±0.5
Ddimer (ng/ml)	691±311*	476±297*†	100±70
DIC score	2.9±1*	2.2±1*†	0
Thrombin antithrombin complex (ug/L)	21.9±18*	13±10*†	2.6±0.9
Prothrombin fragment 1+2 (pmol/L)	284±185*	215±144*†	105±41
P-selectin (ng/ml)	51±17*	36±19*†	28±6
Tissue factor pathway inhibitor (ng/ml)	92±39*	85±24	72±22
Organ dysfunction (SOFA score)	2.3±1.7*	1.8±1.8*†	0

*: Significant compared to the control.
†: Significant difference between group 1 and 2.

Table (4): Correlation between haemostatic parameter and organ dysfunction (SOFA score).

Haemostatic parameters	SOFA score in overt DIC		SOFA score in non overt DIC	
	r	*p* value	*r*	*p* value
Platelet count x 10^9/L	-0.3	0.004	NS	NS
Prothrombin time (seconds)	NS	NS	NS	NS
Partial thromboplastin time (seconds)	NS	NS	-0.2	.01
Fibrinogen (g/L)	-0.4	0.005	NS	NS
Ddimer (ng/ml)	0.3	0.001	NS	NS
DIC score	0.6	0.001	NS	NS
Thrombin antithrombin complex (ug/L)	0.5	0.006	NS	NS
Prothrombin fragment 1+2 (pmol/L)	0.5	0.001	0.5	0.005
P-selectin (n/ml)	0.4	0.03	NS	NS
Tissue factor pathway inhibitor (ng/ml)	0.5	0.001	NS	NS

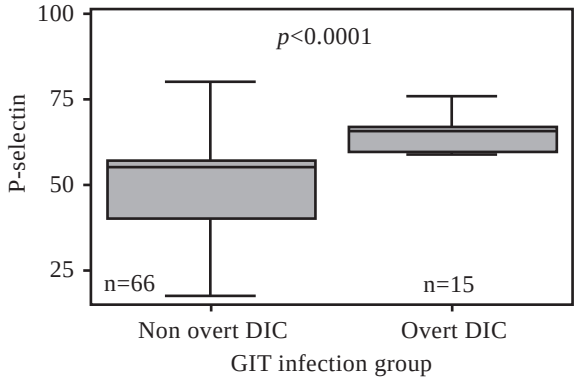

Fig. (1): P-selectin levels in GIT groups.

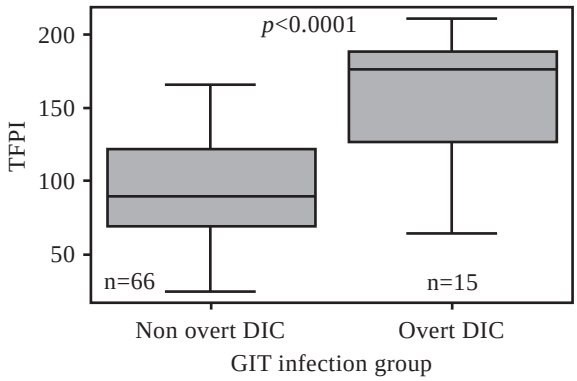

Fig. (2): TFPI levels in GIT groups.

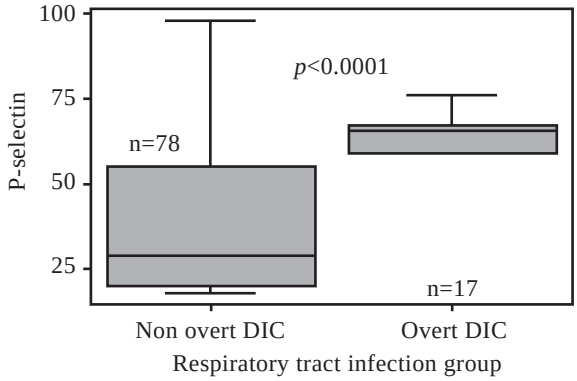

Fig. (3): P-selectin levels in respiratory groups.

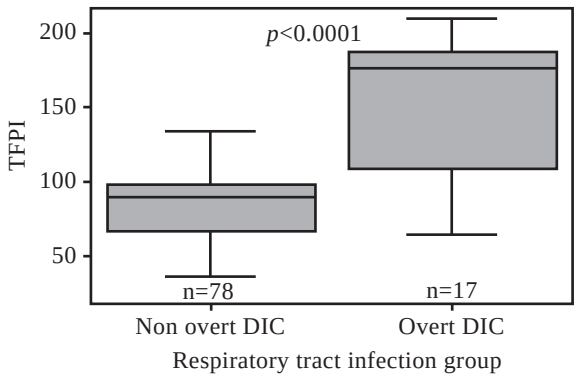

Fig. (4): TFPI levels in respiratory groups.

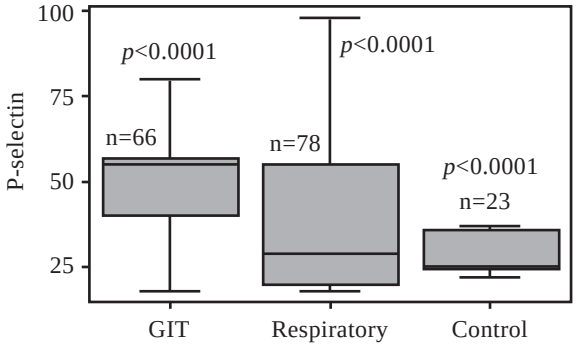

Fig. (5): P-selectin in non overt DIC groups compared to the control.

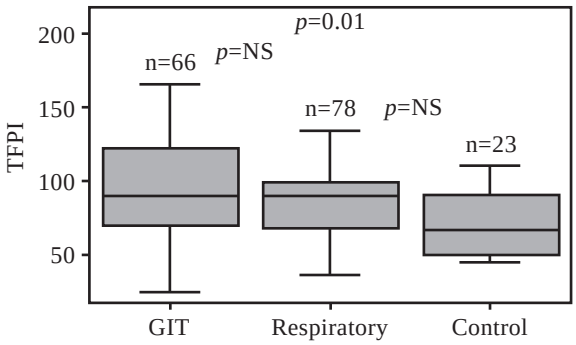

Fig. (6): TFPI in non overt DIC groups compared to the control.

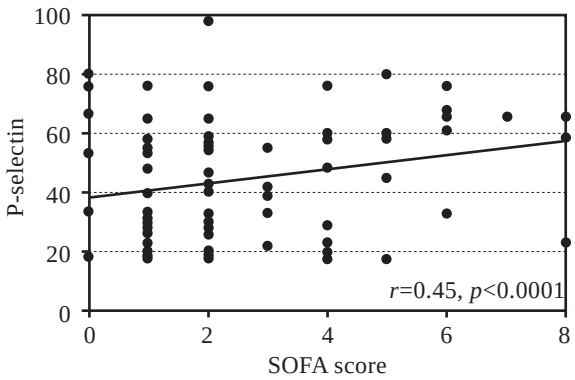

Fig. (7): Correlation between P-selectin and SOFA score in overt DIC.

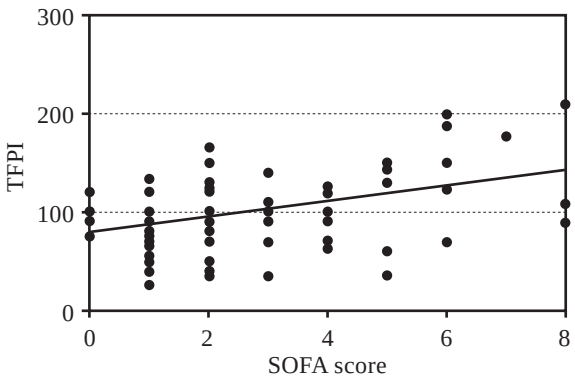

Fig. (8): Correlation between TFPI and SOFA score in overt DIC.

DISCUSSION

Sepsis with acute organ dysfunction is common, frequently fatal, and associated with a significant national health economic burden **[23]**. Abnormalities of coagulation and fibrinolysis are frequently observed in patients with sepsis. Endotoxins released from Gram-positive or negative bacteria can initiate the inflammatory cascade that characterizes sepsis which could change the properties of the vascular endothelium from anticoagulant to procoagulant. During infection and after stimulation with endotoxin or tumor necrosis factor, TF can be induced rapidly on blood mononuclear cells **[24]** and on vascular endothelium **[25]**. Fibrin deposition and complement activation can cause extensive vessel wall damage and may be associated with multiple organ failure **[26]**.

Generalized activation of coagulation depletes the body natural inhibitors including TFPI **[27,28]**. The previous studies have shown controversial results on the plasma TFPI concentrations in patients with DIC (overt and non overt) **[29,30]**. Our results showed that, plasma TFPI was elevated in patients with overt and non overt DIC. Simlar to our finding high concentration of TFPI has been reported in patients with septicemia **[31,32]**. Studies in primates showing that the coagulant response during bacteremia or endotoxemia could be completely blocked by monoclonal antibodies to TF or by infusion of the tissue factor pathway inhibitor (TFPI) which is capable of blocking the coagulant response completely and reducing the cytokine response **[33,34,35]**.

In our study TFPI remained within physiologic levels in non overt DIC in respiratory tract infection. Similar result was reported by Sabharwal et al. and Bajaj and Tricomi **[36,37]** who have studied the plasma levels of TFPI in patients with and at risk for acute respiratory distress syndrome (ARDS). They found that, the mean plasma TFPI levels in the patients at risk for ARDS group did not differ from the normal despite markedly increased TF levels in the patients with ARDS **[38,39]**. To explain the occurrence of non overt DIC in the presence of normal TFPI (as in respiratory group) or elevated TFPI levels (as in GIT infection), we have to take into account the following possibilities. The first, being that TFPI at physiologic concentration inhibits TF/VIIa effectively only after Xa, has been generated. Thus, TFPI does

not sufficiently prevent the coagulation process when continuing generation of TF occurs **[40]**. The second neutrophil elastase, a serine proteinase, cleaves TFPI. This impairs the ability of TFPI to neutralize both factors Xa and TF/VIIa. The third is that more recently Belaaouaj et al. **[41]** proved that matrix metalloproteinase derived from activated leukocytes cleave TFPI but not TF, factor VIIa, and factor Xa. Function analysis further demonstrated that matrix metalloproteinase-mediated cleavage of TFPI was accompanied by considerable loss of anticoagulant and anti-Xa activities **[41,42]**.

P-selectin had been established as a vascular adhesion molecule critical in the inflammatory response, in 1992. Palabrica et al. demonstrated that P-selectin also played a significant role in blood coagulation and thrombosis **[43]**. In our study P-selectin was elevated in patients with non overt DIC in both GIT and respiratory tract infection as well as in overt DIC. In accordance with our result Furie et al. **[44]** reported that P-selectin expression on platelets significantly increased in patients with severe SIRS in comparison with values in normal volunteers. During inflammatory states, intact endothelial cells release VWF and P-selectin from their Weibel-Palade bodies. Both molecules are ligands for GP Ib-IX-V. The newly released VWF binds platelets spontaneously **[45]**. P-selectin up regulate tissue factor generation in monocytes, and activates the elaboration of cytokines in a mechanism that involved P-selectin interaction with its receptor, PSGL **[46]**. A number of reports have secured that over expression of P-selectin can induce a pro coagulant state, that circulating micro particles bearing PSGL-1, the counter receptor for P-selectin, deliver tissue factor to the growing platelet thrombus **[44,45]**.

Our results showed that platelet counts and fibrinogen levels were significantly lower in overt than non overt group. DIC score, plasma levels of D-dimer and thrombin activation markers were significantly higher in patients with overt DIC than those with non overt –DIC and the later is higher compared to the control. In accordance with our results the plasma levels of, prothrombin fragment F1+2, TAT, and D-dimer were reported to be significantly higher in patients with systemic inflammatory response syndrome **[47,48]**. Prothrombin fragment F1+2, TAT reflect intravascular thrombin generation, but do not directly reflect microthrombi formation. D-dimer is considered to be the most useful marker for diagnosis of DIC and pre DIC **[49,50]**. These parameters are useful for the diagnosis not only overt DIC but also of non overt DIC. In our study prothrombin time did not differ significantly in overt and non overt DIC. In accordance with our results Hiedeo et al. **[51]** studied changes in prothrombin time in pre DIC and they concluded that prothrombin time was not a useful marker for diagnosis of pre DIC.

Fourrier et al. **[52]** identified consumptive coagulopathy as a strong predictor of death and multi organ failure in patients with sepsis in our study a significant difference was found in DIC score between overt and non overt DIC also a significant correlation was found between DIC score and SOFA score in overt DIC suggesting that the severity of DIC may be an important determinant of outcome **[47]**. In accordance with our results Toh and Downey demonstrated that, a score of 5 or greater could diagnostically define patients with a poor prognosis from haemostatic dysfunction, independent of developing overt DIC **[20]**. Different study designs by Toh and Downey et al. in different patient populations, found that a worsening coagulopathy augers a worse outcome in patients with severe sepsis and increased development of new organ failure **[22]**.

We use SOFA score to demonstrate the degree of organ dysfunction or failure as a determinant of outcome and we found that it was correlated significantly with TFPI and P-selectin only in overt DIC. To our knowledge there were no similar studies in the literature. Mean SOFA scores in overt DIC was 6.6±1.2 in GIT infection versus 4.8±1.2 in respiratory group. The Initial and highest scores of more than 11 or mean scores of more than 5 corresponded to mortality of more than 80% **[53]**. Other investigators performed serial SOFA scores to allow a more effective representation of the dynamics of illness including the effects of therapy compared with traditional outcome **[53]**. In accordance with our study Moreno et al. demonstrated that the initial SOFA score can be used to quantify the degree of organ dysfunction or failure present on admission **[54]**.

In conclusion, the plasma TFPI concentration and P selectin increases in the majority of the patients with non overt DIC with GIT and respiratory tract infection they are useful for predicting outcome in DIC patients in the ICU. To improve the outcome of DIC patients, there is

a need to establish more diagnostic criteria for non-overt-DIC. Plasma levels of TFPI and P-selectin may be helpful in this respect.

REFERENCES

1- Talmor M, Hydo L, Barie PS. Relationship of systemic response syndrome to organ dysfunction, lenth of stay, and mortality in critical surgical illness. Arch Surg. 1999, 134: 81-87.

2- Sigfrido Rangel-Frausto M. Sepsis: Still Going Strong. Arch Med Res. 2005, 36: 672-681.

3- Murugan R, Paul EM. Management of sepsis during the early golden hours. J Emergency Medicine. 2006, 31: 185-199.

4- Franchini M, Veneri D, Lippi G. Inflammatiom and hemostasis: abidirectional interaction. 2007, 53: 63-67.

5- Sscha Z, Verena S, Bernhard L, Walter AW, Erik H, Hans PK. Factor XIII in severe sepsis and septic shock. Thrombosis Research. 2007, 119: 311-318.

6- Arthur J Chu. Tissue factor mediates inflammation. Archives of biochemistry and Biophysics. 2005, 440: 123-132.

7- Macfarlane SR, Seatter MJ, Kanke T, Hunter GD, Plevin R. Proteinase-activated receptors. Pharmacol Rev. 2001, 53: 245-282.

8- Théorêt JF, Chahrour W, Yacoub D, Merhi Y. Recombinant P-selectin glycoprotein-ligand-1 delays thrombin induced platelet aggregation: a new role for P-selectin in early aggregate. Br J Pharmacol. 2006, 148: 299-305.

9- Liaw PC, Esmon CT, Kahnamoui K, Schmidt S, Kahnamoui S, Ferrell G, Beaudin S, Julian JA, Weitz JI, Crowther M, Loeb M, Cook D. Patients with severe sepsis vary markedly in their ability to generate activated protein C. Blood. 2004, 104: 3958-64.

10- Ten Cate H, Bauer KA, Levi M, Edgington TS, Sublett RD, Barzegar S, Kass BL, Rosenberg RD. The activation of factor X and prothrombin by recombinant factor VIIa in vivo is mediated by tissue factor. J Clin Invest. 1993, 92: 1207-12.

11- Sandset PM, Warn-Cramer BJ, Rao LVM, Maki SL, Rapaport SI. Depletion of extrinsic pathway inhibitor (EPI) sensitizes rabbits to disseminated intravascular coagulation induced with tissue factor: Evidence supporting a physiological role for EPI as a natural anticoagulant. Proc Nat Acad Sci USA. 1991, 88: 708-12.

12- Ariëns R A, Alberio G, Moia M, Mannucci PM. Low levels of heparin-releasable tissue factor pathway inhibitor in young patients with thrombosis. Thromb Haemost. 1999, 81: 203-207.

13- Kaiser B, Fareed J. Recombinant full-length tissue factor pathway inhibitor (TFPI) preventsthrombus formation and rethrombosis after lysis in a rabbit model of jugular vein thrombosis. Thromb Haemost. 1996, 76: 615-620.

14- Wada H,Wakita Y, Nakase T, Shmura M, Hiyoyama K, Nagaya S, Mori Y, Shiku H. Outcome of dissiminated intravascular coagulation in relation to the score when treatment was begun. Thromb Hemost. 1995, 74: 848-852.

15- Kinasewitz GT, Zein JG, Lee GL, Nazir SA, Taylor FB Jr. Prognostic value of a simple evolving DIC score in patients with severe sepsis. Crit Care Med. 2005, 33: 2214-2221.

16- Dhainaut JF, Shorr AF, Macias WL, Kollef MJ, Levi M, Reinhart K, Nelson DR. Dynamic evolution of coagulopathy in the first day of severe sepsis: Relationship with mortality and organ failure. Crit Care Med. 2005, 33: 341-348.

17- Zygun D, Berthiaume L, Laupland K, Kortbeek J, Doig C. SOFA is superior to MOD score for the determination of non-neurologic organ dysfunction in patients with severe traumatic brain injury: a cohort study. Critical Care. 2006, 10: R115.

18- Quick AJ. The prothrombin time in hemophilia and in obstructive jaundice. J Biol Chem. 1935, 109: 73-74.

19- Bajaj MS, Rana SV, Wysolmerski RB, Bajaj SP. Inhibitor of the factor VIIa-tissue factor complex is reduced in patients with disseminated intravascular coagulation but not in patients with severe hepatocellular disease. J Clin Invest. 1987, 79: 1874-1878.

20- Fletcher B Taylor Jr, Toh CH, Hoots WK, Wada H, Levi M. Towards definition, clinical and laboratory criteria, and a scoring system for disseminated intravascular coagulation* on behalf of the scientific subcommittee on disseminated intravascular coagulation (DIC) of the ISTH. Thrombosis and Haemostasis. 2001, 86: 1327-1330.

21- Toh CH, Hoots WK. The scoring system of the scientific and standardisation committee on disseminated intravascular coagulation of the international society on thrombosis and haemostasis: a 5-year overview Journal of Thrombosis and Haemostasis, J Thromb Haemost. 2007, 5: 604-606.

22- Toh CH, Downey C. Performance and prognostic importance of a new clinical and laboratory scoring system for identifying non-overt disseminated intravascular coagulation. Blood Coagul Fibrinolysis. 2005, 16: 69-74.

23- Dellinger R. Inflammation and coagulation: implication for the septic patient. CID. 2003, 36: 1259-1265.

24- Lorente JA, Garcia-Frade LJ, Landin L, de Pablo R, Torrado C, Renes E. A Time course of hemostatic abnormalities in sepsis and its relation to outcome Chest. 1993, 103: 1536-1542.

25- Key NS, Slungaard A, Dandelet L. Whole blood tissue factor procoagulant activity is elevated in patients with sickle cell disease. Blood. 1998, 91: 4216-4223.

26- Moldow CF, Bach RR, Staskus K, Rick PD. Induction of endothelial tissue factor by endotoxin and its precursors. Thromb Haemost. 1993, 70: 702-706.

27- Giyette RE. Hematology: a comprehensive guide to diagnosis and treatment of blood disorders. Los angles: PMIC, 1997.

28- Iversen N, strekerud FG, Abildgaard U. Tissue factor pathway inhibitor (TFPI) in disseminated intravascular coagulation: low levels of the activated factor X– TFPI complex. Blood coagul fibrinolysis. 2000, 11: S91-S98.

29- Bajaj MS, Rana SV, Wysolmerski RB, Bajaj SP. Inhibitor of the factor VIIa-tissue factor complex is reduced in patients with disseminated intravascular coagulation but not in patients with severe hepatocellular disease. J Clin Invest. 1987, 79: 1874-1878.

30- Novotny WF, Brown SG, Miletich JP, Rader DJ, Broze GJ. Plasma antigen levels of the lipoprotein-associated coagulation inhibitor in patients samples. Blood. 1991, 78: 387-393.

31- Sandset PM, Roise O, Aasen AO, Abildgaard U. Extrinsic pathway inhibitor in postoperative/posttraumatic septicemia: Increased levels in fatal cases. Haemostasis. 1989, 19: 189-195.

32- Takahashi H, Sate N, Shibata A. Plasma tissue factor pathway inhibitor in disseminated intravascular coagulation: Comparison of its behavior with plasma tissue factor Thrombosis Research. 1995, 80 (4): 339-348.

33- Carr C, Bild GS, Chang AC. Recombinant E. coli-derived tissue factor pathway inhibitor reduces coagulopathic and lethal effects in the baboon gram-negative model of septic shock. Circ Shock. 1994, 44: 126-137.

34- Camerota AJ, Creasey AA, Patla V, Larkin VA, Fink MP. Delayed treatment with recombinant human tissue factor pathway inhibitor improves survival in rabbits with gram-negative peritonitis. J Infect Dis. 1998, 177: 668-676.

35- de Jonge E, Dekkers PE, Creasey AA, Hack CE, Paulson SK, Karim A, Kesecioglu J, Levi M, van Deventer SJ, van Der Poll T. Tissue factor pathway inhibitor dose-dependently inhibits coagulation activation without influencing the fibrinolytic and cytokine response during human endotoxemia. Blood. 2000, 95: 1124-1129.

36- Sabharwal AK, Bajaj SP, Ameri A, Toricomi SM, Hyers TM, Dahms TE, et al. Tissue factor pathway inhibitor and von Willebrand factor antigen levels in adult respiratory distress syndrome and in a primate model of sepsis. Am J Respir Crit Care Med. 1995, 151: 758-767.

37- Bajaj MS, Tricomi SM. Plasma levels of the three endothelial-specific proteins von Willebrand factor, tissue factor pathway inhibitor, and thrombomodulin do not predict the development of acute respiratory distress syndrome. Intensive Care Med. 1999, 25: 1259-1266.

38- Gando S, Nanzaki S, Morimoto Y, Ishitani T, Kemmotsu O. Tissue factor pathway inhibitor response does not correlate with tissue factor induced disseminated intravascular coagulation and multiple organ dysfunction syndrome in trauma patients. Crit Care Med. 2001, 29: 262-266.

39- Gando S, Kameue T, Morimoto Y, Matsuda N, Hayakawa M, Kemmotsu O. Tissue factor production not balanced by tissue factor pathway inhibitor in sepsis promotes poor prognosis. Crit Care Med. 2002, 30: 1729-17234.

40- Broze Jr GJ. Tissue factor pathway inhibitor and the current concept of blood coagulation. Blood Coagul Fibrinolysis. 1995, 6: S7-S13.

41- Belaaouaj AA, Li A, Wun TC, Welgus HG, Shapiro SD. Matrix metalloproteinases cleave tissue factor pathway inhibitor: Effects on coagulation. J Biol Chem. 2000, 35: 123-128.

42- Gando S, Kameue T, Matsuda N, Hayakawa M, Morimoto Y. Imbalances between the levels of tissue factor and tissue factor pathway inhibitor in ARDS patients. Thrombosis Research. 2003, 109: 119-124.

43- Palabrica T, Lobb R, Furie BC, Aronovitz M, Benjamin C, Hsu YM, Sajer SA, Furie B. Leukocyte accumulation promoting fibrin deposition is mediated in vivo by P-selectin on adherent platelets. Nature. 1992, 359: 848-851.

44- Furie B. P-selectin and Blood Coagulation: It's Not Only About Inflammation Any More. Arterioscler Thromb Vasc Biol. 2005, 25: 877-8.

45- Wang WS, Lin JK, Lin TC, Chiou TJ, Liu JH, Yen CC, Chen PM. Plasma von Willebrand factor level as a prognostic indicator of patients with metastatic colorectal carcinoma. World J Gastroenterol. 2005, 11: 6072-3.

46- Weyrich AS, Elstad MR, McEver RP, McIntyre TM, Moore KL, Morrissey JH, Prescott SM, Zimmerman GA. Activated platelets signal chemokine synthesis by human monocytes. J Clin Invest. 1996, 97: 1525-1534.

47- Okabayashi K, Wada H, Ohta S, Shiku H. Hemostatic Markers and the Sepsis-Related Organ Failure Assessment Score in Patients With Disseminated Intravascular Coagulation in an Intensive Care Unit AM J Hematol. 2004, 76: 225-229.

48- Gando S, Kameue T, Nanzaki S. Participation of tissue factor and thrombin in posttraumatic systemic inflammatory response syndrome. Crit Care Med. 1997, 25: 1820-1826.

49- Pelzer H, Schwarz A, Heinburger N. Determiniutin of human thrombin anti-thrombin complex in plasma with an enzyme-linked immunosorbant assay. Thromb Haemost. 1987, 59: 101-106.

50- Kienast J, Thompson SG, Raskino C, Peltzer H, Fechtrup C, Osterman H, vande loo J. Prothromibin fragment 1+2 and thrombin-antithrombin III complexes in patients with angina pectoris: relations to the presence and severity of coronary atherosclerosis. Thromb Haemost. 1993, 70: 550-553.

51- Hideo W, Nobuo S, Yositaka M, Mikio T, Takahiro N, Minori S, kazuyo H, Masakatu N, Esteban CG, Kasumi D, Mutuyoshi K, Hi9roshi S. Haemostatic molecular markers before the onset of disseminated intravascular coagulation. Am J Hematol. 1999, 60: 273-278.

52- Fourrier F, Chopin C, Goudemand J, et al. Septic shock, multiple organ failure, and dissiminated intravascular coagulation: compared patterns of antithrombin III, protein C, and protein S deficiencies. Chest. 1992, 101: 816-823.

53- Ferreira FL, Bota DP, Bross A, Lot CM, Vincent JL. Serial evaluation of the SOFA score to predict outcome in critically ill patients. JAMA. 2001, 286: 1756-1758.

54- Moreno R, Vincent JL, Matos A, et al. The use of maximum SOFA scores to quantify organ dysfunction/failure in intensive care: results of a prospective, multicentre study. Intensive Care Med. 1999, 25: 686-696.