

## Study of the pathophysiologic role of tissue factor pathway inhibitor in modulating thalassemia induced hypercoagulable state: relationship to oxidative stress

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### ABSTRACT

*In view of the improved care of thalassemic patients which resulted in doubling of life expectancy, additional previously undescribed complications are now being recognized. In particular, a chronic hypercoagulable state has been observed in these patients, yet the actual mechanism of its production has not been fully elucidated. To our knowledge, the tissue factor pathway and its inhibitor have not been studied in thalassemics. The aim of the present work was to estimate tissue factor pathway inhibitor (TFPI) relation to oxidative stress assessed by thiobarbituric acid reactive substances (TBARS). Twenty patients with thalassemia major and ten healthy controls were enrolled in the study. Complete blood picture, serum ferritin, screening tests for coagulation, prothrombin and partial thromboplastin times (PT, PTT) and prothrombin activity (P activity %) have been estimated. The mean TFPI was higher in patients than controls but did not reach statistical significance, TBARS and serum ferritin were significantly higher in patients than controls ( $p=0.000$ ) reflecting the presence of oxidative stress. No relation was found between TFPI and chelating agent (desferrioxamine) intake, splenectomy or TBARS. In conclusion, tissue factor pathway (TFP) as reflected by increased TFPI could be, at least in part incriminated in the thalassemia-induced hypercoagulable state.*

**Key words:** Thalassemia-Hypercoagulability-tissue factor pathway inhibitor-Oxidative stress.

### INTRODUCTION

It is widely accepted that blood coagulation in vivo is initiated during normal haemostasis, as well as during intravascular thrombus formation, when the cell-surface protein, tissue factor (TF); is exposed to the blood as a consequence of vascular injury. In addition to its essential role in

haemostasis, tissue factor may be also implicated in several pathophysiological processes, such as intracellular signaling, cell proliferation, and inflammation. For these reasons the tissue factor, factor VIIa complex has been the subject of intense research focus<sup>(1)</sup>.

The classical cascade/waterfall hypothesis formulated to explain in

vitro coagulation organized the amplification process into the intrinsic and extrinsic pathways. Recent molecular biology and clinical data indicate that tissue factor/factor-VII interaction is the primary cellular initiator of coagulation in vivo. The process of blood coagulation is divided into an initiation phase followed by a propagation phase. The discovery of tissue factor pathway inhibitor further supports the revised theory of coagulation.<sup>(2)</sup>

It is well established that tissue factor is abundantly present in various extravascular tissues, in the adventitia of blood vessels, and in atheroma plaque. Thus in the event of plaque rupture or damage to the blood vessel wall, tissue factor is readily exposed to flowing blood, allowing it to form a complex with circulating factor VIIa in order to activate factor X both directly and indirectly via factor IX. Platelets quickly adhere to the injured site, facilitating localized thrombin formation and subsequent fibrin production. With each new layer of platelets and fibrin that adheres to the injured surface, the exposed tissue factor on the vessel wall, along with the localized circulating factors IX and X that is generated, becomes increasingly isolated from the events near the surface of the growing thrombus. The physical blanketing of an injured surface by platelets and fibrin in addition to the release of the platelet tissue factor pathway inhibitor, prevents factor IXa and factor Xa from diffusing more than a few tens of microns away from the vessel wall, far short of the 3 mm thickness needed for occlusive thrombosis. Thus an alternative factor Xa generating

mechanism must be involved that allows for the formation of prothrombinase activity far away from the vessel wall near the front of a growing thrombus.<sup>(3)</sup>

TF is best known as the primary cellular initiator of blood coagulation. After vessel wall injury; the TF; FVII a complex activates the coagulation protease cascade which results in fibrin deposition and platelet activation.<sup>(2)</sup> Tissue factor deficiency causes embryonic lethality in the mouse and there have been no reports of tissue factor deficiency in humans. These results indicate that tissue factor is essential for life most likely because of its central role of hemostasis. In addition, aberrant tissue factor expression within the vasculature initiates life threatening thrombosis in various diseases, such as sepsis, atherosclerosis and cancers. Finally recent studies have revealed a nonhemostatic role of tissue factor in the generation of coagulation proteases and subsequent activation of protease activated receptors (PARS) on vascular cells. This tissue factor dependent signaling contributes to a variety of biological processes including inflammation, angiogenesis, metastasis and cell migration.

Interactions between plasma and cellular components of the blood and damaged vessel wall promote the activation of the haemostatic process and ultimately thrombosis.<sup>(4)</sup> Experimental studies have demonstrated that inhibitors of TF: factor VII a procoagulant activity are powerful inhibitors of in vivo thrombosis and that this approach usually result in less pronounced bleeding tendency, as compared to

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other more classical antithrombotic interventions.<sup>(5)</sup>

High TF and TFPI levels in hypercoagulability states have been documented in unstable angina and various diseases.<sup>(6)</sup> Tissue factor pathway inhibitor (TFPI) is a plasma kunitz-type serine protease inhibitor, which modulates initiation of coagulation induced by TF. In a factor F(Xa)-dependent feedback system, TFPI binds directly and inhibits the TF-FVII/FVIIa complex. Normally TFPI exists in plasma both as a full length molecule and as variably carboxy terminal truncated forms. TFPI also circulates in complex with plasma lipoproteins. The level and dual inhibitory effects of TFPI on FXa and TF-FVII/FVIIa complex offers insight into the mechanism of various pathological conditions triggered by TF. The use of a selective pharmacological inhibitors has become an indispensable tool in experimental haemostasis and thrombosis research. In vivo administration of recombinant TFPI (rTFPI) in an experimental animal model prevents thrombosis (and re-thrombosis after thrombolysis), reduces mortality from E coli induced septic shock, prevents fibrin deposition on subendothelial human matrix and protects against disseminated intravascular coagulation (DIC), this TFPI may play an important role in modulating TF-induced thrombogenesis and it may also provide a unique therapeutic approach for prophylaxis and/or treatment of various diseases.<sup>(7)</sup>

$\beta$ -thalassemia is a congenital haemolytic disorder that is caused by partial or complete deficiency of  $\beta$ -

globin chain synthesis. The standards of care for thalassaemic patients have improved in recent years, resulting in doubling of the average life expectancy. As a consequence, additional previously undescribed complications are now being recognized. In particular, profound haemostatic changes have been observed in patients with  $\beta$ -thalassaemia major and intermedia, also in patients with  $\alpha$ -thalassaemia.<sup>(8)</sup> This has been evidenced by elevated thrombin-antithrombin complex (TAT), D-dimer<sup>(9)</sup> and prothrombin fragment 1+2 (F1+2).<sup>(10)</sup>

Iron accumulation and iron mediated organ damage consistently increase the morbidity and mortality of thalassaemic patients especially affecting the myocardium and heart. Despite chelation with desferrioxamine or the new oral chelators, still iron overload results in oxidative stress in these patients due to inability to comply sufficiently with the prescribed treatment. This is because the effective chelation regimen is unpleasant and cumbersome and may even alter iron homeostasis.<sup>(11)</sup> Another mechanism of producing oxidative stress in  $\beta$ -thalassaemia is the excess free  $\alpha$  globin chains due to decreased synthesis of  $\beta$  globin which induces oxidative damage to both integral and cytoskeletal proteins. The excess free  $\alpha$  haemoglobin chains also influence red blood cells redox mechanism in  $\beta$ -thalassaemia.<sup>(12)</sup>

The mechanism of the hypercoagulable state in thalassaemia has not been fully elucidated, however evidence from studies of other types of haemolytic anemia such as sickle

cell disease (SCD) in which thrombosis is also a major clinical entity<sup>(13,14)</sup>, may be helpful in understanding the etiology of the latter phenomenon. To our knowledge, the TFPI has not been studied in patients with thalassemia.

The aim of the present work was to investigate the pathophysiological role of the TFPI in modulating thalassemia-induced hypercoagulable state and its possible relation to the oxidative status of the disease.

## **MATERIAL & METHODS**

Twenty consecutive patients with  $\beta$ -thalassemia were enrolled in the study. They were 10 males and 10 females with a mean age of  $19.20 \pm 4.91$  years (range 12-31).

Ten healthy age and sex matched individuals served as control. Patients with thalassemia intermedia were excluded from the study as well as patients having hereditary thrombophilia. As regards chelation therapy, desferrioxamine intake was either regular (the patient receives desferrioxamine according to the conventional guidelines, 5 days/week subcutaneously at a dose of 40 mg/kg/day) or intermittent chelation (the patient receives irregular desferrioxamine, only with blood transfusion which is inadequate and is due to lack of compliance by the patient or due to unavailability of the drug). Patients were clinically examined and a laboratory workup was designed to patients and controls : complete blood count (CBC)<sup>(15)</sup> was determined by a Coulter Counter (Coulter Electronics, Luton, UC), reticulocyte count was determined by

the manual method<sup>(15)</sup>, serum ferritin by ELISA, prothrombin time (PT) and partial thromboplastin time (PTT) were done by the manual method<sup>(15)</sup>, determination of TFPI by ELISA was done<sup>(16)</sup> as well as thiobarbituric acid reactive substances (TBARS) as a marker of oxidative stress.<sup>(17)</sup> Hepatitis viral markers for HBV and HCV were also done .

Tissue factor pathway inhibitor (TFPI) assay was performed using the IMUBIND Total TFPI ELISA kit (product # 849); 222 Railroad Ave. P.O. Box 1165, Greenwich. It is a sandwich ELISA employing a rabbit anti-human TFPI polyclonal antibody as the capture antibody. Diluted plasma samples were incubated in microtest wells precoated with this capture antibody. TFPI was detected using a biotinylated monoclonal antibody specific for the Kunitz domain 1 of TFPI, the subsequent binding of streptavidin conjugated horseradish peroxidase completed the antibody enzyme detection complex which was visualized as a blue colour upon the addition of TMB substrate, sensitivity was increased by the addition of 0.5 M sulfuric acid stop solution; yielding a yellow colour. TFPI levels were determined by measuring sample solution absorbance at 450 nm and then interpolated from the standard curve. This ELISA detected both intact and truncated forms of TFPI.<sup>(16)</sup>

Statistical analysis of data was carried out using SPSS version 10 and p below 0.05 was considered significant.

## **RESULTS**

Clinical examination revealed that ten patients were splenectomized (50%) and ten had variable degrees of splenomegaly (50%). HBs antigen was found in six patients (30%) and HCV in eight patients (40%). No patients had evident thromboembolic events but four patients had evidence of pulmonary hypertension. As regards chelation therapy, desferrioxamine intake was regular in 7 patients (35%), five patients (25%) did not receive any while 8 patients (40%) receive intermittent chelation therapy.

Table (I) shows the haematological data of patients and controls. A statistically significant increase was found in serum ferritin and reticulocyte count in patients versus the control group ( $p=0.014$  &  $p=0.000$  respectively).

A statistically significant decrease in prothrombin activity was observed in patients compared to the control group ( $t = 0.098$ ,  $p= 0.000$ ).

As regards the PTT, no significant change was found on

comparing both groups ( $t = 1.535$ ,  $p = 0.136$ ).

As regards TFPI, higher mean values was found in patient versus control ( $t = 0.687$ ,  $p= 0.498$ ) yet it did not reach statistical significance. As for the TBARS ( $t = 6.115$ ,  $p = 0.000$ ) a statistically significant increase was observed in patients versus the control group.

Concerning TFPI and chelation therapy (desferrioxamine) in thalassemic patients (regular, intermittent or no intake), no statistical differences was observed among patients (table II).

As regards TFPI and spleen (Table III) there was also no statistical differences in between splenectomized or non splenectomized patients although splenectomized patients tended to have higher TFPI.

No correlation was observed between TFPI levels and platelet count (Figure: 1).

**Table (I): Haematological data in patients and control group (mean  $\pm$ SD).**

	Group	N	Mean	$\pm$ SD	Min	Max	t	P
Hb	Patient	20	7.36	1.84	4.20	11.10	-	0.000*
	Control	10	13.38	1.35	11.80	15.20	9.171	
RBC	Patient	20	3.29	0.94	1.72	4.90	-	0.000*
	Control	10	5.07	0.26	4.71	5.40	5.828	
MCV	Patient	20	76.10	13.58	56.00	116.00	-	0.036*
	Control	10	85.81	3.63	81.00	92.00	2.205	
MCH	Patient	20	23.28	6.03	13.40	38.60	-	0.144
	Control	10	26.29	2.60	22.40	29.70	1.503	
MCHC	Patient	20	30.38	2.97	23.90	34.60	-	0.791
	Control	10	30.70	3.47	26.50	34.80	0.267	
WBC	Patient	20	6.95	2.44	3.50	11.90	-	0.515
	Control	10	6.41	1.07	4.80	8.10	0.659	
Platelet	Patient	20	331.15	159.14	98.00	648.00	-	0.214
	Control	10	265.10	48.96	196.00	350.00	1.273	
Reticulocytes	Patient	20	3.45	1.44	1.60	6.80	-	0.000*
	Control	10	0.81	0.26	0.50	1.20	5.701	
Ferritin	Patient	20	1072.65	1239.68	24.00	4300.00	-	0.014*
	Control	10	30.20	9.47	19.00	47.00	2.636	
PTT	Patient	20	43.59	7.26	31.50	57.00	-	0.136
	Control	10	39.90	2.88	36.00	44.00	1.535	
TFPI	Patient	20	175.37	72.51	46.40	280.00	-	0.498
	Control	10	151.62	116.98	57.20	464.00	0.687	
TBARS	Patient	20	8.86	3.55	3.70	12.70	-	0.000*
	Control	10	1.91	0.51	1.30	2.90	6.115	
P Activity%	Patient	20	73.76	2.99	53.1	99	-	0.000*
	Control	10	97.8	1.78	90	100	0.098	

N=number \*significant as compared to control group.

**Table (II): Values of TFPI, TBARS and desferrioxamine intake in thalassemic patients (mean  $\pm$ SD)**

	Desferrioxamine intake	N	Mean	$\pm$ SD	Min	Max	F	P
TFPI	No	5	158.3	56.956	100.0	221.0	0.547	0.588
	Regular	7	6	5	0	0		
	Intermittent	8	198.7	70.852	108.0	280.0		
		4	165.5	85.160	46.40	265.0		
TBARS	No	5	7.56	2.7844	5.30	12.40	0.480	0.627
	Regular	7	8.96	4.2438	4.20	12.70		
	Intermittent	8	9.59	3.5240	3.70	12.40		
		5						

**Table (III): Values of TFPI, TBARS and spleen in thalassemic patients (mean  $\pm$ SD)**

	Spleen	N	Mean	$\pm$ SD	t	P
TFPI	Splenectomy	10	192.16	75.99	1.038	0.313
	Splenomegaly	10	158.58	68.56		
TBARS	Splenectomy	10	9.37	3.46	0.633	0.535
	Splenomegaly	10	8.35	3.75		

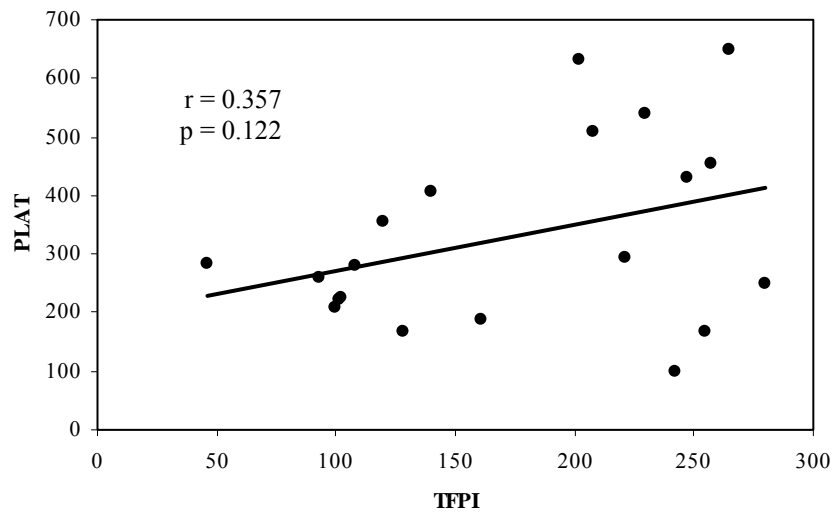


Figure (1): Correlation between TEPI and platelet count.

## DISCUSSION

The blood coagulation system establishes a delicate balance between the procoagulant and the anticoagulant functions of the blood with the vessel wall, thereby guarding against excesses in either direction to prevent unwanted haemorrhage or thrombosis. This is maintained through the controlled expression of tissue factor which is not likely to be expressed on the normal resting endothelium.<sup>(14)</sup>

Activation of vascular endothelium is considered an important facet of inflammation, vasculitis and thrombosis.<sup>(15)</sup> There is a strong evidence of endothelial cell activation in thalassaemic patients, and vascular complications are more frequent.<sup>(16-18)</sup> The infrequent occurrence of significant thrombotic events suggests that thrombosis is

largely a subclinical process, only revealed in autopsy findings.<sup>(8)</sup> The triggering insult is multifactorial; soluble leucocyte adhesion molecules<sup>(19)</sup> and von Willebrand factor have been reported to be elevated in thalasseemics.<sup>(20)</sup>

Moreover, the serum of thalasseemics has been found to induce the expression of these molecules in endothelial cell cultures from normal individuals.<sup>(19)</sup>

It is well known that induction of soluble intercellular adhesion molecule (sICAM), soluble vascular adhesion molecules (sVCAM) & E-selectin is mediated by tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ), Interleukin-1  $\beta$  (IL-1 $\beta$ ) and that these cytokines are elevated in sera of these patients.<sup>(21)</sup>

As a consequence of endothelial cell activation, monocytes, neutrophils and lymphocytes adhere to and

migrate through the endothelium into the tissues.<sup>(22)</sup> In support to this idea, evidence for endothelial activation in  $\beta$ -thalassemia patients was provided in the present work as a significant increase in TFPI, which reflects a compensatory mechanism to overcome the risk for thrombosis in these patients that result from increased tissue factor release from the injured endothelial cells.

In agreement to our findings, de Jonge<sup>(23)</sup> who studied TFP in endotoxaemia stated that TFPI, inhibits coagulation activation in a dose-dependent manner, and that plasma free TFPI antigen is positively correlated to the total amount of FVIIa and TF.<sup>(24)</sup>

In the present study, no statistical difference in TFPi was observed between splenectomized and non-splenectomized patients which agrees with Kyriakou et al<sup>(18)</sup> although our splenectomized patients had higher TFPI levels than the non-splenectomized. This finding could be explained as our patients had thalassemia major and not thalassemia intermedia which is associated with more enhanced capacity to generate thrombin<sup>(25)</sup>, as these patients receive less frequently blood transfusion. In these patients the percentage deformed RBCs is kept undiluted which explains the higher RBCs aggregability and thrombus generation and hence thrombotic events.<sup>(8)</sup>

Similarly, Atichartakarn et al.<sup>(26)</sup> confirmed the presence of markers of in vivo activation of coagulation in splenectomized versus non-splenectomized thalassemic patients. They attributed the hypercoagulability in thalassemic patients to the loss of

red cell deformability which produces increased surface expression of anionic phospholipids, phosphatidyle serine (PS) and phosphatidyle ethanolamine (PE). They provide a phospholipid layer that activates coagulation. It is suggested that the preferential clearing of these abnormal RBCs by the splenic macrophages protects against hypercoagulability in thalassemic patients. Moreover, aged RBCs contain higher amounts of (PS) on the outer leaflet of their membranes compared to young cells and this may serve as a signal for their recognition and removal by the reticuloendothelial system of the spleen.<sup>(8)</sup>

The fact that the PTT was normal in our study could reflect the integrity of the intrinsic system favouring the hypercoagulability to be mostly mediated by tissue factor pathway. In contrast paradoxically, the prothrombin activity was decreased in our patients which is attributed to liver impairment which is common in these patients.

Regarding the oxidative stress in our patients, TBARS were significantly increased in patients compared to the control which agrees with Meral et al.<sup>(27)</sup> who confirmed the elevated TBARS in thalassemic patients as markers of lipid peroxidation. Despite the lack of association between TFPI and TBARS in the present study, yet it does not refute the role of oxidative stress in endothelial activation. This could be explained by the fact that 40% of the patients in the present study did not receive adequate chelation therapy while 35% received regular regimen. Reactive oxygen species initiate nuclear factor Kappa  $\beta$



(NFK $\beta$ ) translocation from the cytoplasm to the nucleus of endothelial cells which switches on the transcription of a large number of genes associated with tissue factor, adhesion molecules<sup>(28)</sup>, cytokines and acute phase proteins.<sup>(29,30)</sup>

The lack of differences in TFPI which was observed in our patients receiving regular, intermittent or no chelation therapy could be explained by heterogeneity of thalassemia in terms of genotype, age, number and type of transfusion, frequency of infection and degree of iron overload.

In addition, our patients with HCV infection had the highest TFPI (data not shown). A possible explanation is that the increased secretion of TFPI may be a protective compensatory mechanism in response to the direct influence of antiphospholipid antibodies in these patients. In agreement to our findings, Adams et al.<sup>(31)</sup> demonstrated a high TFPI in subjects with antiphospholipid syndrome compared to the control, and they confirmed the presence of anti-tissue factor pathway inhibitor activity in these subjects. They added that these antibodies increase TF-like activity in monocytes and endothelial cells, thus altering the balance of the TF pathway with a net increase in thrombin generation and increased thrombotic risk. Moreover, patients with HCV have an immune vasculitis with positive cytoplasmic antineutrophil antibodies that could lead to thrombotic phenomena.

On the other hand no correlation was found between the level of TFPI and platelet count which is known to be higher in splenectomized thalassemic patients. We could

explain this discrepancy by the fact that the trigger that releases TF is not merely through platelets activation and release which is well documented in thalassemic but rather is due to endothelial injury.<sup>(32)</sup>

To the best of our knowledge, this is the first demonstration of an increased TFPI in thalassemic patients reflecting its pathophysiological role in modulating the hypercoagulable state in these patients. It ensues that thalassemic patients exposed to a transient additive thrombotic risk factor (e.g. surgery, pregnancy or immobilization) should receive prophylactic antithrombotic therapy.

The extrapolation of our data offers a potential insight for transfecting the arterial wall with natural inhibitors of tissue factor: factor VIIa complex such as tissue factor pathway inhibitor, which may result in complete inhibition of local thrombosis without increasing the potentially harmful systemic effects. Additional studies are warranted to determine the efficacy and safety of such approaches in thalassemic patients.

## REFERENCES

- 1- **Mackman N (2004).** Role of tissue factor in hemostasis, thrombosis and vascular development. *Arterioscler Thromb Vasc Biol*; 24(6): 1015-22 .
- 2- **Price G C, Thompson SA, Kam PC (2004).** Tissue factor and tissue factor pathway inhibitor. *Anaesthesia*; 59(5): 483-92.
- 3- **Hathcock J (2004).** Vascular biology – the role of tissue

- factor. *Semihematol*; 41(1.suppl1): 30-4.
- 4- **Doshi SN, Marmur ID (2002).** Evolving role of tissue factor and its pathway inhibitor. *Crit Care med*; 30(5): s241-50.
  - 5- **Golino P, Forte L, De Rosa S (2004).** Inhibition of the tissue factor coagulation pathway. *Curr Vac Pharmacol*; 2(4): 319-27.
  - 6- **Cori AM, Fedi S, Pepe G, Falciani M, Rogolino A (2002).** Tissue factor and tissue factor pathway inhibitor levels in unstable angina patients during short –term low – molecular weight heparin administration . *Br J H*; 117(3): 693-8 .
  - 7- **Lwaleed BA, Bass PS (2006).** Tissue factor pathway inhibitor:structure, biology and involvement in diseases. *J Pathol*; 208(3):327-39.
  - 8- **Eldor A, Rachmilewitz EA (2002).** The hypercoagulable state in thalassemia. *Blood*; 99(1): 36-43.
  - 9- **Cappellini MD, Robbiolo L, Coppola R, Manucci AP (2000).** Venous thromboembolism and hypercoagulability in splenectomized patients with thalassemia intermedia. *B J Haematol*; 111: 467-73.
  - 10- **Saleh A, Sadek NA, Badawy RH, El-Bordeny MM, Abdel Razek W (2004).** Prothrombin fragment 1+2 as a marker of Haemostatic activation in thalassemia –induced hypercoagulable state. MS Thesis, Faculty of Medicine, University of Alexandria .
  - 11- **Cohen AR, Galanello R, Pennell J, Cunmugham EV (2004).** Thalassemia. *Hematology*; 1-27.
  - 12- **Capellini MD, Tavazzi D, Duca L, Graziadei G (1999).** Metabolic indicators of oxidative stress correlate with haemichrome attachment to membrane band 3 aggregation and erythrophagocytosis in B-thalassemia intermedia. *BJH*; 104: 504-12.
  - 13- **Setty NY, Kulkarni S, Stewart MJ (2002).** Role of erythrocyte phosphatidylserine in sickle red cell-endothelial adhesion. *Blood*; 99(5): 1564-71.
  - 14- **Tomer A, Harker LA, Kasey S, Eckman JR (2001).** Thrombogenesis in sickle cell disease. *J Lab Clin Med*; 137: 398-407.
  - 15- **Barbaro J, Bain, Imeldo Bates (2001).** Basic haematology techniques. In: Dacie and Lewis. *Practical haematology*, 9<sup>th</sup> ed. Harcourt place. London, New York pp19-46.
  - 16- **Novotny WF, Girard T, Miletich JP (1989).** Purification and characterization of the lipoprotein associated coagulation inhibitor from human plasma. *J Biol Chem*; 264: 18832-7.
  - 17- **Bernheim F, Bernheim MLC, Wilbur KH (1984).** The reaction between thiobarbituric acid and the oxidation products of certain lipids. *L Biol Chem*; 174: 257-64.
  - 18- **Kyriakou DS, Alexandrakis MG, Kyriakou ES, Liapi D, Papadakis A (2001).** Activated
-

- peripheral blood and endothelial cells in thalassemia patients. *Ann Hematol*; 80: 577-83.
- 19- **Iolascon A, Giordano P, Storelli S, Coppola B, Maggio A (2001)**. Thrombophilia in thalassemia major patients: analysis of genetic predisposing factors. *Haematologica*; 86: 1112-3 .
- 20- **Sadek AN, Sultan MM, Badawi HR (2000)**. Role of adhesion molecules in endothelial cell activation in thalassemic patients and their correlation with the clinical finding. *Egypt J Hematol*; 25: 283-92.
- 21- **Butthep P, Rummavas S, Fucharoen S, Bunyaratvey A (2002)**. Increased circulating activated endothelial cells,vascular endothelial growth factor, and tumor necrosis factor in thalassemia. *Am J Hematol*; 70: 100-6.
- 22- **El-Alfy MS, El-Laboudy AM, Abdel Wahab ES (2002)**. Serum adhesion molecules (VCAM-1 and E-selectin ), vWF and nitric oxide in sickle cell anemia and  $\beta$ -thalassemic children. *Egypt J hematol*; 27(1): 45.
- 23- **De Jonge E, Dekkers PEP, Creasey AA (2000)**. Tissue factor pathway dose-dependently inhibits coagulation activation without influencing the fibrinolytic and cytokine response during human endotoxaemia. *Blood*; 95: 1124-9.
- 24- **Hansen IB, Grimsgaard S, Huseby N, Sandset PM, Bonnaa KH (2001)**. Serum lipids and regulation of tissue factor-induced coagulation in middle-aged men. *Thromb Res*; 102(1): 313.
- 25- **Cappellini MD, Robbiolo L, Bottasso BM, Coppola R, Fiorelli G, Manucci AP (2000)**. Venous thromboembolism and hypercoagulability in splenectomized patients with thalassemia intermedia. *B J Haematol*; 111(2): 467-73.
- 26- **Atichartakarn V, Aryurachai K, Chuncgarunee S, Thakkinstian A, Atamasirikul K (2002)**. Relationship between hypercoagulable state and erythrocyte phosphatidylserine exposure in splenectomized haemoglobin E/B-thalassemic patients. *Br J haematol*; 118: 893-8.
- 27- **Meral A, Tuncell P, Surmen-Gur E, Gunay U (2000)**. Lipid peroxidation and antioxidant status in beta-thalassemia. *Pediatr Hemayol Oncol*; 17(8): 687-93.
- 28- **Pawlak K, Naumnik B, Brzosko S (2004)**. Oxidative stress- a link between endothelial injury, coagulation activation and atherosclerosis in haemodialysis patients. *Am J Nephrol*; 24(1): 154-61.
- 29- **Belcher JD, Marker PH, Weber JP, Vercellotti GM (2000)**. Activated monocytes in sickle cell disease: potential role in the activation of vascular endothelium and vaso-occlusion. *Blood*; 96(7): 2451-9.
- 30- **Shebuski RJ, Kilgorek S (2002)**. Role of inflammatory mediators in thrombogenesis. *J*
-

Pharmacol Exp Ther; 300: 729-35.

- 31- **Adams M, Breckler L, Stevens P, Thom J, baker R, Ooytruck R (2004).** Anti-tissue factor pathway inhibitor activity in subjects with antiphospholipid syndrome is associated with increased thrombin generation. *Haematologica*; 89: 985-90.
- 32- **Kamal Mahmoud, Nadia A Sadek, Samia El-belbessy,**

**Mamdouh AG El-Ghandour and S Kadnil (1998).** Study of right and left ventricular functions and determination of the degree of pulmonary hypertension in patients with thalassemia major in relation to iron status and platelets. *Egyptian J Haematol*; 23(2): 243-25.

### دراسة الدور الذي يلعبه مثبط مسار العامل النسيجي في إحداث زيادة القابلية للتجلط في مرض أنيميا البحر الأبيض المتوسط - العلاقة بالإجهاد التأكسدي

فى ضوء التقدم فى العناية بمرضى أنيميا البحر الأبيض المتوسط والذي أحدث زيادة فى العمر الافتراضى لهؤلاء المرضى . ظهرت الآن مضاعفات جديدة لم تكن معروفة فى ما سبق وبالذات زيادة القابلية المزمنة للتجلط والتي لوحظت فى هؤلاء المرضى ولكن الآلية الفعلية لحدوث هذه الظاهرة لم يتم تفسيرها. وعلى حد معرفتنا فإن مسار العامل النسيجي ومثبطه لم يتم دراسته فى هؤلاء المرضى ولذلك كان الهدف من إجراء هذا البحث معرفة علاقة مسار مثبط العامل النسيجي بالإجهاد التأكسدي والذي يتم تقييمه باستخدام المواد النشطة لحامض الثيوبار بيثيوريك. وقد أجرى هذا البحث على ٢٠ مصاب بمرض أنيميا البحر الأبيض المتوسط و ١٠ أشخاص أصحاء استخدموا كمجموعة ضابطة. تم عمل صورة دم كاملة وتحديد نسبة الفيريتين أيضا تم إجراء اختبارات ماسحة للتجلط مثل نسبة البروثرومبين ووقت البروثرومبين الجزئى . وقد أظهرت النتائج أن متوسط نسبة مثبط مسار العامل النسيجي كانت أعلى فى المرضى مقارنة بالمجموعة الضابطة ولكن لم تصل إلى نسبة ذات دلالة إحصائية أما عن المواد النشطة لحامض الثيوبار بيثيوريك فقد كانت مرتفعة بدرجة ذات دلالة إحصائية فى هؤلاء المرضى عند مقارنتهم بالمجموعة الضابطة ( $p = 0.000$ ) وهذا يعكس حالة من الإجهاد التأكسدي. أيضاً لوحظ أنه لم توجد علاقة بين كلاً من مثبط مسار العامل النسيجي وكلاً من: أخذ العقاقير الكالبية للحديد فى الدم (الديسفيرال) أو وجود تضخم فى الطحال أو استئصاله وكذلك نسبة المواد النشطة لحامض الثيوبار بيثيوريك. فى النهاية يتضح أن نسبة مسار العامل النسيجي والتي يعكسها زيادة نسبة المثبط لهذا المسار من الممكن أن يكون لها دور ولو جزئى فى حالات زيادة القابلية للتجلط فى مرض أنيميا البحر الأبيض المتوسط.