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ORIGINAL ARTICLE

CIRCULATING THROMBOTIC AND HAEMOSTATIC COMPONENTS IN PATIENTS WITH CORONARY ARTERY DISEASE

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ABSTRACT

The study aimed to analyze the circulating levels of thrombotic and haemostatic components; tissue factor, tissue factor pathway inhibitor, tissue plasminogen activator and plasminogen activator inhibitor-1 in patients with acute myocardial infarction at presentation (Group 1, n=49), unstable angina and Non-ST elevated MI after treatment (Group 2, n=22), stable angina (Group 3, n=18) and healthy individuals (Group 4, n=31). Significant finding was increase in tissue factor not only in Group 1 (2.0 fold, P=0.001), Group 2 (2.2 fold, P=0.015) but also in Group 3 (1.8 fold, P=0.018) as compared to controls. In Group 1 Plasminogen activator inhibitor-1 increased significantly (35.8%, P=0.02). Tissue factor pathway inhibitor and tissue plasminogen activator demonstrated increase in Group 1 of age<40 years while insignificant changes in elder patients. Increased thrombotic and decreased fibrinolytic conditions in acute myocardial infarction patients were observed. Increase TF in stable angina demonstrates procoagulant status in these patients as well.

KEY WORDS

Acute Myocardial Infarction, Non-ST elevated MI, Thrombotic and haemostatic components, Stable angina.

INTRODUCTION

Plaque disruption and thrombus formation in coronary arteries lead to variable degree of luminal obstruction to the blood flow and can present clinically as unstable angina or acute myocardial infarction (AMI) and lead to sudden death (1). Three major determinants of thrombotic response are (a) the presence of local thrombogenic substrates (2), (b) the local flow disturbances (3) and (c) the systemic thrombotic propensity (1). Thus apart from the local thrombogenic potential even systemic pro-coagulant status may determine the severity of the acute event of thrombosis; one of the severe manifestation of coronary artery disease (CAD).

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Tissue factor (TF) at the upfront of the coagulation pathway plays a crucial role in initiating formation of thrombus after plaque rupture in patients with acute coronary syndromes. Tissue injury disrupts vascular endothelium causing its release into circulating blood and hence activation of coagulation cascades. It activates extrinsic pathway of coagulation and act as cofactor for Factor VII (fVII) and initiates cell surface procoagulant activity. It is also known to activate factor X through intrinsic pathway by activating factor IX, leading to thrombin generation and fibrin formation. Since Suefuji et al (4) reported the role of TF in AMI, there have been many studies conducted on determining the status of plasma TF and AMI (5-9) with contradictory findings.

Tissue plasminogen activator (tPA) activates plasminogen to plasmin which degrades fibrin resulting in fibrinolysis. Although literature documents elevated levels of plasma tPA to be

associated with AMI (10-15) decreased levels of tPA have also been reported to be associated with AMI patients who survived the attack and further suggests that these values could be predictive in determining the outcome (16).

Thus in the present project we aim to analyze the circulating levels of TF responsible for thrombus formation and tPA that attacks the coagulation and brings about fibrinolysis, and their inhibitors tissue factor pathway inhibitor (TFPI) and plasminogen activator inhibitor-1 (PAI-1) respectively in patients with AMI at presentation and stable angina patients on regular treatment and correlate with the degree of severity of CAD. Group of patients with unstable angina and Non ST elevated MI patients (NSTEMI) (not thrombolysed) who were stabilized with intense medical treatment after 4-5 days of the acute episode were also included to see the effect of the treatment.

MATERIALS AND METHODS

Subjects in the age group of 25- 70 years were selected for this study. CAD patients were angiographically verified with at least one major coronary artery having 50% of stenosis. They were further divided into stable angina, unstable angina, NSTEMI and AMI as per their clinical history and examination. AMI patients were with prolonged chest pain for more than 30 minutes and ST segment elevation more than 0.1 mV on at least two adjacent leads. NSTEMI patients were with elevated cardiac marker levels. Unstable angina patients were those having prolonged chest pain at rest with ST segment deviation on 12 lead ECG with normal cardiac marker levels and stable angina patients were with typical exertional angina and positive stress test. Exclusion criteria were diabetes, valvular heart disease, known cardiomyopathy, malignancy, liver diseases, renal insufficiency as well as subjects with current use of anti-inflammatory (except Aspirin) or immunosuppressive drugs. A group of healthy individuals were included under control group who were normotensives (SBP/DBP≤135/85 mmHg) with no clinical symptoms or complaints of any disease or on any medications. As per the selection criteria in each group subjects were recruited with their informed consent. Information regarding their demographic status, clinical history, family history and medication taken was noted down in detail. The ethical committee of Sir H. N. Hospital and Research Centre and Rajawadi Municipal Hospital approved the study protocol.

Peripheral blood samples were collected in plain bulbs, bulbs containing Na- EDTA and in Na- fluoride anticoagulants. Blood samples of AMI patients were collected at presentation within six hours of the acute symptoms before administering of thrombolysing therapy (Group 1, n=49). These patients were with first episode of chest pain and had neither known past history of such clinical symptoms nor were on any known medications for the same. Blood sample of patients with unstable angina and NSTEMI patients who were stabilized after treatment (Group 2, n=22) and stable angina (Group 3, n=18) were collected on the day of the coronary angiography just before coronary intervention. For Group 2 it was 4-5 days of the acute event. As fibrinolytic activity in blood follows a diurnal rhythm, only those patients whose blood sample was collected in the morning hours were included. Twelve hour fasting morning sample was collected from the Control group (n=31). One aliquot of serum separated from the plain bulb was used for biochemical investigations. Na-EDTA blood was sent for hematological investigation. Those subjects with fasting glucose more than 110mg/dl; serum transaminases, blood urea nitrogen (BUN) or creatinine levels beyond normal range and an abnormal ECG were excluded from controls. The other aliquot of serum was stored at -80°C.

The estimation of soluble levels of TF, TFPI-1, tPA, PAI-1 and hsCRP was carried out using the commercially available enzyme- linked Immuno Sorbent assay (ELISA) kits obtained from Immubind American Diagnostica®. The minimum detectable levels of TF, TFPI, TPA and PAI-1 ELISA kits were 10pg/ml, 0.180ng/ml, 1.0ng/ml and 0.125ng/ml respectively. The intra assay coefficients of variations were 8.2%, 8.6%, 5.2% and 7.5% and inter assay coefficient of variations were 4.8%, 9.5%, 7.6% and 9.2% of TF, TFPI, TPA and PAI-1 respectively. The assay for TF measured TF and TF/fVII complexes. TFPI ELISA detected both intact and truncated forms of TFPI, as well as complexes with TF and fVIIa. tPA ELISA measured human tPA antigen in plasma, single- and two-chain tPA as well as tPA/PAI-1 complexes. The PAI-1 ELISA assay measured latent (inactive) and active forms of PAI-1, as well as tPA/PAI-1 and uPA/PAI-1 complexes. Serum hsCRP was analyzed using enzyme immunoassay test kit from BioCheck, Inc. Foster City (CA) with minimum detectable level of 0.1mg/L. The intra and inter assay coefficient variation of assay were 4.25% and 5.95% respectively.

Results are expressed as frequency and percentages. Continuous variables are calculated as mean±SD. Comparison of significance of difference between two groups is carried out by student's unpaired 't'- test and among more than two groups by ANOVA. For parameters showing skewed distribution median with 25^{th} and 75^{th} percentile are expressed. Non-parametric tests, such as Mann Whitney- U test is performed for comparing significance between two

medians and Spearman Correlation to demonstrate correlation.

RESULTS

It can be seen from the demographic data (Table 1) that the average age of patients under Group 1, Group 2 and Group 3 increased by 13.7%, (non-significant [NS]) 27.1% (P=0.001) and 29.1% (P=0.001) respectively than that of the control group. The systolic Blood Pressure (SBP) of all the patient groups was higher as compared to the Control group while in stable angina group diastolic blood pressure (DBP) was also significantly elevated by 10.54% (P=0.001). Table 2 depicts the levels of TF, TFPI, tPA, PAI-1 and hsCRP in AMI patients at presentation (Group 1), unstable angina and NSTEMI patients after treatment (Group 2) and stable angina patients (Group 3) as compared to controls.

There was significant increase in TF of 2.0 fold (P=0.001), PAI-1 of 35.8% (P=0.02) and hsCRP of 2.0fold (P=0.019) in AMI patients of Group 1 at presentation as compared to controls. TFPI and t-PA levels showed insignificant changes as compared to the controls (Table 2). Table 3 depicts data of Group 1 patients of < 40 years and ≥ 40 years of age. Group 1 AMI patients below 40 years (younger AMI patients) demonstrated similar trend with respect to TF and PAI-1 as seen overall while there was although non-significant but increase in tPA (34.7%), and TFPI (50.7%) as compared to respective controls as well. Elder AMI patients (age group ≥ 40 years) as compared to respective controls demonstrated significant increase in TF (1.8fold, P=0.035) and insignificant changes in TFPI and tPA; similar to that seen overall however the increase in PAI was only 24.9% (NS). hsCRP trend remained similar as overall in both the age groups as compared to respective controls.

There was increase in TF (2.2 fold, P=0.015), TFPI (27.6%, NS), tPA (35.9%, P=0.044) and hsCRP (71.4%, NS) while PAI-1 decreased by 28.8%. (P=0.001) in unstable angina and NSTEMI patients after treatment as compared to controls (Table 2). In this group there was strong correlation of TFPI with hsCRP (rs=0.668, P=0.003). Age matched analysis with respective controls (age ≥ 40 years) demonstrated similar trend as overall (Data not shown). As compared to Group 1 AMI patients at presentation, Group 2 patients after treatment demonstrated insignificant change with respect to TF, hsCRP and non-significant but moderate increase in TFPI (42.1%) (Figure 1a), tPA (28.8%) and significant decrease in PAI-1 (47.5%, P=0.001) (Figure 1b). Age matched analysis between two groups demonstrated similar trend (Data not shown).

In stable angina patients there was significant increase of 1.8 fold (P=0.018) in TF and non-significant decrease of 25.1% in TFPI as compared to controls. tPA was increased by 22.2% (NS) while PAI-1 was decreased by 26.0% that was statistically significant (P=0.001) and median value of hsCRP was almost similar to controls (Table 2). Analysis of stable angina patients

Table 1: Demographic Data

	Controls	Group 1	Group 2	Group 3	P value (ANOVA)
No. (male/female)	31(14/17)	49(36/13)	22 (19/3)	18 (11/7)	
Age (Years)	44.6±7.57	50.7±10.9	56.7± 10.2	57.6±10.2	0.001
Age group (<40 /≥40 years)	7/24	5/44	1/21	1/17	
SBP (mmHg)	115.5±9.72	131.2±27	131.7± 11	131.7 ±3.2	0.003
DBP (mmHg)	78.7±5.7	81.2±24.4	79.4 ±6.8	87.0 ±1.7	NS
Total Cholesterol (TChol) (mmol/l)	5.1± 0.84	4.68 ±1.1	4.47 ±0.85	4.24 ± 1.1	0.015
HDL-Cholesterol (mmol/l)	1.23± 0.26	1.04± 0.2	1.06 ±0.21	1.00 ± 0.19	0.002
TChol /HDL-Cholesterol	4.2±0.67	4.5±0.76	4.2± 0.8	4.61±0.01	NS
Triglyceride(mmol/l)	1.34± 0.54	1.57±0.76	1.57± 0.71	1.46± 0.5	NS
LDL-Cholesterol (mmol/l)	3.30± 0.67	2.88±0.81	2.48± 0.63	2.87± 1.07	0.004
Smokers	5 (16.1%)	15 (30.6%)	2 (8.69%)	2 (11.1%)	
Alcohol	5(16.1%)	9 (18.4%)	3 (13.04%)	1 (5.6%)	
Hypertension	0	10 (20.4%)	12 (52.2%)	6 (33.3%)	
Diabetes mellitus	0	11 (22.4%)	8 (34.7%)	2 (11.1%)	

NS: Non-significant

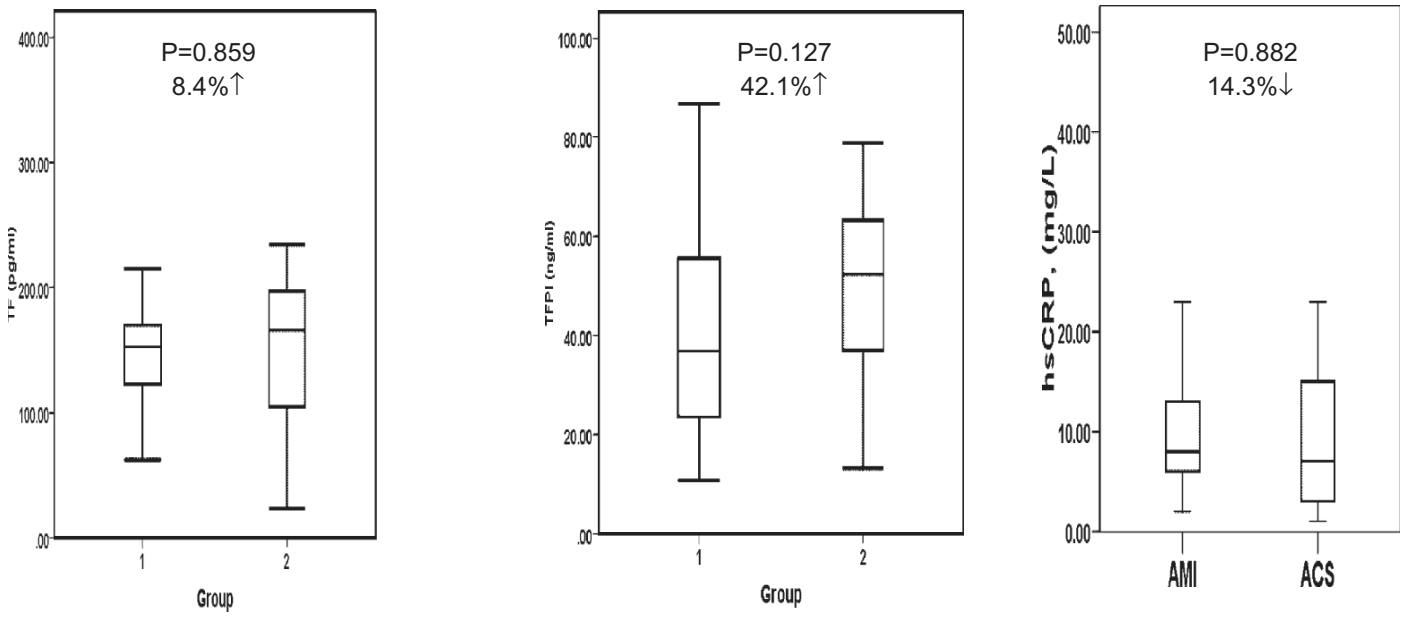

Fig 1a: Circulating levels of TF, TFPI and hsCRP in unstable angina and AMI patients after treatment of the acute event (Group 2) as compared to AMI patients at presentation (Group 1)

with age matched controls (age ≥ 40 years) demonstrated similar findings (Data not shown).

Table 4 and Table 5 depict the data of smoking and alcohol consumption habit of Group 1 AMI patients with respective controls wherein the trend remained similar as overall except PAI-1 was significantly elevated of only non smokers and of patients without alcohol consumption with respective controls.

Table 2: Hemostatic and Thrombotic Parameters in Controls and Coronary Artery disease Patient Groups

	Controls (n=31)	Group 1 (n=49)	Group 2 (n=22)	Group 3 (n=18)
TF (pg/ml)	74.00 (44/130)	152.7 (118/190) 2.0fold ↑ P=0.001	165.6 (96.7/197.8) 2.2fold↑ P=0.015	136.5 (117/165) 1.80 fold↑ P= 0.018
TFPI (ng/ml)	41.0 (30/46.8)	36.8 (22/57.8) 10.24%↓ NS	52.3 (35.6/67.2) 27.6%↑ NS	30.7 (24/48.6) 25.1%↓ NS
tPA (ng/ml)	10.23 (7.9/15)	10.8 (9.2/13.2) 5.5%↑ NS	13.9 (9.8/19.7) 35.9%↑ P=0.044	12.5 (10.1/16.8) (22.2%↑) NS
PAI-1 (ng/ml)	61.20 (54/73.6)	83.10 (56/130) 35.8%↑ P=0.02	43.6 (33.3/55.3) 28.8%↓ P=0.001	45.3 (36.7/54) 26.0%↓ P= 0.001
hsCRP (mg/l)	3.5 (3.0/7.0)	7.0 (5/13) 2.0fold↑ P=0.019	6 (2/14) 71.4%↑ NS	4 (3/14) 14.28%↑ NS

NS: non-significant

Within controls with respect to smoking or alcohol consumption the difference in the levels between presence and absence of these risk factor did not reach statistical significance. Within Group 1, AMI patients with smoking habit demonstrated significant (P=0.017) decrease of 27.2% in TFPI levels as compared to AMI patients without smoking habit. On the other hand AMI Patients without smoking habit as compared to with smoking habit and AMI patients without alcohol habit as compared to with alcohol habit demonstrated moderate increase in PAI of 41.3% (NS) and of 35.2% (P=0.029) respectively. In this group the median of TF, tPA and hsCRP did not deviate between presence and absence of risk factors. As there were only two smokers each in Group 2 and Group 3 and with alcohol habit three patients in Group 2 and one in Group 3, the analysis of the data of these risk factors for these groups was not carried out. With respect to the presence and absence of hypertension and diabetes within the patients groups the difference in the levels of parameter studied did not reach statistical significance. Analysis with respect to diabetes for Group 3 was not carried out as there were only two patients with diabetes mellitus.

Table 3: Hemostatic and Thrombotic Parameters in Group 1 with respect to age

	<40 years		≥40 years	
	Controls (n=7)	Group 1 (n=5)	Controls (n=24)	Group 1 (n=44)
TF(pg/ml)	52.0 (44/130)	150.7 (117.5/357) 2.9 fold↑ P=0.073	89 (41.5/134.5)	158 (109.5/195.6) 1.8 fold↑ P=0.035
TFPI (ng/ml)	30 (23.6/48.8)	45.2 (16/99.3) 50.7%↑ NS	41.2 (33.6/46.1)	36.5 (24.4/54.4) 11.4%↓ NS
tPA (ng/ml)	8.02 (6.21/13.6)	10.8 (9.48/15.4) 34.7%↑ NS	10.3 (8.7/17.9)	10.8 (9.1/13.2) 5.1%↑ NS
PAI-1 (ng/ml)	56.8 (52.4/76.6)	81.8 (49.7/186.9) 44.0%↑ NS	66.7 (56.5/73.5)	83.3 (57.8/ 131.3) 24.9%↑ NS
hsCRP(mg/l)	3 (2/6)	5 (1/8) 66.7%↑ NS	4 (2.25/6.75)	7 (3/11) 75%↑ NS↑

NS: non-significant

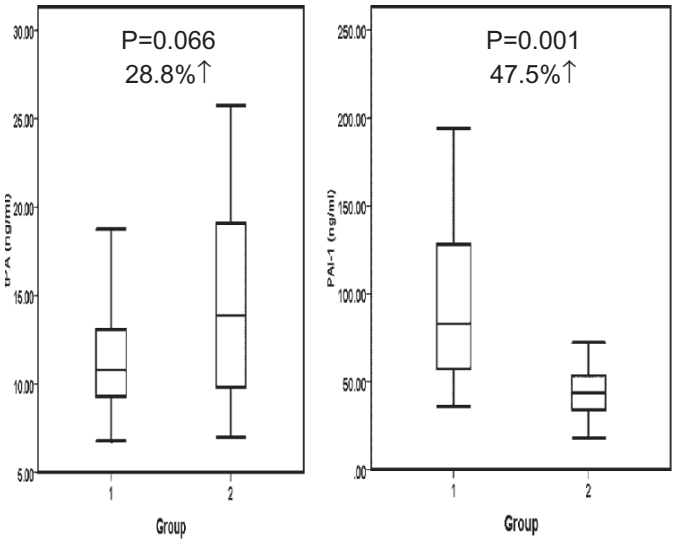

Fig 1b: Circulating levels of tPA and PAI-1 in unstable angina and AMI patients after treatment of the acute event (Group 2) as compared to AMI patients at presentation (Group 1)

DISCUSSION

In the present study we have analyzed TF and tPA and their inhibitors TFPI and PAI-1 respectively in blood sample of AMI patients collected within 6 hours of the symptoms and before any thrombolytic therapy given (Group 1), unstable angina and NSTEMI after 4.5 days of the event on treatment (Group 2), stable angina patients (Group 3) and healthy individuals (Controls). Significant finding was increase in TF not only in Group 1 and Group 2 but also in Group 3 as compared to controls. PAI-1 significantly increased in Group 1 while it was significantly reduced in the other two patient groups. TFPI and tPA demonstrated increase in Group 1 of age<40 years while insignificant changes in elder patients. Although marginal but significant rise in tPA and non significant rise in TFPI was seen in Group 2.

TF, formerly known as thromboplastin, is expressed in both vascular and nonvascular cells (17). In the vessel wall, TF is constitutively expressed in subendothelial cells such as vascular smooth muscle cells leading to rapid initiation of coagulation when the vessel is damaged (18). Also plaque rupture leads to exposure of highly procoagulant plaque content to the circulation and it may also contribute to the increased TF plasma levels. Corresponding to this pathogenesis there was increased levels of TF in AMI patients at presentation (Group 1). In contrast, endothelial cells and monocytes do not express TF under physiological conditions; as a consequence, there is no appreciable contact of cellular

Table 4: Hemostatic and Thrombotic Parameters of Group 1 with respect to smoking habits

	Non-smokers		Smokers	
	Controls (n=26)	Group 1 (n=34)	Controls (n=5)	Group 1 (n=15)
TF (pg/ml)	74.0 (46/130)	142 (120/267) 1.9 fold↑ P=0.001	67 (10/150)	134 (111/164) 1.8 fold↑ NS
TFPI (ng/ml)	41.3 (30/48.8)	42.2 (31/79.6)	36 (30.9/41.1)	26.0 (17.7/46.7) 27.7%↓ NS
tPA (ng/ml)	10.2 (7.84/14.8)	10.6 (8.94/15.7)	10.3 (5.68/16.8)	11.6 (9.5/14.3) 12.6%↑ NS
PAI-1 (ng/ml)	60.8 (52.2/78.2)	89.3 (61.9/146.2) 46.9%↑ P=0.001	70.8 (58.5/72.4)	66.3 (53.2/ 84.4) 6.35%↓ NS
hsCRP (mg/l)	3.5 (2.75/6.25)	7 (2.0/10.0) 2.0fold↑ NS	3.5 (1.0/5.5)	6 (4/10) 71.4%↑ NS↑

Table 5: Hemostatic and Thrombotic Parameters of Group 1 with respect to Alcohol habit

	Without Alcohol Consumption		With Alcohol Consumption	
	Controls (n=26)	Group 1 (n=40)	Controls (n=5)	Group 1 (n=9)
TF (pg/ml)	74.0 (44/130)	140 (111.5/206.8) 89.2%↑ P=0.001	75 (10/150)	135 (123/191) 1.8 fold↑ NS
TFPI (ng/ml)	38.4 (30/45.9)	38.3 (26.9/54)	42 (35.5/55.6)	33.2 (19.2/48.8) 20.9%↓ NS
tPA (ng/ml)	10.3 (7.84/15.2)	10.7 (9.1/15.7)	10.2 (6.3/17.3)	10.3 (8.5/11.9)
PAI-1 (ng/ml)	66.7 (53.8/74.4)	84.9 (59.3/142.6) 27.3%↑ NS	60.6 (54/78.1)	62.8 (48.9/ 78.8)
hsCRP (mg/l)	3.5 (2.0/6.0)	6.5 (3.0/15.3) 85.7↑ P=0.037	4.5 (2.3/6.8)	8 (3/9) 77.7%↑ NS↑

TF with the circulating blood. In response to various stimuli, however, TF expression and activity can be induced in these cells by cytokines, growth factors, and biogenic amines. In conditions like acute coronary syndrome apart from vascular cells, these cells and aggregating platelets have been suggested to be source of the elevated levels of circulating TF (19). This may have contributed to the increased levels of TF observed in NSTEMI and unstable angina patients of Group 2 in spite of the stabilization of their symptoms.

In the present study TF levels were also elevated in stable angina patients of Group 3 and the rise was similar to the extent as seen in Group 1 and Group 2 patients. Apart from the surface bound form in vascular cells and leukocytes, TF is also detected in the bloodstream, referred to as circulating or blood-borne TF (20). This form of TF is mainly associated with microparticles originating from vascular smooth muscle cells, leukocytes or platelets (21, 22) and atherosclerotic plaques in the early stages of atherogenesis (18). This could also contribute to the increased levels of circulating TF in these patients. Recently, an alternative spliced form of TF has been discovered, which is soluble, circulates in the blood and exhibits procoagulant activity (23). Cytokines stimulate its expression and releases from endothelial cells (24). Spliced TF is not bound to microparticles and appears to represent a distinct form of circulating TF; as such, it may have an important role in thrombus propagation (23). These studies on blood-borne TF imply that activation of coagulation, contrary to traditional belief, may be initiated and propagated without contact of the blood to the extravascular space and increase the procoagulant status of blood of these patients.

Role of TFPI is the attenuation of the thrombogenicity of the atherosclerotic plaque. It has a dual inhibitory function; it inhibits the complex FactorVIIa/TF (25) and directly inhibits Factor Xa by binding at or near its active serine site (26). Several reports have demonstrated the co-localization of TFPI and TF in atherosclerotic plaque (27-29). The secreted TFPI from monocytes and most vascular wall cells may be associated within plaque through the binding with proteoglycans and other TFPI binding protein on the cells (30, 31). It has been reported in literature to be elevated in AMI patients (5, 7-9). Our results indicate increase levels in younger AMI patients while insignificant changes in the levels of plasma TFPI in elder AMI patients as compared to controls. Depletion of TFPI may lower the threshold by which TF induces intravascular coagulation. Thus the in-significant changes in TFPI levels at the time of the acute event correspond to the formation of thrombus and thus AMI. In case of Group 2 and 3 there was marginal increase and decrease in TFPI respectively as compared to controls. As synthesis and secretion of TFPI is enhanced by heparin (32, 33), it may have caused increase TFPI in Group 2 patients on treatment. Literature reports elevated levels in unstable angina patients (5, 34-37) and marginal increase in case of stable angina patients (7). Moreover in our study we observed significant decrease in TFPI in AMI patients who had added risk factor of smoking.

tPA is localized in endothelial cells in arterial vessel walls and is released upon stimuli; such as, venous occlusion, physical exertion, stress. In our study there was increase in tPA levels in younger AMI patients while there was no change in tPA levels observed in elder AMI patients of Group 1 at presentation as compared to respective controls. In literature a study by Wang et al (38) analyzing the association between vulnerability of plaque assessed with intravascular ultrasound (IVUS) and plasma levels of fibrinolytic biomarkers demonstrated that plasma levels of tPA was significantly reduced in patients with thin-cap fibroatheroma than in patients with non-thin cap fibroatheroma and in their study decrease in tPA level was associated with plaque vulnerability. Rupture or erosion of this thin cap fibroatheroma with superimposed thrombus is the most common cause of myocardial infarction. On the other hand an occlusion test carried out by Hoffmeister et al (39) and Yan et al (40) demonstrated that endothelial tPA reserve capacity is reduced during acute phase and becomes

normalized during follow-up. Similar to this finding in the present study significant rise in tPA levels was observed in unstable angina and NSTEMI patients stabilized on treatment of Group 2. Moreover at early stages of atherosclerosis tPA mass concentration has also been reported to be elevated (41). Similar to this finding we observed although non significant but elevated levels of tPA in stable angina patients.

PAI-1 prevents fibrinolysis and thus accelerates thrombus formation. Immunohistochemical staining of coronary artery specimens (42) and mRNA expression (43) studies have demonstrated increased expression of PAI-1 in unstable plaques. Increased PAI-1 levels in AMI patients have been documented in the literature (44, 45). Our results demonstrated moderate but significant increase in plasma PAI-1 levels in AMI patients at presentation of Group 1 and significant decrease in Group 2 and Group 3 patients as compared to the controls, thus correlating increased PAI-1 levels with onset of the acute event. Further, remarkable increase in PAI-1 levels in non-smoker, non-alcoholics and younger AMI patients of Group 1 demonstrate presence of a risk factor otherwise in a low risk population. Literature documents elevated circulating concentrations of PAI-1 in young men at increased risk for recurrent infarction (45).

CRP is demonstrated to promote coagulation by enhancing the expression and activity of TF and reducing that of TFPI (46-48), stimulating PAI-1 and inhibiting tPA activity (49). In the present study although TF, PAI as well as hsCRP were increased of Group 1 AMI patients, they did not demonstrate corresponding correlation with hsCRP. In fact unstable angina and NSTEMI patients of Group 2 demonstrated strong positive correlation of hsCRP with TFPI. As already discussed synthesis and secretion of TFPI is enhanced by heparin (32) and further decrease in TFPI due to CRP is demonstrated to be counteracted by low molecular weight heparin anticoagulant therapy (33). Thus still persistent inflammation in these patients on one hand and elevated TFPI due to treatment on other hand may have demonstrated its strong positive correlation with hsCRP.

Small sample size and lack of follow-up of AMI patients of Group 1 and thus correlation of elevated TF and PAI-1 levels at acute phase with any adverse cardiovascular outcome on follow-up of the same patients could not be studied. However we compared the data of unstable angina and NSTEMI patients stabilized on treatment (Group-2) with that of AMI patients at presentation (Group 1). In this group although there was equal rise in TF as in Group 1, increase in TFPI, tPA and decrease in PAI-1 levels were observed as compared to Group 1.

Thus our findings are in favor with that of the hypothesis of imbalanced hemostatic mechanism with increased levels of TF and PAI-1 in AMI at presentation. Although younger AMI patients (<40 years) were protected by increased TFPI and tPA levels, they still demonstrated increased TF and PAI-1 levels as compared to respective control. In the elder age group increased TF and PAI-1 and insignificant changes in tPA and TFPI as compared to respective controls correlate with the acute event of MI. TFPI required for the inhibition of TF and tPA for activation of plasminogen were insufficient to prevent the formation of thrombus, which lead to AMI. Thus from our study apart from increase TF, circulating TFPI level could be considered as a marker for AMI in patients with increased risks, such as smoking while PAI-1 was found to be a good indicator of thrombosis in younger patients, in non-smokers and in patients without alcohol consumption which are otherwise at low-risk. Further, increase in TF in stable angina patients demonstrates presence of elevated blood borne TF and thus increased procoagulant status in these patients as well. TF exposed from ruptured plaque is the actual trigger but systemic procoagulant status also plays important role. Independent of cellular TF, blood borne soluble TF may play a role in the propagation of thrombosis which also needs monitoring in early atherosclerotic conditions.

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