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ROLE OF PLASMA PAI-1 AND ITS CORRELATION WITH OTHER CARDIAC BIOMARKERS IN ST ELEVATED ACUTE MYOCARDIAL INFARCTION IN YOUNG PATIENTS

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ABSTRACT

Background: Prevalence of younger age Acute Myocardial Infarction (AMI) is increasing worldwide. This study was aimed to ascertain plasma PAI-1 as an independent biomarker in STEMI, Quantification and Establishing the indicative evidence range of Plasma plasminogen Activator inhibitor-1(PAI-1)and ascertaining it's correlation with CK-MB in young south Indian ethnics. **Methodology:** This cross sectional study was conducted at MGMGH, Trichy. Study subjects includes 40 Patients with typical chest pain, shows ST Elevation in ECG, rise in CK-MB and without any other risk factors of AMI. 40 age and sex matched control subjects were studied at the same time. Plasma PAI- 1 was assayed within six months of sample collection. Analysis of Serum Urea, Creatinine, Glucose, Lipid Profile, CK-MB and others risk factors of AMI was done on admission. Statistics was analyzed using SPSS - 19.0. **Results:** There was a positive significance association observed in plasma PAI-1 ($P \leq 0.001$), Serum Urea: $P \leq 0.001$, Serum Glucose: $P \leq 0.04$, Serum AST: $P \leq 0.001$, Serum CK-MB: $P \leq 0.001$ and Serum HDL: $P \leq 0.008$ between patients and control subjects. The Mean and SD of plasma PAI-1 for patients and Controls are 3450.76 ± 1406.68 and 1966.03 ± 1406.68. Furthermore an inverse association observed between plasma PAI-1 and HDL level. **Conclusion:** This study statistically confirmed the independent association between STEMI and plasma PAI-1 and established its analytical range as 3000-5000 pg/ml, wherein, it is 1000- 2000 pg/ml for controls also observed the inverse association of Plasma PAI-1 with serum HDL levels.

KEYWORDS: PAI-1, CK-MB and PAI-1, young AMI, STEMI.

INTRODUCTION

Cardiovascular disease is the most important cause of morbidity and mortality throughout the world and ST elevation myocardial infarction (STEMI) the most common cause of death in developing countries like India.[1] About 9% of new events occur in patients under 45 years of age without any traditional and nontraditional risk factors. It is estimated that a genetic element is involved in some 20%-60% of these cases.[2] Most myocardial infarctions are caused by a disruption in the vascular endothelium associated with an unstable atherosclerotic plaque that stimulates the development of an intracoronary thrombus, which results in coronary artery occlusion and reduced blood flow to cardiac tissue. Certainly, it is known that fibrinolytic activity is reduced in patients under 45 who suffer an acute myocardial infarction (AMI).[3]

Plasminogen activator inhibitor type-1 (PAI-1) is the main physiological inhibitor of the activity of the fibrinolytic system.[4] It achieves this via[1] inhibition of tissue plasminogen activator (tPA)[2] inhibition of the inhibitor of the urokinase type activator (uPA). An increase in plasma concentration due to genetic and acquired conditions like young AMI, Stroke and Metabolic syndrome are therefore associated with thrombotic events.[5] Over expression of PAI-1 may also promote development of weak plaques with thin fibrous caps due to inhibition of both u-PA receptor- and integrin-mediated cell adhesion and migration. In addition together, increased plasma PAI-1 levels have been reported in survivors of myocardial infarction (MI) compared with the general population. Therefore, PAI-1 might play an important role in the pathogenesis of CAD.

An increase in the plasma concentration of PAI-1 is Associated, associated with higher mortality and the chance of a second AMI in patients under 45years of age.[6] Different populations around the world show variation in terms of the allelic frequencies of 4G and 5G, with the 4G allele seen more commonly in Asian (59%),[7] Caucasian (51%).[8] Spanish (47%) [9] and Indian (54%)[10] populations. This leads to variations in the plasma concentration of PAI-1 between populations, as well as in its interaction with other regulating factors such as triglycerides, glucose, insulin, hypertension, and smoking.[11,12] This contributes to differences in susceptibility to the development of cardio vascular disease among different populations, which is more common among American Indians (15%-20%) and Caucasians (20%-25%) than among Africans (1%-5%).[13]

High levels of PAI-1 in Indians are reported in association with hypertriglyceridaemia and hyperinsulinaemia. This combination promotes thrombosis by impairing fibrinolysis.[4] Indian relevance of PAI-1 with other AMI marker was studied and showed with positive association with PAI-1 polymorphism.[24,25,26] The PAI-1 promoter polymorphism (4G/5G) and plasma PAI-1 as an early marker of STEMI/AMI was reported a decade ago but no study has been reported from India, in particular, south India. The variation in the association of PAI-1 on AMI has been observed in ethnic, age and gender groups. Therefore, there is a need to assess the relationship between carriage of the 4G or 5G alleles, PAl-1 production, and the development of STEMI in Indian patients aged 45 years or younger. Consequently, the quantification of PAI-1 in plasma and detection of 4G or 5G alleles polymorphism is essential for early diagnosis and prevention of fatal complications of AMI in younger Indians. Hence this study is more relevant in the context of region wise data creation to support nationalized early diagnostic and therapeutic measures. In some studies from India, the percentage of patients below the age of 45 years suffering from acute myocardial infarction (AMI) is reported as high as 25-40%.[14,15] The risk-factor evaluation must start earlier.

AIM AND OBJECTIVE OF THE STUDY

Aim of this study is to ascertain role of PAI-1 as an independent bio-chemical marker for the Indian young MI patient's age less than 45 years. The objectives of the study are, to establish the analytical range of PAI-1 in plasma to Myocardial Infarction of young patients, to find out association of plasma PAI-1 with other risk factors of MI and to check the connection of CK-MB with PAI-1.

The outcomes of this study facilitate early diagnosis of end stage diseases and early intervention which then prevent mortality, morbidity and social stigma of the society caused by AMI.

METHODOLOGY

I. Materials and Methods

This cross-sectional study was conducted at Mahatma Gandhi Memorial hospital, Trichy, Study subjects includes 40 Patients (sample size calculated by conventional statistical formula) with acute myocardial infarction who had typical chest pain, shows electrocardiographic changes (ST Elevation) and a transient rise in cardiac enzymes to more than twice the upper, Age less than 45 years. Control subjects were 40 healthy men and women who came with some patients and healthy volunteers' age less than 45 years, during May 2016 to March 2017.

II. Inclusion criterias for this study are

Age less than 45 years, Admitted within 24 hours of chest pain, ECG shows ST segment Elevation

III. Exclusion criterias for study are

Case and Control Subjects With, Renal Disease, Severe (Neuro) Psychiatric Problems, Life Expectancy Less Than One Year, Known Diabetic Patients, Known Hypertensive Patients, Known History of Thromboembolic Disorders, Smokers, Alcoholic, History of Previous Coronary Artery Disease, Obese Individuals, Every individual completed a questionnaire concerning the presence of cardiovascular risk factors such as smoking and alcohol consumption. For patients, all questions referred to the period before their myocardial infarction. The Quetelet index was derived by dividing weight (kilograms) by squared height (meters2). Persons were considered obese if their Quetelet index exceeded 30 kg/m2.

IV. Sample collection

Under sterile condition, 6ml of peripheral venous blood was withdrawn using sterile disposable syringes from all the study subjects. The 4ml patient EDTA sample, centrifuged at 2500 rpm for 20 minutes and plasma was separated and 500µl was stored in sterile 2 ml Eppendorf and stored at-20^0C for PAI-1 (ELISA KIT-KOCH3071) Estimation which was assayed within six months of sample collection. Remaining plasma was transferred to another Eppendorf tube for the analysis of Blood Glucose, Urea, Creatinine, Total Cholesterol, Triacylglycerol, and HDL. Alanine transferase (ALT), Aspartate transferase (AST), Serum Electrolytes, Creatine Kinase-MB (CK-MB).

V. Measurement of PAI-1 Antigen by ELISA

PAI-1 antigen was measured in Case and control subjects with an enzyme Linked Immune Sorbent assay, according to the manufacturers instruction. **Human PAI-1 ELISA Kit** Catalog No. KHC3071 & KHC3072, Pub. No. MAN0014692 Rev 1.0.

RESULT AND STATATISTICS

Statistical analysis

For standard statistical analysis of the data's, Statistical products and service Solutions (SPSS) package were used. The biochemical parameters between Myocardial infarction cases and healthy controls were tested by using student's t test. The frequency of Genotype distribution between cases and controls were Compared using Chi-square ($\chi 2$) test. In logistic regression analysis, Odds ratio with two tailed p values and 95% Confidence intervals (CI) were calculated. Level of significance for p-value was set at point < 0.05. If p <0.001 shows strongly significant. Hardy- Weinberg law was followed and the frequency of genotype were tested for Hardy – Weinberg equilibrium. Pearson correlation was used to compare PAI-1 with other parameters. Levine's Test used to check Equality of Variances between patients and control

Table 1: Minimum, Maximum and Mean age and SD of the Patients and Control groups.

Group	No of cases	Minimum Age	Maximum Age	Mean Age	Std. Deviation
Patients	40	18.00	43.00	36.48	5.26
Control	40	19.00	42.00	32.33	6.09

Table 2: Levine's Test for Equality of Variances for different risk factors of AMI with plasma PAI-1.

Variable	Group	Mean	Std. Deviation	Std. Error Mean	P value (P<0.05 significant)
SBP	Patients	106.97	9.18	1.59	0.906
	Control	106.67	11.36	1.97	
DBP	Patients	74.24	8.3	1.44	0.871
	Control	74.55	6.6	1.15	
BMI	Patients	23.38	1.98	0.34	0.205
	Control	22.80	1.67	0.29	
UREA	Patients	36.91	6.19	1.07	0.000
	Control	31.18	5.73	0.99	
SUGAR	Patients	126.42	38.04	6.62	0.003
	Control	103.79	14.22	2.47	
CREATININE	Patients	0.942	0.16	0.02	0.443
	Control	0.973	0.14	0.025	
AST	Patients	122.09	452.44	78.76	0.261
	Control	31.94	10.44	1.81	
ALT	Patients	160.97	654.39	113.91	0.273
	Control	33.85	7.25	1.26	
APTT	Patients	25.33	6.17	1.065	0.513
	Control	24.58	2.45	0.42	
NA	Patients	138.79	3.40	0.59	0.547
	Control	135.69	17.68	3.07	
K	Patients	3.967	0.43	0.07	0.240
	Control	3.912	0.28	0.04	
CHOL	Patients	168.39	29.00	5.04	0.532
	Control	176.39	25.67	4.46	
TGL	Patients	169.03	59.07	10.28	0.532
	Control	158.03	81.46	14.18	
VLDL	Patients	33.80	11.81	2.05	0.532
	Control	31.60	16.29	2.8363	
LDL	Patients	95.89	24.36	4.24	0.320
	Control	103.06	33.07	5.75	
HDL	Patients	38.70	3.869	0.67	0.002
	Control	41.73	3.843	0.66	
CKMB	Patients	138.58	108.014	18.80	0.0165
	Control	133.24	192.761	63.18	
PAI-1	Patients	3450.76	1406.68	244.87	0.000
	Control	1966.03	878.74	152.97	
PT	Patients	16.94	13.193	2.29	0.090
	Control	12.89	1.651	0.287	

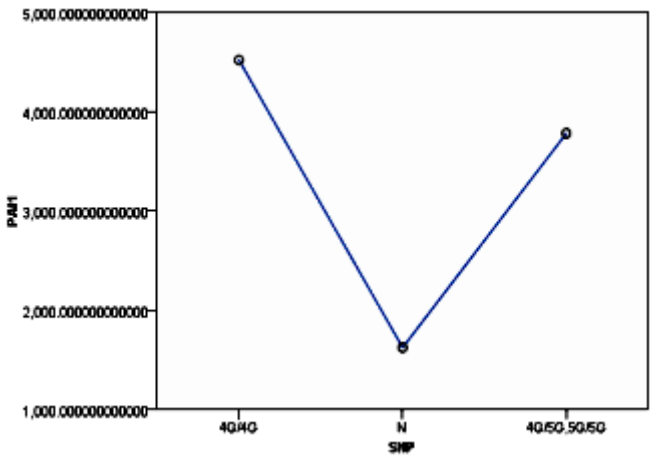

Figure 1: Distribution of Plasma PAI-1 in Patients and Controls.

DISCUSSION

Plasminogen activator inhibitor type 1(PAI-1) is the main inhibitor of both urinary-type (uPA) and tissue-type(tPA) Plasminogen activators. The increased plasma levels of PAI-1 causes reduction in plasma fibrinolytic activity, mainly, which is associated with coronary heart disease (CHD)[16] and recurrent myocardial infarction.[4] Endothelial injury can stimulate PAI-1 expression and facilitate thrombosis.[17] Hereditarily determined variability in PAI-1 expression has been recommended as a risk factor for coronary atherogenesis and thrombosis.[6] The human PAI-1 gene is present on chromosome 7q21.3-q22, and it is susceptible for several polymorphisms which have been described in various studies.[18]

Table 3: Shows significant association of risk factors between patients and Control in relation to plasma PAI-1 Mann-Whitney U and Wilcoxon W- rank tests.

	SBP	Sex	DBP	BMI	Urea	Sugar	Creat	AST	ALT	PT	APTT	NA^+	K^+	CHO	TGL	VLDL	LDL	HDL	CK-MB	PAI-1
Mann-Whitney U	528	379	541	436	279.5	387.5	450	296.5	255	477	534.5	532	455	456	443	443	454	338	91	205
Wilcoxon W	1089	940	1102	997	840.5	948.5	1011	857.5	816	1038	1095	1093	1016	1017	1004	1004	1015	899	652	766
Z	0.22	2.74	0.04	1.39	3.4	2.01	1.2	3.18	3.7	0.87	0.12	0.161	1.15	1.13	1.3	1.3	1.16	2.66	5.8	4.35
Asymp. Sig.(2-tailed)	0.8	0.006	0.96	0.16	0.001	0.04	0.2	0.001	0.13	0.38	0.89	0.872	0.24	0.25	0.19	0.19	0.2	0.008	0.001	0.001

Table 4: Pearson Correlations between serum CK-MB, plasmaPAI-1.

		CKMB	PAI-1
CKMB	Pearson Correlation	-	0.019
	Sig. (2-tailed)		0.881
PAI-1	Pearson Correlation	0.019	-
	Sig. (2-tailed)	0.828	0.000

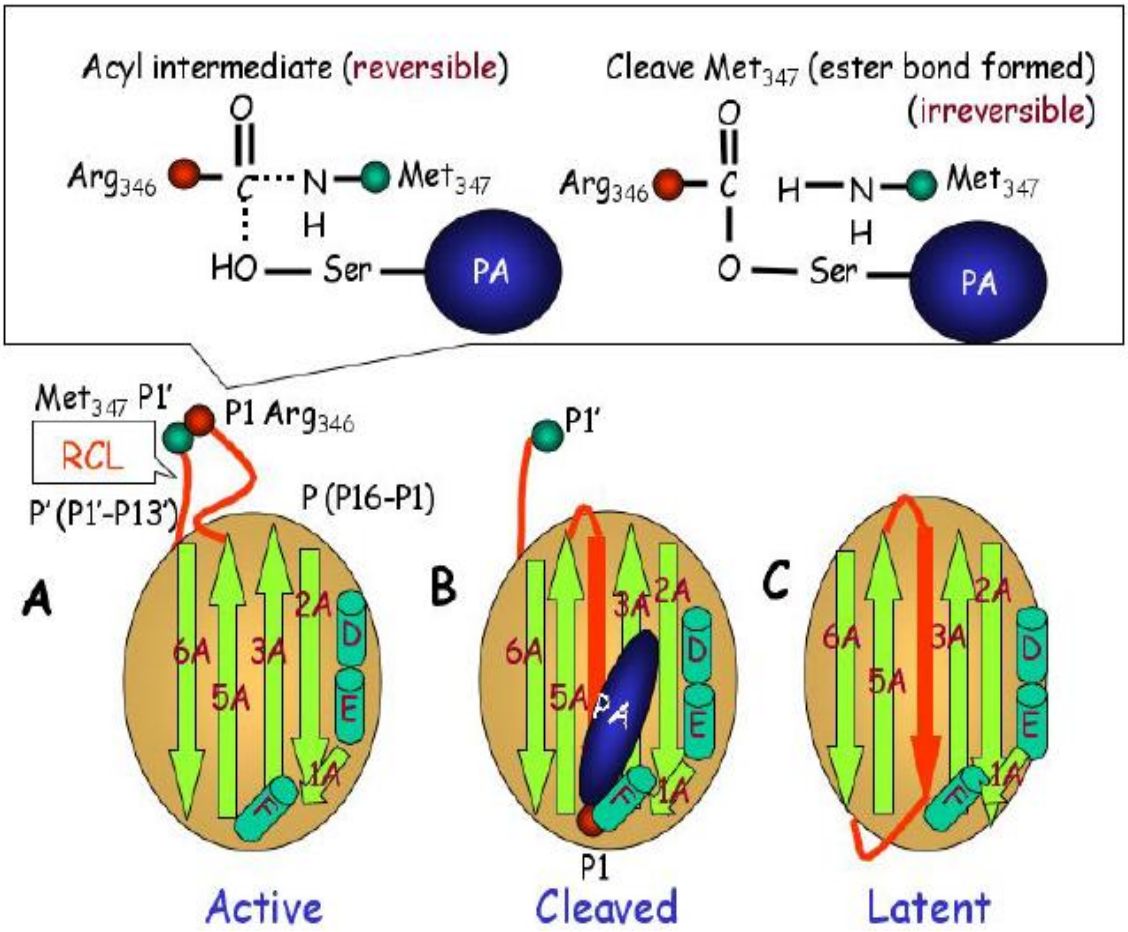

Figure 2: (A):PAI-1contain an exposed Reactive center loop and an Active site Arg- 346-Met-347 that interact with target protease.(B) Once the acyl intermediate is formed the P1-P1' bond is cleaved and P1-PA complex is translated.(C) In the latent PAI-1 form, the entire amino terminus of the RCL(i.e.P1-P16) is inserted into β-sheet A.

Conformational States and Mechanism of action of PAI-1

PAI-1 exists in several conformations like, Active, Inactive Forms and Latent Stages. The native form of PAI-1 is shown as a ribbon structure. PAI-1 is produced by different cell types including liver cells, smooth muscle cells (SMC), adipocytes, and platelets and is released into circulation. The active form spontaneously converts to the latent form with a half-life of 1 hr.[19] The latent form also inter convertible into the active form by treatment with negatively charged phospholipids, denaturants or vitronectin. Latent PAI-1 can also be reactivated in vivo. It is the only SERPIN that can reversibly switch between the active and latent conformational states. Recently, the three-dimensional structure of the latent form of PAI-1 was also determined. In this structure, the entire amino terminal side of the reactive center loop is inserted as the central strand into sheet A [figure3]. The three dimensional change accounts for the increased stability of latent PAI-1 and the lack of inhibitory activity. In addition to the latent form of PAI-1, oxidation of one or more critical methionine residues within active PAI-1 gives second inactive form of PAI-1.[20] Oxidized PAI-1 causes rapid conformational change to a structure that is distinct from both active and latent PAI-1. Oxidative inactivation of PAI-1 may be an important mechanism for the regulation of the Plasminogen Activation system. Oxygen radicals produced by neutrophils and other cells could inactivate PAI-1 and therefore allow the generation of plasmin activity at sites of infection or in areas of tissue remodeling. As a result of the unique labile structure of PAI-1, immunological methods for determining PAI-1 concentrations can vary by >10-fold, depending on the specific mix of conformations present and the specificity of the indicator antibodies. Thus PAI-1 antigen measurements should be interpreted with care and a functional "bioimmunoassay" is to be preferred. PAI-1 in plasma or in the extracellular matrix is stabilized by vitronectin. In solution, vitronectin-bound PAI-1 complex is approximately twice as stable as unbound PAI-1, and on extracellular matrix, the half-life is reported to be >24 hrs. Very small amounts of Latent PAI-1 are present in normal fresh plasma, and most of the PAI-1 in whole blood and platelets. Even though, platelets contain vitronectin, which can potentially function to reactivate latent platelet PAI-1. Reports from various clot lysis studies suggest that platelet PAI-1 may

be a major factor in the resistance of platelet-rich thrombi to thrombolysis.PAI-1 also binds to heparin with high affinity. This glucosaminoglycan- binding property of PAI-1 serves as an additional mechanism to anchor PAI-1 to the matrix.[36] But heparin does not influence functional activity of PAI-1.

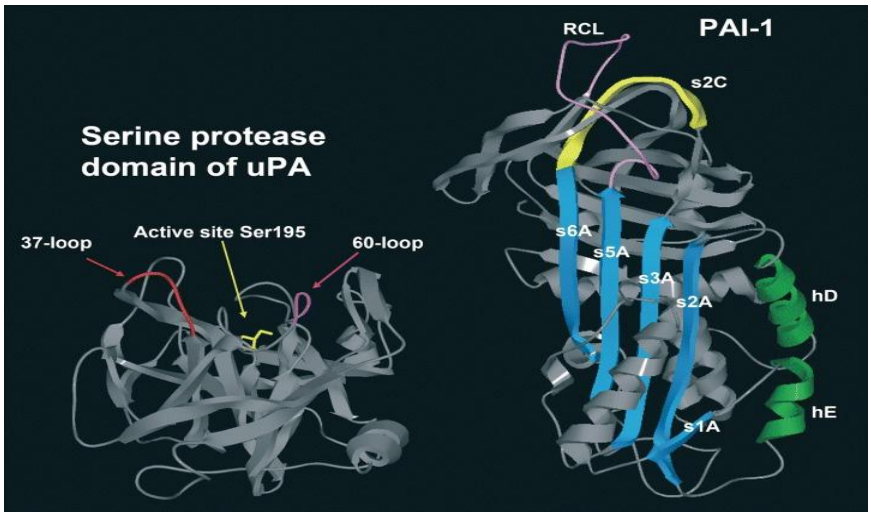

Figure 3: Three dimensional Structure of PAI-1 which shows active Site and Position of various amino acid.

Several studies have found an association between high plasma PAI-1 levels and AMI. Whether this is causal or a consequence of atherosclerosis or tissue damage remains unclear.

This study shows the distribution of age of patients and control subjects detailed in table 1 and values are Mean Age and SD of Patients:36.48 ±5.26, Mean Age of Controls: 32.33 ± 6.09, Minimum and maximum age for patients was 18 and 43 years, Minimum and Maximum age for controls are 19 and 42 years.

Levene's Test for Equality of Variances in Table 2 shows details of positive association of various risk factors for AMI with plasma PAI-1 of between patients and control subjects with its Mean, SD and P values. Mean and standard deviation of serum Glucose for patients and Controls are 126.42±38.04 and 103.79 ±14.22 ($P \leq 0.03$), Mean and standard deviation of serum Urea for patients and Controls were 36.91±6.19 and 31.18±5.73 ($P \leq 0.001$), Mean and standard deviation of serum HDL for patients and Controls were 38.70± and41.73 ±3.843 ($P \leq 0.002$), Mean and standard deviation of serum CK-MB for patients and Controls were 138.58±108.014 and 133.24± 192.761 (≤ 0.016.), Mean and standard deviation of plasma PAI-1 for patients and Controls were 3450.76±1406.68 and1966.03±1406.68 ($P \leq 0.001$).

The mean and SD of plasma PAI-1 for patients and Controls are 3450.76±1406.68 and 1966.03±1406.68 (table2) and there was a positive significance variation of plasma PAI-1($P \leq 0.001$) observed between patients and controls. Plasma PAI-1 level in individuals AMI range from 3000-5000 pg/ml Plasma PAI-1 where in the control group is 1000-2000 pg/mg (figure 1). Plasma PAI-1 shows significant changes. Further, a positive significant changes between patients and controls in Serum Urea: $P \leq 0.001$, Serum Glucose: $P \leq 0.04$, Serum AST: $P \leq 0.001$, Serum HDL: $P \leq 0.008$, Serum CK-MB: $P \leq 0.001$, Plasma PAI-1: $P \leq 0.001$ were also observed (table 2). As per table 4, Serum CK-MP shows negative correlation with Plasma PAI-1 ($P \leq 0.828$). Some authors have reported an association between 4G/5G polymorphism in the promoter region of PAl-1 and the development of AMI.[13] Inconsistency in PAI-1 plasma concentrations has been reported in different ethnic groups around the world[21] and it was 5000pg/ml for Indian euthenics. Festa et al[22] reported the ethnic differences in the distribution of 4G/5G polymorphism to be a determining factor in the plasma concentration of PAI-1. In the present work, plasma PAl-1 concentrations measured in post-STEMI were highest as reported by Serrano Rios et al in patients with metabolic syndrome. Such increases in PAl-1 have been associated with AMI, Panahloo et al report that they can remain high for six months.[20] These findings have the same opinion with the proposal of Sobel et al[23] that the over expression of PAI-1 leads to a reduced smooth muscle fiber content in atherosclerotic plaques, inducing a reduction in the amount of collagen and extracellular matrix proteins, a reduction in resistance to atheroma, the development of a vulnerable plaque, and its eventual breakage and consequent AMI.

Further, an increased concentration of PAI-1 favors a state of hypofibrinolysis via the inhibition of tPA and therefore a reduction in the transformation of Plasminogen into plasmin, a key enzyme in the regulation of the fibrinolytic system. It might, therefore, be hypothesized that the 4G allele is associated with high concentrations of PAI-1 and accordingly with two mechanisms that favor the onset of an AMI: the formation of vulnerable plaques and a reduction in fibrinolysis. This could be of particular interest in explaining the pathophysiological mechanisms behind STEMI in young patients.

The study showed that the high plasma PAI-1 levels will be a carrier even in healthy person among the young population and various other cardiac parameters like CK-MB glucose, urea, Creatinine, Lipid Profile, AST, ALT, PT, APTT, Sodium and Potassium in 40 young patients age less than 45 years admitted with STEMI in ICU with no positive history of any other known risk factors of AMI and equal number of age and sex matched control subjects. Furthermore, the study ascertained the association of cardiovascular risk factors with PAI-1 levels. It also showed an inverse association of PAI-1 antigen levels with the HDL-cholesterol levels. Hence, it is concluded that the PAI-1 level is associated with the risk of AMI. The study confirmed that there won't be any association between PAI-1 antigen levels in control subjects, whereas, there is a positive association between plasma PAI-1 antigen level in AMI patients.

Hence it is concluded that the detection of this high plasma PAI-1 along with other risk factors may, therefore, be useful in primary prevention. The outcomes of this study facilitate an early diagnosis of AMI, an end stage diseases, and establishment of its treatment scheme. This will help to avoid social stigma of the young AMI patients and prevention of sudden death of young individual due to AMI.

CONCLUSION

Studies have shown an association of PAI-1 might play an important role in the pathogenesis of CAD. The results concludes that the independent association between STEMI and the plasma PAI-1 among Indian euthenics. This study established an analytical range of PAI-1 in plasma in AMI of Indian young patients was 3000-5000 pg/ml and for the Normal reference range is 1000-2000 pg/mg. This shows a positive association between Plasma PAI-1 antigen levels and AMI. It also showed an inverse association of PAI-1 antigen levels with the HDL-cholesterol levels. Furthermore, this study anticipated correlation plasma PAI-1 with other risk factors of MI and found a positive relationship with Urea, HDL, and glucose. There was no significant connection between PAI-1 in Sex Serum AST, ALT, Creatinine, PT, APTT Serum sodium and Potassium levels. The other important conclusion is that there was no connection between CK-MB and PAI-1.

The outcomes of this study facilitate an early diagnosis of AMI, an end stage diseases, and establishment of its treatment scheme. This will help to avoid social burden of the young AMI patients and prevention of sudden death of young individual due to AMI.

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Conflict of interest

Nil

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