Journal of Rawalpindi Medical College (JRMC); 2019;23(1): 25-29

Original Article

Comparison of levels of von Willebrand factor and ADAMTS13 in Patients of Myocardial Infarction and Healthy Controls

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Abstract

Background: to determine the levels of von Willebrand factor (vWF) and ADAMTS13 in patients of myocardial infarction and healthy controls

Methods: In a comparative study, the samples were collected from 45 myocardial infarction patients and 45 age and sex matched healthy controls. Total 5ml blood was collected in tri-sodium citrated vials. The determination of VWF was carried out by chromogenic assay on Stago and ADAMTS13 assay was carried out by standard ELISA kit. The Mann Whitney U test was used to determine the median difference between two groups (patients and control).

Results; Patients with MI had higher median plasma level of VWF (161%) as compared to healthy controls (120%). The median plasma level of ADAMTS13 of control and patients was 56.54 U/L and 18.80 U/L respectively. The difference in median plasma levels of VWF & ADAMTS13 between groups was statistically significant ($p < 0.001$

Conclusion: Plasma VWF and ADAMTS13 had a significant association with MI patients. There exists an inverse correlation between plasma VWF and ADAMTS13 levels in MI patients, but this correlation was very weak in control group.

Key Words: Von Willebrand Factor, ADAMTS13, Myocardial Infarction, Elisa, Stago

Introduction:

VWF is a plasma glycoprotein synthesized by endothelial cells and megakaryocytes. Plasma VWF binding to platelets is important for the normal platelet adhesion and aggregation. The ADAMTS13 regulates VWF multimeric composition in the plasma by cleaving the large VWF multimers into smaller forms which are less thrombogenic. This metalloproteinase enzyme ADAMTS13 is synthesized mainly by hepatocytes, megakaryocytes and endothelial cells. High levels of VWF and low level of ADAMTS13 in the plasma are associated with an increased risk of recurrent Myocardial Infarction (MI).Hemostatic mechanism of human body relies on different factors and one of the most crucial ones is the Von Willebrand factor (VWF).[1,2] It contributes significantly to fibrinolysis, hemostasis and regulation of vascular wall permeability.[3] For platelet aggregation and adhesion, VWF is a major determinant and it acts as a bridge between collagen molecules of sub-endothelium at the site of blood vessel injury. It also serves as a carrier molecule for factor VIII and its plasma concentration is approximately 10µg/ml. [4,5]

Thrombotic diseases in human beings can take place due to decreased activity of ADAMTS13. [6,7] It circulates in plasma at a concentration of approximately 1 µg/ml. [8] Ultra large VWF multimers are absent in plasma of healthy people and remain in WPB. [9] After endothelial cell activation or an injury, these ultra large VWF multimers are discharged from WPB into the blood circulation. In the process of discharge, the ULVWF multimers transiently remains attached to endothelial surface, where ADAMTS13 cleaves them into smaller as well as less active VWF multimers. [10] There is a high prevalence of spontaneous micro thrombi in the vasculature of patients with thrombotic thrombocytopenic purpura which have markedly decreased the plasma level of ADAMTS13. [11] ADAMTS13 plays an important role in an inactivation of VWF during its release.[12]

Myocardial Infarction (MI) is one of the most common CVD manifestations. The risk factors which are more strongly associated with MI include hypertension, diabetes, hypercholesterolemia, smoking and physical activity.[13-15] After the risk factor analysis in patients of myocardial Infarction (MI), it has been found that high plasma level of VWF are accompanied with an increased risk of the recurrent Myocardial Infarction

(MI). [16] VWF serves as a prognostic index of future cardiovascular risk in the general population and in asymptomatic patients with established coronary artery disease (CAD). [17]

Pathophysiologic evidence suggests that VWF is not only a marker but also a mediator of cardiovascular disease events. [18] It is produced and released by vascular endothelial cells in response to variety of stimuli which are associated with acute ischemic syndromes including inflammatory cytokines, hypoxia, thrombin and adrenaline. [19,20] Its central role in thrombogenesis makes it a promising target for research into new therapies which exclusively inhibit the VWF. A small number of studies have investigated the association of ADAMTS13 with MI by contradictory outcomes. The studies investigating the ADAMTS13 antigen levels during the acute phase (0-14 days) of MI has observed the decreased levels of ADAMTS13.[21]

Patients and Methods

In this comparative study 90 subjects were recruited and divided in 2 groups . Myocardial infarction patients and age and gender matched healthy individuals as controls. The patients above 45 years with first attack of myocardial infarction were included. These males and females patients were enrolled from outpatient department of Punjab Institute of Cardiology (PIC) Lahore with 3-6 months post MI history. The diagnosis was confirmed by taking detailed medical history, enzymes levels (T/I) and electrocardiogram findings. The study was completed in one year after approval from the ethical review committee and advance studies and research board of University of Health Sciences, Lahore. The patients were informed about the research and a written consent was taken from each enrolled patient. The blood samples were taken in sodium citrate vials and platelet poor plasma was separated by centrifugation for 15 min at 2500g-3000g at room temperature. The plasma for VWF and ADAMTS13 were stored at -80 ˚C. The plasma was thawed at 37˚C for not more than 5 minutes prior to testing. Plasma levels of VWF were determined using commercially available Immuno-Turbidimetric assay of VWF (STA-LIATEST VWF: Ag, REF 00518, Stago, France) (22). The plasma ADAMTS13 level was determined by using commercially available ELISA kit (Human ADAMTS13WF-cp ELISA kit, CATALOG# 12759, Glory Science, USA) (23). The statistical analyses were performed using SPSS version 20.0.

Results

In present study, 90 subjects were recruited, 45 myocardial infarction patients and 45 healthy controls. Among 45 patients, there were 37 males and 8 females and in 45 healthy controls, there were 36 males 9 females with a male to female ratio of 4:1. The mean age of the patients was (53.18 ± 7.75), slightly higher than in controls (52.04 ± 6.75) and the mean difference was not statistically significant (p = 0.461). The mean BMI of the MI patients (24.5 ± 1.4) was significantly higher than in controls (22.9 ±1.4) and we found statistically significant difference between patients and controls (p < 0.001) (Table-1). The clinical characteristics of MI patients showed that the hypertension (53.3%) was most commonly observed risk factor followed by diabetes mellitus (35.6%), history of smoking (20.0%) and hypercholesterolemia (6.7%) (Figure-2).Considering the findings related to plasma levels of VWF and ADAMTS13, the Mann Whitney U test showed that the median level of VWF in patients was significantly higher as compared to healthy controls (p < 0.000) and the median levels of ADAMTS13 in patients were significantly lower as compared to control (p < 0.000) (Table-2). Spearman Rho Correlation test was used to determine the correlation between plasma levels of VWF and ADAMTS13 in both healthy controls and patients with MI and a significant negative correlation was found in MI patients (Correlation Coefficient = -0.382, p = 0.010) whereas, in controls, a positive and weak correlation was observed which was statistically not significant (Correlation Coefficient = 0.120, p = 0.433) (Table-3 and Figure-3)..

Table-1: Distribution of age, and BMI of study subjects in both groups

		Controls	Patients	*p*-value
Age (Years)	Mean ± SD (Min–Max)	52.04 ± 6.75 (45 – 70)	53.18 ± 7.75 (45 – 72)	0.461
Gender	Male	36	37	>0.999
	Female	9	8	
BMI (kg/m^2)	Mean ± SD (Min–Max)	22.9 ±1.4 (20.2– 25.5)	24.5 ± 1.4 (21.3– 27.8)	< 0.001

SD = Standard Deviation, BMI = Body Mass Index

The Odd Ratio (OR) was significantly higher in 3rd and 4th quartile whereas in 2nd it was on lower side for VWF using the 1st quartile as the reference group. We found an increased risk of MI in 3rd and 4th quartile which showed that there was a strong association of VWF between MI patients and controls. The OR was significantly higher for ADAMTS13 in 1st, 2nd and 3rd

quartile using the 4th quartile as the reference group. We found an increased risk for MI in 1st, 2nd and 3rd quartile which showed that there was a strong association of ADAMTS13 between MI patients and healthy controls (Table-4). Out of 45 myocardial infarction subjects, 9 were smokers, 23 were hypertensive, 15 had history of diabetes and 3 had high cholesterols levels

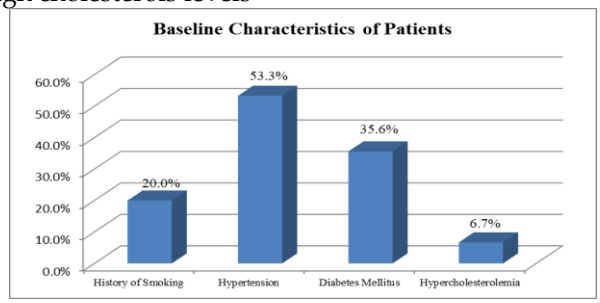

Figure 1:Baseline characteristics of patients

Table-2:- Comparison of Median Plasma Levels of VWF and ADAMTS13 in Study Subjects

Variables	Patients (45)		Controls (45)		Z-score	*p*-value
	Median	(Min-Max)	Median	(Min-Max)		
VWF (%)	161.0	51-420	120.0	48-178	-5.021	0.000
ADAMTS13 (U/L)	18.80	1.17-107.78	56.54	3.38-746.28	-4.196	0.000

Table-3: Correlation of Plasma levels of ADAMTS13 and VWF in Patients and Controls

Study Subjects	Plasma level of VWF %	Plasma level of ADAMTS13 U/L	Spearman Rho Correlation	*p*-value
Patients	161.0	18.80	-0.382	0.010
Controls	120.0	56.54	0.120	0.433

Table-4: ADAMTS13 and VWF levels in relation to risk of MI

Quar-tiles	VWF	Pat ients	Cont rols	Tot al	OR	Lower	Upper
Q1	48-114	3	19	22	Ref	Ref	Ref
Q2	115-141	8	15	23	3.281	0.623	17.285
Q3	142-167	16	7	23	29.368	4.653	185.369
Q4	168-420	18	4	22	41.217	5.679	299.167
	Total	45	45	90			
Quar-tiles	ADAMTS13						
Q1	1.17 – 11.07	16	6	22	26.667	4.727	150.428
Q2	11.08-35.28	13	10	23	13.000	2.445	69.131
Q3	35.29-76.59	14	9	23	15.556	2.906	83.256
Q4	76.59-746.28	2	20	22	Ref	Ref	Ref
	Total	45	45	90			

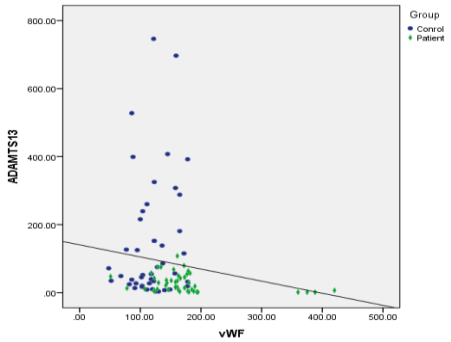

Figure-2: The plasma VWF levels of controls and patients were plotted against ADAMTS13 plasma levels. The plasma VWF & ADAMTS13 levels in controls (blue) and MI patients (green). A significant negative correlation was found in patients whereas, in controls, there was weak correlation in plasma levels of VWF and ADAMTS13.

Discussion

Myocardial infarction (MI) is the most important manifestation of CVDs and is one of leading cause of mortality.[24] The outcomes have been improved in patients of MI with identification and management of modifiable risk factors. [25] The role of VWF and ADAMTS13 as easily measured and potentially modifiable risk factors has been an interesting area of research. The present study was done to explore the role of VWF and ADMATS13 as potential clinical markers and predictors for MI and its recurrence.

The findings of the current study suggests that patients with MI have higher median plasma level of VWF (161.0%) as compared to healthy controls (120.0%). Meta-analysis of some already published studies has shown that higher circulating level of VWF is an independent risk factor for development of coronary artery disease (CAD). [26-28] A study on MI patients reported that plasma levels of VWF were found to be higher in patients as compared to healthy controls. [29]

The plasma levels of VWF were correlated with plasma levels of ADAMTS-13 in our group of MI patients, although we found a significant value (p = 0.010) but they were inversely and very weakly correlated (Correlation Coefficient = -0.382). In control subjects, there was a positive and a weak correlation among plasma levels of VWF and ADAMTS-13 (Correlation Coefficient = 0.120) but it was statistically not significant (p = 0.433). It means that when the plasma level of one variable increases, the level of other variable decreases and vice versa. Combined risk of having both high VWF and low ADAMTS13 are higher than that of the individual risk factor.[4,21]

Low ADAMTS13 levels are associated with MI, as is shown in our results and these findings are same as from the GLAMIS case-control study. [30] Other researchers did not find any association among low ADAMTS13 and MI. [31] Present study results showed a relatively weak association of low ADAMTS13 plasma levels with MI. Similar to that of current study, another study conducted in Glasgow reported, a lack of association between plasma levels of VWF and ADAMTS13 in controls. They had the opinion that plasma ADAMTS13 levels hardly affect plasma VWF levels (Crawley et al., 2011). A previous study carried out by Mannucci and his colleagues reported that a moderate degree of inverse correlation exists between plasma ADAMTS13 and VWF levels in MI patients. These findings are in line with that of current study. [32]

The findings of the current study suggests that the mean age of patients with MI was 53.18 ± 7.75 years. In a large study mean age of MI patients was 60.7±12.8 years.[33] The higher mean age of MI patients might be due to dietary, environmental and genetic factors of western populations. The reason for lower mean age of MI patients in our part of the world might be due to the consumption of more fatty diet and sedentary life style.

In present study, hypertension was the most commonly observed clinical sign followed by diabetes mellitus, history of smoking and hypercholesterolemia in MI patients. A very large study carried out in eight Middle Eastern countries has reported that the diabetes mellitus, hypertension and hypercholesterolemia were attributed risk factors which may have driven healthy subjects towards cardiac diseases. [34]

In the current study subjects, hypertension was observed in large number of patients (53.3%). It is an important risk factor leading to atherogenesis and the development of risk plaques which turn in thrombosis and vessel blockage and is responsible for the development of acute myocardial infarction (AMI). [35] The number of diabetic patients in present study was 15. In diabetes mellitus, obesity is common particularly in type-2 diabetes mellitus and is associated with an increased risk of CVD. [36] Smoking which is attributed to ischemia may also cause the increment in disease burden and in this study about 20.0% population was found to be smoker. Nicotine, carbon monoxide (CO) and oxidant gases of cigarette smoke can lead to CVD.[37] The risk of atherosclerosis and atherosclerosis-related disorders like coronary, peripheral vascular and cerebrovascular diseases is raised in the presence of hyperlipidemia. [38-40]

Conclusion

1.Risk of MI is increased by high VWF and low ADAMTS13 plasma levels and these both results mutually suggest that an imbalance between the enzyme and its substrate may plays a role in the formation of occlusive thrombi in a coronary artery.

2.The ability to differentiate patient subtypes or those at risk of an event, on the basis of VWF levels, can be explored.

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Key for Contribution of Authors : A= Conception/ Study/ Designing /Planning; B= Experimentation/Study conduction;C=Analysis/Interpretation/ Discussion; D= Manuscript writing;E= Critical review;F= Facilitated for reagents/Material/Analysis