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ORIGINAL ARTICLE

Role of High VWF and Low ADAMTS-13 in Ischemic Stroke

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ABSTRACT

Aim: To measure levels of VWF and ADAMTS-13 in patients of ischemic stroke and healthy controls.
Method: Blood samples of forty five patients and forty five controls of age 30-65 years were taken from medical or neurology wards of Lahore General Hospital. VWF antigen assay was determined by Immuno-Turbidimetric method and quantitative ADAMTS-13 antigen assay was measured by ELISA.
Results: The mean level of plasma VWF antigen of controls was 114.0±49.7% and mean plasma level of VWF antigen in patients was 137.0±54.3%. The mean plasma VWF antigen level was significantly elevated among patients as compared to controls (p=0.011). The mean ADAMTS-13 antigen level of controls was 151.7± 87.9 U/L and mean ADAMTS-13 antigen level of patients was 107.9±106.7 U/L. Our study also found that mean ADAMTS-13 was significantly lower in patients as compared to controls (p=0.018).
Conclusion: In conclusion, Vwf and ADAMTS-13 antigen assays have an important role in pathogenesis of clot formation in patients of ischemic stroke.
Keywords: Low ADAMTS, ischemic stroke, high WWF

INTRODUCTION

In adults, Stroke causes serious cognitive and physical long-term disability and it is ranked fourth in United states among all causes of death. Prevention of future cardiovascular events is a major goal in care of those patients who are at increased risk due to history of stroke[1].

WHO MONICA criteria defines stroke as a sudden onset of focal (or global) disturbance of cerebral function lasting >24 hours (unless interrupted by surgery or death) with no apparent nonvascular cause. Recurrent stroke is defined as an episode of stroke that occurs >28 days after the last one[2]. Incidence is approximated 250/100,000 annually, whereas every year, new cases are almost 350,000. The prevalence reported in study by TH Jafar in 2006 was 4.8% which was similar in women and men. There is multifactorial etiology of stroke, and particularly to prevent recurrent stroke, therapeutic actions which are focused on vascular risk factors, have reduced the risk of recurrence of stroke, as well as the risk of peripheral vascular disease or any other coronary event[3]. Hemostasis is a process to prevent hemorrhage by arresting and keeping the blood within the damaged vessel walls. It is a complex process that is dependent on the complex interaction of platelets, plasma coagulation cascades, fibrinolytic proteins, blood vasculatures and cytokine mediators[4].

VWF is a plasma glycoprotein which plays its role in haemostasis by binding to FVIII, to other glycoproteins on the platelet surface and to connective tissue constituents. In circulation, VWF stabilizes factor VIII[5]. VWF is formed in different dimensions, denoted to as VWF multimers, which constitute low, middle, high, and ultra-large forms of molecular-weight[6]. Platelets play an important role in cardiovascular events such as MI and stroke and VWF is critical for platelet activation so VWF may affect the risks of cardiovascular disease. Additionally, it is suggested by evidence that VWF can play a vital part in atherogenesis because it directly contributes in plaque formation and thus affect CVD risk[7]. VWF has been assumed to be linked with ischemic stroke[8]. VWF-cleaving protease, ADAMTS 13, regulates the functional capacity of VWF in blood, as VWF reactivity towards platelets is directly proportional to its multimeric size[9].

Hypothesis is made that there is a role of ADAMTS13 in arterial thrombosis for the reason that low ADAMTS13 action will effect in less degradation of ULVWF multimers and thereby in increased VWF activity[10]. Reports from several studies describe that risk of ischemic stroke is increased by low ADAMTS-13 levels[11]. It is described in previous articles that the consequence of deficiency of VWF is increased vascularity and encourages maturing of new vessel; therefore, the importance of VWF in angiogenesis after brain ischemia is well established. This assumption that VWF and cleaving protease ADAMTS13 influence angiogenesis and maturation has led to a new window of research that will support in therapeutic intervention of these cases[12].

Rationale: In view of above sited literature, a case control study was planned. Levels of VWF and ADAMTS-13 were measured in patients of ischemic stroke and healthy controls. These hemostatic parameters were studied together for the first time. It will be helpful in secondary prevention of cerebrovascular events. So, disease burden in our country can be reduced by simple, cost effective measures. Mortality and morbidity of patients will be reduced.

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MATERIALS AND METHODS

Study Design: Case control study was done in Post Graduate Medical Institute Lahore and patients were enrolled from Lahore General Hospital after permission from Ethical Committee. Forty five patients and forty five controls of age 30-65 years were taken by convenient sampling method. Patients taken were admitted in medical or neurology ward with hemiparesis, hemiplegia, monoplegia, aphasia, vision loss/ visual field defects within 24hrs of presentation, and confirmed on having ischemic stroke on CT scan. Patients/attendants and controls were informed about the study. Written and verbal consent was taken & comments were marked on specified questionnaire.

Von Willebrand Factor Antigen Assay: Von Willebrand Factor (VWF) is a large plasma glycoprotein which plays an essential role in normal hemostasis by binding & stabilizing factor VIII and protects it from proteolysis. Commercially available Immuno-Turbidimetric assasay VWF kit (STA-LIATEST VWF:Ag, REF 00518, Stago, France) was used to determine VWF plasma levels. This test was performed on coagulation analyser STA COMPACT, Stago, Made in France. Change in turbidity of a microparticle suspension which is measured by photometry is the basic principle of this assay. The increase in absorbance is a function of the level of VWF present in the sample to be tested. As soon as the samples were loaded the analyser automatically at 540 nm tested the VWF:Ag assay of the plasmas. Reference range for VWF was 77-97%.

Quantitative ADAMTS-13 Antigen Assay: Using commercially available ELISA kit Plasma ADAMTS-13 level was determined (Human ADAMTS-13VWF-cp ELISA kit CATALOG# 10592, Glory Science).This test was performed on ELISA Stat fax 2100, Made in France. To coat microtitre plate, acquire purified ADAMTS-13 antibody to determine quantitative level of Human ADAMTS-13 level in the test sample, then develop solid-phase antibody and add up ADAMTS-13 to wells. To form antibody, antigen, enzyme, antibody-complex, combine ADAMTS-13 antibody with labeled HRP. Add TMB substrate solution after complete washing. When HRP enzyme was catalyzed, TMB substrate shows blue colour. By adding a stop solution, this reaction is stopped and the colour change is determined at 450nm.

RESULTS

The current study consisted 45 controls and 45 patients. Among 45 healthy controls, there were 28 (62.2%) males and 17(37.8%) females whereas in ischemic stroke group, there were 21(46.7%) males and 24 (43.35) females. The mean level of plasma VWF antigen of controls was 114.0 ± 49.7% and mean plasma level of VWF antigen in patients was 137.0±54.3%. The mean plasma VWF antigen level was significantly higher among patients as compared to controls (p = 0.011).

The mean ADAMTS-13 antigen level of controls was 151.7±87.9U/L and mean ADAMTS-13 antigen level of patients was 107.9±106.7U/L. It was found that the difference in mean ADAMTS-13 antigen levels between both groups was statistically significant (p = 0.018).

Normal level of VWF: Ag was 77-97%. Out of 90, 44(48.9%) subjects had high plasma VWF antigen levels. Chi square test revealed a significant association between raised plasma VWF antigen levels and groups. Out of 45 ischemic stroke patients, 28(62.2%) patients had raised VWF levels whereas in controls only 16(35.6%) subjects had raised levels. There was statistically significant p-value of 0.011.

	Mean plasma VWF antigen				
	Mean±SD (%)	**Median (Inter-Quartile Range)**	**Minimum**	**Maximum**	**p- value**
Patients	137.0 ± 54.3	115.0 (90.0 – 200.0)	77	235	0.011
Controls	114.0 ± 49.7	91.0 (81.0 – 140.0)	65	240	

	Mean ADAMTS-13				
	Mean±SD (U/L)	**Median (IQR)**	**Minimum**	**Maximum**	**p- value**
Patients	107.9 ± 106.7	102.9 (11.1 – 186.1)	1.40	350.2	0.018
Controls	151.7 ± 87.9	154.0(105.8 – 194.3)	3.30	350.0	

	Plasma VWF antigen levels (%)		Total
	Normal	**High**	
Controls	29 (64.4%)	16 (35.6%)	45 (100.0%)
Patients	17 (37.8%)	28 (62.2%)	45 (100.0%)
p-value	0.011		

Normal level of ADAMTS-13:Ag was 10U/L-800U/L. Out of 90, 31(34.4%) subjects had low ADAMTS13 antigen levels. Chi square test revealed that there is an association low ADAMTS13 antigen levels and groups. Out of 45 ischemic stroke patients, 21(46.7%) patients had low ADAMTS13 levels whereas in controls only 10(22.2%) subjects had low levels. The difference of low ADAMTS13 antigen levels between patients and controls was statistically significant (p-value 0.015.

	ADAMTS13 antigen levels		Total
	Low	**Normal**	
Controls	10 (22.2%)	35 (77.8%)	45 (100%)
Patients	21 (46.7%)	24 (53.3%)	45 (100%)
p-value	0.015		

To see the correlation between plasma levels of ADAMTS-13 and plasma levels of VWF in study groups (patients & controls) Spearman Rho correlation test was applied. Weak -ve correlation was seen in Ischemic stroke patients (**r** = -0.144, p-value = 0.344) and in controls (**r**=-0.046, p-value = 0.7).

			Plasma level of ADAMTS-13
Controls	Plasma level of VWF	Pearson Correlation**(r)**	- 0.046
		p-valuea	0.765
Patients		Pearson Correlation**(r)**	-0.144
		p-valuea	0.344

DISCUSSION

Ischemic stroke is known as a main reason of death and disability around the world. Almost 795,000 individuals yearly in the united states suffered a new episode or recurrent episode of stroke[13]. At place of vessel injury, platelet aggregation is mandatory for normal hemostasis but leads to myocardial infarction and ischemic stroke as well. Currently, acute stroke treatment depends on thrombolysis.

Our study also revealed that patients of ischemic stroke had raised mean plasma level of VWF:Ag as that of controls. The mean difference between the groups was determined by Mann Whitney U test. Test results showed that difference in mean level of plasma VWF antigen between groups was statistically significant (p = 0.011). Out of 90, 44 (48.9%) subjects had high plasma VWF antigen levels. Chi square test revealed that there is an association raised plasma VWF antigen levels and groups. These results were in line with study done by Hanson et al., who demonstrated that increased levels of VWF are associated with recurrent stroke[14].

ADAMTS-13 has antithrombotic function as it cleaves VWF in smaller multimers which are less active. In this study, our objective was to measure particularly the association of ADAMTS-13 and ischemic stroke. Our study revealed that ADAMTS-13 level was low in ischemic stroke patients as compared to controls. These results were in accordance with the previous study which showed that low ADAMTS-13 activity was linked with the ischemic stroke risk and upgraded the precision of risk predictions of IS other than usual risk factors[15]. Findings of another study implicate reduced ADAMTS-13 activity as a risk factor for pediatric arterial ischemic stroke[16].

One of the previous studies exhibited that growing levels of VWF and declining levels of ADAMTS-13 were risk factors for ischemic stroke comparable to that of present study[17].

In fact, different studies revealed that low ADAMTS-13 antigen and activity levels were linked with an increased risk of

ischemic stroke. Some researchers had proven in addition that both low ADAMTS-13 levels and raised VWF levels granted an even higher risk[18]. This is the first local study conducted to measure the risk of Ischemic stroke conferred by the combination of both high VWF and low ADAMTS13 antigen levels in patients and controls.

CONCLUSION

This study highlights a remarkable relation among increased VWF:Ag and low ADAMTS-13 levels and the existence of ischemic stroke in opposite directions and support the concept that VWF and ADAMTS-13 perform a strong function in pathogenesis of thrombus formation.

Conflict of interest: Nil

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