Role of fibrinolytic markers in acute stroke

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ABSTRACT

Introduction: The fibrinolytic system plays an important role in normal haemostasis and endothelial function. This study was conducted to compare three fibrinolytic markers, i.e. plasminogen, tissue-plasminogen activator (t-PA) and plasminogen activator inhibitor type-I (PAI-I) between acute stroke and stable non-stroke patients and to investigate the clinical significance of these markers.

Methods: A prospective study was done for a one-year period upon obtaining ethical approval from the local institution. 106 non-stroke individuals from general outpatient clinics (control group) and 51 acute stroke patients were selected. All subjects were tested for t-PA and PAI-I levels using the enzyme immunoassay technique (Biopool TintElize®) and for plasminogen level by colorimetric assay (HemosIL™). They were followed up over a period of three months for survival and neurological recovery.

Results: Only the mean t-PA level was significantly higher in acute stroke patients compared to the control group, including after adjusting for confounders (using ANCOVA). There was no statistical association between the three fibrinolytic markers and the age, gender, stroke subtypes, number of risk factors, functional impairment, survival and neurological recovery. We observed that all the eight patients who died within one month of stroke onset had high levels of t-PA. An association between high t-PA antigen and acute stroke was found during a cerebrovascular event with a 4.6-fold odds ratio compared to non-stroke controls.

Conclusion: High t-PA antigen in acute stroke patients probably indicates a poor prognosis. Its value as a marker for monitoring and prognostication needs to be evaluated as a routine clinical practice.

Keywords: acute stroke, plasminogen, plasminogen activator inhibitor-I (PAI-I), tissue plasminogen activator (t-PA)

Singapore Med J 2009; 50(6): 604-609

INTRODUCTION

The fibrinolytic system is responsible for the degradation of the solid-phase fibrin network that constitutes the major protein component of the thrombus. Fibrinolysis is initiated by either one of the serine proteases urokinase-type plasminogen activator or tissue-plasminogen activator (t-PA). This fibrinolytic process is mainly controlled by two homologous proteins – plasminogen activator inhibitor type-1 (PAI-1), that rapidly complexes with t-PA, and α^2-antiplasmin, which acts as a specific and rapid inhibitor of plasmin.[1]

The relationship between stroke and the fibrinolytic variables has been investigated in several studies. In one study, t-PA antigen concentrations were measured in baseline plasma samples from 88 healthy men who subsequently had a first-ever stroke and from 471 participants (control group) who remained free of cardiovascular disease during five years of follow-up. The study reported that mean baseline t-PA concentrations were significantly higher among men who later had strokes than in the controls.[2] In another study, high levels of t-PA antigen in ischaemic stroke patients were significantly higher than age-matched healthy control subjects.[3]

In the acute phase, stroke patients had significantly higher PAI-1 antigen concentration levels compared to the control subjects.[4] However, it has been reported that the genetic make-up for increased PAI-1 expression protects against stroke. These especially involve the PAI-1 4G/5G polymorphism because the alleles are the real determinants of PAI-1 expression.[5,6] The objective of this study was to investigate whether changes in fibrinolytic markers consistently present in acute stroke patients (as reported by previous researchers) and its contribution to the immediate clinical outcomes of the disease, i.e. the recovery potential and survival (independent of ischaemic volume and haemorrhagic size). It is important to know the status of these fibrinolytic markers in our local stroke patients and compare the results with other studies, as there may be differences in the disease process due to environmental, genetic or other factors.

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Table I. Modified Rankin scale.*

Grade	Description
0	No symptom at all.
1	No significant disability despite symptoms: able to carry out all usual duties and activities.
2	Slight disability: unable to carry out all previous activities but able to look after own affairs without assistance.
3	Moderate disability: requires some help, but able to walk without assistance.
4	Moderately severe disability: unable to walk without assistance, and unable to attend to own bodily needs.
5	Severe disability: bedridden, incontinent and requires constant nursing care and attention.
6	Death.

* adapted from van Swieten et al, 1988.[9]

METHODS

A comparative study was conducted over a one-year period in 2006. The participants were patients diagnosed with acute stroke, admitted to medical wards in our hospital. The control subjects were non-stroke individuals from various general outpatient clinics. Verbal and written consent were obtained from all the participants in this study. The study was approved by the hospital ethical committee.

Patients for the acute stroke group were selected based on the following criteria: (1) age > 45 years, male and female; (2) acute stroke, both ischaemic and haemorrhagic stroke; and (3) a time window of seven days from the onset of stroke. The exclusion criteria for the study group were patients who received anticoagulant/thrombolytic agents or developed a stroke due to one of the following complications: infective endocarditis or atrial fibrillation; haematological disorders, including antiphospholipid syndrome; brain tumour or metastatic tumour; and trauma or head injury. The selection criteria for the control group were based on the following: (1) non-hospitalised subjects; (2) age > 45 years, male and female; and (3) no previous history of stroke or transient ischaemic attack.

Subjects aged > 45 years were selected for both groups based on a study showing that most strokes occur when individuals are between 45 and 74 years of age.[7] All the subjects in this study were checked for the presence of stroke risk factors, which include hypertension (HPT), hyperlipidaemia (HPL), diabetes mellitus (DM) and ischaemic heart disease (IHD), obtained from their medical records. The presence of HPT was included when the blood pressure documented was ≥ 140/90 mmHg on two different occasions or when there was evidence of taking antihypertensive medication administered by the treating doctor. DM was present if the subjects had been informed of the diagnosis by the treating doctor and the medical records showed hyperglycaemia requiring a diet, oral medication or insulin treatment. A risk of IHD was included when the subject was receiving treatment or when the medical records documented the diagnosis. HPL was included if the subject had been informed about the diagnosis by the treating doctor and was on lipid-lowering agents.

Stroke patients usually present at the hospital within one week, if not immediately; therefore a one-week time window following stroke onset was accepted for this study. Furthermore, the fibrinolytic variables were expected to retain their levels during this period. One study showed that the levels of t-PA measured in stroke patients during the acute period (less than two weeks) were not significantly different from those measured after two weeks.[8]

The level of disability on admission was assessed using the modified Rankin Scale (MRS), as shown in Table I.[9] The MRS assessment was based on information gathered from the patients' medical records after being reviewed by a neurologist. Grade 0–1 was categorised as being functionally independent, Grade 2–3 was classified as being moderately dependent and Grade 4–5 was considered as being severely dependent. A favourable outcome by MRS was defined as a score of either ≤ 1 or ≤ 2.

The patients' outcome was studied prospectively at approximately three months after the stroke event when they attended the neurology clinic on their appointment day. The outcome was expressed as alive or dead at the said time. Patients who were alive were assessed for overall impairment using the MRS within this period. The MRS assessment was done prospectively, and retrospectively through medical records or by interview if they did not attend the clinic on their appointment day. Patients who did not return to the hospital were contacted and interviewed through the telephone. Patients were considered to be lost to follow-up if they did not return within three months after discharge or did not attend the neurology clinic on the given appointment date and could not be contacted via telephone.

Once the subjects consented, blood samples were collected by venipuncture into a trisodium citrate anticoagulant container. The samples were collected and processed according to the National Committee for Clinical Laboratory Standards guidelines. The tests for t-PA and PAI-1 antigen were conducted using Biopool TintElize® by enzyme immunoassay technique (Trinity Biotech PLC,

Table II. Demographical and clinical data of patients and controls.

	No. (%) of stroke patients (n = 51)	No. (%) of controls (n = 106)
Gender		
Male	21 (41.2)	52 (49.0)
Female	30 (58.8)	54 (50.9)
Ethnicity		
Malays	49 (96)	82 (77.4)
Chinese	2 (4)	24 (22.6)
Premorbidity		
Hypertension	42 (82.3)	72 (67.9)
Diabetes mellitus	19 (37.2)	44 (41.5)
Hyperlipidaemia	8 (15.7)	45 (42.5)
Ischaemic heart disease	10 (19.6)	5 (4.7)
No. of risk factors		
0	5 (9.8)	At least one risk factor present in each subject*
1	15 (29.4)	
2	17 (33.3)	
≥ 3	14 (27.5)	
Stroke subtype		
Ischaemic	42 (82.3)	
Haemorrhagic	9 (17.7)	

*The details of the number of risk factors in the control group are not available.

IDA Business Park, Bray, Wicklow, Ireland). The test for plasminogen activity was done by coagulation analyzer ACL 9000 using HemosIL™ (Instrumentation Laboratory Company SpA, Milano, Italy). The median level of t-PA in healthy individuals between 55 and 64 years of age is 8.6 ng/ml and 7.6 ng/ml for males and females, respectively.[10,11] The normal value of PAI-1 antigen in human platelet-poor plasma has been reported to be in the range of 4–43 ng/ml (18 ± 10 ng/ml with a correlation to PAI-1 activity of r = 0.80).[12] The range for plasminogen activity in normal subjects has been reported to be between 75% and 160%.[13]

All data was analysed using the Statistical Package for Social Sciences (SPSS) version 12.0 for Windows (SPSS Inc, Chicago, IL, USA). Plasminogen, t-PA and PAI-1 levels were numerical variables, therefore mean values were used. The differences in the means of the two groups were analysed using an independent *t*-test. Analysis of covariance (ANCOVA) was used to test the difference in the means of the two groups after controlling for confounders. Chi-square and Fisher exact test were used for categorical variables. The degree of association was presented by odds ratio (OR) and its 95% confidence interval (CI) as appropriate. Mann-Whitney U test was used in the analysis of non-normal distribution data.

RESULTS

There were 106 control subjects and 51 acute stroke cases altogether. In the control group, the age ranged between 50 and 84 years, with a mean of 59.9 years, whereas in the stroke group, the age ranged from 47 to 89 years, with a mean of 65.4 years. The demographical data of both groups are shown in Table II. Among the control group, the subjects possessed at least one risk factor for stroke.

The range, mean and standard deviation of all the three markers and the comparative means of all variables between both groups are shown in Table III. The mean t-PA concentrations were significantly higher for cases than for controls (14.76 vs. 10.93), with a p-value of 0.008, whereas there was no significant difference in the mean plasminogen (96.21 vs. 99.35) and PAI-1 (36.82 vs. 31.82) levels between the two groups, with p-values of 0.340 and 0.143, respectively. After controlling for possible confounders (adjusted mean using ANCOVA controlling for age, HPT, HPL, DM and IHD), t-PA was still significantly different between the stroke and control groups, with a p-value of 0.034. The association between the level of t-PA and the occurrence of stroke was tested using the χ^2 test, and the degree of association was presented by OR and its 95% CI as appropriate. A significant association was found between the high level of t-PA antigen and stroke occurrence with a 4.6-fold OR (95% CI 2.1–9.7; p = 0.000).

The degree of disability by MRS showed that most of the stroke patients were classified as Grade 4 (41%, n = 21). This was followed by Grade 5 (27.5%, n = 14), Grade 2 (15.7%, n = 8), Grade 3 (11.8%, n = 6) and Grade 1 (3.9%, n = 2). The majority of the patients were categorised in the severe disability group by MRS. However, there was no significant association between the levels of the three fibrinolytic markers and the severity of disease (functional impairment), i.e. mild to moderate disability (Grade 1–3) and severe disability (Grade 4–5) with a p-value of 0.732, 0.659 and 0.942 for t-PA, PAI-1 and plasminogen, respectively. Patients with the cerebral infarction subtype (n = 42) did not differ from the intracerebral haemorrhage subtype (n = 9) in the median plasminogen (100.5 vs. 101.0), median t-PA (12.5 vs. 13.0) or median PAI-1 (31.3 vs. 21.4), with a p-value > 0.05 by Mann-Whitney U test (non-parametric distribution).

There was no statistical association between the levels of the three fibrinolytic markers and age and gender (p > 0.05). There was also no association with the number of risk factors, i.e. one, two or more risk factors for DM, HPT, IHD and HPL (p = 0.323, 0.325 and 0.144, respectively, for t-PA, PAI-1 and plasminogen). Among the 51 stroke patients, four died during hospitalisation, one had ischaemic stroke and another three patients suffered from intracerebral bleeding. Four patients were reported to have died, between a few days and one month after being discharged from hospital. Two patients were

Table III. Comparison of plasminogen, t-PA and PAI-1 values between the stroke and control groups.

	Stroke Mean ± SD (range)	Control Mean ± SD (range)	Mean difference (95% confidence interval)	p-value*
Plasminogen (%)	96.21 ± 21.5 (47.2–145.0)	99.35 ± 17.9 (63.1–146.0)	3.14 (−9.6–3.3)	0.340
t-PA (ng/ml)	14.76 ± 9.84) (4.5–53.2)	10.93 ± 9.4 (0.2–81.4)	4.37 (1.16–7.59)	0.008
PAI-1 (ng/ml)	36.82 ± 21.9) (5.7–114.4)	31.82 ± 14.4 (6.7–74.3)	4.99 (−1.73–11.73)	0.143

* Independent *t*-test, $p < 0.05$ significant at 95% confidence interval.
SD: standard deviation

initially classified as functionally independent without significant impairments (Grade 1). However, these two patients' recovery could not be assessed accurately since their clinical findings were mild at presentation (Grade 0–1).

The recovery status could not be ascertained in 20 patients, who were lost to follow-up. Thus, an assessment of recovery could be done in only 21 patients (after excluding the death cases and the two patients with mild presentation). Information on the recovery status was also obtained by contacting these patients via telephone if they did not return to the hospital or clinic within three months of hospitalisation. The recovery status was assessed based on MRS. Out of these 21 patients, 14 had improved from Grades 4, 3 and 2 to Grade 0–1, whereas the other seven patients showed no clinical improvement based on their MRS grade at presentation. We did not find any significant difference in the recovery status among the stroke patients and the levels of the three fibrinolytic markers ($p = 1.000$ using Fisher exact tests).

A survival assessment was done from the available 21 patients at approximately three months post-stroke. Four patients died during hospitalisation and another four died at home within one month. All of them showed high t-PA levels and the majority presented with haemorrhagic stroke. However, our statistical analysis found no significant relationship between the levels of the three fibrinolytic markers (t-PA, PAI-1 and plasminogen) and the survival of the stroke patients, $p = 0.076$, 0.379 and 1.000, respectively. The mean t-PA level for patients who were lost to follow-up was higher than that of the patients who were being followed up ($p = 0.002$). However, there was no difference for the other two fibrinolytic markers.

DISCUSSION

High levels of both t-PA and PAI-1 have been observed in patients with a history of stroke.[2-4,14] In this comparative study, we found that among the three fibrinolytic markers, only the t-PA antigen level differed significantly between stroke patients and control subjects with similar risk factors. After adjustment for relevant confounders, i.e. premorbid conditions including age, HPT, DM, HPL and IHD, t-PA was still shown to be significantly different between the two groups. A population-based case-control study has shown that a high t-PA antigen level is independently associated with an increased risk of ischaemic stroke in non-diabetic females.[8]

In this study, an elevated level of t-PA was not found to be a protective factor as it was initially thought to be (by controlling the clot extension). The t-PA antigen assay used in this study detected both free t-PA and t-PA in complex with PAI-1. When the t-PA antigen was analysed with enzyme immunoassay, both free t-PA and t-PA/PAI-1 complex with its inhibitors were determined. A higher level of t-PA could indicate a decrease in fibrinolytic activity due to the inactivation of t-PA/PAI-1 complex. One study investigated whether t-PA, PAI-1 and tPA/PAI-1 complex could predict a first stroke. It reported that tPA antigen and t-PA/PAI-1 complex were independently associated with the development of a first stroke, especially haemorrhagic stroke.[15] This finding supports the hypothesis that disturbances in fibrinolysis do occur even before the first cerebrovascular event.

New insights from transgenic mice models have shown that the involvement of PAI-1 in stroke patients is more complicated because PAI-1 is involved in both harmful and protective steps in arterial disease aetiology.[16-19] A recent finding has shown a protective effect of 4G allele against stroke, suggesting that the involvement of PAI-1 in stroke patients is through a mechanism not related to fibrinolysis.[20] It is known that gender, blood pressure and body build influence the fibrinolytic variables.[11] In this study, no association was found between gender and the levels of the fibrinolytic markers. One study found a higher PAI-1 antigen level among women compared to men in South Asian ischaemic stroke patients.[21] Reduced fibrinolysis or an abnormal fibrinolytic system has been shown to be related

to hypertriglyceridaemia and coronary heart disease.[22] It has been reported that the plasma plasminogen level is decreased in the ischaemic cerebral vascular group and it was significantly different when compared to the control group.[23] Only five patients in this study showed low plasminogen levels and this was not due to a secondary cause, e.g. thrombolytic therapy.[13]

This study had a few limitations, including a small number of follow-up cases for statistical analysis. The findings from this study could be affected as a result of the small sample size. Only a few patients with intracerebral haemorrhage were analysed here. It was therefore difficult to ascertain the impact of the fibrinolytic markers and stroke subtype. The association of fibrinolytic variables among different ethnic groups (Malay and Chinese) were not analysed in this study as the majority of the subjects were Malay and therefore the contribution of the genetic factor could not be determined. However, based on the statistical analysis, no association was found between the mean t-PA levels in Chinese and Malays in the control group, $p = 0.172$. This finding probably indicates that a high t-PA level among the stroke patients was a genuine finding that is not related to the race of the study cohort.

The higher t-PA level in the patients who were lost to follow-up compared to those who were followed up was an important finding. Although there was no information to confirm whether these patients did not do well based on their high t-PA levels, t-PA could be a useful marker to be considered for future studies. For our data analysis, normal reference ranges for the fibrinolytic markers were based on the published data. Establishing normal reference ranges for fibrinolytic markers requires a set of criteria for the selection of subjects from the local population as they are influenced by many factors, including age, gender, smoking, etc.

In conclusion, the level of t-PA antigen was significantly different between acute stroke patients and the control group in our study cohort. The finding is consistent with the reported data in the literature. All patients who died within one month of onset possessed a high level of t-PA. Although the t-PA level is not routinely measured in stroke and high-risk patients, its value as a marker for monitoring and prognostication needs to be further evaluated in clinical practice.

ACKNOWLEDGEMENTS

We are grateful to Mr Madhavan Ramankutty (Haematology Laboratory Scientific Officer) for his advice on the laboratory methods, and to the University Short Term Grant (USM 304/PPSP/6131408) for supporting this study.

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