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An Investigative Study of Prothrombin Time, Activated Partial Thromboplastin Time and Antithrombin Levels in Patients with Ischaemic Stroke

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Background: The haemostatic system maintains a balance between prothrombotic and antithrombotic components in the body. The role of this system in ischaemic stroke is not certain.
Objective: Prothrombin time (PT) and Activated Partial Thromboplastin Time(APTT) are widely used screening tests to assess the haemostatic cascade. We sought to evaluate the relevance of these test and antithrombin (AT) levels in patients with ischaemic stroke.

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Methods: AT, platelet count, PT and APTT tests were carried out for 65 patients with ischaemic stroke and 65 controls with no history of stroke.
Results: The mean age of stroke subjects was 60.4 ± 12.3yrs. PT and APTT in the test and control groups were similar and Antithrombin levels did not show significant difference. But there was a significant association between functional AT deficiency and diabetes mellitus in the ischaemic stroke group. A significant negative correlation existed between functional AT activity and prothrombin and between AT Ag levels and APTT
Conclusion: There was no difference in the Antithrombin levels, PT and APTT values in patients with ischaemic stroke and those without a history of stroke and carrying out these tests routinely to assess hypercoagulable states in ischaemic stroke patients may not have any diagnostic value in our population.

Keywords: Antithrombin; Activated partial thromboplastin time; prothrombin time; Ischaemic stroke.

1. INTRODUCTION

The haemostatic system coordinates a delicate balance of pro-coagulant and anticoagulant proteins which maintain blood in the fluid state within the blood vessel and initiate clot formation to seal up the vessel upon injury, while ensuring clot dissolution after healing. The absence or amplification of components of this system may lead to inappropriate clotting and thrombotic conditions but its role in the pathogenesis of ischaemic stroke is not well defined. Components of the haemostatic system include the blood vessels, platelets, coagulation factors, natural anticoagulants and fibrinolytic proteins [1].

Platelets promote primary haemostasis and also provide the scaffold required for the progression of the coagulation cascade [1]. Platelets count and activation are used to assess platelet activity [2]. The coagulation cascade underlies the concept of the blood coagulation system and have been traditionally classified into the intrinsic and extrinsic pathways made up of clotting factors which converge to form thrombin through a series of interconnecting enzymatic reactions [3]. Thrombin cleaves circulating fibrinogen into insoluble fibrin and activates factor XIII, which covalently crosslinks fibrin polymers found in the platelet plug which stabilizes the clot and forms a permanent secondary haemostatic plug.

In the laboratory, the two basic coagulation tests used to assess the extrinsic and intrinsic coagulation pathways are prothrombin time (PT), and activated partial thromboplastin time(APTT) respectively. (4) The extrinsic pathway involves the transmembrane receptor tissue factor (TF) and plasma factor VII/VIIa while the intrinsic pathway is triggered by internal damage and involves coagulation factors in the intrinsic and common pathway (XII, XI, IX, X, VIII, II and I). Abnormal plasma levels or activity of these clotting factors are expected to cause dysregulation of the pathways which manifest as increased or shortened PT or APTT [4]. Some previous studies have found a significant correlation between the shortening of the APTT and increased risk of thromboembolism [5].

Antithrombin regulates coagulation by inhibiting thrombin (IIa), factor Xa, and to a lesser extent, factors IXa, XIa, XIIa, and factor VIIa/tissue factor. (3) Its capacity to restrict coagulation through various interactions makes it one of the main natural anticoagulant proteins.

A Stroke is a sudden loss of neurologic function resulting from focal (or global) disturbance of cerebral blood flow due to ischaemia or haemorrhage and may lead to permanent neurologic damage or death. Traditional risk factors for stroke include: hypertension, obesity, and diabetes mellitus among others, however, hypercoagulable states are recognized risk factors also and account for up to 1% of all patients with ischaemic stroke and up to 4% of young adults with stroke [6].

To ascertain the role of hypercoagulable states in ischaemic stroke, we assessed the PT and APTT of patients with ischaemic stroke, their association with antithrombin levels and the usefulness of these basic coagulation tests in screening ischaemic stroke patients.

The aim of this study is that it will contribute to knowledge on improving stroke management if it ascertain that these basic coagulation test are deranged in ischaemic stroke patients and also further studies can elucidate the role of anticoagulation in these patients.

2. MATERIALS AND METHODS

This was a descriptive, cross-sectional study carried out at the neurology clinic and stroke

ward of a Tertiary hospital in, Benin City, Edo State, south-south Nigeria. Adult patients with ischaemic stroke along with age- and sex-matched controls (healthy blood donors attending the blood donor clinic, and staff volunteers) were consecutively recruited for the study.

The Sample size was calculated from the formula for a cross-sectional study using prevalence of antithrombin deficiency by Soare et al. [7]. A total of 130 participants aged 18 years and above were consecutively recruited, comprising of sixty-five patients with ischaemic stroke (confirmed by cranial computerized tomography (CT scan)) verses sixty-five healthy controls.

2.1 Exclusion Criteria

Participants on oral anticoagulant therapy, Subjects with liver disease (confirmed by deranged liver function test) and a vascular disease were excluded from the study.

2.2 Methods

Demographic features, history of stroke risk factors and drugs were obtained by means of an interviewer-administered questionnaire as well as from patients' case notes.

Blood collection and laboratory analysis: Venous blood was collected into an EDTA bottle for analysis of platelet count (Full blood count) using a haematology Analyser (Erma Inc. PCE-210), heparin bottle for liver function test (LFT) and 3.2% sodium citrate bottles which were separated within 1hour by centrifugation at 2500rpm over 20 minutes and plasma supernatant extracted for PT and APTT (using Sysmex coagulometer (CA-560, Sysmex Europe GMBH, Bornbarch) while second aliquot for functional and antigen antithrombin assay was frozen at -80°C and analyzed in batches using TECHNOCHROM AT111 kit 39T (manual method) by Technoclone GmbH, Brunner str. 67, 1230 Vienna, Austria. LOT: 0531B00.02. REF: 5340225 and Enzyme-linked immunoassay test kit- AssayMax Human AT111 ELISA (Assaypro, 30 Triad South Drive St Charles,MO 63304, USA. LOT: 021371433. Catalog: EA3303-1).

Statistical analysis of data: Data was analyzed using SPSS version 18. Data were summarized as mean, frequency and percentages where appropriate. Chi-square, Fisher's test and student's t-test were used to compare the difference in proportions between non-parametric variables and parametric variables. Pearson correlation coefficient was used to correlate AT levels with PT and APTT. P value <0.05 was considered significant.

3. RESULTS

Sixty-five subjects with ischaemic stroke and 65 controls participated in the study and had mean age of 60.4 ± 12.3yrs and 59.0 ± 14.1yrs respectively. The stroke subjects included 42 (64.6%) males and 23(35.4%) females as against 45 (69.2%) males and 20(30.8%) females in the controls. Details of the demographic parameters and risk factors for stroke are presented in Table 1.

Table 1. Demographic Parameters and Identifiable Risk Factors in Study Participants

Variables	Ischaemic Stroke (n =65)	Controls (n = 65)
Age		
(Mean ± SD)	60.4 ± 12.3	59.0 ± 14.1
Sex		
Males	42 (64.6)	45 (69.2)
Females	23 (35.4)	20 (30.8)
Identifiable Risks		
Hypertension	46 (70.8)	29 (44.6)
Diabetes Mellitus	14 (21.5)	2 (3.1)
Smoking	3 (3.6)	2 (3.1)
Alcohol intake	17 (26.2)	10 (15.4)
Positive family history of Stroke	6 (9.2)	0 (0.0)
Previous Stroke	13 (20)	0 (0.0)
Previous VTE	1 (1.5)	0 (0.0)

Table 2 shows that the stroke subjects had higher platelet count but not statistically significant and the PT and APTT in test and control groups were similar.

As shown in Table 3, the mean functional AT levels, AT Ag levels and AT Ag(%) of the stroke and control groups were 93.36 ± 21.38, 282.97 ± 47.52 and 101.29 ± 2.36 respectively while those of the controls were 93.36 ± 19.88, 278.28 ± 49.59 and 99.19 ± 27.73 respectively. There was no statistically significant difference in the means between the patients and control groups.

Table 4 depicts no significant correlation between platelet count and AT. However, there was a significant negative correlation between functional AT activity and prothrombin time (r=-0.412; p- value = 0.001) and also between AT Ag levels (%) and APTT in the stroke subjects (r=-0.258; P = 0.038).

Table 2. Platelet count, Prothrombin time (PT) and Activated partial thromboplastin times (APTT) of Study Participants

Variables	Ischaemic Stroke (n =65)	Controls (n = 65)	P Value
Platelet (x10 ^{9}/l)	197.92 ± 64.81	186.35 ± 54.49	0.269
PT (secs)	13.68 ± 2.47	13.34 ± 1.60	0.356
APTT (secs)	37.11 ± 3.14	37.86 ± 1.43	0.081

Table 3. Antithrombin levels in Study Participants

Variables	Ischaemic Stroke (n =65)	Controls (n = 65)	P value
Functional AT (%)	93.36 ± 21.38	93.36 ± 19.88	0.998
AT Ag LEVEL (µg/l)	282.97 ± 47.52	278.28 ± 49.59	0.583
AT Ag (%)	101.29 ± 2.36	99.19 ± 27.73	0.641

Table 4. Correlation between Antithrombin, Platelet, Prothrombin time and APTT in Subjects with Ischaemic Stroke

		Platelet	Prothrombin Time	APTT
Functional AT	R	-0182	-0.412	-0.175
	P value	0.147	0.001*	0.163
AT Ag Level	R	-0.047	0.013	-0.232
	P value	0.712	0.915	0.065
AT Ag (%)	R	-0.029	-0.009	-0.258
	P value	0.820	0.945	0.038*

**Significant (p<0.05)*

Table 5. Comparism of demographic variables and risk factors for stroke in ischaemic stroke patients with and without AT deficiency

Variables	Ischaemic Stroke with AT def. (%)	Ischaemic Stroke without AT def. (%)	P Value
Age (Mean ± SD)	65.0 ± 0.0	60.3 ± 1.2	0.597
Sex			
Males	1 (1.5)	41 (63.1)	
Females	1 (1.5)	22 (33.8)	1.000
Total	2 (3)	63 (96.9)	
Identifiable Risk			
Hypertension	1 (1.5)	46 (70.8)	0.480
Diabetes Mellitus	2 (3.1)	12 (18.5)	0.044*
Smoking	0 (0.0)	3 (4.6)	1.000
Alcohol Intake	1 (1.5)	16 (24.6)	0.458
Positive family history of stroke	0 (0.0)	6 (9.2)	1.000
Previous Stroke	0 (0.0)	13 (20.0)	1.000
Previous VTE	0 (0.0)	1 (1.3)	1.000

**significant (p<0.05)*

There was a significant association between functional AT deficiency and diabetes mellitus in the ischaemic stroke group (Table 5).

4. DISCUSSION

The role of platelet count, PT and APTT in screening for hypercoagulability in ischaemic stroke was assessed in this study. The mean platelet counts of both study groups was in the mid range which may not pose a risk for hypercoagulability. This view aligns with the study of Yang et al that established a correlation between extremes of platelet counts and pathogenesis of ischaemic stroke but not mid-range values [8]. Low platelet count results from platelet activation and consumption associated with atherothrombosis which propagates recruitment of inflammatory complexes involved in stages of atherogenesis while increased platelet counts were associated with elaboration of platelet associated-prothrombotic substances [8,9].

We found that the PT and APTT tests were not deranged in patients with ischaemic stroke. In agreement with this, another study on COVID-19 patients with ischaemic stroke did not find any relationship between hypercoagulability and the severity level of ischemic stroke [10]. In contrast, a previous study had reported shortened APTT values in acute ischaemic stroke patients [11]. The disparity may be attributable to the time of assessment of the patients. Due to late presentation by our patients our study occurred in the post-acute stroke period unlike the other study which was carried out during the acute stroke event. Acute phase responses could affect the components of the coagulation pathway erratically with normalization in the chronic period and haemostatic disturbance could be a contributor and not a cause [12]. Diagnostic yield of coagulation tests in ischaemic stroke may require more specific coagulation tests which can reflect the complexity of the haemostatic impairment and also adjust for the timing of the stroke event. Also, it is important to control preanalytical errors from difficult or poor blood collection which cause cellular damage and release of tissue factors and commonly cause shortened APTT [13]. In the index study care was taken to avoid traumatic venipuncture and tourniquet stasis during blood collection. This was done by collecting blood from prominent veins and removing the tourniquet immediately the needle was inserted into the vein.

Our finding of normal antithrombin levels have been upheld by Hashem et al but refuted by Spiegelenberg et al who found lower levels of antithrombin activity especially in younger patients with recurrent stroke [14-16]. Differences in study population as seen with younger age group and timing of stroke event could account for these differences. Our finding aligns with the suggestion that antithrombin and other natural anticoagulants may not be involved in arterial thrombosis including ischaemic stroke, unlike its implication in venous thromboembolism. However, time-dependent changes in antithrombin activity may occur following acute events including stroke. So, levels might decline initially and then rebound in the following days accounting for the finding in the index study [17]. Antithrombin deficiency other hypercoagulable states are relatively more common in younger ischaemic stroke patients unlike older patients where traditional risk factors are dominant. The ischaemic stroke patients we studied, were mostly in the middle age group, and traditional risk factors included age, hypertension and diabetes mellitus. previous and family history of stroke would have predisposed them more to stroke rather than deficiency of antithrombin.

There was a significant association between functional AT deficiency and diabetes mellitus in the ischaemic stroke group. This is in keeping with the observations of Wang et al. [18]. This has been attributable to hyperglycaemia-induced conformational changes in antithrombin causing low heparin affinity, loss of anticoagulant activity and impaired secretion. Low AT levels in diabetes mellitus have also been reported in another study and this was opined to result from excessive consumption following coagulation activation [19]. The differences in antithrombin levels could result from timing of the study following acute stroke event.

We found a negative correlation between AT levels, PT and APTT. This is similar to the reports of Fisher, who suggested that a similar mechanism may account for the reduction in both procoagulant and anticoagulant proteins during acute thrombotic episodes [20]. Similarly, Gissel et al reported low levels of FVIII, FV, FX, prolonged APTT and higher AT levels in previous stroke patients compared to acute stroke patients [21]. This he attributed to the consequences of activation of coagulation and fibrinolysis in the acute and chronic phases of cerebral thrombosis.

5. CONCLUSION

PT and APTT tests may not have diagnostic value in ischaemic stroke patients and routine assessment with them may not be justifiable. Antithrombin may not play a significant role in ischaemic stroke in our population, but patients with diabetes mellitus may be assessed for additional risk of AT functional deficiency.

With regard to the study limitations, small sample size may limit generalization of the study findings.

6. RECOMMENDATIONS FOR FURTHER STUDY

Future studies with larger sample sizes and incorporation of more specific coagulation tests, such as D-dimer or thrombin generation assays, could provide a more comprehensive assessment of hypercoagulable states. Longitudinal studies to track changes in haemostatic factors over time or interventional studies to test the efficacy of targeted treatments for hypercoagulability would also provide clearer guidance for future research efforts.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Authors hereby declare that NO generative AI technologies such as language models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of manuscripts.

ETHICAL APPROVAL AND CONSENT

University of Benin Teaching Hospital Ethics and Research Committee was obtained for the study. Written informed consent was obtained from the participants or their relatives.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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