ORIGINAL ARTICLE

Influence of Acute Phase Biomarkers on Mortality and Functional Outcome in Adults with Acute Ischemic Stroke in North-western Nigeria

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ABSTRACT

Background: Stroke play a leading role in emergency admissions in developing countries and has contributed immensely to the huge financial burden to both the patient and the society especially in sub-Saharan Africa. Assessment of some routine acute phase biomarkers may aid in predicting mortality and functional outcomes in acute ischemic stroke. **Objectives:** To assess the role of routinely estimated C-reactive protein (CRP), Erythrocyte sedimentation rate (ESR), and platelet count in predicting mortality and short-term functional outcomes among patients with AIS in Kano, North western Nigeria. **Methods:** A case-control study where one hundred and twenty acute ischemic stroke patients and sixty age and sex matched healthy controls, were enrolled over a period of 10 months. Data on socio-demographic information and clinical characteristics were collected. Blood samples for acute phase biomarkers; C-reactive protein (C-RP), Erythrocyte sedimentation rate (ESR), and platelet count were collected and analyzed. The patients were followed up for 30-days and data on mortality and functional outcome using Modified Rankin Scale (MRS) was collected and analyzed using SPSS version 20.0 (IBM Armonk, NY). **Results:** The mean age of patients and their controls were 61.9±11.8 years and 61.8±12.4years and were predominantly of female gender (cases, 70/120 vs controls, 35/60). The mean of CRP, ESR, and platelets count for the cases and controls were 77.6±23.7 mg/L vs. 6.3± 2.8 mg/L, 53.4±30.2mm/hr vs. 12.2± 8.1mm/hr and 182.5±19.1/µL vs 251.4± 53.8/µL respectively. NIHSS was shown to independently predict 30-day mortality (Adjusted HR 1.10; 95% CI:1.02-1.18), p=0.011) after AIS. Additionally, elevated SBP, DBP, NIHSS and C-RP were shown to be associated with a poor 30-day functional outcome. **Conclusion:** The study showed that elevated levels of serum C-RP and ESR and a reduced platelet count predict higher mortality and poor functional outcomes in acute ischemic stroke. Assessment of some inexpensive routinely estimated acute phase biomarkers will guide therapy and help reduce overall mortality and improve short term functional outcomes in our resource-challenged environment.

Key words: Biomarkers, mortality, functional outcome, ischemic stroke

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Introduction

Stroke is a non-communicable disease with significant global socioeconomic consequences.[1]It is also a leading cause of increased morbidity and mortality, which is projected to worsen especially in developing countries over the next few decades.[2]Stroke account for 10.8% of all causes of mortality globally, with figures showing that 2.4% of all emergency admissions in Nigeria were due to stroke, and its case fatality at 7days, 30 days and 6 months were 28%, 40%, and 46% respectively.[3,4]

Inflammatory response led to increase in the serum levels of haematological acute phase biomarkers involved in the pathogenesis of acute ischemic stroke, which may contribute to overall mortality and short-term outcomes.[5,6]Those acute phase

biomarkers involved in stroke include, ESR, C-RP, fibrinogen, ferritin, serum amyloid protein A, alpha-1-antichymotrypsin, alpha-1-antitrypsin, haptoglobulin, alpha-1 acid glucoprotein, ceruloplasmin, and complements (C3, C4). Some of these markers are readily available, cheap, and routinely measured even in our resource challenged environment. Short term outcomes amongst acute stroke patients can be predicted early and the inflammatory process attenuated, using their serum or blood levels as a guide.[7]

C-reactive protein is an annular pentameric protein found in plasma synthesized in the liver and increases in response to an inflammatory process.[8]Its secretion follows elaboration of Interleukin-6 (IL-6) that is produced by both macrophages and T-cells. It usually binds to lysophosphatidyl choline expressed on surface of dead and dying cells, which is expressed as an antigen and this activates the complement system via C1Q complex. Association between the serum level of C-RP with the severity and outcomes in acute ischemic stroke has been found in previous studies. [9,10,11,12]

Erythrocyte sedimentation rate (ESR) is a non-specific measure of inflammation, which tends to increase in most inflammatory conditions.[13]Elevated ESR values were observed soon after stroke, which showed the relationship between the degree of acute phase response in the early phase of ischemic stroke and extent of local brain damage.[14]

Platelets play an important role in the pathogenesis of ischemic stroke by facilitating the formation of thrombi at local sites of vascular intimal damage or ruptured atherosclerotic plaques. Studies have shown that platelets count are lower in ischemic stroke patients when compared to the control groups.[15,16]There is paucity of data on the role of routinely estimated acute phase biomarkers in predicting mortality and short-term outcomes among patients with AIS in our environment. The data generated will help guide use of available therapy and the development of novel treatments targeting these biomarkers by physicians managing these patients in the future.

Methods

Study Design

A case-control study where the influence of some routinely estimated hematological acute phase biomarkers on 30-day mortality and functional outcomes of patients with AIS was conducted after due approval by the hospital research ethics committee. The study enrolled all consecutive patients 18 years and above and/or their proxies in the case of those with aphasia or altered consciousness who consented to participate in the study with clinical and radiological diagnosis of acute ischemic stroke, which occurred within 7 days. Patients with clinical or radiologic diagnosis of hemorrhagic stroke, stroke in pregnancy, developed stroke while on admission for other medical conditions (heart failure, chronic infections, malignancies, connective tissue diseases etc.) or patients taking medications (NSAIDs, statins, steroids) that are known to affect C-RP, ESR or platelet counts were excluded. The primary end point was either survival at 30 days or mortality from stroke. Patients were closely followed up to monitor progress using phone numbers provided in the questionnaire to call the patient directly (if conscious and not aphasic) or their relations (if patient is aphasic) and this was done at least twice weekly in order to enquire for any new onset symptom until the 30 days has elapsed. For those that did not survive the 30 days' period, information was gotten via the telephone call of the date and time of death from the date of onset of acute stroke and the possible cause of death. On the 30th day, the patients were brought to the hospital and their functional outcome was assessed using theMRS.[17]For those that could not make it to the hospital the assessment was done via the telephone and activities of daily living and dependence were asked. Demographic information and blood samples of healthy volunteers (including spouses, caregivers and relatives of stroke patients, civil servants, and healthy relatives of patients visiting the general outpatient department of the hospital) matched for age and gender were enrolled as controls. Volunteers with febrile illness or other chronic ailments like chronic liver disease and those on drugs (steroids, statins, and Aspirin) known to affect biomarkers were excluded. For every case and control, venous blood was taken from the ante-cubital vein using standard aseptic procedure. The samples were then placed in EDTA specimen bottles for platelets count and ESR, and a clot and gel activator specimen bottle for C-RP and were appropriately labelled. The blood samples were then transported to the laboratory and processed immediately for ESR and platelet counts, or were

centrifuged and serum for C-RP was obtained and stored at -200 degrees Centigrade for onward analyses. The samples and reagents were allowed to stand at room temperature and where gently mixed(followed by standard, enzyme conjugate, buffer, substrate and stop solutions), and absorbance was read on ELISA reader at 450nm within few minutes after adding the stop solution and a graph was plotted to obtain the values of CRP in mg/l.

For ESR assay the well mixed blood was transferred into the reservoir and the diluent added to the blood until it reaches the blood-filled level and with the cap securely placed, and the reservoir gently mixed manually by about 10 complete inversions. The blood–diluent mixture was then allowed to return to the bottom section of the reservoir by placing it at 900 to the horizontal and timing started immediately. After 60-minutes, the red cell column has slowly settle at the bottom leaving clear plasma above, which is measured in mm/hour. The estimated value for females was calculated using the formula (age + 10/2) while for males the formula (age/2) was used.

Platelet count was performed on blood in the EDTA bottle analyzed within two hours of collection for both the cases and controls using Automated Hematology Analyzer (Swelab Alfa, Boule medical diagnostic Stockholm, Sweden), which uses electrical impedance principle.[18]

Data Analyses

Data collected onto questionnaires were entered and analyzed using SPSS version 20.0 (IBM Armonk, NY). Summaries for categorical demographic variables were made using frequencies and percentages, while continuous demographic variables were presented as means and standard deviation or median and interquartile range for parametric and non-parametric variables respectively. Cox regression (proportional hazard model) was used to determine the effect of variables on mortality. Survival analysis(Kaplan-Meier) was used to estimate time to event (i.e. death) since the subjects were continuously enrolled at different times with variable lengths of follow-ups. Multivariate logistic regression was used to determine the predictors of functional outcome. P value of less than or equal to 0.05 was considered to be statistically significant throughout the study.

Results

A total of120 patients with ischemic stroke and 60matched controls who satisfied the inclusion criteria were enrolled over a period of 10months.Majority of the study subjects (62.7%)were married and were predominantly of female gender (cases, 70/120 vs. controls, 35/60). Stroke was uncommon (8.3%)below the age of 46 years (table 1). The mean age of patients and their controls was 61.9±11.8 years vs. 61.8±12.4years and the estimated serum CRP, ESR, and platelets count for the cases and control were 77.6±23.7 mg/L vs. 6.3± 2.8 mg/L, 53.4±30.2mm/hr vs. 12.2± 8.1mm/hr and 182.5±19.1/µL vs. 251.4± 53.8/µL respectively (table 2).

Cox regression identified NIHSS as the only variable found to truly predict mortality after 30-days of stroke patients (table 3). Survival analysis showed the time (30-days) to event(death) with majority of deaths occurring within the first 10 days after stroke (the highest case fatality at day 8) and the longest survival interval between days 20 to 25 (figure 1).

Patients with elevated SBP (OR;1.09, P=0.030), DBP (OR;0.85, P=0.024), NIHSS (OR;1.73, P=0.003) and serum C-RP (OR;1.09, P=0.040) were shown to have poor 30-day functional outcome among patients with acute ischemic stroke (table 4).

Table 1: Socio-Demographic Characteristics of Study Participants

Variables	Cases (N=120) Frequency (%)	Control (N=60) Frequency (%)	Chi square	P-value
Age (years)			0.109	0.947
18-45	10 (8.3)	5 (8.3)		
46-65	55 (45.8)	29 (48.3)		
≥66	55 (45.8)	26 (43.3)		
Gender			0.001	0.562
Male	50 (41.7)	25 (41.7)		
Female	70 (58.3)	35 (58.3)		
Marital status			9.023	**0.029***
Single	0 (0)	3 (5.0)		
Married	72 (60.0)	41 (68.3)		
Divorced	2 (1.7)	0 (0)		
Widowed	46 (38.3)	16 (26.7)		
Educational level			3.929	0.269
Informal	61(50.8)	29 (48.3)		
Primary	12 (10.0)	7 (11.7)		
Secondary	18 (15.0)	15 (25.0)		
Tertiary	29(24.2)	9 (15.0)		
Ethnicity			2.204	0.531
Hausa	89 (74.2)	44 (73.3)		
Fulani	21(17.5)	14 (23.3)		
Igbo	5 (4.2)	1 (1.7)		
Yoruba	5 (4.2)	1 (1.7)		

*Significant at the 0.05 level (2-tailed)

Table 2: Clinical Characteristics of Study Participants

Variables	Cases (N=120) (Mean±SD)	Control (N=60) (Mean±SD)	t-test	P-value
Blood pressure (mmHg)				
Systolic Blood Pressure	147.9±28.6	132.1±16.1	4.71	**<0.001***
Diastolic Blood Pressure	93.4±14.1	87.3±8.1	3.50	**0.001***
Capillary Random Blood Glucose (mmol/L)	6.9±1.2	6.7±1.2	0.92	0.36
Axillary Body Temperature (^{0}C)	36.5±0.2	36.4±0.2	2.26	**0.03***
30-day Functional Outcome among survivors using MRS (n=90) (Median±IQR)	3.0(2.0)	NA	NA	NA
C-RP (mg/L)	77.7±23.7	6.3±2.8	23.37	**<0.001***
Platelets (x10^9/L)	182.5±19.1	251.4±53.8	-9.61	**<0.001***
ESR (mm/hr)	53.4±30.2	12.2±8.1	13.95	**<0.001***

*Significant at the 0.05 level (2-tailed). NA= Not applicable, IQR-Interquartile Range

Table 3: Predictors of 30-day mortality in patients with acute ischemic stroke

Variable	Crude HR	P-value	Adjusted HR	P-value
Age	0.99 (0.95-1.04)	0.931		
Gender	0.40 (0.11-1.49)	0.175		
Pulse	0.99 (0.95-1.04)	0.892		
SBP	1.00 (0.97-1.02)	0.989		
DBP	1.03 (0.97-1.10)	0.231		
RBG	1.80 (1.05-3.08)	**0.030***	1.26 (0.89-1.79)	0.184
Temperature	1.22 (0.24-6.23)	0.805		
NIHSS	1.22 (1.01-1.46)	**0.032***	1.10 (1.02-1.18)	**0.011***
GCS	1.20 (0.85-1.71)	0.289		
MRS	1.22 (0.83-1.73)	0.225		
C-RP	0.97 (0.91-1.03)	0.414		
ESR	1.01 (0.97-1.05)	0.463		
PLT	0.99 (0.96-1.02)	0.678		

*Significant at the 0.05 level (2-tailed). HR-Hazard Ratio, SBP-systolic blood pressure, DBP-Diastolic blood pressure, RBG-Random blood glucose, GCS-Glasgow coma score, MRS- Modified Rankin score, CRP-C-reactive protein, ESR-Erythrocyte sedimentation rate, PLT-platelet count

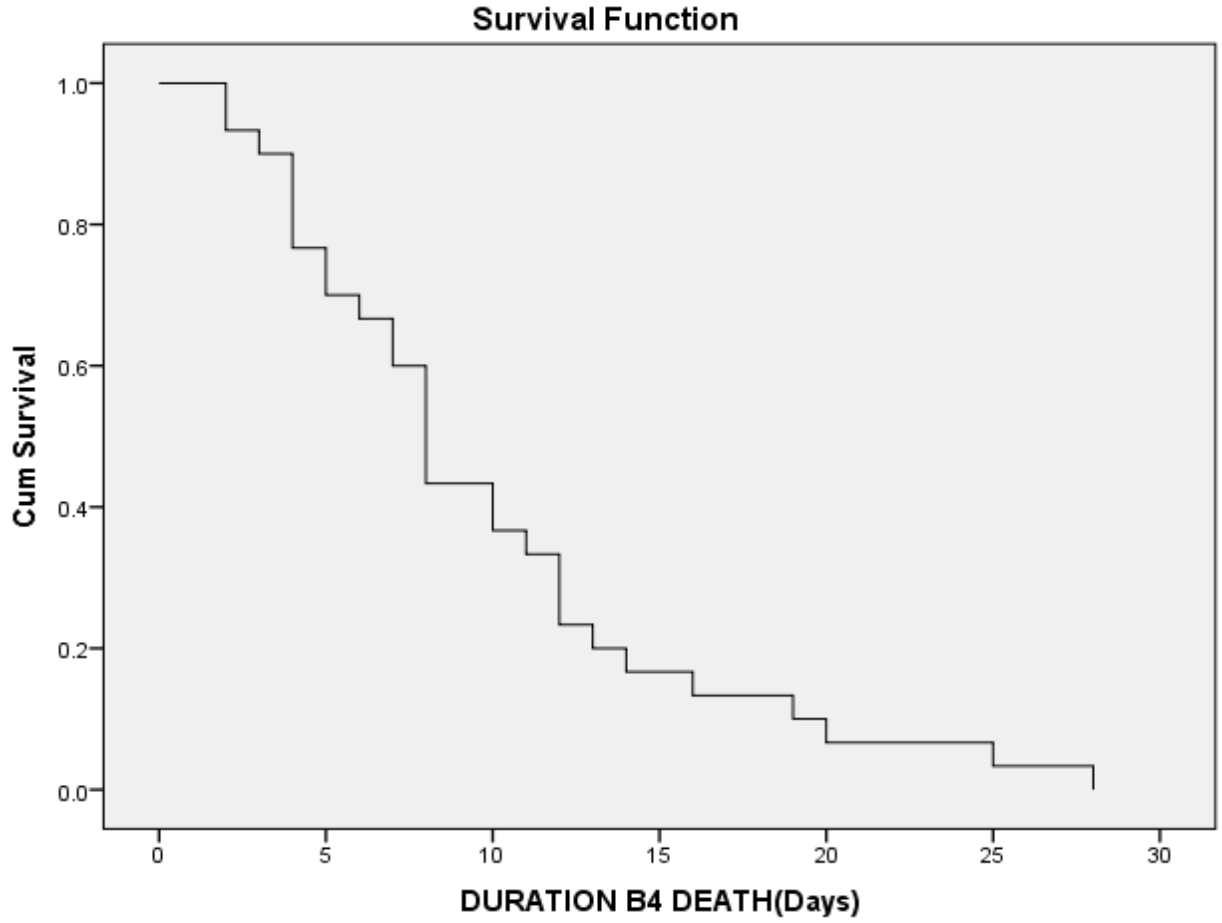

Figure 1: Survival plot for patients with acute ischemic stroke observed over 30 days

Table 4: Predictors of 30-day functional outcome using MRS

Variables	Unadjusted OR	P-value	Adjusted OR	P-value
Age	0.10	0.751	1.04	0.302
Sex	3.45	0.063	1.57	0.653
Pulse	4.66	0.031	1.00	0.824
SBP	12.82	**<0.001***	1.09	**0.030***
DBP	0.04	0.831	0.85	**0.024***
NIHSS	100.4	**<0.001***	1.73	**0.003***
GCS	80.26	**<0.001***	0.89	0.861
C-RP	60.77	**<0.001***	1.09	**0.040***
ESR	58.49	**<0.001***	0.97	0.598
PLT	0.12	0.726	0.96	0.252

*Significant at the 0.05 level (2-tailed) OR- Odd ratio

Discussion

The study present study shows that serum C-RP, ESR and PLT measured on admission can predict 30 days' mortality and functional outcome in patients with AIS. Several studies carried in African and non-African countries have shown increased serum C-RP and ESR values to be associated with increased mortality.[20,21,22,23] One explanation offered for this is the association between elevated C-RP and ESR levels and the large size of infarct which will cause a larger area of brain necrosis and will worsen the functional outcome due to neurological complications. Appearance of inflammatory cells within the damaged tissue after cerebral ischemia suggests that an inflammatory response plays a major role. Furthermore, the mean platelets count in stroke patients who died was lower compared to those who survived which was shown to be associated with poorer functional outcomes.[24,25]Thesefindings implies that the higher the mean C-RP and ESR levels in stroke patients the more the 30-day mortality, and the lower the platelets count the higher the 30-day mortality.

Our findings also indicated that NIHSS can predict the time to event (death) in AIS. Additionally, the time to event analysis showed death occurred more frequently in the first 10 days following an ischemic stroke, which is in agreement with studies conducted by Stegmayer who reported the highest mortality in the first week after stroke among Europeans.[26,27] Early death within the first seven days were attributed to the direct effects of neurological damage from inflammatory cascade, while deaths between 7-30 days mainly from complications of immobility.[8]

Limitations to the study included measurement of the acute phase biomarkers only once during their admission, which could be associated with generalized interpretation of findings. Serial measurements during hospitalization and even at home after discharge from the hospital would have been more representative in order to monitor the level of variability that may be encountered in early and intermediate phases of acute ischemic stroke. Furthermore, logistical challenges due to lack of a dedicated neuro-intensive care or acute stroke unit in our facility (with facilities for thrombolytic therapy) for the management of acute stroke could possibly influenced early death and poor functional outcomes.

Conclusion

The study showed that common and easily measurable acute phase biomarkers can be used to strongly predict early mortality and functional outcomes following stroke, even in resource challenged settings. Therefore, these markers may be added to the standard of care for AIS in our environment, because of the substantial clinical benefits at a much lower cost.

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None

Conflicts of Interest

None

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