Plasma Glial Fibrillary Acidic Protein, D-Dimer and S100β Protein: A Panel for Differential Diagnosis of Acute Stroke

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Abstract: Objectives: To explore the diagnostic utility of glial fibrillary acidic protein (GFAP), S100β protein and d-Dimer for differentiation between cases of acute stroke compared to CT findings as a gold-standard diagnostic modality. Patients & Methods: The study included 80 patients; 51 males and 29 females with mean age of 49.6±6.8 years. Mean time lapsed since occurrence of symptoms till sampling was 8.3±2.7; range: 2-12 hours. All patients underwent clinical injury severity evaluation using the National Institute of Health Stroke Scale (NIHSS), neuroimaging and gave blood samples for ELISA estimation of plasma levels of GFAP, S100β protein and d-Dimer. Results: Radiodiagnosis depended on computed tomography (CT) alone in 54 patients, magnetic resonance imaging (MRI) alone 17 patients and both CT and MRI in 9 patients and defined intracranial hemorrhage (ICH) in 12 patients, ischemic stroke (IS) in 31 patients, transient ischemic attack (TIA) in 19 patients and stroke mimic (S mimic) attack in 18 patients. Mean NIHSS score of ICH patients was significantly higher in patients had ICH and IS compared to TIA and S mimic patients. Mean at admission plasma levels of S100β protein and GFAP were significantly higher in ICH patients compared to all other patients and in IS patients compared to those had TIA and S mimic with significantly higher plasma levels of GFAP and significantly lower S100β in TIA compared to S mimic patients. Mean at admission levels of d-Dimer were significantly higher in IS patients compared to other groups. Stepwise regression and ROC curve analyses revealed that high GFAP and S100β levels are specific predictors for ICH, while high GFAP and d-Dimer could differentiate between acute IS from S mimics and TIA. Conclusion: High plasma levels of GFAP and S100β protein in association with short time lapsed till presentation and high clinical severity score could identify cases of hemorrhagic stroke, while high plasma levels of d-Dimer and GFAP in association with high clinical severity score could identify IS cases among cases of non-hemorrhagic stroke, so a panel of the three parameters; GFAP, d-Dimer and S100β protein could be used as a differentiating modality among cases of stroke and could be applied wherever neuroimaging facilities are unavailable or if patient's transfer is hazardous.
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1. Introduction

Neurological emergencies are common and frequently devastating. However, full evaluation in the acute setting often requires advanced diagnostics, and treatment frequently necessitates transfer to specialized centers. Delays in diagnosis and/or treatment may result in worsened outcomes therefore optimization of diagnostics is critical ***(Brown et al., 2004; Rosamond et al., 2008)***.

To date, the non-contrast CT has served as the main diagnostic tool. Although brain parenchymal changes visible on CT provide valuable prognostic information, they provide limited insight into the potential for tissue salvage in response to reperfusion therapy, such as thrombolysis. Moreover, while CT technology is readily available in most cities, rural settings and developing countries may not have rapid guaranteed access to this diagnostic tool or the skilled personnel to interpret the images. Even when CT is available, time is dedicated to the acquisition and interpretation of the images before treatment can begin, causing an unavoidable and highly variable delay in therapy ***(Butcher & Emery, 2010)***.

Serum biomarkers were widely applicable for evaluation of patients had various forms of acute illness especially those with misleading clinical and inconclusive radiological findings and for prognosis of patients with severe illness ***(Rallidis et al., 2006; Svoboda et al., 2007)***. Identifying a biomarker or panel of biomarkers of cerebral ischemia would have a major impact on the care of stroke patients by facilitating early management decisions and individualization of care ***(El Husseini & Laskowitz, 2010)***.

Serum biomarkers related to pathways of hemostasis, oxidation and inflammation, or alterations in glial and neuronal proteins, have been identified.

Protein S100 is an acidic, disulfide-linked, dimeric, calcium-binding, low molecular weight protein. The beta subtype of this protein exists in astrocyte cells in relatively high concentrations. Rises in serum concentrations of S100B have been shown to relate to clinical evidence of central nervous system damage ***(Foerch et al., 2005)***.

The astrocytic glial fibrillary acidic protein (GFAP) is the principal intermediate filament protein found in the cytoskeleton of mature astroglia ***(Hergenroeder et al., 2008)***. Previous studies showed a delayed release of the GFAP into the serum in patients with ischemic stroke, reaching maximum concentrations between days 2 and 4 ***(Herrmann et al., 2000; Foerch et al., 2003)***. Because of the more sudden disruption of the blood–brain barrier and the resulting brain damage ***(Foerch et al., 2009)***, so it is hypothesised that GFAP would immediately be detectable in serum in the hyperacute phase of ICH, but not in ischemic stroke, and thus could be useful as a rapid diagnostic marker for ICH in acute stroke patients. The present study aimed to explore the diagnostic utility of GFAP, S100β protein for differentiation between the two entities of acute stroke compared to CT findings as a gold-standard diagnostic modality.

2. Patients & Methods

The present study was conducted at Neurology and Clinical Pathology Departments Al-Dar Hospital, Quba, KSA. After obtaining written fully informed consent from patients or their nearest relatives, 80 patients presented with new neurological symptoms consistent with acute stroke and had no recent history of trauma were enrolled in the study. Ten volunteers who passed the preliminary test for blood donation and within cross-matched age and sex were included in the study to donate blood samples as control group for serum levels of estimated markers.

Neurological assessment

Stroke was defined as persistent neurological deficit lasting <24 hours felt to be of vascular etiology and associated with compatible neuroimaging studies; intracerebral hemorrhage was diagnosed based on CT, transient ischemic attack (TIA) was defined as transient focal neurological deficits believed to be of ischemic vascular etiology but with clinical symptoms lasting <24 hours, or stroke mimic defined by historical, radiographic, or laboratory evidence of an underlying nonvascular medical condition resulting in a neurological deficit. Clinical injury severity was judged using the National Institute of Health Stroke Scale ***(NIHSS, 2003)***.

Neuroradiological Examinations

All neuro-radiological examinations were based on cranial CT and/or MRI scans. All scans were performed in standardized slices without contrast enhancement soon after admission. Lesions were evaluated with respect to lesion topography and territories of vascular supply on the basis of ***Damasio & Damasio (1989)***.

Neurobiochemical Examinations

Venous blood samples were collected at admission in Na citrated tube in a ratio of 9 vol. blood: 1 vol. Na citrate, plasma was separated by centrifugation and stored at –80 ^{o}C till ELISA estimation of plasma levels of S-100β protein, ***(Missler & Wiesmann, 1995)***, GFAP ***(Missler et al., 1999)*** and d-Dimer ***(Pittet et al., 1996)***.

Statistical analysis

Obtained data were presented as mean±SD, ranges and ratios. Results were analyzed using Wilxocon Ranked (Z-test) test for unrelated data. Regression analysis, Stepwise method, was used to evaluate the predictability of a panel of the three laboratory parameters alone and in combination for the CT pathological findings. Specificity of these predictors was evaluated using the receiver operating characteristic (ROC) curve analysis judged by the area under the curve (AUC) compared versus the null hypothesis that the AUC=0.5. Statistical analysis was conducted using the SPSS (Version 10, 2002) for Windows statistical package. P value <0.05 was considered statistically significant.

3. Results

The study included 80 patients; 51 males (63.8%) and 29 females (36.2%) with mean age of 49.6±6.8; range: 39-71 years. Mean time lapsed since occurrence of symptoms till sampling was 8.3±2.7; range: 2-12 hours, but was significantly ($p<0.05$) shorter in patients had ICH compared to others. Radiodiagnosis depended on CT alone in 54 patients (67.4%), MRI alone 17 patients (21.3%) and both CT and MRI in 9 patients (11.3%).

Clinical and radiological work-up defined 12 patients had ICH, 31 patients had ischemic stroke, 19 had TIA and 18 had stroke mimic attack. The mean NIHSS score of patients had ICH was 13.3±5.4, while was 11.6±2.8 in patients had IS and was significantly ($p<0.05$) in patients had ICH and IS compared to those had TIA and S mimic with non-significant difference between ICH and IS patients and between TIA and S mimic patients, (Table 1).

Mean at admission plasma levels of S100β protein and GFAP were significantly higher in patients had ICH compared to all other patients and in patients had IS compared to those had TIA and S mimic with significantly higher plasma levels of GFAP and significantly lower S100β in patients had TIA compared to those had S mimic. On contrary, mean at admission levels of d-Dimer were significantly higher in patients had IS compared to other patients' groups that showed non-significant inter-group difference, (Table 2, Fig. 1).

Table (1): Patients' presenting data

		ICH	IS	TIA	S mimic	Total
Number		12 (14.9%)	31 (38.8%)	19 (23.8%)	18 (22.5%)	80
Age (years)		46.5±5.6	51.6±12.4	49.9±5.1	47.8±4.4	49.6±6.8
Gender; M:F		8:4	22:9	11:8	10:8	51:29
Time till sampling (hrs)		4.9±2	8.2±2.7*	9.9±2.1*	9.2±2.2*	8.3±2.7
NIHSS score		13.8±3.9	11.6±2.8	8.8±1.7*†	4.6±2*†	9.7±3.9
Mode of radiologic diagnosis	CT	7 (58.3%)	21 (67.7%)	14 (73.7%)	12 (66.7%)	54 (67.5%)
	MRI	3 (25%)	7 (22.6%)	3 (15.8%)	4 (22.2%)	17 (21.3%)
	Both	2 (16.7%)	3 (9.7%)	2 (10.5%)	2 (11.1%)	9 (11.2%)

ICH: intracranial hemorrhage IS: ischemic stroke TIA: transient ischemic attack S mimic: Stroke mimic
CT: Computed tomography MRI: magnetic resonance imaging *: significant versus ICH †: significant versus IS

Table (2): Mean (±SD) at admission plasma markers levels

	Control (n=10)	ICH (n=12)	IS (n=31)	TIA (n=19)	S mimic (n=18)
S100β (ng/ml)	24.5±9 (11-42)	115.2±31* (69.3-165.3)	74.4±27.4 *† (34.1-131.9)	49.5±24.8*†‡ (24.7-109.3)	68.2±17.6*†‡# (32.9-98.1)
d-Dimer (ng/ml)	0.152±0.03 (0.11-0.21)	0.61±0.23*‡ (0.31-0.93)	1.04±0.46* (0.5-2.3)	0.51±0.23*‡ (0.23-0.97)	0.71 ±0.39*‡ (0.25-1.78)
GFAP (ng/ml)	3.48±1.76 (1.6-6.5)	89.7 ±18.6* (64-118)	58.7 ±16.9* † (36-78)	31.8 ±10.1*† ‡ (24-65)	17.8 ±4.8*†‡# (9-31)

ICH: intracranial hemorrhage IS: ischemic stroke TIA: transient ischemic attack S mimic: Stroke mimic
*: significant versus control †: significant versus ICH ‡: significant versus IS #: significant versus TIA

The presence of ICH showed a negative significant correlation, (r=-0.326, p=0.003) with the time lapsed till arrival to the hospital, but showed a positive significant correlation with NIHSS score determined at time of admission, (r=0.451, p<0.001), plasma levels of S100β protein, (r=0.569, p<0.001) and GFAP, (r=0.657, p<0.001). On contrary, the presence of IS among non-hemorrhagic strokes showed a positive significant correlation with severity score, (r=0.516, p<0.001), plasma levels of d-Dimer, (r=0.579, p<0.001), S100β protein, (r=0.348, p=0.004) and GFAP, (r=0.537, p<0.001).

Concerning the presence of ICH as constant factor, regression analysis defined plasma GFAP level as the constant significant predictor for the presence of ICH, followed by plasma S100β protein and lastly plasma d-Dimer level. As regards diagnosis of IS among non-hemorrhagic strokes, plasma d-Dimer level was found to be the constant significant predictor followed by GFAP, while plasma S100β protein was the least diagnostic. To verify the significance of combined estimation for the diagnosis of ICH among stroke cases, estimated serum GFAP level alone showed the highest significance, (F=59.371, p<0.001), followed by a panel of GFAP and S100β, (F=44.437, p<0.001) and lastly the panel of the three parameters, (F=43.512, p<0.001). However, for IS diagnosis among non-hemorrhagic strokes, d-Dimer alone showed the highest significance, (F=33.260, p<0.001), followed by a panel of d-Dimer and GFAP, (F=32.779, p<0.001).

ROC curve analysis defined that the shorter the time till admission and the more severe clinical presentation; the more likelihood for the presence of ICH. Thus both clinical data are significantly suggestive of ICH as a diagnosis, while GFAP and S100β levels could be considered as significant specific predictors for the presence of ICH, (Fig. 2). For differentiation of IS among other types of non-hemorrhagic stroke, high plasma d-Dimer and GFAP levels in association with high clinical severity score could be considered as significant specific predictors for IS among other types of stroke, (Table 3, Fig. 3).

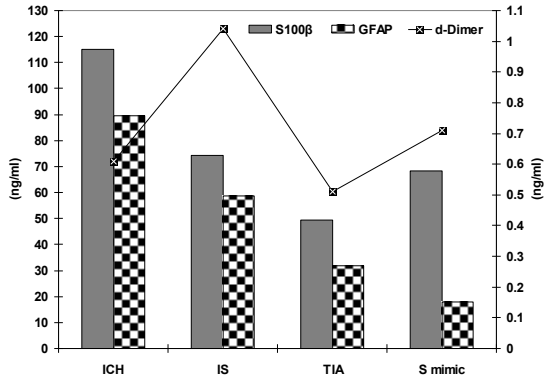

Fig. (1): Mean plasma levels of estimated parameters in patients categorized according to radiologic findings

Table (3): ROC curve analysis for the specificity of estimated parameters for presence of ICH on neuroimaging

		AUC	SE	Significance	Asymptotic 95% CI	
					Lower Bound	Upper Bound
For presence of ICH among strokes	Time till admission	0.240	0.089	0.004	0.065	0.414
	HINSS score	0.825	0.054	<0.001	0.719	0.931
	GFAP	0.995	0.024	<0.001	0.909	1.002
	S100β	0.891	0.048	<0.001	0.796	0.986
	d-Dimer	0.409	0.083	>0.05	0.247	0.571
For IS among non-hemorrhagic stroke	Time till admission	0.474	0.072	>0.05	0.333	0.615
	HINSS score	0.812	0.054	<0.001	0.706	0.919
	GFAP	0.851	0.044	<0.001	0.764	0.939
	S100β	0.693	0.064	=0.006	0.567	0.819
	d-Dimer	0.809	0.063	<0.001	0.686	0.932

ICH: intracranial hemorrhage IS: ischemic stroke NIHSS: National Institute of Health Stroke Scale
GFAP: glial fibrillary acidic protein AUC: area under curve SE: standard error CI: confidence interval

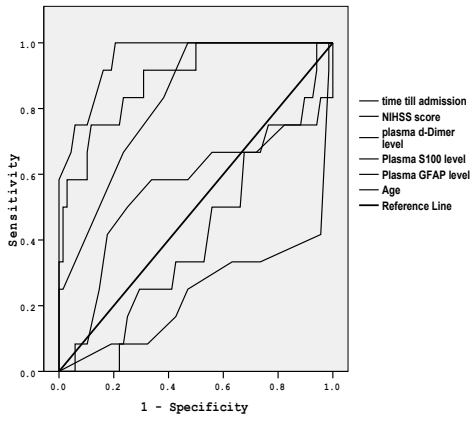

Fig. (2): ROC curve analysis of evaluated parameters as predictors for diagnosis of ICH among stroke cases

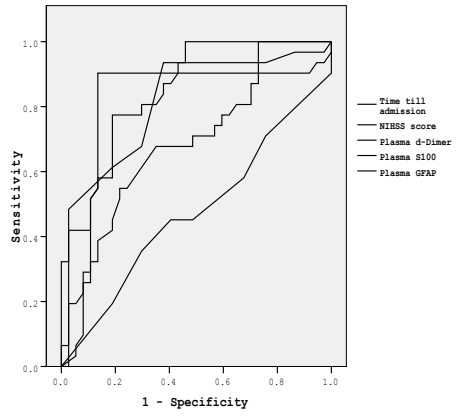

Fig. (3): ROC curve analysis of evaluated parameters as predictors for differentiation between cases had IS among non-hemorrhagic stroke cases

4. Discussion

The term "stroke" comprises all diseases in which a region of the brain is transiently or continuously affected by ischemia or hemorrhage, and/or in which there are pathologies in the vessels nourishing the brain ***(Kmietowicz, 2009)***. Besides being a serious health problem due to its high mortality and morbidity, stroke is a very significant social and economical problem ***(Olai et al., 2009)***.

Brain imaging has been indispensable for the reliable differentiation between ICH and cerebral ischemia in acute stroke. Consequently, cause specific management of acute stroke patients is not possible in the prehospital setting. For instance, according to current recommendations, raised blood pressure should be treated more aggressively in patients with ICH than in those with ischemic stroke. Mechanical ventilation is more often required and neurosurgical treatment may be indicated in ICH patients. In addition, very early haemostatic treatment may offer the possibility of arresting hematoma growth, which is mainly responsible for clinical deterioration within the first few hours ***(Qureshi et al., 2001; Mayer et al., 2005 a&b)***. Thus a simple diagnostic test applicable in the prehospital phase would be helpful for triage and to optimize the subsequent hospital management, so the present study aimed to evaluate the diagnostic yield of estimation of plasma levels of GFAP, S100β protein and d-Dimer for differentiation between cases of acute stroke.

Mean at admission plasma levels of S100β protein and GFAP were significantly higher in patients had ICH compared to all other patients and in patients had IS compared to those had TIA and S mimic with significantly higher plasma levels of GFAP and significantly lower S100β in patients had TIA compared to those had S mimic. On contrary, mean at admission levels of d-Dimer were significantly higher in patients had IS compared to other patients' groups that showed non-significant inter-group difference.

These data point to the following facts: firstly the plasma levels of the three markers were elevated in

stroke patients, irrespective of the underlying pathology and go in hand with ***Kaneko et al. (2009)*** who found serum GFAP was significantly higher in cardiac arrest patients with a poor outcome at 12 and 24hrs without therapeutic hypothermia and at 48hrs with therapeutic hypothermia and was a specific predictor of poor neurological outcome at 6 months with or without therapeutic hypothermia treatment after the return of spontaneous circulation. ***Shinozaki et al. (2009)*** tried to determine whether is the early and accurate serum predictor of neurological outcome; protein S100β or neuron-specific enolase within 24h after cardiac arrest and found serum S100β and NSE in "poor outcome" group were higher than those in "favorable outcome" group, but S100β was found more reliable as an early predictor of poor neurological outcome within 24h after cardiac arrest than NSE and can be applied clinically.

Secondly, the increased levels of the three parameters implies a fact that a combined pathology is a possibility, such attribution goes in hand with ***Wang et al. (2009)*** who reported that the frequency of acute symptomatic cerebral infarctions in patients with spontaneous supratentorial ICH is high and is associated with longer hospitalization and worse outcome. ***Jeon et al. (2009)*** suggested that new micro-bleeds can develop rapidly after acute ischemic stroke and baseline micro-bleeds and severe small vessel disease are predictors for the development of new micro-bleeds.

Combined high GFAP and S100β levels could specifically differentiated between hemorrhagic and non-hemorrhagic acute stroke, while high GFAP and d-Dimer could differentiate between acute IS from S-mimic and TIA. These findings were in line with that previously reported in literature; ***Dvorak et al. (2009)*** found between 2 and 6 h of stroke onset, serum GFAP was significantly higher in ICH patients than in IS patients and serum GFAP values were significantly correlated with intracranial hematoma volume. ***Bernard et al. (2010)*** identified D-dimer as candidate biomarker for etiology and prognosis in childhood-onset arterial ischemic stroke. ***Isenegger et al. (2010)*** found low D-dimer levels in the first few hours make a cardioembolic stroke unlikely, and may be useful to guide further investigations.

The diagnostic yield of S100β is still controversial, in the current study plasma S100β level was found to be correlated with the presence of hemorrhage and also with the presence of ischemic insult, a finding indicating its relation to the presence of brain injury irrespective of the mechanism of injury without differentiating power, a finding supported that previously reported by ***Und et al. (2009)*** who found no differences in S100 and NSE levels between patients with ischemic stroke or intracranial hemorrhage. On the other hand, S100β protein level when estimated in combination with GFAP could differentiate between hemorrhagic and ischemic stroke. These data were in line with ***James et al. (2009)*** who found serum S100β and brain natriuretic peptide levels in the first 24 h after injury accurately predict neurological function at discharge after supratentorial ICH.

Concerning the presence of ICH as constant factor, regression analysis defined plasma GFAP level as the constant significant predictor presence of ICH and ROC curve analysis defined that the shorter the time till admission and the more severe clinical presentation; the more likelihood for the presence of ICH, while GFAP and S100β levels could be considered as significant specific predictors for the presence of ICH. In hand with these findings, ***Foerch et al. (2012)***, reported that plasma GFAP analysis performed within 4.5 h of symptom onset can differentiate ICH and ischemic stroke.

Conclusion

It could be concluded that high plasma levels of GFAP and S100β protein in association with short time lapsed till presentation and high clinical severity score could identify cases of hemorrhagic stroke, while high plasma levels of d-Dimer and GFAP in association with high clinical severity score could identify cases ischemic stroke among cases of non-hemorrhagic stroke, so a panel of the three parameters; GFAP, d-Dimer and S100β protein could be used as a differentiating modality among cases of stroke and could be applied wherever neuroimaging facilities are unavailable or f patient's transfer is hazardous.

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