Original Article

Assessment of S100β Biomarker in Acute Ischemic Cerebrovascular Stroke Patients with Hypertension

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ABSTRACT

Background: Cerebrovascular stroke (CVS) is the third leading most common cause of death in the world. Role of antihypertensive drugs and nitric oxide donors such as glyceryl trinitrate (GTN) in acute ischemic cerebrovascular stroke varies in their effects on cerebral autoregulation (CA). Assessment of biochemical marker as S100β protein is an important diagnostic tool. **Objective:** The aim of this study is to assess the role of transdermal glyceryl trinitrate (GTN) patch in the management of acute ischemic CVS and to evaluate the role of S100β protein as a prognostic marker in acute ischemic CVS. **Methods:** Forty acute ischemic CVS patients with hypertension were included. They were divided in to two groups, Group (A); 20 patients maintained on their anti-hypertensive treatment, and Group (B); 20 patients received GTN nitro dermal patch 5mg. All cases were subjected to, clinical evaluations by European stroke scale, assessment of S100β on third day and after 14 days of stroke onset and brain CT. **Results:** There was no significant statistical difference between patient groups as regard clinical stroke evaluation on third day on stroke onset but there was significant statistical difference between group (A) and (B) after 14 days of stroke. There was highly significant statistical difference in the serum level of S100β in group (A) and (B) on third day of stroke onset. **Conclusion:** Using GTN nitro dermal patch is a promising solution in management of hypertension in acute ischemic CVS and S100β may help in the prediction of its prognosis. **[Egypt J Neurol Psychiat Neurosurg. 2014; 51(2): 153-158]**

Key Words: Acute ischemic CVS, Hypertension, Transdermal GTN, S100 β.

INTRODUCTION

Cerebrovascular stroke (CVS) remains a major cause of long-term disability. Hypertension presents in more than 75 % of patients at presentation with acute ischemic stroke.[1]

Cerebral autoregulation (CA) is defined as the ability of the brain to maintain relatively constant CBF despite changes in cerebral perfusion pressure (CPP).[2] CA is impaired in the presence of moderate to severe cerebral ischemia.[3] Calcium channel blocker is one of antihypertensive drugs which reduces cerebral perfusion in parallel with their effect on blood pressure (BP).[4] Angiotensin modifying drugs, such as captopril and perindopril (angiotensin-converting enzyme inhibitors) and losartan (angiotensin receptor antagonist) did not seem to alter cerebral blood flow (CBF) or middle cerebral artery blood velocity.[5]

Similarly, nitric oxide donors such as sodium nitroprusside and glyceryl trinitrate (GTN) maintain regional cerebral blood flow.[6] It was further shown that the administration of a vasodilating agent would often dilate vessels in normal regions and shunt blood from the ischemic area. This paradoxical phenomenon is called the intracerebral steal. Thus significant reductions in BP after transdermal GTN are not associated with changes in CBF or cerebral perfusion pressure or cerebral steal in patients with recent stroke, this renders it an important agent in management of acute ischemic cerebrovascular stroke.[7]

At present, the absence of a widely available and sensitive diagnostic laboratory test for acute cerebral ischemia remains a significant limitation in the diagnosis and prediction of outcome of stroke. The evaluation of blood-borne biochemical markers of tissue injury appears recently as a new diagnostic and prognostic tool.[8] Several monitoring techniques have been developed based on measuring the levels of various proteins, including neuron specific enolase, myelin basic protein, glial fibrillary acidic protein, and S100 protein.[9]

S100 protein is an acidic calcium-binding protein, its molecular weight is 21,000μg, and it constitutes a major component of the cytosol, predominantly in astroglial cells. It was termed S100 because it is partially soluble in a 100% saturated solution of ammonium sulfate. The protein consists of two subunits (α and β). S100α is found in striated muscles, kidneys and heart and S100β is found in high

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concentration in glial and schwann cell. Elevations of S100β in cerebospinal fluid (CSF) and serum were reported in various forms of acute brain damage. Concentration of S100β in CSF is a sensitive marker of brain damage after head trauma, cerebral hypoxia, cerebral bleeding and ischemic stroke.[10]

Post stroke S100β serum concentrations were reported to correlate significantly with the size of infarcted brain areas. As it is released from dead astroglail cells followed by a leakage of the protein through an impaired blood-brain barrier. The peaks of S100β serum levels were observed at the second and the third days after stroke onset.[11]

Aim of work: to assess the role of transdermal GTN patch in the management of acute ischemic cerebrovascular stroke patients with hypertension and to evaluate the role of S100β protein as a prognostic marker, in the patients groups in correlation with clinical and radiological evaluation.

SUBJECTS AND METHODS

This is case control study included 40 patients with acute cerebrovascular ischemic stroke and hypertension, 18 of them are males and 22 are females with mean age ±SD (57.65±12.16). Patients were divided into two groups 20 patient maintained on their antihypertensive treatment group (A) and 20 patient received GTN nitrodermal patch 5mg. Group (B). Patients were selected from the inpatient of neurology department, outpatient clinic and emergency room department of Al-Zahraa University Hospital. Healthy 20 subjects of matched age and sex are selected to act as a control group.

We excluded those suffering from previous cerebrovascular stroke, recent acute infection, collagen disorders, hematological disorders, liver and renal diseases, myocardial infarction, unstable angina, left ventricular failure and contraindication to nitrate therapy.

All patients were submitted to the following: full medical history and general medical examination, neurological assessment including history and complete neurological examination, evaluation of stroke by European stroke scale to assess the neurological deficit. This scale was done within 48hours from the onset of stroke and repeated after 14 days for each patient to detect the prognostic outcome of stroke, as regard group B they received GTN nitrodermal patch 5mg for 16 hour daily then removed 8 hours for 6 days, follow up of blood pressure for both groups, routine laboratory investigations, assessment of laboratory biomarkers; serum S100β on the third day and repeated after14 days of stroke onset and assay of S100β protein was done by Enzyme Linked Immunosorbant assay kit (ELISA) and brain computed axial tomography (CT) (Philips Tomoscan LX); it was done for all patients within 48hours from the onset of stroke and repeated after 14 days. It was done to detect; type of stroke (ischemic only), site of the lesion and size of the lesion.

Statistical Analysis

The data were collected, coded and SPSS (statistical package for social science), windows version 11.5 was used for analysis. Data were expressed as number and mean ±standard deviation (SD). We also used analysis of variance (ANOVA); a single test used to collectively indicate the presence of any significant difference between several groups (several arithmetic means). Quantitative data were analyzed by using student t-test of significance. Correlation coefficient (r) test to correlate between the data of the different groups. $P<0.05$ is considered significant and $p<0.001$ is considered highly significant.

RESULT

This study was conducted on 40 patients (18 male and 22 female) the mean of their age was 57.65±12.16, divided into two groups. Group (A); 20 patient maintained on their antihypertensive treatment. Group (B); 20 patient received GTN nitrodermal patch 5 mg.

There was no significant statistical difference between patients used antihypertensive drugs (group A) and patients used GTN patch (group B) as regard clinical stroke evaluation according to European stroke scale on the third day of stroke onset (p value >0.05) but there was significant statistical difference between group A&B after 14 days of stroke onset it was better in group B than group A (p value <0.05) (Table 1).

There was decrease in mean arterial BP (MAP) in patient used antihypertensive drugs group A as well as in those using GTN patch group B. The decrease was more in patient used GTN patch than those maintained on their antihypertensive drugs but of no significant statistical difference (p value >0.05) (Table 2). There was highly significant statistical difference as regard the level of S100β between patient groups and control group ($p<0.001$) (Table 3).

There was highly significant statistical difference in the serum level of S100β in group A and B of patients in comparison with control group on 3rd day and after 14 days of stroke onset (p value <0.001). Also there was highly significant statistical difference in the serum level of S100β between group A and group B on 3rd day of stroke onset (p value <0.001) but there was no significant statistical difference in the serum level of S100β between group A and group B after 14 days of stroke onset (p value >0.05) (Table 4).

There was significant statistical difference between means of S100β in different sites of insults it was higher in subcortical regions then capsular then brain stem and the lower level was in the lacunar stroke (p value <0.05) (Table 5).

There was significant statistical difference between group A and B as regard the size of infarction was larger in group A than group B (p value <0.05), also there was significant statistical difference between group A and B as regard outcome of stroke (the European stroke scale after 14 days) it was better in group B than in group A (P value <0.05) (Table 6).

There was a highly significant positive correlation between the level of S100β and the size of infarction at the 3rd and 14th days of stroke onset in the patient groups (p<0.001) (Table 7).

Table 1. European stroke scale results of patient groups (A and B) according to the time of examination.

	G(A)			G(B)			Independent t-test	
	Range	Mean	±SD	Range	Mean	±SD	t	P-value
European scale (on 3rd day)	54 - 93	80.1	10.99	75 - 90	83.9	4.79	1.418	0.164
European scale(14th day)	70 - 100	89.6	7.24	80 - 98	94.65	4.89	2.585	0.013*

*Significant at p<0.05

Table 2. Comparison between patient groups (A and B) as regard BP.

Variable	Group A		Group B		Independent t-test	
	Mean	±SD	Mean	±SD	t	P-value
Day 1	117.32	14.13	113.18	13.62	0.941	0.353
Day 2	113.49	10.29	106.93	16.54	1.506	0.140
Day 3	108.59	12.70	104.63	13.84	0.943	0.352
Day 4	105.74	13.06	106.36	16.25	0.133	0.895
Day 5	107.19	13.75	106.80	21.59	0.068	0.946
Day 6	109.89	9.20	101.46	18.23	1.845	0.073

Table 3. The level of S100β in the studied patient groups versus the control group.

Groups	S100β			Independent t-test	
	Mean	SD	Range	t	P-value
Patient groups	524.13	120.37	350 – 842	16.939	0.000*
Control group	65.2	11.72	48 – 90		

*Significant at p<0.01

Table 4. Comparison between patient groups A and B and control group as regard S100β in the 3rd day and after 14 days of stroke onset.

Variable	Group A Antihypertensive		Group B GTN patch		Control		P-value
	Mean	±SD	Mean	±SD	Mean	±SD	
S100β 3rd day (ng/L)	574.75	128.8	473.5	88	65.2	11.7	$p_1 < 0.001^*$ $p_2 < 0.001^*$ $p_3 < 0.001^*$
S100β 14th day (ng/L)	317.6	137	347.2	65.6	65.2	11.7	$p_1 > 0.05$ $p_2 < 0.001^*$ $p_3 < 0.001^*$

*Significant at p<0.01, p_1: Comparison between Antihypertensive group and GTN group, p_2: Comparison between Antihypertensive group and Control group, p_3: Comparison between GTN group and Control group

Table 5. Comparison between the level of S100β and site of infarction in patient groups.

Site	S100β		One-Way ANOVA	
	Mean	±SD	F	P-value
Cortical-Subcortical	582.40	161.31	3.211	0.002*
Capsular	565.78	133.29		
Lacunar	472.44	66.50		
Brain stem	456.00	10.58		

* Significant at p<0.01

Table 6. Comparison between patients groups A and B as regard size of infarction, European stroke scale after 14 days of stroke onset.

Variable	Group A		Group B		Independent t-test	
	Mean	±SD	Mean	±SD	t	P-value
CT-scan 14th day (cm)	1.77	0.65	1.09	0.71	2.234	0.038*
European scale at 14th day	89.47	7.21	95.65	4.82	-2.253	0.036*

* Significant at p<0.05

Table 7. Correlation between the level of S100β and the size of infarction among patient groups (A and B) on the 3rd and 14th days of stroke onset.

Variable	Size of infarction	
	r	P-value
S100β at 3rd day (ng/L)	0.799	0.000*
S100β at 14th day (ng/L)	0.899	0.000*

* Significant at p<0.01

DISCUSSION

The management of high BP in acute ischemic stroke is highly controversial because of a lack of reliable evidence from randomized, controlled trials. In spite of the controversy, the evidence that blood pressure in acute stroke should be treated when it is very high level.[12]

There was no significant statistical difference between patients used GTN patch (group A) and patients used other antihypertensive drugs (group B) as regard clinical stroke evaluation by European stroke scale on the third day of stroke onset but there was significant statistical difference between group A and B after 14 days of stroke onset.

This can be explained that GTN nitrodermal patch act as anti-inflammatory through oxygen free radicle activation and it is not associated with changes in CBF or cerebral perfusion pressure or cerebral steal in patients with recent stroke.[7]

Our study found that GTN is useful in the control of hypertension during acute stage of stroke than other antihypertensive drugs, there was decrease in the MAP in both groups but it was more in patients used GTN (group B) than those used various types of antihypertensive drugs (group A). The current study found that the decrease of BP was more in the 2nd and 6th day of stroke, with good outcome of patients according to European stroke scale.

Our findings are in partial agreement with Philip and colleagues[13] who stated that GTN significantly lowered BP by 13.0/5.2 mmHg at day 1 and 9.3/5.0 mmHg at day 8. The lesser reduction at day 8 than day 1 can be explained by tolerance to GTN was developing. This result agrees with Ankolekar and colleagues[14] who reported that GTN might be beneficial if given very early after stroke.

However, this result disagrees with Schrader and colleagues[15] who stated that BP in the first week and functional status at onset of stroke and at 3 months were similar between treatment and placebo groups. This can be explained by the difference in the selection of patient groups.

Schrader and colleagues[15] explained his results by that acute ischemic stroke, is associated with dysfunctional cerebral autoregulation so that cerebral perfusion becomes dependent on systemic BP. However, GTN release NO, which is a potent modulator of cerebrovascular reactivity, especially in collateral vessels.[16] Vascular nitric oxide levels are low in stroke, so collateral vessels may not be maximally dilated. Hence, CBF might be held constant with GTN if moderate reductions in systemic BP were counterbalanced by increases in collateral blood supply, which would be potentially beneficial in acute stroke.[17]

Our mean value of S100β is highly significant in patients groups than control because it is sensitive marker of brain damage.[10]

Our samples are collected within the first three days of stroke onset because the peaks of S-100β serum levels were observed at days 2 and 3 after stroke onset.[18] A possible explanation for this gradual increase could be that cellular injury resulting from cerebral ischemia is a gradual process.[19]

Our results agree with that of Foerch and colleagues[20] who stated that S100β measurements between 12 and 24 h after stroke onset predict a size of infarction with the highest degree of accuracy.

In our study there was significant statistical difference between means of S100β as regard sites of insults it was higher in cortical subcortical regions then capsular then brain stem and the lower level was in the

lacunar stroke. These results was in agreement with Kim and colleagues[21] who reported non-measurable S100β concentrations in patients with small subcortical lesions, and this is consistant largely with our study in patients with lacunar infarction who had normal levels of serum S100β subunit and who improved markedly and showed excellent clinical improvement on basis of neurological and functional outcome.

In our study, there was a highly significant statistical positive correlation between S100β and the size of infarction at the 3rd day and after 14 days of stroke onset in the patient groups A and B. This is explained by the presence of S100β in serum is the consequence of combined leakage out of necrotic glial cells and passage through an impaired blood-brain barrier, indicating severe ischemic cell injury. Large infarction zones generally lead to serious neurological deficits in the acute stage of stroke and severe functional impairment.[19]

Our findings are in agreement with Brea and colleagues[11], who reported that serum S100β levels are significantly raised in patients with ischemic stroke and haemorrhagic stroke; S100β levels are significantly related to infarct size and clinical outcome.

Conclusion

Our study showed that GTN patch can be useful as an antihypertensive medication for the hypertensive patients who developed acute ischemic cerebrovascular stroke. Added to its antihypertensive effect, it can improve the clinical scales and the size of infarction in those patients. The serum level S100β was positively correlated with the size of infarction adding an indirect laboratory confirmation for the promising role of GTN patch in acute ischemic cerebrovascular stroke. Further studies are warranted to investigate the role of GTN patch in the management of hemorrhagic cerebrovascular stroke. Nevertheless, single S100β obtained 48 and 72 hours after ischemic stroke onset could be applied as an easily accessible and valid surrogate marker to evaluate the size of infarction and monitor the effect of GTN patch or any other future antihypertensive medications to be used in acute cerebrovascular stroke.

[Disclosure: Authors report no conflict of interest]

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الملخص العربى

تقييم دور بروتين إس 100 بيتا في مرضي السكتة الدماغية الإرتوائية الحادة المصابون بارتفاع ضغط الدم

تعتبر السكتة الدماغية ثالث أكبر سبب يؤدي إلى الوفاة في العالم ويعتبر ارتفاع ضغط الدم الموجود في أكثر من 75٪ من مرضى السكتة الدماغية الإرتوائية الحادة من أهم الأسباب التى ترتبط بشكل مستقل بقلة نسبة التحسن وتكرار الحالة في وقت مبكر.

وقد وجد أن العقاقير التي تخفض ضغط الدم في مرضى السكتة الدماغية الحادة تختلف في تأثيرها على تدفق الدم إلى المخ.

وقد قامت بعض الأبحاث بدراسة مدى فاعلية لاصقة ثلاثى نترات الجلسرين عبر الجلد لتقليل ضغط الدم أثناء السكتة الدماغية الحادة وما يصاحب ذلك من تغيرات في الإمداد الدموى للمخ.

وقد برزت أهمية اكتشاف مجموعة من الدلالات المعملية لتشخيص السكتة الدماغية ومدى تأثيرها على خلايا المخ مثل بروتين إس 100 بيتا في مرضي السكتة الدماغية حيث أنه يمثل أحد الدلالات الحاسمة السريعة والدقيقة التي تعبر بدقة عن حجم الإصابة.

العينة وطرق البحث: لقد تم إجراء الدراسة على 40 من مرضى السكتة الدماغية الإرتوائية الحادة المصابون بارتفاع ضغط الدم. ولقد تم تقسيم المرضى الى مجموعتين أساسيتين مجموعة (أ) 20 من المرضى يعالجون بأدوية الضغط المختلفة، مجموعة (ب) عشرون من المرضى يعالجون باستخدام لاصقة ثلاثى نترات الجلسرين، ومجموعة ضابطة وتشمل عشرون من الأصحاء.

أساليب البحث: وقد خضع جميع الأفراد إلى ما يلي: التاريخ الطبي الكامل، والفحص الطبي العام و الفحص العصبى. الفحوص المخبرية الروتينية. نسبة بروتين إس 100بيتا بالدم فى اليوم الثالث واليوم الرابع عشر من حدوث السكتة الدماغية. أشعة مقطعية على المخ.

ولقد أبرزت الدراسة النتائج الآتية:

- أن لاصقة ثلاثي نترات الجلسرين تخفض ضغط الدم في السكتة الدماغية الإرتوائية الحادة بدون أي تأثير علي تدفق الدم إلي المخ أو زيادة أعراض السكتة الدماغية وذلك بالمقارنة بأدوية الضغط الأخري.
- قد وجد أن مآل السكتة الدماغية الإرتوائية الحادة كان أفضل في المرضي الذين قاموا باستخدام لاصقة ثلاثي نترات الجلسرين بالمقارنة بالذين قاموا باستخدام أدوية الضغط الأخري .
- أنه يوجد علاقة طردية واضحة بين مستوي بروتين إس 100بيتا بالدم وحجم الجلطة المخية في الأشعة المقطعية في اليوم الثالث واليوم الرابع عشر من حدوث السكتة الدماغية الإرتوائية الحادة.