Reflex Plasma Renin Activity, Plasminogen Activator Inhibitor and Glomerular Filtration Rate in Patients with Ischemic Cerebral Stroke

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ABSTRACT

Background: Short-term evolution following ischemic stroke can associate acute renal insufficiency as a possible complication which is frequently overlooked and underestimated. Objective: We aimed in this prospective study to estimate this incidence and to test the probable affection of plasma renin reflex sensitivity (PRRS). Methods: Twenty one consecutive cerebral ischemic patients hospitalized with a C.T. confirmed diagnosis of ischemic stroke. Seventeen control normal persons were used for comparison. Recumbent, standing plasma renin activities (PRA), PRRS, plasminogen activator inhibitor and creatinine clearance (Ccr) were estimated at admission of the patients and two weeks later.

Results: The Ccr was 68±8.6 & 66±6.4 ml/min. at admission and two weeks later respectively. It was significantly lower than that of control group (103±4.0 ml/min.). PRRS had decreased significantly at admission in comparison to control persons (1.24±0.15 and 2.30±0.26 ng/ml respectively), but it had increased significantly 2 weeks later in comparison to either that at admission or of control persons (4.86 ng/ml). The decreased Ccr at admission was significantly and negatively correlated with the decrease in the sensitivity of the reflex PRA.

Conclusion: Acute ischemic stroke patients had a reduced Ccr at admission and 2 weeks later, the sensitivity of reflex PRA had changed significantly from a significant decrease at admission to a significant increase 2 weeks later. This finding may be suggested as a sensitive visceral neurovegetative parameter for further studies to be added to the currently used somatosensory parameters of NIHSS (national Institute of Health Stroke Scale) for follow up of ischemic stroke accidents. (Egypt J Neurol Psychiat Neurosurg. 2010; 47(2): 297-302)

Key Words: Cerebral ischemic stroke, creatinine clearance, plasma renin reflex sensitivity.

INTRODUCTION

Several reports have indicated chronic kidney disease to be an additional independent and powerful predictor for stroke outcome and activation of the renin angiotensin system (RAS) has been linked with an increased risk of myocardial infarction and stroke1. Also based on a retrospective analysis of a large population in the immediate period following stroke Covic et al.2, reported that acute kidney function diminution may develop as a possible complication. The authors included the acute kidney injury as a risk factor in the survival analysis. However, this association is frequently overlooked and underestimated in clinical trials. And there are still no studies describing a cerebral-renal physiological axis which could explain the reported stroke associated acute kidney alteration, and how could a chronic kidney disease be a powerful predictor for stroke outcome.

In this work, we aimed at confirming the incidence of this acute kidney function alteration in our ischemic stroke patients and studying our suggested alteration and/or incrimination of the physiological reflex neuronal mechanism of renal renin secretion in a probable cerebral-renal interaction in stroke patients. This is together with suggested plaminogen activated inhibitor integration in this interaction since the RAS can influence the fibrinolytic balance3.

SUBJECTS AND METHODS

The study was approved by the committee of Medical Research Ethics and was carried out at neurology department, Mansoura University hospital and medical school; Egypt.

Study population:

This is a prospective study conducted at Mansoura university hospital. The study included all consecutive cases with a CT- confirmed diagnosis of ischemic stroke at the neurology department. A total of 21 patients were eligible for the study. All patients included in the study were followed for two weeks. These patients were studied in comparison to 17 volunteer normal persons.
Inclusion criteria and stroke definition:
In this study, we included all patients aged around sixty years that presented in Mansoura Emergency Neurology Department with first ever stroke between February 2008 and January 2009. Stroke was defined as rapidly developing clinical signs of focal disturbance of cerebral function, lasting more than 24 h with no apparent cause other than vascular origin. Ascertainment of each case was based on the medical history, clinical neurological examination by a neurologist, CT scan to confirm the diagnosis and to determine the type and evolution of stroke at admission at the first 24 hours and at the end of the two weeks of follow up. Local investigators used the National Institute of Health Stroke Scale (NIHSS) to assess the neurologic deficits of the patients at baseline and at their follow up. All 21 patients were characterized as being completely independent according to this scale. Eight patients had no detectable CT scan lesion at time of admission in spite of stroke diagnosis based on the neurological examination; these patients had the detectable CT scan lesion by the scan done at the end of two weeks of follow up. This observation had been previously reported. According to the hospital protocol, radio contrast media were not used; considered unnecessary; for all cases. All patients were under treatment with antiplatelets, nootropics, antioxidants and some patients received anticoagulants during the two weeks of follow up.

Exclusion criteria:
Patients were excluded if they had a hemorrhagic stroke; had a history of cardiovascular or renal diseases or diabetes or with a pre-stroke drug treatment for a systemic disease.

Clinical variables:
Details of neurological status and disabilities, and current drug use were recorded. Other relevant clinical data, including ECG and CT scan results, were also collected. Because of the short duration (The first two weeks of stroke), the NIHSS neurological scale follow up was considered outside the scope of this study. However, with the objective of recording the conscious state of our patients along the two weeks of the study we followed Glasgow coma scale. All patients were having either 14 or 15 score of the good consciousness along the two weeks of follow up.

Laboratory data, including creatinine and glucose concentration on admission, were collected. In addition to:

1. Definition of acute kidney function diminution:
In this study acute kidney function diminution was defined by estimation of the creatinine clearance (Ccr) which indicates the value of the glomerular filtration rate (GFR) both at the patient admission and after two weeks of follow up. Change of the Ccr after two weeks compared to that estimated at admission confirm the acute post stroke alteration of the GFR. This approach could compensate absence of prestroke information about serum creatinine in some patients.

Creatinine clearance was estimated through the usual equation:

\[ \text{Urine creatinine mg/dl urine collected for 24 hrs} \]
\[ \times \text{Urine volume collected for 24 hrs} \]
\[ \text{Serum creatinine mg/dl serum} \times 1440 \]

2. Estimation of reflex plasma renin activity (PRA): The patient coming recumbent to the emergency room within the first 12 hours of the stroke was consulted. Venous blood sampling was done at the end of one hour of recumbence. Following this period of recumbence another venous blood sample was taken at the end of 15 minutes of standing. The difference between the estimated PRA of the two samples indicates the sensitivity of the baroreceptor mediated reflex renin secretion by the juxtaglomerular apparatus of the kidneys. The increase in serum PRA by standing indicates the efficiency of the reflex renin secretory activity of the kidneys. The PRA was determined by radioimmunoassay (RIA) kit (Hyphen Medical, USA).

3. Determination of plasminogen activator inhibitor (PAI): It was determined in plasma using ELISA kit (Hyphen Medical, USA).

Statistical Analysis:
The data obtained were parametric. They are presented as mean ± SEM. One-way ANOVA analysis of data was done followed by post hoc test of Tukey. Paired Student t-test was done when appropriate in comparing the infarction size and in comparing recumbent and standing PRA of the same patient. Pearson correlation statistical analysis was done for detection of a probable significance between two different parameters. A P value of < 0.05 value was considered significant.

RESULTS

Seventeen control group normal persons were having a GFR of 102.9±4.1 ml/min., a recumbent PRA of 6.2±0.6 which reflexly increased to 8.5±0.7 ng/ml by standing, a difference which was significant, but the value of the reflex increase was positively significantly proportional to the recumbent position PRA. Together with a PAI of 20.5±1.2 ng/ml. On the other hand, 17 patients of 21 admitted with a diagnosed ischemic stroke were of a left hemispheric localization and the remaining four patients were having a right hemispheric localization. At admission all patients had a significant decrease in GFR in comparison to control normal persons (68±8.6 and 103±4.0 ml/min. respectively. This degree of diminution was detectable also (66±6.4 ml/min) at the end of the second week after admission (Table 1).
Recumbent PRA measured at the first 6-12 hours of admission of these patients was significantly higher than of the control normal persons (11.0±0.6 versus 6.2±0.6 ng/ml). It had increased more and more at the end of the second week of admission (30.0±1.4 ng/ml), an increase which was markedly significant in comparison to the level measured at admission. PRA measured at the end of 15 minutes of standing following 6-12 hours of recumbence was having the same developing significant increases like these of the recumbent PRA (Table 1). In both normal control persons and ischemic stroke patients standing had induced a significant increase in PRA in comparison to the recumbent PRA at either admission or two weeks later.

Reflex PRA indicated by the difference between standing and recumbent PRA was 1.24±0.10 ng/ml in ischemic stroke patients at admission which was significantly less than that of the control normal persons (2.30±0.37 ng/ml). However this reflex PRA was significantly and markedly increased at the second week measurement (4.86±0.37 ng/ml) either in comparison to that of the admission value or to that of the control normal person group. Only, admission value of the standing induced reflex PRA was significantly negatively correlated with GFR measured at admission of the ischemic stroke patients. This correlation had not been detected at the second week of admission (Table 1). PAI had increased significantly by ischemic stroke to the same degree at either admission or at the second week measurement in comparison to the control normal persons.

Eight of the 21 ischemic stroke patients were having a non detectable C.T. scan lesion of either hemisphere and the lesion had been detected in all 8 patients at the left hemisphere by the end of the second week. The C.T. detected lesion of the 21 patients was significantly increased in size at the second week in comparison to the size measured at admission of the 13 patients.

Table 1. Reflex plasma renin activity, plasminogen activator inhibitor and glomerular filtration rate in patients with ischemic cerebral stroke.

<table>
<thead>
<tr>
<th></th>
<th>Control Normal Person</th>
<th>Admission</th>
<th>Two Weeks Later</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (ml/min)</td>
<td>103±4.0</td>
<td>68±8.6 *</td>
<td>66±6.4 *</td>
</tr>
<tr>
<td>RPRA (ng/ml)</td>
<td>6.2±0.6</td>
<td>11.0±0.6 *</td>
<td>30±1.4 A*</td>
</tr>
<tr>
<td>SPRA (ng/ml)</td>
<td>8.5±0.7†</td>
<td>12.2±0.6 †</td>
<td>35.0±1.4 A†</td>
</tr>
<tr>
<td>REFLEX PRA (ng/ml)</td>
<td>2.30±0.26</td>
<td>1.24±0.15 †</td>
<td>4.86±0.37 A*</td>
</tr>
<tr>
<td>PAI (ng/ml)</td>
<td>20.5±1.2</td>
<td>40.5±1.1 †</td>
<td>42.7±1.2 A</td>
</tr>
<tr>
<td>Infarction area (Cm²)</td>
<td></td>
<td>0.296±0.09</td>
<td>0.562±0.12 †</td>
</tr>
</tbody>
</table>

GFR: glomerular filtration rate; RPRA: recumbent plasma rennin activity; SPRA: standing plasma rennin activity
Reflex PRA: reflex plasma rennin activity; PAI: plasminogen activator inhibitor

Data were expressed as Mean±SEM

1- One-way ANOVA analysis of data was done followed by post hoc test of Tukey.
2- *< 0.05 in comparison to control normal persons & ∆ in comparison to Admission value
3- † < 0.05 in comparison to control normal persons
4- ‡< 0.05 significant negative correlation with GFR at admission.

DISCUSSION

This clinical study confirm the post stroke acute diminution of the renal function as indicated by the decrease in the GFR which was significantly marked at the first 24 hours of admission in those patients with detected CT hemispheric infarction. This incidence of the acute diminution of renal function is in accordance with the observation of Covic et al. They have explained this acute alteration of the kidney function by the particular characteristics of the stroke-prone population, elderly individuals, associating multiple cardiovascular co morbidities frequently treated with multiple drug associations, and usually with impaired renal function. Our patients were around sixty of age; and they had no history of neither associated cardiovascular co morbidities or impairment of the renal function. Interestingly this impairment of renal function seems to be not reversible since our patients had no improvement of the renal function by the end of the two weeks of follow up (Table 1).

Since activation of the RAS has been linked with an increased risk of myocardial infarction and stroke, we have evaluated both tonic (recumbent) and reflex (standing) PRA. The difference between recumbent and standing levels indicates the baroreceptor reflex mediated renin release. The cardiopulmonary reflex represents a powerful mechanism for the direct control of renin secretion by the juxtaglomerular cells and under most physiological circumstances this control outweighs the control exerted by the arterial baroreceptors during assumption of the upright posture. Reflex renin secretion is a complex physiological activity modulated by the supramedullary centers in addition to the medullary ones. This visceral reflex function could be altered by

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stroke like other somatoskeletal hemiplegic alterations\textsuperscript{15}. The RAS is important in the hemodynamic regulation and the modulation of renal function\textsuperscript{16}. In addition angiotensin II (AII) is recently implicated in stroke induced cerebral tissue necrosis\textsuperscript{17,18}.

Interestingly, our results indicate that, at admission and two weeks later, stroke increases significantly PRA in the recumbent state, indicating an increase in the tonic stimulation of renin secretion. Reflex renin secretion at admission is inhibited compared to that of control normal persons and stimulated at the second week measurement. The detected significant correlation between GFR and the decreased reflex PRA at admission indicates the link between the Reflex PRA and the developing renal insufficiency. Also the significant increase in the infarction area by the end of the second week could be related to the significantly increased reflex PRA.

The significant rise in PRA in focal cerebral ischemia is in agreement with the results of Dai et al.,\textsuperscript{(17)} who had shown that AII, the effector peptide of RAS, might be centrally increased and involved in the initiation and regulation of processes occurring during brain ischemia. Also, Walther et al.\textsuperscript{(18)}, had found that central AII was increased 24 hours after permanent MCA occlusion. They found also that AI knockout mice showed smaller infarct area 24 hours after permanent MCA occlusion in comparison to wild type. In contrast, a larger infarct area was noted in the angiotensinogen over expressing mice 24 hours after permanent MCA occlusion in comparison to wild type. Peripherally stimulated RAS could also have central effects through area postrema. It is known also that the area postrema lacks a blood-brain barrier and contains AII receptors. The neurons of the area postrema project to structures such as the nucleus tractus solitarius, the dorsal motor nucleus of the vagus, the ventrolateral medulla, and the lateral parabrachial nuclei, all of which have important roles in cardiovascular regulation\textsuperscript{19}.

Lamina terminalis region of the brain is rich in AI receptors and is neurally linked to the renal sympathetic nerves\textsuperscript{20}. McKinley et al.\textsuperscript{21}, suggested that Angiotensin-receptive neurons in the lamina terminalis may initiate inhibitory influence on renal renin secretion, which is probably signaled to the kidney as a reduction in renal sympathetic nerve activity. During stress, there is loss of normal inhibitory impulses from higher cortical centers to the subcortical centers in the hypothalamus regulating the autonomic activity resulting in an attenuation of baroreflex activity with consequent increase in arterial blood pressure and heart rate\textsuperscript{22}. In the acute stage of cerebral ischemia there is marked stimulation of these hypothalamic centers which elicited a peculiar association of sympathetically mediated visceral changes\textsuperscript{16}. One of the signs of sympathetic excitation is the tonic activation of the renin producing juxtaglomerular cells\textsuperscript{24}. Also, in stress state, there is massive release of cortical neurotransmitters associating the acute stage of stroke with enhancement of the tonic activity of the subcortical autonomic centers\textsuperscript{25}. So, our patients at admission would be stressed with an increased tonic sympathetic activity explaining the increased recumbent PRA. The activated inhibitory cortical cardiovascular modulatory centers would inhibit the subcortical reflex modulatory cardiovascular centers explaining the inhibited reflex PRA at admission. But two weeks later, the enhanced reflex PRA could be explained by the probable loss of cortical modulatory function on reflex PRA due to ischemic destruction of lamina terminalis\textsuperscript{21}, insula and amygdale\textsuperscript{15}.

Our result of renal dysfunction and probably cerebral morbidity (increased infarct size at the end of the second week) may correlate with a suspected endothelial dysfunction induced by this enhanced post stroke plasma renin activity. Mac Walter et al.\textsuperscript{24} reported that patients with end-stage renal disease have increased arterial stiffness independent of other risk factors for atherosclerosis. They observed that, even among patients with mild to moderate renal insufficiency, there was increased central artery stiffness suggesting that renal dysfunction adversely affected small and large arteries which may contribute to cardiovascular morbidity and mortality. By this increased arterial stiffness, they had related it as a profound etiological mechanism of the predictable mortality after acute stroke in renal patients.

Sokol et al.\textsuperscript{25} reported that RAS had a role in the regulation of homeostasis and tissue response to injury in both CNS and the peripheral tissue via the activity of AII. Vascular and hematologic effects induced by AII including endothelial dysfunction, vascular structural changes, inflammation, homeostasis and fibrinolysis may increasingly link to the occurrence of cerebrovascular events\textsuperscript{26}. Another hypothesis for the role of RAS as a risk factor in the development of cerebrovascular diseases, away from atherosclerosis and hypertension, is focused on the influence of AII on the production and release of reactive oxygen species\textsuperscript{27}. The oxidative stress, which is selectively stimulated via the AI receptors subtype and most likely reduced by AT2 receptors stimulation, has a central role in the development of cerebrovascular diseases\textsuperscript{28}. Moreover, AII is capable of increasing the expression of a number inducible transcription factors including c-Fos and c-Jun via stimulation of AI receptors in the brain. It seems likely that increased expression of these transcription factors represents part of the genetic program mediating apoptosis in brain following focal ischemia\textsuperscript{29}.

AII acts at multiple sites in the kidney; directly as a vasoconstrictor on both afferent and efferent arterioles and also as a major regulator of reabsorption from the proximal tubule, thus altering the signal that reaches the macula densa\textsuperscript{30}. Importantly, it is also a strong modulator of the magnitude of the TGF response; this effect occurs at the afferent arteriole and is apparent in the absence of AII-dependent vasoconstriction\textsuperscript{31}. Thus modulation by AII of
both myogenic and TGF-mediated autoregulation is at least conceptually independent of its vasconstrictor effect. So, the regulation of GFR and RBF at low perfusion pressures results from AII-mediated efferent constriction combined with autoregulatory afferent vasodilatation. These may explain the decrease in creatinine clearance and hence the GFR as a result of the increase of PRA noted in our research together with the direct effect of AII on the mesangial tissue.

PAI is significantly increased by stroke to the same degree in all groups of stroke as measured at admission or at the end of the two weeks of follow up. Absence of difference in the observed values at admission and at the end of two weeks of follow up of stroke may exclude its incrimination or integration in the two weeks evolution. Meanwhile it could be a consequence of the more elevated values of PRA in this group of patients. PAI-1 is considered to be a key molecule in thrombotic vascular diseases. The plasminogen activator system protects against intravascular thrombosis and PAI balance its actions. Bradkinin also contributes to this balance by inducing the expression of tissue plasminogen activator (t-PA). ACE inhibitors decrease PAI-1 levels and increase bradykinin levels, thus favoring fibrinolysis. This was suggested as one of the mechanisms responsible for the benefit of ACE inhibition after myocardial infarction. Increased plasma levels of PAI-1 are positively correlated with the risk of developing coronary artery disease as well as the extent of coronary sclerosis, restenosis, myocardial infarction, and deep vein thrombosis. PAI-1 also seems to play a role in renal diseases. It has been reported that elevated PAI-1 levels are associated with nephritic syndrome and the hemolytic uremic syndrome in patients with hemolytic uremic anemia, glomerular fibrin deposition is found. Clinical studies suggest that PAI-1 is the circulating inhibitor of fibrinolysis in this syndrome and that the normalization of elevated PAI-1 activity correlates with improvement in renal function. Intervention with PAI-1 function might be an important future tool for additional therapeutic strategies in vascular occlusive diseases. At the same time PAI could be a product of the activity of RAS. All can modulate hemostasis and fibrinolysis through its ability to induce the expression of PAI-1.

In conclusion, acute ischemic stroke had an association of an acute diminution the renal function which was maintained along the two weeks of follow up of the 21 patients. The decreased GFR was associated with a significant decrease in the plasma renin reflex sensitivity at admission but was inversely associated with a significant increase in this reflex sensitivity at the end of the two weeks of follow up. This biphasic alteration of this reflex mechanism could indicate its integration in the acuteness of the developed diminution of the renal insufficiency. Also this reflex plasma renin sensitivity may be used as a sensitive neurovegetative index of the cerebro-renal affection of acute ischemic stroke and may be used to stratify risk and target (cerebral & renal) interventions, e.g. the use of angiotensin-converting enzyme inhibitors and angiotensin receptor blockers in ischemic stroke.

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