High Sensitivity C-Reactive Protein and its Gene Polymorphism in Acute Ischemic Stroke

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ABSTRACT

Background: Elevated C-reactive protein (CRP) level is considered to be a predictor of ischemic stroke and CRP genetic polymorphism is reported to be associated with elevated CRP level. Objective: The aim of this work was to study the relation between high sensitivity CRP (hs-CRP) as well as CRP gene polymorphism and ischemic stroke. Methods: Forty-one ischemic stroke patients and forty normal subjects were included in this study. Patients were evaluated clinically and radiologically. Laboratory investigations, hs-CRP assay and genetic testing of CRP gene were done for both patients and control groups. Results: Serum hs-CRP level was significantly high in ischemic stroke patients compared to control subjects and high CRP level was significantly associated with male gender, hypertension, D.M., hyperlipidemia, total anterior circulation syndrome (TACI), large sized infarction on CT examination and poor stroke outcome. In human CRP G1059C polymorphism genotype, GG allele was the most common one in both patients and control groups (95%, 100%) with no significant difference, as GC allele is less common (only two patients), and CC allele was absent in both groups, with no significant association between GG allele and high hs-CRP. Conclusion: hs-CRP level is elevated in acute ischemic stroke and it is intimately associated with different vascular risk factors and prognostic factors. Human CRP G1059C polymorphism especially GG allele did not influence the serum level of hs-CRP and did not show any association with ischemic stroke. [Egypt J Neurol Psychiat Neurosurg. 2010; 47(3): 373-379]

Key Words: Ischemic stroke – High sensitivity C-reactive protein – Polymorphism

INTRODUCTION

Atherosclerosis, one of the major causes of ischemic cerebrovascular disease is considered to involve the inflammatory system. C-reactive protein (CRP) widely known to be an inflammatory marker was detected in atherosclerotic plaques and was recently reported to be involved in the development of atherosclerosis¹². Elevated plasma CRP level may be an atherothrombotic biomarker that provides additive prognostic information about not only coronary heart disease⁶ but also cerebral and peripheral arterial disease⁷. Moreover, elevated CRP level could be a marker that predicts the risk of ischemic stroke in elderly⁸.

The realization that CRP genetic polymorphism does exists and that it directly and predictably influences steady-state blood CRP level could be of substantial clinical importance⁹.

This work aimed at studying the high sensitivity-CRP (hs-CRP) assay as an inflammatory marker of stroke risk and valuable additional diagnostic and prognostic tool. Also, to study genetic association between CRP gene polymorphism and ischemic stroke.

SUBJECTS AND METHODS

Subjects
The participants were recruited from Neurology Department, Beni-Sueif University Hospital and Cairo University Hospital during the period from April 2006 to December 2008. Forty-one patients (group I) (24 males and 17 females) with acute first ever ischemic stroke (within first 48 hours of stroke onset) diagnosed clinically and by computed tomography (CT) were included in the study. Patients’ age ranged from 45 to 76 years with a mean age of 57.9±9.1 years. Excluded from the study were patients with hemorrhagic stroke diagnosed by CT, and patients with cerebral embolism of cardiac origin diagnosed...
clinically and by ECG, and echocardiography. Also, patients with cancer and autoimmune disease were excluded. No participants had a history of peripheral arterial occlusive disease, myocardial infarction, recurrent stroke or any acute or chronic infection at the time of sampling.

Forty healthy subjects (group II) (21 males and 19 females) were enrolled as control subjects, their age ranged from 45 to 71 years (mean age of 55.5±6.6 years). Excluded from this group, subjects with history of stroke or myocardial infarction, rheumatic heart diseases, autoimmune diseases. Diabetic and hypertensive persons or persons with any acute or chronic infection at the time of sampling were also excluded.

METHODS

Patients were evaluated by clinical examination and were clinically classified using Oxfordshire Community Stroke Project Classification (OCSP) into lacunar infarction syndrome (LACI), total anterior circulation infarction syndrome (TACI), partial anterior circulation infarction syndrome (PACI) and posterior circulation infarction syndrome (POCI).

Assessment of stroke severity and level of impairment using the National Institute of Health Stroke Scale (NIHSS), which is a standardized neurologic stroke severity scale developed to quantify stroke patients’ deficits in clinical trials. A score of 0-4= minor stroke, 5-15 = moderate stroke. 15-20 = moderate /sever stroke and 21-42=sever stroke.

Assessment of functional outcome using different stroke scales: Modified Rankin Scale (MRS)(10), which is an efficient global functional outcome index after stroke. It is a 7 grade scoring system with 0 corresponding to persons having no symptoms and 6 being dead.

Glasgow Outcome Scale (GOS)(11), a 5 point scale that reflects disability and handicap. A score of 1-2 is considered good outcome, whereas a score of 3-6 is considered poor one.

The outcome functional scales were done for patients one month after stroke onset.

Laboratory investigations were done and they included routine laboratory profile and specific laboratory investigations including: (a) hs-CRP serum level using automated chemiluminescence using Immulite analyzer (immunometric assay). Normal values of serum hs-CRP are 0.14 mg/dl to 1.1 mg/dl, serum hs-CRP was done in the first 48 hours of stroke onset. (b) Genetic profile: mutation detection of CRP gene was done using RFLP

(Restriction Fragment Length Polymerase) technique. The human CRP gene was mapped to chromosome 1q21. The CRP 1059 G/C polymorphism could be detected by digestion of endonuclease Mae III(12).

Computed tomography of the brain was done using the Toshiba Express/XS System, initially and follow-up after 72 hours if needed. The scans were conducted in the axial imaging planes. The axial cuts were normally at 10 mm. Size of infarction was detected as follows: small infarction: <1 cm, moderate sized infarction: 1-3 cm and large sized infarction: >3 cm.

The control group underwent the same routine and specific laboratory tests (hs-CRP and genetic testing).

Statistical Methods

Quantitative data were summarized as means and standard deviations. Categorical data were summarized as percentages. Qualitative data were compared by Chi-square or Fisher’ exact tests according to the expected frequencies. Persons Correlation Coefficient, which is a test to measure the strength of the linear relationship between two variables was used. A 5% probability level (p<0.05) was considered statistically significant.

RESULTS

Hypertension was found in 18 patients (43.9%), twenty-two patients were cigarette smokers (53.7%), TIAs in 2 patients (4.9%) and family history of stroke in 6 patients (14.6%). Hypercholesterolemia was found in 15 patients (36.6%), elevated blood sugar in 15 patients (36.6%) and hyperuricemia in 11 patients (26.8%).

Clinically LACI syndrome was diagnosed in 6 patients (14.6%), TACI in 2 patients (4.9%), PACI in 33 patients (80.5%) and no patients had POCI syndrome.

Assessment of stroke severity by NIHSS revealed mean of 8.6±3.7 with 36 patients (87.8%) having minor and moderate stroke and 5 patients (12.2%) having moderate/sever and sever stroke.

Clinical functional outcome scales showed mean MRS of 3.1±1.2 with 23 patients (56.1%) with good outcome, and GOS mean of 2.3±0.8 with 24 patients (58.5%) with good outcome.

CT results showed 6 patients (14.64%) had small sized infarction, 18 patients (43.9%) had medium sized infarction and 17 patients (41.46%) had large sized infarction.
Specific Laboratory Results:

(a) Serum high sensitivity CRP levels (hs-CRP):
Elevated serum hs-CRP was found in 22 patients (53.7%) while normal serum hs-CRP was found in 19 patients (46.3%). Whereas all subjects in control group had normal serum hs-CRP levels. There was a highly significant difference between acute stroke patients and control subjects regarding the mean level hs-CRP (p-value <0.01) (Table 1).

(b) CRP genotyping:
Human CRP G1059C polymorphism genotype results revealed that (GG allele) (the common allele) was found in 39 patients (95%) and all persons of control group, while (GC allele) (the less common allele) was found in 2 patients only and absent in control group. The third rare allele (CC) was absent in both groups. In patients having the homoyzous allele GG, the mean CRP level was 1.9±1.0 mg/dl whereas the mean level of CRP in patients having GC allele was 0.52±0.4 mg/dl (Table 2).

Relation of hs-CRP level and risk factors and CT findings:
1. Male patients had significantly higher mean level of hs-CRP (3.1±2.0 mg/dl) than female patients (1.7±1.1 mg/dl).
2. Hypertensive patients had significantly higher mean level of hs-CRP (3.7±3.7 mg/dl) than normotensive patients (P-value = 0.02). Also patients with hypercholesterolemia and high blood sugar were significantly associated with elevated hs-CRP (2.4±2.2 mg/dl), (4.3±1.3 mg/dl) respectively.
3. However, smoking, TIAs, +ve family history of stroke, hyperuricemia, elevated WBCs count did not reach a significant level.
4. According to OCSP: patients with TACI had significantly higher serum level of hs-CRP (3.5±0.5 mg/dl) than patients with either PACI or LACI (2.3±0.5 mg/dl) (0.5±0.4 mg/dl) respectively.
5. Patients with large infarction had highly significant mean level of CRP (4.5±2.9 mg/dl) than patients with small and medium sized infarction (0.5±0.4 mg/dl) (Table 3).

Correlation study between hs-CRP serum level and different laboratory risk factors and outcome scales (Table 4):
1. There was positive correlation between hs-CRP and different laboratory risk factors, which reached statistical significance regarding cholesterol level only.
2. Negative correlation between hs-CRP and HDL serum level (did not reach significant value).
3. Also positive correlation was present between hs-CRP level and different assessment and outcome scales (NIHSS, GOS and MRS). Correlation with GOS reached significant value and that with MRS reached a highly significant value.

Table 1. Serum high sensitivity CRP levels in group I (patients with acute stroke) and group II (Healthy controls)

<table>
<thead>
<tr>
<th>CRP</th>
<th>Group I</th>
<th>Group II</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Minimum</td>
<td>0.01</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Maximum</td>
<td>15.7</td>
<td>0.67</td>
<td>0.001*</td>
</tr>
<tr>
<td>Mean±SD</td>
<td>2.1±0.9</td>
<td>0.3±0.2</td>
<td></td>
</tr>
</tbody>
</table>

CRP C-reactive protein, SD standard deviation
* Significant at p <0.01

Table 2. hs-CRP levels according to CRP polymorphism in group I (patients with acute stroke)

<table>
<thead>
<tr>
<th>CRP polymorphism</th>
<th>Serum hs-CRP (mean±SD) mg/dl</th>
</tr>
</thead>
<tbody>
<tr>
<td>1059GG</td>
<td>1.9±1.0</td>
</tr>
<tr>
<td>1059 GC</td>
<td>0.52±0.4</td>
</tr>
<tr>
<td>1059CC</td>
<td>Absent</td>
</tr>
</tbody>
</table>

CRP C-reactive protein, SD standard deviation
Table 3. hs-CRP levels and size of infarction in group I (Patients with acute ischemic strokes)

<table>
<thead>
<tr>
<th>Infarction size</th>
<th>Large infarction</th>
<th>Small and medium infarction</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP (mean±SD)</td>
<td>4.5±2.9</td>
<td>0.5±0.4</td>
<td>0.006**</td>
</tr>
</tbody>
</table>

CRP C-reactive protein, SD standard deviation
* significant at p<0.01

Table 4. Correlation between hs-CRP level and laboratory risk factors and clinical assessment and outcome scales in patients with acute ischemic strokes

<table>
<thead>
<tr>
<th>Laboratory risk factors and different assessment and outcome scales</th>
<th>“r” value</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol level</td>
<td>0.312</td>
<td>0.04*</td>
</tr>
<tr>
<td>Triglycerides level</td>
<td>0.039</td>
<td>0.806</td>
</tr>
<tr>
<td>Fasting blood sugar</td>
<td>0.146</td>
<td>0.361</td>
</tr>
<tr>
<td>HDL</td>
<td>-0.175</td>
<td>0.274</td>
</tr>
<tr>
<td>LDL</td>
<td>0.104</td>
<td>0.517</td>
</tr>
<tr>
<td>Uric acid</td>
<td>0.113</td>
<td>0.482</td>
</tr>
<tr>
<td>ESR</td>
<td>0.090</td>
<td>0.575</td>
</tr>
<tr>
<td>WBCs count</td>
<td>0.004</td>
<td>0.982</td>
</tr>
<tr>
<td>NIHSS</td>
<td>0.157</td>
<td>0.328</td>
</tr>
<tr>
<td>GOS</td>
<td>0.337</td>
<td>0.031*</td>
</tr>
<tr>
<td>MRS</td>
<td>0.441</td>
<td>0.004**</td>
</tr>
</tbody>
</table>

ESR erythrocyte sedimentation rate, GOS Glasgow Outcome Scale, HDL high density lipoprotein, LDL low density lipoprotein, MRS Modified Rankin Scale, NIHSS national institute of health stroke scale, WBC white blood cells
* significant at p<0.05 ** significant at p<0.01

DISCUSSION

There is much evidence to suggest that neuro-inflammatory mechanisms play an important role in ischemic injury, and that interruption of these processes can result in improved neurological outcomes.13

In this study there was a highly significant increase in the hs-CRP serum level in the first 48 hours after stroke onset and this was in accordance with some preceding studies14,15. A finding which can be explained by the fact that, inflammation plays a central role in all phases of atherosclerosis, from the initial recruitment of circulating leukocytes to the arterial wall to the rupture of unstable plaques, which results in the clinical manifestations of the disease. Hs-CRP may be involved in each of these stages by direct influencing processes like complement activation, apoptosis, vascular cell activation, lipid accumulation and thrombosis.16

On the other hand, DiNapoli et al.17 concluded that, there is no sufficient evidence to recommend measurement of hs-CRP in the routine evaluation of cerebrovascular disease risk in primary prevention. As hs-CRP is simply a marker reflecting inflammatory state, any factor that alters the relationship between its concentration and inflammation may distort interpretation.

Roquer et al.3 recently demonstrated that many biomarkers of inflammation are elevated years in advance of first ever thrombotic stroke and that these same biomarkers are highly predictive of recurrent stroke.

With regards to cerebral vessels increased biomarkers of inflammation, including hs-CRP have been associated with increased stroke risk as well as an increased rate of atherosclerosis progression in the carotid vessels.4,6

Serum hs-CRP level was higher in acute ischemic stroke men than women. This was supported by the study of Devaraj et al.18 who stated that elevation of hs-CRP level was an independent risk factor for future ischemic stroke in men not in women. On the other hand, clinical evidence has shown that endogenous estrogen protects the development of atherosclerosis, and has anti-inflammatory action in premenopausal women.20

The significantly higher mean levels of serum hs-CRP in hypertensive patients observed in this study can be explained on the basis of the relation between atherosclerosis as a chronic inflammatory...
Inflammation is known to play a major role in the development of hypertension. The association between CRP and diabetes is well established, with diabetes being a key risk factor for cardiovascular disease. CRP is a marker of inflammation, and higher levels of CRP are associated with increased cardiovascular risk. CRP levels are influenced by various factors, including age, gender, and smoking status. Smoking has been shown to increase CRP levels, and this might be explained by the production of reactive oxygen species and the activation of inflammatory pathways.

Some other polymorphisms are associated with CRP levels and are related to diabetes. The CRP 1059 C>G polymorphism, for example, is associated with higher CRP levels in smokers and in patients with diabetes. The CRP 1059 C>G polymorphism is in high linkage disequilibrium with the CRP gene promoter polymorphism (−1131 A>G), which is associated with hyperglycemia and diabetes. The CRP 1059 C>G polymorphism is also associated with an increased risk of cardiovascular disease, and it is predicted that individuals with the C-allele will have higher CRP levels than those with the G-allele.

Conclusion:
Elevated hs-CRP level is significant in acute ischemic stroke and associated with multiple risk and prognostic factors however, its genetic polymorphism did not influence its level.

[Disclosure: Authors report no conflict of interest]
REFERENCES


الملخص العربي

قياس عالي الحساسية والتعدد الجيني لبروتين سي التفاعلي والسكته الدموية الحادة

يهدف هذا البحث إلى دراسة نسبة بروتين سي التفاعلي والتعدد الجيني له في حالات السكتة الدماغية الحادة.

أجريت هذه الدراسة على واحد وأربعين مريضا يعانون من الجمطة المخية الحادة 17 رجل و42 سيدة تراوحت أعمارهم بين 24 إلى 76 سنة بوسط عمرها 57.9 ± 9.1 سنة وأربعين آخرين من الأصحاء كمجموعة مقارنة تم تقييم المرضى وإليكميا مع عمل أشعة مقطعية على المخ، الفحوصات العملية الروتينية، قياس عالي الحساسية لبروتين سي التفاعلي بالدم، والتعدد الشكمي الوراثي لجين 1059 لبروتين سي التفاعلي لكلا من المرضى والأصحاء.

وقد أظهرت النتائج أن نسبة القياس عالي الحساسية لبروتين سي التفاعلي أعلى في المرضى عن الأصحاء وذات دلالة إحصائية.

العلاقا هناك علاقة ذات دلالة إحصائية بين نسبة بروتين سي التفاعلي بالدم بالمرضى الذكور منهم وارتفاع ضغط الدم والسكر، وارتفاع الكوليسترول بالدم والمرضى الذين عانون من المركب المخية لحمية لاحتباس تام في الدورة المخية الأمامية تمت بتقليد مقياس أكسفورد شاهير، والمرضى الذين عانون من ناتج سي، تمت لمقياس التقييم الوظيفي المختلفة للكشف الدموية المستخدمة في البحث أيضا مع مرضي الجمطة المخية كبيرة الحجم بالأشعة المقطعية. كما وجد أن الصفة الجينية GGC لجين 1059 لبروتين سي التفاعلي وجدت في 95% من المرضى وفي 100% من الأصحاء بينما الصفة الجينية GCC وجدت في 5% من المرضى، مع عدم وجود علاقة ذات دلالة إحصائية بين الصفة الجينية GGC وارتفاع نسبة بروتين سي التفاعلي بالدم.