Neopterin in Carotid Artery Atherosclerosis and Cerebrovascular Ischaemic Events

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ABSTRACT

Introduction and aim of the study: Artery-to-artery atherothromboembolic ischaemic insults in the carotid artery domain may be determined by vulnerable (high-risk) features of carotid atherosclerotic plaque. Intra-plaque inflammation is the main event rendering it vulnerable. The principal players in that process are the activated macrophages. Neopterin is a specific product of activated human macrophages. This study was designed to assess serum neopterin level in carotid atherosclerosis, its relation to atherosclerotic plaque morphology, and to ipsilateral ischaemic cerebral events.

Subjects and Methods: The study comprised 100 patients (62 males, and 38 females, with age range of 50-78 years) with brain computerized tomography scan-proven carotid artery territory ischaemia, and ultrasound-proven carotid ipsilateral atherosclerotic plaque. Age/sex matching 20 healthy subjects were included as control group. Migraine, sympathomimetic drug intake, coagulopathy, and cerebral cardiogenic embolism were excluded. In addition to routine laboratory tests, serum neopterin level was assessed for all subjects. Results: Vulnerable carotid atherosclerotic plaque was found ipsilaterally in 80% of patients. 94% of patients with multiple cerebral ischaemic events had ipsilateral vulnerable plaque. Serum neopterin level was found to be significantly higher in patients with carotid atherosclerotic plaque compared to healthy control (p<0.001). The level was found to be more significantly higher in those with vulnerable plaques than in those without (p<0.001), and further higher, yet non-significantly, in patients with multiple cerebral ischaemic events than in patients with a single event. Conclusion: Carotid artery atherosclerotic plaque morphology can be a determinant of cerebral ischaemic events' risk. It can be studied peacefully non-invasively by ultrasonography. Serum neopterin level is a biochemical marker that can indicate carotid atherosclerosis presence and severity, and may assess the efficiency of its therapy.

INTRODUCTION

Stroke is the third most common cause of death and the most important cause of disability among adults¹,²,³. Of the heterogenous entity of stroke 80% are ischaemic, and almost 50% of the latter are due to large artery atherothrombosis⁴. Ischaemic strokes and transient ischaemic attacks (TIAs) are frequently caused by cerebral embolism from an atherothrombotic plaque or thrombosis at the site of plaque rupture⁵.

Atherosclerosis is a diffuse and progressive process with a variable distribution and clinical presentation that is dependent on the regional circulation involved⁶. All types of atherosclerotic plaques with a high likelihood of thrombotic complications and rapid progression should be considered as vulnerable plaques underlying the cause of most clinical events⁷. Plaque vulnerability has opened new avenues in the field of atherothromboembolic stroke, and this vulnerability is influenced by genetic factors, infiltration with inflammatory cells (macrophages, lymphocytes), secretion of metalloproteinases and cytokines, inflammatory neovascularization, formation of a lipid core with thin fibrous cap fragilization and apoptosis, platelet activation, and thrombus formation which are all interrelated⁸,⁹. Chronic inflammation in atheromatous plaques is well-documented⁹. Macrophages are the key cells in the development and growth of atherosclerotic plaques¹⁰,¹¹. They release proteolytic and oxidizing enzymes, and secrete transforming growth factor beta, metalloproteinases, and pro-inflammatory cytokines, which induce the recruitment of more inflammatory cells into the plaques. This vicious cycle results in plaque progression and disease exacerbation. Neopterin is a specific product of activated human macrophages, produced during the course of their activation. Neopterin level is a biochemical marker that can indicate carotid atherosclerosis presence and severity, and may assess the efficiency of its therapy. (Egypt J. Neurol. Psychiat. Neurosurg., 2009, 46(1): 185-192)
agents. These agents turn the low-density lipoprotein (LDL) of intima into oxidized LDL (oxLDL). Macrophages take up oxLDL (foam cells). The toxic oxLDL leads to death and lysis of macrophages, fibroblasts, smooth muscle cells and endothelium, all of which make up an advanced plaque with a lipid-rich necrotic core with high likelihood of rupture and thrombus formation. Vulnerable atherosclerotic plaques have an increased number of both macrophages and activated lymphocytes. Serum markers of inflammation are elevated in patients with atherosclerosis, and the level independently predicts subsequent events.

Neopterin seems part of the pro-inflammatory armature of the activated human macrophage. Plasma neopterin originates as the oxidation product of 7,8-dihydroneopterin secreted by γ-interferon stimulated macrophages within atherosclerotic plaques, and is increasingly being used as a marker of inflammation during clinical management of patients with a range of disorders including atherosclerosis. An involvement of the nonspecific immune system in atherogenesis is suggested by the increased plasma neopterin concentrations in atherosclerotic patients.

Aim of the study
This study was designed to assess serum neopterin level in patients with carotid atherosclerosis as a possible biochemical marker for high-risk features of carotid plaque (vulnerable plaque), and to study their relation to ipsilateral cerebral ischaemic events.

SUBJECTS AND METHODS

Subjects
From patients presented to Neurology Department, Faculty of Medicine, Zagazig University with history suggestive of carotid artery territory transient ischaemic attacks (TIAs) and/or chronic stable ischaemic stroke, this study comprised 100 patients (62 males, and 38 females) whose high-resolution B-mode ultrasound of the carotid arteries revealed ipsilateral atherosclerotic plaque(s).

Age/sex matching 20 healthy subjects (12 males, and 8 females) were also included in the study as control group.

Methods
For all subjects the following was carried out:

Clinical assessment:
- Detailed history taking and thorough general and neurological examination to exclude neurological insults outside carotid artery domain.
- Cardiological assessment (including echocardiography when indicated) to exclude cardiac causes of cerebrovascular ischaemic events.

History of multiple unilateral strokes, of unilateral TIAs, and of stroke(s) with TIA(s) in the same vascular territory, with the exclusion of history suggestive of migraine, coagulopathy, or cardiological lesion, and with no sympathomimetic drugs’ intake were all suggestive of cerebral artery-to-artery atherothromboembolism.

In patients with hemiparesis/ hypothesis, the finding of aphasia and/or ipsilateral amaurosis fugax was suggestive of carotid domain lesion.

There was stress on history of current smoking habit, and on exclusion of history of head/neck surgery, significant trauma, or irradiation.

Carotid artery gentle palpation for weak/absent pulse, and auscultation for bruit were carried out for all patients.

Hypertension was defined as systolic blood pressure (SBP) ≥ 140 mm Hg, diastolic blood pressure (DBP) ≥ 90 mm Hg, or use of antihypertensive medications, diabetes mellitus as fasting glucose level of ≥ 126 mg/dL, a nonfasting glucose level of ≥ 200 mg/dL, or use of insulin or oral hypoglycaemic agents, and hypercholesterolaemia as serum total cholesterol ≥ 220 mg/dL or use of antihypercholesterolaemic medications.

Radiological assessment:
- Computerized tomography (CT) scan of the brain to confirm the diagnosis of carotid artery territory infarct, or TIA(s).

For diagnosis of carotid domain ischaemic stroke not due to perforator arterial occlusion the following were required: neurological deficit consistent with carotid artery territory lesion, identification of culprit infarct(s) on CT scan in carotid territory of more than 1.5 centimeter (cm) in diameter, and exclusion of non-ischaemic lesions.

History of episode(s) of sudden-onset unprovoked focal neurological dysfunction in carotid domain (including retinal ischaemia), that last less than 24 hours, with free CT of the brain, in addition to exclusion of history suggestive of migraine, seizure disorder, coagulopathy, or cardiological lesions, and exclusion of sympathomimetic drugs' intake, were required for diagnosis of carotid domain TIA(s).

Carotid arteries high-resolution B-mode ultrasound assessment to confirm carotid atherosclerosis, and exclude non-atherosclerotic carotid disease as aneurysms, arteritis, carotid dissection, coils and kinks, fibromuscular dysplasia, radiation, and vasospasm.

A high-resolution ultrasonographic system (Toshiba - SSA - 270A) with a high-resolution transducer 7.5 MHZ was used. Plaque was defined as a focal protrusion 50% greater than the surrounding area and localized along the extracranial carotid tree (internal carotid artery/bifurcation or common carotid artery). Carotid plaque was classified by surface characteristics, echogenicity, and texture. Surface characteristics were defined as irregular (height variations of >0.4 mm), and ulcerated (a discrete depression of >2 mm in width extended into the media). Lesion echogenicity was characterized as hypoechoic, isoechoic, hyperechoic, or calcified. Lesion texture was classified as homogeneous or heterogeneous. In case of multiple focal lesions, the largest lesion on each side was measured. High-risk (vulnerable) plaque was defined as presence of irregular or ulcerated surface or hypoechoic or heterogeneous plaques that occupied >50% of the total plaque volume. The remaining plaques were defined as stable plaques. When >1 type of plaque was detected in an individual, the plaque risk was determined by the more severe type.

**Laboratory assessment:**

- Laboratory tests including lipid profile, blood sugar, liver function tests, renal function tests, complete blood picture with prothrombin time (PT), partial thromboplastin time (PTT), and bleeding time.

- Assessment of serum neopterin level by enzyme linked immunosorbent assay (ELISA). The ELItest® Neopterin-Screening is a competitive enzyme immunoassay specifically adapted for the indication of screening of blood donors for quantitative determination of neopterin in serum and plasma using coated microtitre plates.

### RESULTS

Table (1) shows that patients' age mean value ± SD (years) was 62.7±8.9, and 62 of them were males, and 38 of them were females. Hypertension was found in 83% of them, diabetes mellitus in 75%, hypercholesterolaemia in 50%, and 28% of them were current smokers. Ipsilaterally to the patient's carotid artery domain ischaemic event, vulnerable carotid atherosclerotic plaque was found in 80%, and stable plaque in 20%.

Table (2) shows that out of the 36 patients with a single carotid artery domain ischaemic event, 20 patients (55.6%) had an ipsilateral vulnerable carotid atherosclerotic plaque, and 16 patients (44.4%) had an ipsilateral stable one. While out of the 64 patients with multiple events, 60 patients (94%) had an ipsilateral vulnerable plaque, and 4 patients (6%) had an ipsilateral stable one.

Table (3) shows that mean value ± SD of serum neopterin level (nmol/l) was 5.64±2.05 in control group (number=20), and 20.18±7.67 in patients (number=100), which is significantly higher (p<0.001).

Table (4) shows that mean value ± SD of serum neopterin level (nmol/l) was 14.28±5.03 in patients with stable carotid artery atherosclerotic plaque (number=20), and 21.52±7.42 in patients
with vulnerable carotid artery atherosclerotic plaque (number=80), which is significantly higher (p<0.001).

Table (5) shows that mean value ± SD of serum neopterin level (nmol/l) was 18.13±7.60 in patients with a single carotid artery domain ischaemic event(number=36), and 21.24±7.50 in patients with multiple events (number=64) which is insignificantly higher.

**Table 1.** Clinical and carotid ultrasonographic findings of patients.

<table>
<thead>
<tr>
<th>The variable</th>
<th>Finding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean value ± SD)</td>
<td>(62.7± 8.9) ( range 50-78 years)</td>
</tr>
<tr>
<td>Sex</td>
<td>Males 62 %, females 38%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>83%</td>
</tr>
<tr>
<td>Hypercholesterolemia</td>
<td>50%</td>
</tr>
<tr>
<td>Diabetes Mellitus</td>
<td>75%</td>
</tr>
<tr>
<td>Current smoking</td>
<td>28%</td>
</tr>
<tr>
<td>Ipsilateral carotid stable plaque</td>
<td>20%</td>
</tr>
<tr>
<td>Ipsilateral carotid vulnerable plaque</td>
<td>80%</td>
</tr>
</tbody>
</table>

**Table 2.** Number of cerebral ischaemic events (stroke and/or TIA) and morphology of ipsilateral carotid atherosclerotic plaque.

<table>
<thead>
<tr>
<th>Patients with a single cerebral ischaemic event (n = 36)</th>
<th>Patients with multiple cerebral ischaemic events (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number (%) of patients having ipsilateral vulnerable plaque</td>
<td>20 (55.6%)</td>
</tr>
<tr>
<td>Number (%) of patients having ipsilateral stable plaque</td>
<td>16 (44.4%)</td>
</tr>
</tbody>
</table>

**Table 3.** Serum neopterin level (nmol/l) in patients and healthy control.

<table>
<thead>
<tr>
<th>Control group n = 20</th>
<th>Patients group n = 100</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value ± SD of serum neopterin level (nmol/l)</td>
<td>5.64± 2.05</td>
<td>20.18± 7.67</td>
<td>27.01</td>
</tr>
</tbody>
</table>

**Table 4.** Serum neopterin level (nmol/l ) and carotid atherosclerotic plaque morphology.

<table>
<thead>
<tr>
<th>Patients with vulnerable plaques (n = 80)</th>
<th>Patients with stable plaques (n = 20)</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean value ± SD of serum neopterin level (nmol/l)</td>
<td>21.52 ± 7.42</td>
<td>14.28 ± 5.03</td>
<td>5.20</td>
</tr>
</tbody>
</table>

**Table 5.** Serum neopterin level (nmol/l) and number of ischemic cerebral events (stroke and/or TIA).

<table>
<thead>
<tr>
<th>Patients with a single cerebral ischaemic event</th>
<th>Patients with multiple cerebral ischaemic</th>
<th>t</th>
<th>P</th>
</tr>
</thead>
</table>
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| DISCUSSION |

Table (1) shows that vulnerable (high-risk) carotid atherosclerotic plaque was found ipsilaterally in 80% of patients. This finding may be explained by the inclusion and exclusion criteria used in patients' selection, which increased the likelihood of artery-to-artery atherothromboembolism as a cause for stroke in patients included in the study. This high likelihood of artery-to-artery atherothromboembolism in the study may also explain that 94% of patients with multiple cerebral ischaemic events had ipsilateral vulnerable plaque as shown in table (2). So, measurements other than carotid intima/media thickness, such as carotid atherosclerotic plaque surface, echogenicity, and texture features, assessed by ultrasonography, may be powerful determinants of stroke risk.

B-mode ultrasonography has been a useful noninvasive technique for identifying high-risk plaque features. Identification of vulnerable atherosclerotic plaque features was found to be highly associated with a recent history of TIA or stroke. Irregular or ulcerated plaque surface morphology has been correlated with advancing stenosis and independently with ischemic stroke in angiographic studies.

Thus, the morphological features of carotid atherosclerotic plaque, other than the mere carotid artery stenosis, may be additional and more sensitive indicators needed to identify carotid artery lesions associated with a higher risk of stroke.

The vulnerability of the carotid plaque morphology has been recognized as an important predictor for stroke.

Table (3) shows that serum neopterin level was found to be significantly higher in patients with carotid atherosclerotic plaque compared to healthy control (p<0.001). Serum neopterin level was not only higher in patients with carotid atherosclerotic plaque (Table 3), but it was also found to be rather more significantly higher in patients with features of vulnerable (high-risk) plaques than in those without (p<0.001) (Table 4). Although table (5) showed higher, yet non-significant, serum neopterin level in patients with multiple cerebral ischaemic events than in patients with a single event, serum neopterin level still can represent a kind of biochemical reflection (marker) of the mere presence of carotid atherosclerosis, and of its high-risk (vulnerability) features as well.

Neopterin was found to act as pro-oxidant, enhancing oxidant damage and triggering apoptosis in a number of different cell types. Earlier, serum concentrations of neopterin were found to be significantly higher in subjects with carotid atherosclerosis than in those without, and were closely correlated with the extent of carotid atherosclerosis.

The finding of a significantly higher serum neopterin level in patients with carotid atherosclerosis, and of the rather significantly more higher level in patients with vulnerable carotid atherosclerotic plaque, may be explained by the fact that neopterin is basically a product of the stimulated macrophages inside the atherosclerotic plaque, which are responsible for the active inflammatory process that renders these plaques vulnerable, unstable, and more likely to fragment leading to cerebral ischaemic events. So, inflammation is what is going on inside a carotid atherosclerotic plaque. Thus, the more intra-plaque inflammation is active, the more vulnerable (high-risk) the plaque is, and the more neopterin is honestly rising.

Earlier reports have argued with the ongoing intra-plaque inflammation that can explain this significantly higher serum neopterin level in patients with carotid atherosclerosis. These findings are an inspiration for using serum neopterin level as a marker for the presence and grading of carotid atherosclerosis.

In a preliminary report neopterin plasma level was suggested as a parameter in activity staging of atherosclerotic patients.

Even the idea of using serum neopterin level to assess the success of therapeutic interventions for carotid atherosclerosis does not seem so weird, where measurements of plasma neopterin levels are already used as a clinical tool to assess efficacy of treatments for a range of infections including malaria.
Conclusion
This study confirms the notion that carotid artery atherosclerotic plaque morphology can be a powerful determinant of cerebral ischaemic events' risk. And this morphology can be peacefully studied by the non-invasive B-mode ultrasound. This may not only improve sorting out patients for medical or surgical treatment, but may also help monitoring disease progression and evaluating the efficacy of its therapeutic interventions.

The study also presents plasma neopterin level as a possible biochemical marker for carotid atherosclerotic disease and its severity (plaque high-risk features). Being a product and a reflection of the active inflammatory process inside the vulnerable carotid atherosclerotic plaque, neopterin plasma level may be a future tool to assess the success of medical treatment hypothesized to control and stabilize carotid atherosclerotic plaque.

REFERENCES


الملخص العربي

نيوبتريس في تصلب الشريان السباتي والحادثات الدماغية الوعائية الانتهاضية

تعتبر الصفات الشكلية لتصفيات تصلب الشريان السباتي والمرضى للنوات (الصفح عالية الخطورة) من محددات الإصابات الانتهاضية بأجزاء الدماغ التي بيدا الشريان السباتي بالدمع. وقد وجد أن خلايا البلاسمة الكبيرة في اللاعب الرئيسي في عملية الانشطار داخل هذه الصفح مما يجعل منها صفاح عالية الخطورة، وأن هذه الخلايا هي مصدر مادة النيوبتريس في مصل الدم. وفقت حزعته هذه الدراسة قياس مستوى النيوبتريس في مصل الدم في مرضى تصلب الشريان السباتي ودراسة علاجها بالصفح الشكلية لتصفيات التصلب والاضمات الدماغية الانتهاضية على نفس جهة الصفح.

اشتملت الدراسة على مدار مريض بالاحتشاء في مناطق الشريان السباتي مستثنى بالخصى البياني المحوري الحاسوي والذين أظهروا بخصوص المواد فوق الوضوية صفاح تصلب الشريان السباتي على نفس جهة الاحتشاء، وكان مدى أعمارهم من 50 إلى 78 سنة، منهم 62 ذكر و 38 أنثى، وتشمل الدراسة أيضًا على 20 من الأصحاء كمجموعة ضابطة تجذب مع مجموعة المرضى في العمر والنوع، وقد تم استعداد الإحصاء بالشفقة وأعراض الفلل السمية لجلطة الدماغ وأعراض زيادة تجلط الدم وتعاطي العقاري المنشأية للجهاز العصبي السمنبي من مجموعة المرضى.

وقد أجري للمرضى والمجموعة الضابطة التحليل العملية الروتينية بالإضافة إلى قياس مستوى النيوبتريس في مصل الدم، وقد وجدت صُفاح تصلب الشريان السباتي للنوات (الصفح عالية الخطورة) على نفس جهة الإصابات الدماغية الانتهاضية في 80% من المرضى عمومًا، وأيضاً على نفس جهات في 94% من المرضى المصابين باختيارات متعددة، وقد وجد مستوى النيوبتريس مرتفع ارتفاعًا ذا قيمة إحصائية في مصل الدم الدماغي بالنسبة للمجموعة الضابطة، وفي مجموعة المرضى وجد مرتفع من الارتفاع في مستوى النيوبتريس ذو قيمة إحصائية فين أظهروا صافح تصلب الشريان السباتي العرضي للنوات (الصفح عالية الخطورة) عن بقية المرضى، وقد وجد المستوى أعلى في المرضى ذوي الاحتشادات المتعددة ولكن دونا قيمة إحصائية.

ومن خلال هذه الدراسة وجد أن الصفح الشكلية لتصفيات تصلب الشريان السباتي من الممكن أن تكون محددة لخطر الإصابة بالحادثات الدماغية وذلك ممكن دراستة بشكل عم ودون اختراق للجسم عن طريق المواد فوق الوضوية، وأن مستوى النيوبتريس في مصل الدم يمكن أن يكون مؤشرًا كيميائيًا حيويًا على وجود مرض تصلب الشريان السباتي وعلي مدى شدة، وأعرازه يستخدم تقييم فعالية علاج.