DOI: https://doi.org/10.5455/JPMA.3194

Research Article

Pentraxin-3 level in subarachnoid hemorrhage: Is it a prognostic factor?

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Abstract

Objectives: The current study was planned to investigate the relationship of serum level of pentraxin-3 with various clinical and neurological scales and scores.

Methods: The prospective case-control study was conducted at the Emergency Department of the Ondokuz Mayis University, Samsun, Turkey, from March 2013 to June 2014, and comprised subarachnoid haemorrhage patients and healthy. Pentraxin-3 levels were measured from serum samples and compared with sub-groups of the various scales and scores used in the study. Data was analysed using SPSS 15.

Results: Of the 77 subjects, 40(52%) were patients and 37(48%) were controls. Pentraxine-3 levels in the cases were significantly higher than the controls (p<0.001). Among the cases, pentraxine-3 level of the Glasgow Coma Scale sub-group was significantly different between the severe and mild categories (p=0.048). Likewise, pentraxine-3 levels were significantly different in terms of Fisher scale in patients with minor haemorrhage compared to those with massive haemorrhage (p=0.026). Also,
pentraxine-3 levels were significantly higher in patients who died compared to those who fully recovered (p=0.042).

**Conclusion:** There was found to be a relationship between pentraxine-3 level and the clinical severity of subarachnoid haemorrhage patients.

**Key Words:** Pentraxin-3, Subarachnoid haemorrhage, Glasgow coma scale, Fisher scale, Glasgow outcome scale.

**Introduction**

Spontaneous non-traumatic subarachnoid haemorrhage (SAH) involves bleeding directly into the subarachnoid space; usually from a ruptured artery or vein.\(^1\) The incidence of SAH is higher in females compared to males, and rises with increasing age in both genders. Spontaneous SAH peaks between the ages of 45 and 64 years, and over 45% of annual SAH cases occur in this age group.\(^2\) Intracranial aneurysm is the most common cause of SAH (85%).\(^3\)

The severity of the initial haemorrhage, cerebral vasospasm, re-haemorrhage, and surgical complications are the most significant causes of morbidity and mortality in SAH.\(^4\) Cerebral vasospasm starts on the third day and peaks on the sixth to tenth days.\(^5\)

One of the theories accepted in the pathophysiology of vasospasm is that it is triggered by a spasmogenous formed as result of degradation of extravasated erythrocytes and blood platelets.\(^6\)

It is reported that neurogenic and classic inflammatory responses as well as spasmogenous agents have a role in the pathogenesis of vasospasm following SAH.\(^6,7\)

The collection of blood in the subarachnoid space results in the activation of the inflammatory response within the first 48 hours, prompting increased neutrophil and macrophage migration to the subarachnoid space.\(^8\) Furthermore, it is reported in literature that increases in substance P and calcitonin gene-related peptide concentrations in the cerebro-spinal fluid (CSF) occur after SAH.\(^9\) Reagents histamine, bradykinin and
hydroxytryptamine, which are known to be responsible for the neurogenic inflammatory response, and endothelin-1 (ET-1) result in the failure of the blood-brain barrier.\textsuperscript{7}

Pentraxins (PTX-3), proteins that are produced in the mononuclear phagocyte cells, and bone marrow-originated dendritic cells are components of natural humoral immunity and belong to the acute phase reactant super family.\textsuperscript{10-12} The concentration of PTX-3 increases rapidly in the initial 6-8 hours after the stimulus in proportion to the stimulus and severity of the disease.\textsuperscript{10}

The current study was planned to investigate the relationship of serum level of PTX-3 with various neurological scales and scores.

**Patients and Methods**

The prospective case-control study was conducted at the Emergency Department (ED) of the Ondokuz Mayis University, Samsun, Turkey, from March 2013 to June 2014. After getting approval from the institutional ethics committee, the sample size was calculated at 95\% confidence level, 100\% testing power and moderate effect size. Patients with suspicion of non-traumatic SAH who presented at the ED within the first 24 hours after onset of symptoms were included. A group of healthy controls was also enrolled. The diagnosis of SAH patients with clinical suspicion was made by computerised tomography (CT) in the ED. Also, lumbar puncture (LP) was used for patients who were not diagnosed with CT. After the diagnosis, the patients were followed up in the neurosurgery department.

Pregnant women, patients with renal, liver and heart failure, and acute inflammation were excluded.

Scales and scores used for clinical and neurological testing included the Hunt-Hess scale (HHS)\textsuperscript{13} and Fisher scores,\textsuperscript{14} which indicated SAH severity, the Glasgow coma scale (GCS),\textsuperscript{15} which assessed the level of consciousness, and the Glasgow Outcome Scale (GOS),\textsuperscript{16} which revealed the neurological results.
On presentation, GCS scores of the patients were further divided into three sub-groups depending on their level of consciousness: subgroup A: GCS=3-8; subgroup B: GCS=9-13; and subgroup C: GCS=14-15. HHS was divided into two subgroups: mild = 1-3; severe = 4-5. The Fisher score, which shows the volume of the haemorrhage, was calculated based on the patients’ CT scans thus: Grade I = no SAH detected; Grade II = diffuse or vertical layer of subarachnoid blood <1mm thick; Grade III = Localised clot and/or vertical layer within the subarachnoid space >1mm thick; Grade IV = intracerebral haemorrhage (ICH) or intraventricular haemorrhage (IVH) with diffuse or no SAH. Diagnosis of Grade 1 SAH, according to the Fisher score, was made by CT angiography and LP. The duration of hospitalisation for each patient was recorded, and their clinical evaluation at the time of discharge was based on GOS thus: Group I = dead; Group II = persistent vegetative state; Group III = severely disabled; Group IV = moderately disabled; Group V = good recovery.\textsuperscript{16} The relationship between the clinical scoring through GCS, HHS and GOS, and radiological grading by Fisher score of the sub-groups was analysed in comparison with PTX-3.

Analyses of haemogram (leucocytes, haemoglobin, platelets, and haematocrit) and biochemical parameters (creatinine, blood urea nitrogen [BUN], alanine aminotransferase [ALT], aspartate aminotransferase [AST], glucose, sodium, potassium, creatin kinase, and troponin) were performed for each patient. The blood samples were placed into tubes that did not contain anticoagulant, and centrifuged at 1000 xg for 15 minutes to obtain the serum. PTX-3 analysis was then performed on the serum samples. The concentration of PTX-3 was determined using the enzyme-linked immunosorbent assay (ELISA) method following the manufacturer’s instructions (Catalog No. SK00101-01, Adipo Bioscience, USA).

Data was analysed using SPSS 15. Testing power was 100%, according to PostHoc analysis. Shapiro-Wilk test was used to evaluate normal distribution. When comparing the two groups, independent samples t test was performed for parameters of normal distribution, and the Mann Whitney U test was performed for parameters of non-normal distribution. Kruskal Wallis test was used when comparing more than two groups or sub-
groups. The results were expressed as median (interquartile range [IQR]). The relationship between the variables was analysed by Spearman’s correlation analysis, and the level of significance was set at p<0.05.

Results

Of the 77 subjects, 40(52%) were patients and 37(48%) were controls. There were 16(40%) males among the cases with a median age 51.31 (IQR: 13.15), and 24(60%) were females with a median age of 52.39 (IQR: 25.00). The overall median age of the cases was 51.94 (IQR: 26.25) years. Among the controls, there were 19(51.35%) males with a median age of 59.63 (IQR: 11.00) years, and 18(48.65%) were females with a median age of 55.06 (IQR: 5.75) years. The overall median age of controls was 57.41 (IQR: 6.00) years. There were no significant differences between the groups with regard to age or gender (p>0.05).

No significant correlation was found between the number of leukocytes and PTX-3 levels in the patients (p=0.813). A significant difference was evident between PTX-3 levels of the controls and the patients (p<0.001). PTX-3 value of the control group was the same as that determined in healthy persons (<2 ng/mL) (Table 1).

There was a significant difference between the groups GCS sub-groups were compared (p=0.048) (Figure 1).

When the PTX-3 levels of HHS mild and severe subgroups were compared, there was no significant difference between them (p=0.111).

There was a significant difference between PTX-3 levels in those with minor and massive haemorrhage (p=0.026) (Figure 2).

In GOS terms, there was a significant difference between those who died and those who fully recovered (p=0.042). No significant differences were found among the other groups (Figure 3).

A positive correlation was found between the duration of hospitalisation and increased PTX-3 levels (p=0.026).
Discussion

Cerebral vasospasm occurring after acute aneurysmal SAH (aSAH) is one of the most feared complications of SAH. The aetiology of vasospasm post-SAH is multi-factorial, and many independent mechanisms play a role in its pathophysiology. The phagocytosis of blood clots begins 48 hours after the occurrence of SAH. Free iron ion in the environment causes free radical formation and lipid peroxidation. Haemoglobin also binds to nitric oxide (NO), reducing its efficiency or directly inhibiting guanylate cyclase.

ET-1 synthesis in leukocytes is induced after the blood clot in the subarachnoid space is haemolysed. ET-1 contributes to the development of vasospasm by causing vasoconstriction and exerting a proliferative impact on endothelium and smooth muscle cells. PTX-3 levels increased approximately three times more in the SAH group than in the control group in the current study. The increased levels of PTX-3 in patients with low GCS and high GOS scores highlights the relationship between the clinical state of the patients and their PTX-3 levels. Furthermore, the positive correlation between the duration of hospitalisation and PTX-3 levels further supports this relationship. In addition, high PTX-3 levels in patients with a high Fisher score underline the relationship between the haemorrhage volume and PTX-3.

Spontaneous SAH is related to early brain damage, which is the primary cause of mortality in these patients. Early brain damage plays a significant role in cerebral vasospasm pathogenesis, and the activation of inflammatory pathways features in the pathogenesis of brain damage that develops secondary to SAH. The deposition of peri-vascular immune complex indicates that the inflammatory process is active in the large vessels of the brain. Moreover, a relationship exists between the changes in cerebral blood flow and an increase in leukocyte adhesion molecules and cytokines in the serum and CSF in SAH patients. Schneider et al. reported that pro-inflammatory characteristics in CSF were evident in SAH patients long before the occurrence of cerebral vasospasm, and an increase in the deposition of leukocytes and leukocyte-endothelium interaction in CSF was present from the second day after the occurrence of
SAH. Furthermore, rolling leukocytes were significantly increased on the sixth day, and sticking leukocytes on the second and fourth days. In the same study, there was an increase in the migration of monocytes in the CSF through endothelium cells during the first 12 days post-SAH. In another study conducted by Romero et al., an increase in C-reactive protein (CRP) concentration was observed in patients with aSAH, which was measured during the first three days after presentation at hospital. In the same study, a positive correlation was identified among HHS, Fisher scores, and serum CRP levels while there was a negative correlation between the serum CRP levels and GCS scores of the patients at the time of presentation. In addition, it is reported that there was a negative correlation among vasospasm, GOS score, Modified Rankin Scale, and the CRP. In a study, greater numbers of leukocytes and neutrophils were detected in the blood of patients who developed vasospasm. Furthermore, there was a positive correlation between the increased number of neutrophils and matrix metallopeptidase 9 (MMP-9) content, indicating that the resource of MMP-9 was neutrophils. In the same study, the increased number of neutrophils predicted a poor clinical recovery during the initial three-month period. A correlation was evident between the increase in CSF and serum MMP-9 concentrations and poor clinical course, but no relationship was found with vasospasm. Those results show that the number of neutrophils was increased in SAH and that inflammation mediators were also increased along with the rise in the number of neutrophils. Our study also revealed a rise in PTX-3 levels, which indicates an increase in acute inflammation. However, no correlation was found between the number of leukocytes and the PTX-3 level. This result suggests that the resource of PTX-3 in SAH is not leukocytes. As the PTX-3 levels increased independently, it may be useful in the first 24 hours as a biomarker for the diagnosis of SAH.

Processes other than inflammation also play a significant role in the development of brain damage secondary to haemorrhage. In a study performed by Chun-Chen Chiu et al., a relationship was found between the higher D-Dimer levels and mortality in the patients with intracerebral haemorrhage (ICH). Furthermore, a midline shift is seen on the brain in CTs of patients with higher D-Dimer levels, and the ICH score was also higher in these
In terms of the level of PTX-3 between patients who died and those who recovered completely with respect to GOS, there were significant differences in our study, too. At the same time, hospital stay was also longer in patients who had higher PTX-3 level. This shows that high PTX-3 level in SAH patients can be a good indicator in predicting mortality and determining hospital stay.

Conclusions
PTX-3 levels increase during the first 24 hours post-SAH. Furthermore, there is a relationship between the Fisher score, GCS, GOS, and the levels of PTX-3 in SAH patients. Thus, although further studies are required to validate the findings, it is suggested that PTX-3 may be useful as a biomarker in the diagnosis of SAH within the first 24 hours after presentation.

Disclaimer: None.
Conflict of Interest: None.
Source of Funding: The Ondokuz Mayis University, Turkey.

References


| Table 1: Demographic and laboratory features of patients and control groups. |
|-------------------------------------------------|-----------------|---------|
| Patients (n=40) | Control (n=37) | p       |
| Gender          |                 |         |
| Female (n, %)   | 16 (40)         | 18 (51.40) | > 0.05 |
| Male (n, %)     | 24 (60)         | 19 (48.60) | > 0.05 |
| Median Age (IQR) |                 |         |
| Female          | 52.39 (25.00)   | 55.06 (5.75) | > 0.05 |
| Male            | 51.31 (13.15)   | 59.63 (11.00) | > 0.05 |
| PTX-3           | 3.53 (0.68-10.40) | 1.3 (0.61-1.83) | < 0.001 |
| Laboratory      |                 |         |
| Leucocytes (1000/uL) | 12.95 (1.44-36.00) |         |
| Hb (g/dL)       | 13.05 (9.20-17.00) |         |
| Plt (1000/uL)   | 234 (102-398)   |         |
| Glucose (mg/dL) | 160 (94-400)    |         |
| BUN (mg/dL)     | 14 (4-52)       |         |
| Creatinine (mg/dL) | 0.72 (0.20-5.70) |         |
| ALT (U/L)       | 19 (10-79)      |         |
| AST (U/L)       | 22 (13-110)     |         |

IQR: Inter quartile range, PTX-3: Pentraxin-3 (ng/mL), Hb: Haemoglobin, Plt: Platelets, BUN: Blood urea nitrogen, ALT: Alanin aminotransferase, AST: Aspartate aminotransferase.

Figure 1: Comparison of Pentraxin-3 (PTX-3) levels among Glasgow Coma scale (GCS) subgroups of the subarachnoid haemorrhage (SAH) patients.

*p= 0.048, when compared with the control group.
Figure 2: Comparison of Pentraxin-3 (PTX-3) levels in Fisher scale sub-group of subarachnoid haemorrhage (SAH) patients.
*p=0.026, when compared with Grade I-II.

Figure 3: Comparison of Pentraxin-3 (PTX-3) levels in Glasgow Outcome Scale (GOS) sub-group of subarachnoid haemorrhage (SAH).
*p=0.042, when compared with Group V.