Serum Levels of Monocyte Chemoattractant Protein-1 Correlate with Poor Clinical Grades in Cerebral Aneurysms

Abdolkarim Rahmanian1, Navideh Mohebali1, Ali Haghnegahdar1, Eskandar Kamali Sarvestani2, Ali Razmkon1, Juri Kivelev3, Fahim Baghban1

1Department of Neurosurgery, 2Department of Immunology, Shiraz Medical School, Shiraz University of Medical Sciences, Shiraz, Iran, 3Department of Neurosurgery, Helsinki University Central Hospital, Topeliuksenkatu, Finland

ABSTRACT

Background: Ruptured cerebral aneurysms (ICAs) are the most common non-traumatic cause of subarachnoid hemorrhage (SAH) that is associated with life threatening complications such as Vasospasm, Infarction, and Hydrocephalus (HCP). The active participation of macrophage/monocyte-mediated inflammatory response in the pathogenesis of cerebral aneurysm as labeled with Monocyte Chemoattractant Protein-1 (MCP-1) is suggested. Objective: To measure the serum level of MCP-1 in ruptured CAs in different time intervals. Methods: We measured the serum levels of MCP-1 in SAH patients who had CAs and compared it with that of MCP-1 in two control groups: including patients with SAH without CAs, and the normal population of blood donors. We also measured the MCP-1 levels in patients with CAs one week afterward to evaluate the effect of treatment. Serum level of MCP-1 was measured by a commercial ELISA assay. Results: Mean serum MCP-1 level in patients with SAH and CAs was 188.2168 Pg/ml and 331.3982 Pg/ml in the normal population. There was no statistically significant difference between serum levels of MCP-1 on the first (mean=188.2168 Pg/ml) and 7th days after SAH onset (mean=171.8450 Pg/ml) (p=0.739). Serum level of MCP-1 increased significantly as Glasgow Coma Scale decreased (p=0.078) and Hunt and Hess score increased (p=0.089). Conclusion: Our results did not show an increasing MCP-1 serum level in patients with aneurysmal SAH. There was a relationship between poor clinical grade and MCP-1 levels in patients with CAs. MCP-1 may be a local inflammatory marker for cerebral aneurysms without systemic manifestation.

Keywords: Intracranial Aneurysm, Monocyte Chemoattractant Protein 1, Serum Level

**INTRODUCTION**

Subarachnoid hemorrhage (SAH) accounts for 5% of strokes. The aneurysm rupture is the most common cause of spontaneous SAH (1). After SAH, cerebral arteries may undergo spasm resulting in poor cerebral perfusion and ischemia, which in turn may cause infarction. Circulation of cerebrospinal fluid may be impeded as blood becomes organized into scar tissue that blocks the subarachnoid space causing hydrocephalus. The reported mortality after SAH is about 40% within the first 30 days. Among those who survive, less than 25% have a good functional outcome.

Histological studies suggest that cerebral aneurysm walls rupture as a consequence of matrix degeneration and de-cellularization, which may be the result of direct vascular stress (2). The presence of macrophages, T cells, B cells, immunoglobulin antibodies, and activated complement in cerebral aneurysm walls, especially in the ruptured aneurysms, indicates that active innate and adaptive immunologic reactions are associated with aneurysm formation and rupture (2). Up-regulated expression of the pro-inflammatory chemokine monocyte chemoattractantprotein-I (MCP-I) in the aneurysm wall has also been observed (2). Even though MCP-1 mRNA has not been detectable in normal arteries, it was often observed in the intima of the aneurysmal walls and appeared to be expressed in the cytoplasm of the fibroblast cells that are assembled with monocyte-like cells. The extent of inflammatory cell infiltration and complement activation appeared to be higher in the ruptured rather than unruptured aneurysms (2).

Elevation of serum MCP-1 was seen in hemodialysis patients as well (3). According to previous studies, a role for MCP-1 in neurological injury was assessed, implying that it may act as a biomarker of poor outcome in the sera of patients with vasospasm following aneurysmal subarachnoid hemorrhage (4). Although these data imply that inflammation is involved in the process of aneurysm progression or rupture, it is still unknown whether inflammation is the primary process causing the initiation and progression of disease or it is a secondary reaction accompanied by disease process. At present, treatment modalities for cerebral aneurysms are confined to surgical obliteration of aneurysms by clipping or coiling. If we could disclose more detailed mechanisms of inflammatory reactions involved in the progression of cerebral aneurysms, specific anti-inflammatory treatments would be the first choice for the patients with cerebral aneurysms in future (1). A number of articles evaluate the role of MCP-1 in CAs but serum level of MCP-1 has not been evaluated in any of them. Because monitoring the serum level is more convenient, less expensive and safer than tissue sampling and it is assessable before the rupture of aneurysms, we decided to evaluate the serum level of MCP-1 in CAs. The main hypothesis of this study was to find if serum levels of MCP-1 are significantly higher in ruptured aneurysms, so it can be used as a less-invasive screening test in diagnosis, or may be used as a target for future innovative treatments.

**MATERIALS AND METHODS**

**Subjects.** This is a case control study for evaluating the serum level of monocyte chemoattractantprotein-I (MCP-1) in ruptured cerebral aneurysms. Measuring of MCP-1 in CAs but serum level of MCP-1 has not been evaluated in any of them. Because monitoring the serum level is more convenient, less expensive and safer than tissue sampling and it is assessable before the rupture of aneurysms, we decided to evaluate the serum level of MCP-1 in CAs. The main hypothesis of this study was to find if serum levels of MCP-1 are significantly higher in ruptured aneurysms, so it can be used as a less-invasive screening test in diagnosis, or may be used as a target for future innovative treatments.
1 was done in 3 groups for 4 times. The samples were obtained from patients who referred to Shiraz Namazi hospital from May 2011 to May 2013 with definite diagnosis of subarachnoid hemorrhage (SAH). SAH was established with brain CT scan. Following admission, CT Angiography of the brain was done for all of them. Patients who had CAs were selected as the case group (labeled as group A) and patients who had no evidence of CAs were selected as the first control group (labeled as group B). Group B included patients with non-aneurysmal causes of SAH, most commonly labeled as benign peri-mesencephalic SAH. Samples of the 3rd group were taken from voluntary blood donors in the blood bank center (labeled as group C). Another blood sample was taken from patients in group A to be compared with the first sample in order to evaluate the role of therapeutic management in the serum level; this dataset was labeled as group D (Table 1).

### Table 1. Characteristics of the groups in the study.

<table>
<thead>
<tr>
<th>Type of Group</th>
<th>Time of Sampling</th>
<th>SAH</th>
<th>ICAs</th>
<th>Number</th>
</tr>
</thead>
<tbody>
<tr>
<td>A  case group</td>
<td>First 48 hr from onset</td>
<td>+</td>
<td>+</td>
<td>22</td>
</tr>
<tr>
<td>B  control group 1</td>
<td>First 48 hr from onset</td>
<td>+</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>C  control group 2</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>22</td>
</tr>
<tr>
<td>D  case group</td>
<td>1 week after first sample (after surgery)</td>
<td>+</td>
<td>+</td>
<td>22</td>
</tr>
</tbody>
</table>

**Inclusion Criteria.** Patients in this study were selected based on the following inclusion criteria: 1) patients who were alive with a Glasgow Coma Scale (GCS) score higher than 3 and with present stem reflexes, 2) time between the onset of SAH and serum sampling was not longer than 48 hours, 3) there was no need for any emergency neurosurgical intervention before serum sampling.

**Exclusion Criteria.** Exclusion criteria were: 1) patients with any history of inflammatory, rheumatoid and tumoral disease, 2) those who deceased before aneurysmal clipping operation, 3) patients with traumatic SAH, 4) those with other causes of SAH except CAs and prepontine hemorrhage such as toxins, blood disease, neoplasm, infections, 5) patients with a history of anticoagulant therapy, and 6) patients with normal CTA that had aneurysm in the follow up Conventional Angiography. In both groups, consent form was taken from the patient or first degree relatives.

**Sampling and Data Collection.** Data were recorded in data collection forms including: 1) demographic information such as age and sex, 2) SAH information such as the time of onset, time of admission, GCS Score, Fisher Grading, Hunt and Hess (H&H) grading, 3) aneurysm information such as location, size, number, shape, and 4) operation information such as date, duration, bleeding. Groups A and B were the same in management except in the plan for surgery in group A. We did not rule out CAs in group B completely until the follow up angiography was done during the first 14 days. We managed all SAH patients to see if they have CAs in the follow up angiography, therefore, all of therapeutic managements were similar to that of documented CAs in group A. About 5 ml blood sample was taken from each patient in the clot tube and sent to the Autoimmune Diseases Research Center in Shiraz University of Medical Sciences.
within 3 hours. We allowed the samples to clot for 30 minutes at room temperature; then, centrifugation of the samples was done for 15 minutes at 1000 xg; and, after separation of the serum, the samples were stored at \( \leq -20^\circ C \) until MCP-1 was measured. Group C was matched in demographic information with group A. Group C was considered as the second control group. Screening of group C for the history of previous CAs or other inflammatory and tumoral disease was performed (Table 1). The samples in this study were selected based on the MCP-1 plate; we could check MCP-1 on 96-well plates, 8 of which were used for standardization. After completion of the sampling, the serum level of MCP-1 was measured by Enzyme Linked Immuno Sorbent Assay (ELISA) (Human CCL2/MCP-1 Quantikine R&D Systems kit, USA) according to manufacturer’s recommendations (5). The sensitivity of the test was 10 Pg/ml.

**Statistical Analysis:** The comparison of data was done between groups using the Statistical Package for Social Sciences, version 18.0 (SPSS Inc., Chicago, IL, USA). Comparisons of serum level between groups were made using One-way ANOVA test. Paired-sample \( t \)-test, one-sample \( t \)-test, Fisher test, Kruskall-Wallis and Mann-Whitney tests were applied for the analysis of data. All the tests were performed with confidence interval (CI) of 95% and \( p \)-value \( \leq 0.05 \) was considered as significant.

**RESULTS**

We gathered the data from 3 groups with a total number of 66 and with 22 patients in each group (34 male and 32 female). Mean age was 53.83 with \( p=0.549 \). Distribution of age and sex in the 3 groups was almost the same. Patients in groups A and B were evaluated for clinical and radiological findings. Three parameters were assessed: H&H grade, Fisher Grading and GCS score (Table 2).

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>55 ± 11.2</td>
<td>51.6 ± 11</td>
<td>54.9 ± 12.2</td>
<td>0.549</td>
</tr>
<tr>
<td>GCS</td>
<td>12.1 ± 3.6</td>
<td>13.6 ± 2.1</td>
<td>-</td>
<td>0.078</td>
</tr>
<tr>
<td>H&amp;H</td>
<td>2.1 ± 1.3</td>
<td>1.5 ± 0.9</td>
<td>-</td>
<td>0.089</td>
</tr>
<tr>
<td>Fisher</td>
<td>3 ± 1</td>
<td>2.4 ± 0.9</td>
<td>-</td>
<td>0.052</td>
</tr>
</tbody>
</table>

H&H, Fisher and GCS were compared between groups A and B using one-way ANOVA test. Mean H&H was higher in group B compared with group A with no significant relationship (\( p=0.089 \)). Mean GCS was higher in group A compared with group B again with no significant relationship (\( p=0.078 \)).

In group A, 2 patients had 2 CAs and 1 had 3 CAs for whom clipping was done in one procedure but in 2 different surgical positions. With summation of multiple and simple CAs, we had 25 CAs that distribution was as below (Figure 1).
Distribution of CAs location was broad and most of them were Anterior communicating artery (A-Com) and middle cerebral artery (MCA) and posterior communicating artery (P-Com). We analyzed MCP-1 level in these 3 locations with Kruskall-Wallis test and there was no statistical relationship between them (p=0.350). Figure 2 shows the results of serum sampling in the four groups. MCP-1 serum level was not significantly higher in group A than group B (p>0.05). It was also unexpectedly lower in group A and B than group C. Mean MCP-1 level decreased after one weak but there was no significant statistical relationship between them (p=0.930).

The relationship between MCP-1 level and clinical and radiological grade of patients was evaluated by linear Pearson Correlation. In group A, the correlation between GCS and MCP-1 had p=0.001 and in group B, p value was 0.717, so there was a significant relationship between GCS and MCP-1 in the case group. This means that if GCS was better, the MCP-1 level was lower but this result was not seen in SAH without CAs. In group A, correlation between H&H and Fisher scale with MCP-1 level was also analyzed with Pearson Correlation test. This table indicates that MCP-1 level was higher in patients with poor grade and no significant relationship existed between them. Fisher scale was analyzed in the same manner; MCP-1 levels in group A & B and in group A mean MCP-1 level were higher in patients with poor Fisher scale but in both
groups, there was no significant relationship between Fisher scale and MCP-1 level (Table 3).

**Table 3. Correlation of GCS and H&H and Fisher with MCP-1 level.**

<table>
<thead>
<tr>
<th>Group</th>
<th>MCP-1 level in group</th>
<th>Pearson Correlation</th>
<th>Sig. (2-tailed)</th>
<th>N</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td></td>
<td>0.599</td>
<td>0.003</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.161</td>
<td>0.473</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-0.647</td>
<td>0.001</td>
<td>22</td>
</tr>
<tr>
<td>B</td>
<td></td>
<td>-0.075</td>
<td>-0.119</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.082</td>
<td></td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.741</td>
<td>0.599</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.717</td>
<td></td>
<td>22</td>
</tr>
</tbody>
</table>

Based on the results, there was no relationship between age and MCP-1 with Pearson correlation test (p=0.968); also, no relationship was found between sex and MCP-1 level using Mann-Whitney test (p=0.482).

**DISCUSSION**

About 85% of cases of subarachnoid hemorrhage are caused by rupture of saccular aneurysms. Three to 18% of patients suffering from SAH die before hospitalization and mortality of hospitalized SAH patients depends on factors such as initial hemorrhage re-bleeding and medical complications. Current evidence on the pathogenesis of cerebral aneurysms suggests that arterial wall proteolysis by matrix metalloproteinases, apoptosis, and chronic inflammation play a key role in disease progression. Up-regulated expression of the proinflammatory chemokine monocyte chemoattractant protein-1 (MCP-I) in the aneurysm wall has also been observed. Even though MCP-I mRNA was not detectable in normal arteries, it was often observed in the intima of aneurysmal walls and appeared to be expressed in the cytoplasm of the fibroblast cells that assembled with monocyte-like cells (1).

The role of MCP-1 in the inflammatory processes in the body and its inhibition for reduction of inflammatory response and decrease of the severity of disease was demonstrated in many studies. For example, in an animal model for atherosclerosis (6), experimental autoimmune encephalomyelitis (7), peripheral endotoxin insult (8), lethal endotoxiaemia (9), acute septic peritonitis (10) and cerebral aneurysm (11). Many inflammatory pathways and mediators are involved in aneurysm formation. MCP-1, Ets-1 and NFκB have been suggested to have a critical role in CAs formation by the induction of an inflammatory response (12).

The monocyte chemoattractant protein-1 (MCP-1/CCL2) is a member of the C-C chemokine family, and a potent chemotactic factor for monocytes. MCP-1 regulates the migration and infiltration of the monocytes, memory T lymphocytes, and natural killer (NK) cells. It is worth mentioning that MCP-1 is among the most studied members of
the chemokine family, and has been shown to be a potential intervention point for the treatment of various diseases, including multiple sclerosis, rheumatoid arthritis, atherosclerosis, and insulin-resistant diabetes (13). In approving the role of MCP-1 in inflammatory process, regression of CAs with blocking MCP-1 was seen (14) or decrease of serum level of MCP-1 with treatment of inflammatory disease (15), elevation of circulating level of MCP-1 in another focal disease (16) were shown. Elevation of circulating levels of MCP-1 in other diseases such as Ischemic Stroke, Myocardial Infarction (16) and hemodialysis patients was shown. According to the previous studies, the role of MCP-1 in neurological injury was assessed and implied that it may act as a biomarker of poor outcome in the serum of patients with vasospasm following aneurysmal subarachnoid hemorrhage. However, for approving the MCP-1 role in inflammatory process of CAs, regression of CAs with blocking MCP-1 was seen (11) and dramatic decrease of serum level of MCP-1 was shown with treatment of diseases such as Kawasaki (15). The activity of coils coated with MCP-1 in promoting inflammatory intra-aneurysmal tissue healing was assessed and MCP-1 releasing coils promoted significantly greater aneurysm tissue in-growth than bare platinum or PLGA-only coils (17).

According to the role of MCP-1 in the aneurysm formation and its high level in aneurysmal tissue, we decided to measure serum MCP-1 level in patients with ruptured CAs and correlate it with two control groups: first with SAH without CAs and second with the general population. Also, we measured MCP-1 level in CAs patients after one week and compared it with the level on admission and before surgery. Distribution of age and sex between groups was the same. There was no relationship between age and sex with MCP-1 level (p=0.968 and 0.482). Most of the patients were admitted within 2 days after SAH onset and most of them were operated within 3 days after SAH onset. Most of the CAs were A-com aneurysm (40%). There was no relationship between MCP-1 level and location of CAs (p=0.350).

MCP-1 level in groups A and B was lower than that of group C (control group) (p<0.001) but this relationship is opposite to what we expected. We expected that serum MCP-1 level in CAs patients, according to high tissue level, to be higher than in patients without CAs. In another study in type 2 diabetic patients, serum level of MCP-1 was not elevated and no correlation was seen between local and systemic level. Mean MCP-1 level was higher in SAH with CAs than SAH without CAs but the difference was not significant (p=0.409). It means that we could not find any relationship between increasing of serum MCP-1 level and CAs mean. MCP-1 level was decreased after one week of medical and surgical treatment but again not statistically significant. A significant relationship may be acquired with increase of sample size in groups A and B. GCS score was lower in group A than group B. H&H and Fisher score was higher in patients in group A as compared to group B. This means that neurological condition in patients with only SAH was better than patients with SAH and CAs. In patients with CAs, MCP-1 level was higher in patients with lower GCS scores and poor H&H grade (p=0.001 and 0.003), indicating that MCP-1 is higher in patients with worsened neurological condition that may show the role of MCP-1 in severity of patients’ condition. The same significant result was not seen between Fisher score and MCP-1 level but the mean level of MCP-1 was higher in higher Fisher score probably owing to the small sample size.

One of our patients in group A had 3 aneurysms; the results of statistical analyses were not reliable and needed a larger number of cases. Because the types of aneurysms were
broad and we had only one patient in some aneurysm location, we could not correlate demographic and clinical and radiological information with aneurysm location. According to these results, we suggest that elevation of MCP-1 was a local inflammatory process, and its serum level did not correlate with that of local process; we can not use MCP-1 serum level as a marker of CAs in diagnosis and evaluation of CAs patients. One limitation of the study is the small number of the groups to reach statistical significance. Performing the study on both tissue and serum of the patients simultaneously, as well as CSF may also be different options in reaching promising results. Another limitation of the study was the universal administration of corticosteroids in all patients with SAH after admission to hospital. Some studies have noted the effect of corticosteroids on decreasing MCP-1 levels in human subjects (18). Therefore, it seems logical to measure the serum level of the marker before administration of such drugs in future studies.

In conclusion, our results did not show an increased MCP-1 serum level in patients with aneurysmal SAH. A larger study, including CSF data with more controlled conditions before use of any drug such as corticosteroids may be necessary to better test such hypothesis. The relationship between poor clinical grade and MCP-1 level may suggest that MCP-1 is a local inflammatory marker for cerebral aneurysms without systemic manifestation.

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