Change of Plasma High Sensitive —C reactive protein levels in climbers

JMAJ 49(11-12): 358–364, 2006

Shintaro Suzuki,*1–3 Yuji Kiuchi,*2,4 Tetsuya Nemoto,*3 Kenta Kobayashi,*3 Hidekazu Ota*2

Abstract

Rationale Acute mountain sickness (AMS) is a high altitude illness. Previous studies report that levels of inflammatory indicators are higher at high altitudes. Therefore, inflammation is important for AMS.

Objectives We examined whether slight inflammation in mountain climbers could be detected with plasma levels of high sensitive C-reactive protein (hs-CRP). Next, we studied the relations of clinical parameters of plasma hs-CRP levels in AMS patients.

Methods (A) 32 healthy subjects were recruited to climb the mountain. We collected their blood in Tokyo (about 40 m above sea level) and in Mt. Kitadake Clinic (about 2,900 m above sea level). We measured plasma hs-CRP levels in their samples. (B) Next, we collected blood from 21 climbers diagnosed with AMS. We also measured hs-CRP levels in AMS patients and examined their relationship to clinical parameters of AMS.

Results Plasma hs-CRP levels of healthy subjects after climbing the mountain were significantly higher than before climbing (914±272 ng/ml vs 299±86, P<0.05). Plasma levels of hs-CRP were much higher values in AMS patients (2,433±831). They were correlated with AMS score (P<0.001, r=0.658), and symptomatic duration (P<0.001, r=0.691).

Conclusion These results showed slight inflammation existed in healthy climbers. Moreover, it was demonstrated plasma hs-CRP levels were related to clinical parameters of AMS. Therefore, hs-CRP was suggested to be an available and objective marker that could be used to evaluate the severity of AMS as an inflammatory disease.

Keywords Cytokine, High altitude medicine, High sensitive c-reactive protein, Mountain climbing, Stress failure

Introduction

Trekking and mountain climbing are popular leisure activities with middle aged to elderly people in Japan, because of the recent fitness boom. However, many climbers have been injured or suffered illness when climbing. The most important matter for amateur climbers is to prevent high altitude illness, especially acute mountain sickness (AMS). AMS is characterized by non-specific symptoms and a few physical findings. The main symptoms are headache, anorexia, nausea, vomiting, fatigue, dizziness and sleep disturbance, although all of them must not be present for diagnosis. These symptoms typically appear 6–12 hours after arrival at high altitude; over 2,500 m above sea level.1,2 High altitude cerebral edema (HACE) and high altitude pulmonary edema (HAPE) are severe forms of AMS. The incidences of HACE and HAPE are much lower than AMS, but they are poten-
Since there are no high mountains over 4,000 m in Japan, the high altitude illness that occurs is AMS in most cases.

Past studies show that hypobarism, hypoxia and excessive exercise may cause AMS. However, the pathophysiology of AMS has not been fully clarified. Its fundamental pathophysiology is supposed to be disorder in the capillary vascular wall. Oxidative changes and subsequent inflammatory response are thought to be involved in its progress. Previous studies evaluated AMS as an inflammatory disease by observation of the blood levels of several inflammatory markers such as inflammatory cytokines, chemokines, white blood cells count and C-reactive protein (CRP). However, no researchers have evaluated the plasma CRP levels in AMS patients with high-sensitive assays, but with only conventional assays. With high-sensitive CRP (hs-CRP) assays, we can detect inflammatory changes in earlier phases of diseases than usual assays, so that hs-CRP assays are not being used to evaluate the prognosis of lifestyle diseases in clinical metabolic medicine.

Mild to moderate grade AMS can usually be resolved by descending the mountain. Therefore, studies on mild to moderate grade AMS have not progressed. Furthermore, as previous studies on AMS were usually performed with a passive ascent to high altitude or by simulation tests in a hypobaric chamber, they might not necessarily reflect the current state of AMS patients. In particular, the relation between mild to moderate grade AMS and inflammation in the early phase of AMS is unknown.

In this study, we examined whether the plasma levels of hs-CRP were a possible marker of the early phase of AMS in two independent studies. First, we evaluated the change of plasma levels of hs-CRP in healthy climbers between before and just after climbing a mountain. Next, we examined the plasma levels of hs-CRP in AMS patients who consulted our mountain clinic and evaluated the relation between plasma levels of hs-CRP and known clinical parameters in AMS.

### Material and methods

#### Participants and subjects

Mt. Kita is the second highest mountain in Japan (3,192 m above sea level, in Yamanashi Prefecture). In 1985, Showa University School of Medicine established the clinic on Mt. Kita (about 2,900 m) for climbers suffering from diseases and injuries, and it is open every summer from July to August. Each summer, about 250–300 climbers visit the clinic, and two-thirds of them are AMS patients.

We examined two independent studies as follows (A and B).

**Study (A):** We recruited 40 healthy subjects to climb the mountain. Of these, 2 subjects withdrew, and as 6 persons had several symptoms that could possibly be caused by a high altitudinal environment, they were excluded from the study. So, a total of 32 subjects climbed the mountain route to Mt. Kita Clinic in safety. They did not complain of AMS symptoms and did not have any abnormal findings. They consisted of 20 men and 12 women ranging in age from 20 to 26 years old (Table 1). All subjects resided at altitudes <100 m above sea level. As for past history, 2 persons had remittal bronchial asthma, and 5 persons had allergic rhinitis. One subject had a past history of Kawasaki disease, but it was in remission. One person was a current smoker.

The plan of the climb is shown in Fig. 1A. On the first day, they arrived at the foot of Mt. Kita (1,529 m) from Tokyo by train and bus. They stayed overnight in a lodge and started climbing from 6:00 the next morning. Everyone climbed to the clinic via the same climbing route decided...
by us. Although they did not pass through the peak of Mt. Kita, it took from 7 to 9 hours. They ascended about 1,371 m per day. The altitude from Tokyo was about 2,860 m. It was good weather throughout the program. The weight of each person's luggage was less than 10 kg.

They were permitted to take food and drink from Tokyo to the clinic. However, they were instructed to avoid certain foods, supplements, and medicine such as vitamins reported to have anti-oxidative effects, for at least 1 week before the collection of blood in Tokyo. We allowed them to take medicine for therapy. There were no restrictions on the times of urination and defecation. No one inhaled oxygen during or after climbing.

Study (B): Twenty-one AMS patients participated in our second study. They climbed the mountain on foot and consulted our clinic when they complained of symptoms. Each climber gave his or her informed consent. They consisted of 8 men and 13 women, ranging in age from 34 to 66 years with an average age of 52.2 ± 1.8 years old (Table 2). Physicians performed clinical examinations on them and diagnosed them with AMS. Collection of blood samples was performed in the clinic. As patients climbed in various parties, their climbing routes differed. We divided them into two groups according to whether or not they reached the peak of Mt. Kita (3,192 m) (Fig. 1B). Fourteen patients via route A reached the peak. Fifteen patients had recurrent episodes of high altitude illness. Five patients had a past history, such as myoma uterus, gout, allergic dermatitis, allergic conjunctivitis and chronic sinusitis. Two persons were current smokers. No patients smoked or drank alcohol while climbing the mountain.

The study was approved by the ethics committee of Showa University School of Medicine and informed consent was obtained from all subjects.

**Collection, separation and store of blood sample**

Blood samples (9 ml) were drawn from the
antecubital vein. All the blood samples were taken using sterilized plastic syringes, and placed in plastic tubes including EDTA. Within 10 min, plasma and serum were separated from each sample by centrifugation and immediately frozen at −30°C until measurement. Frozen samples were transported to a laboratory in Showa University in a cooler to avoid defrosting.

Subjects of Study (A): Baseline blood sampling was performed 2–3 weeks before climbing in Tokyo (about 40 m). Blood was drawn from 14:00–16:00, before dinner. This timing was matched to the arrival time at the clinic. In the mountain, drawing of blood was performed within 1 hour after they arrived at Mt. Kita Clinic.

Subject of Study (B): Blood was drawn from AMS patients after medical examination in the clinic.

Measurement of plasma levels of hs-CRP
We requested SRL Co. (Tokyo, Japan) to measure hs-CRP in 500 µl plasma samples. It was performed according to the method of latex agglutination assay with Behring Nephelometer and N Latex CRP (Dade Behring). The lower limit of this test was 50 ng/ml (0.05 mg/dl). When measured values were above the limits, they were expressed as the actual values.

The assays for CRP are mainly used for detecting infection and inflammatory diseases. The upper reference value of the conventional CRP assay is mostly 0.2 to 0.5 mg/dl. The precision of the assay with the low-normal range is not satisfactory. High sensitivity assay for CRP has recently become commercially available and is used for predicting cardiovascular disease and managing metabolic syndrome. This assay is 40 times as sensitive as usual assays. The normal range of hs-CRP in Japanese is thought to be from 1,000 to 10,000 ng/ml (from 0.1 to 1 mg/dl). In some clinical cases, plasma levels of hs-CRP have been reported to be useful for the diagnosis of inflammatory disease such as infection or acute coronary syndrome in the super-early phase.

Calculating of body mass index (BMI)
As hs-CRP is affected by obesity and hyperlipemia, we also evaluated body mass index, total serum cholesterol levels and triglycerides levels in all subjects.

BMI as an indicator of obesity was calculated with height and body weight. BMI was expressed as body weight [kg]/(height [m])². Obesity was defined as over 25 kg/m².

Measurement of total serum levels of cholesterol (TC) and triglyceride (TG)
Serum levels of total cholesterol and triglyceride were measured using E-test WAKO (WAKO, Japan) and spectrophotometer (HITACHI, Japan).

Measurement of serum levels of interleukin-8 (IL-8), interleukin-6 (IL-6) and vascular endothelial growth factor (VEGF)
For only subjects of Study (A), serum levels of IL-8 and VEGF were measured by the methods of enzyme linked immunosorbent assay (ELISA) using Quantikine (R&D, Minneapolis, USA) ELISA kit. Serum levels of IL-6 were measured by the methods of high sensitivity ELISA using the same kit. The reference values of IL-8, IL-6, and VEGF were respectively 8.0 pg/ml, 2.41 pg/ml, and 38.3 pg/ml.

Evaluation of the Lake Louise score (AMS score)
All patients who consulted our clinic were scored for AMS according to the interview form called the Lake Louise Consensus scoring system (Lake Louise Consensus Questionnaire (LLCQ) translated into Japanese. This questionnaire comprised 8 items to ask climbers the degree of AMS symptoms such as headache, gastrointestinal symptoms, fatigue, dizziness and insomnia etc. Each item is scored with 0, 1, 2 or 3 points. The presence of AMS was defined as a cumulative score ≥5 points out of 23 for the total Lake Louise score. The system is usually called AMS scoring. We used the AMS score for evaluating the severity grade of AMS.

Noninvasive measurement of arterial oxygen saturation (SaO₂)
For subjects of Study (B), SaO₂ was measured noninvasively with a pulse oxymeter finger probe (PULSOX 7, Minolta, Japan), three times for each patient, and the mean values were recorded.

Statistical analyses
STAT view Ver5.0 (SAS Institute; Cary, NC) was used for the statistical analysis. Paired t-test was used for comparison of the plasma levels of hs-CRP and the serum levels of IL-6, IL-8.
and VEGF between before and after climbing. Pearson’s correlation coefficient was used between the plasma levels of hs-CRP and other AMS parameters. All values are shown as mean ± SEM. A $P$ value <0.05 was considered statistically significant.

**Results**

**Study (A) with healthy subjects**

Plasma levels of hs-CRP in healthy subjects after climbing were significantly higher than those before climbing (914 ± 272 ng/ml vs 299 ± 86, $P<0.05$) (Table 3). Serum total cholesterol and triglyceride levels were within normal limits (TC 146.7 ± 6.0 mg/dl, TG 93.77 ± 11.87 mg/dl), and did not correlate with their plasma levels of hs-CRP. The mean value of BMI was 20.7 ± 1.4 kg/[m]², and did not correlate with hs-CRP. Furthermore, there was no correlation with age or gender. All participants were checked by a physician and no infectious symptoms were found.

In levels of IL-6, IL-8 and VEGF in the serum of healthy climbers, no significant difference was seen between after and before climbing. However, the serum levels of IL-8 tended to be higher after climbing than those before climbing (16.0 ± 9.5 pg/ml vs 10.5 ± 2.67 pg/ml, NS). Serum levels of IL-6 also tended to be higher after climbing than those before climbing (1.21 ± 0.25 pg/ml vs 0.88 ± 0.40 pg/ml, NS). Serum levels of VEGF showed no difference between before and after climbing (300.2 ± 32.7 pg/ml vs 296.1 ± 52.7 pg/ml, NS). There was no correlation between plasma hs-CRP levels with either of the above serum cytokines levels.

**Study (B) with AMS patients**

The plasma levels of hs-CRP in AMS were significantly higher than those in healthy climbers after climbing (2,433 ± 831 ng/ml vs 914 ± 272 ng/ml, $P<0.05$). Most AMS patients did not have lifestyle diseases, but 1 person was being treated for gout. Total amounts of cholesterol and triglyceride of the patients were TC 194.9 ± 9.7 mg/dl, TG 81.1 ± 10.3 mg/dl, respectively. Calculated BMI was 21.8 ± 2.6 kg/[m]² in AMS patients (Table 2). Four patients showed abnormal data on lipid metabolism and 1 patient was obese, but their plasma hs-CRP levels did not correlate with blood lipid levels.

AMS was diagnosed by physical examinations and AMS score with LLCQ. In this study, we diagnosed AMS when the AMS score was higher than 5. The average AMS score of patients was 6.3 ± 0.2. The highest AMS score in the patients was 8. Plasma levels of hs-CRP in AMS patients significantly correlated with their AMS scores ($P=0.008$, $r=0.658$) (Fig. 2).

Some climbers complained of symptoms while climbing and other climbers developed AMS after climbing. The duration from onset of symptomatic AMS varied. The average duration calculated with clinical records, was 6.4 ± 0.8 hours. Plasma levels of hs-CRP in AMS patients significantly correlated with the duration of AMS ($P=0.003$, $r=0.691$) (Fig. 3). Plasma levels

---

**Table 3** Plasma levels of inflammatory indicators of healthy subjects

<table>
<thead>
<tr>
<th>(ng/ml)</th>
<th>Before</th>
<th>After</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>hs-CRP</td>
<td>914 ± 272</td>
<td>299 ± 86</td>
<td>&lt;0.05</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>(pg/ml)</th>
<th>Before</th>
<th>After</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>IL-6</td>
<td>1.21 ± 0.25</td>
<td>0.88 ± 0.40</td>
<td>NS</td>
</tr>
<tr>
<td>IL-8</td>
<td>16.0 ± 9.5</td>
<td>10.5 ± 2.67</td>
<td>NS</td>
</tr>
<tr>
<td>VEGF</td>
<td>300.2 ± 32.7</td>
<td>296.1 ± 52.7</td>
<td>NS</td>
</tr>
</tbody>
</table>

Mean ± S.E.M.
of hs-CRP did not correlate with age, gender, body temperature, SaO₂, or other clinical parameters.

Discussion

Mountain climbing increases serum levels of inflammatory mediators such as cytokines and chemokines in mountain climbers. For example, IL-1β, IL-6, IL-8, tumor necrotizing factor-α and CRP have been reported to be increased in peripheral blood and related to high altitude diseases.\textsuperscript{5,18-21} Previous studies with conventional assays showed plasma levels of CRP elevated some days after climbing or passive movement to highlands.\textsuperscript{9,18,19} However, these studies did not show slight inflammation caused by climbing in the early term. In this study, we studied the plasma levels of hs-CRP in mountain climbers including AMS patients just after climbing. In this study, we studied the plasma levels of hs-CRP in mountain climbers including AMS patients just after climbing. Our results show that the plasma levels of hs-CRP in healthy subjects after climbing are significantly higher than those before climbing. These findings indicate the possibility of slight inflammation in healthy subjects in the early term after climbing.

Our results also suggest that plasma hs-CRP levels are more sensitive to evaluating slight inflammation caused right after climbing than serum IL-6, IL-8, or VEGF levels. Previous studies reported that the increase of plasma levels of IL-6 and VEGF after exposure to high altitude were greater than the other proinflammatory cytokines.\textsuperscript{19-21} However, in this study, inflammatory cytokines or chemokines levels might be too small to evaluate in the early phase, in healthy climbers without complaint. Inflammatory mediators are likely to be less activated in high altitudinal illness than systemic inflammatory disease, since they are reported to be usually around normal limits.\textsuperscript{9}

Furthermore, we examined plasma hs-CRP levels in mild to moderate grade AMS patients who had climbed the mountain on foot. In past studies, most subjects were patients with severe AMS or HAPE who were carried to a hospital at low altitude, where they were examined and treated. In contrast, blood was drawn just after diagnosis of mild to moderate grade AMS in this study, and our results reflected the current status of the patients. Plasma hs-CRP levels in AMS patients were found to be much higher than those in healthy subjects after climbing, although precise comparison is difficult because of differences in biological background (e.g. age or gender) between healthy subjects and patients. Therefore, we supposed that measurement of CRP was suitable for detection and diagnosis of AMS in the early term. We speculated that production of CRP began immediately after exposure to a high altitudinal environment and that AMS aggravated inflammation in climbers and promoted CRP production, while there was a possibility that plasma hs-CRP was increased partly due to excessive exercise during climbing. Also, the plasma levels of CRP in AMS patients were significantly correlated with AMS score and the symptomatic duration of AMS, suggesting possible correlation between the plasma levels of CRP and the severity of AMS. Therefore, hs-CRP may be an objective marker for evaluating the severity of AMS.

The etiology of AMS has been not been clarified. Complementary hypoventilation, impaired gas exchange, fluid retention and redistribution are thought to be involved.\textsuperscript{22} Also, the elevation of intracranial pressure is often observed in cases of severe AMS and HACE. However, patients even in these cases felt better by descending, and usually did not see the doctor after convalescence, so little clinical data has been accumulated. However, some pathophysiological features of HAPE, the severity type of AMS, have been speculated from the data of inpatients suffering from HAPE as follows. Human vessels were injured by rapid exposure to a high alti-
tudinal environment such as hypobarism and hypoxia, and by endurance exercise, resulting in endothelial injury and capillary leakage, so-called “stress failure.” Edema, inflammation and oxidative change were consequently raised in various organs.1,2,7,22–24 Those events that occurred in the gastro-intestine, lung and brain were considered to lead to AMS symptoms. Acetazolamide as a diuretic was thought to be effective for edema of AMS. However, at present, there is no specific remedy for AMS without symptomatic treatment and rapidly descending. Further studies on the pathophysiology of high altitude illness are required to improve the safety of mountain climbing.

Acknowledgements

We are grateful to the members of Showa University Mt. Kitadake Clinic, medical students, nursing college students, and volunteer medical doctors.

References