

## Original Article

# An elevated serum level of nesfatin-1 may predict poor outcomes in patients with aneurysmal subarachnoid hemorrhages

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**Abstract:** Objective: To investigate the role of nesfatin-1 in patients with aneurysmal subarachnoid hemorrhage (aSAH). Method: A retrospective study was performed. There were 97 aSAH patients treated in Shenzhen People's Hospital from October 1, 2014 to December 30, 2016 in this study. Patient demographics, neurological presentations, in hospital complications, and clinical outcomes were recorded in detail. Serum samples on admission were collected and quantitative assay techniques were used to detect the serum level of nesfatin-1. The association between nesfatin-1 and the clinical outcome was analyzed using a multivariate regression analysis. In addition, we used a receiver operating characteristic (ROC) curve investigate the value of nesfatin-1 in predicting poor outcomes. Results: Among the 97 included patients, 72 patients achieved good outcomes and 25 patients achieved poor outcomes. The serum concentration of nesfatin-1 in patients with poor outcomes was higher than patients with good outcomes. After analyzing with multivariate regression analysis, we found that GCS, Hunt-Hess III-V, DCI, and an elevated level of nesfatin-1 were significantly associated with poor outcome. The area under the ROC curve of Fisher grade, GCS, Hunt-Hess and nesfatin-1 were 0.721, 0.748, 0.808, and 0.852, respectively. Conclusion: Elevated serum levels of nesfatin-1 significantly correlated with the clinical severity of aSAH patients. Serum nesfatin-1 in aSAH patients might be a potential biomarker for predicting the outcome of patients with aSAH.

**Keywords:** Subarachnoid hemorrhage, Nesfatin-1, biomarker, outcome

## Introduction

Aneurysmal subarachnoid hemorrhage (aSAH) is a fateful neurological disorder which is caused by the rupture of an intracranial aneurysm. aSAH accounted for 5%-10% of all strokes and its estimated annual incidence is about 9/100,000 person/year [1]. It was reported that 90 day case-fatality rates of patients with aSAH was still as high as 29% in 2010 despite of an obvious decline in case-fatality compared with 1988 [2]. The World Federation of Neurological Surgeons grade (WFNS), the Hunt-Hess grade, and postoperative complications (such as cerebral vasospasm (CVS), delayed cerebral ischemia (DCI)) are reported to be independent risk factors associated with the poor outcomes of patients with aSAH. But their values in predicting poor outcomes weren't very high and were sometimes inaccurate.

Many molecules are involved in the pathological process after aSAH and some of them played important roles in causing neurological complications. In recent years, more and more studies found that inflammatory biomarkers [3-5] (C-reactive protein (CRP), interleukin 6 (IL-6), Neutrophil), Thromboelastography parameters [6] and other cytokines [7] were significantly associated with the poor outcomes of aSAH patients. Using biomarkers to predict the outcome of patients with aSAH might be more reliable, and previous studies have demonstrated their incomparable roles in predicting prognosis.

Nesfatin-1 is an anorexigenic peptide derived from the calcium and DNA-binding protein, nucleobindin2 (NUCB2) [8]. Nesfatin-1 can be found in cerebrospinal fluid and in the hypothalamic paraventricular nucleus [9]. Recently, so-

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me studies revealed that serum nesfatin-1 was significantly associated with clinical severity and could be used as a new biochemical marker of various diseases, including polycystic ovary syndrome [10], chronic obstructive pulmonary disease [11], preeclampsia [12], and traumatic brain injury [13]. That nesfatin-1 acts as a biochemical marker for many diseases might be partly due to its role in the inflammatory cascade of diseases. The inflammatory response in brain tissue after subarachnoid hemorrhage has been observed and a previous study indicated that the level of nesfatin-1 in peripheral blood was elevated in aSAH patients. Until now, little was known about the prognostic role of nesfatin-1 in patients with aSAH. So we performed this retrospective study to explore the prognostic role of nesfatin-1 in patients with aSAH.

### Method

#### *Patients*

This retrospective study was performed in the Department of Neurosurgery of Shenzhen People's Hospital from October 1, 2014 to December 30, 2016. This study was approved by the Ethics Committee of Shenzhen People's Hospital and informed consents from all patients included were signed by their relatives. The inclusion criteria were: (1) Diagnosed with a subarachnoid hemorrhage using head computed tomography (CT), and (2) A responsible intracranial aneurysm was present on the head and found using CT angiography (CTA) or digital subtraction angiography (DSA). The exclusion criteria were: (1) Time from symptom onset to hospitalization > 72 hours; (2) Peri-mesencephalic non-aneurysmal SAH or SAH caused by head trauma, vascular anomaly, vascular malformation and moyamoya disease. All patients were treated with a therapeutic regimen including hemostatic, prophylactic, anti-epilepsy, and nimodipine drugs. All patients received coiling or clipping within 72 hours of admission.

#### *Data collection*

The demographic information of the patients included was collected. The details of the accompanying diseases were acquired by questioning the patients or their relatives in detail. The Glasgow Coma Scale (GCS) and the Hunt-Hess grade were used to assess the neurological status of the patients included. The Fisher Grade according to the presentations on the

head CT on admission was used. Postoperative complications including neurological (DCI, re-bleeding, epilepsy) and systematic complications (pneumonia, urinary tract infection (UTI)) were all recorded in detail. A head CT was routinely performed for all patients on the first day and at any time during the hospital stay if the patients suffered a clinical deterioration.

#### *Definition*

Delayed cerebral ischemia: DCI is defined as a unexplained neurological deterioration or a new infarct on head CT after clipping or coiling but does not refer to surgery-related cerebral ischemia. A neurological infection is defined as: if patients had complications, such as encephalitis or meningitis during a hospital stay. A poor outcome is defined as a Glasgow Outcome Scale (GOS) score of 1-3, and a good outcome was defined as a GOS score of 4-5 at 6 months.

#### *Blood samples*

Blood samples were collected on admission. All samples were centrifuged at 3000 rpm for 5 min, and the supernatant fluid was stored at -80°C for future assays. ELISA kits (ELISA Kit for Nesfatin-1 (CEA242Hu, Wuhan USCN business Co., Ltd.)) were used to detect the serum level of nesfatin-1. All operations were performed according to the manufacturer's instructions.

#### *Statistical analysis*

The mean, the standard deviation, and the median were used for the continuous variables and percentage was used for the categorical variables. A *t* test was used for comparisons between two groups and a one-way ANOVA analysis was used for three or more group comparisons. A box-plot was used to present the levels of Nesfatin-1 between different groups. Univariate and multivariate regression analyses were used to analyze the independent risk factors of poor outcomes. Receiver operating characteristic (ROC) curves were used to access the variables' prediction abilities. The statistical analyses were conducted using SPSS 21.

### Results

#### *Baseline information*

There were 97 patients included in this study. There were 35 males and 62 females. The aver-

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**Table 1.** Baseline characteristics

| Variables                         | N (%)            |
|-----------------------------------|------------------|
| Male (%)                          | 35 (36.082)      |
| Age (y)                           | 58.226 ± 10.453  |
| Hypertension                      | 23 (22.680)      |
| Smoking                           | 11 (11.340)      |
| Blood glucose (mmol/l)            | 9.34             |
| Systolic arterial pressure (mmHg) | 152.441 ± 22.237 |
| GCS                               | 11.453 ± 3.609   |
| Hunt-Hess III-IV                  | 31 (31.959)      |
| Fisher III-IV                     | 53 (54.639)      |
| Intraventricular hemorrhage       | 18 (18.557)      |
| Admission time (h)                | 13.340           |
| Anterior circulation artery       | 86 (77.477)      |
| Clipping                          | 88 (79.279)      |

age age was 58.226 ± 10.453 years old. There were 28 patients with hypertension, 11 patients with diabetes, and 23 patients who smoked. Among these patients, 31 patients were Hunt-Hess grade III-IV and 53 patients were Fisher grade III-IV. In all, there were 111 intracranial aneurysms, 88 of which received microsurgical clipping. The basic information of the included patients is presented in **Table 1**.

### *Serum levels of nesfatin-1 in aSAH patients*

The patients with poor outcomes had higher levels of nesfatin-1 when compared with the patients with good outcomes (11.342 ± 1.826 vs 9.118 ± 0.278,  $P < 0.01$ , **Figure 1A**). Also, the patients with DCI had higher levels of nesfatin-1 than the patients with non-DCI (11.736 ± 1.844 vs 8.842 ± 2.629,  $P < 0.01$ , **Figure 1B**). Because nesfatin-1 plays an important role in the process of inflammation, we performed an additional analysis. But the results revealed that there was no difference between patients with infectious diseases and patients with non-infectious during their hospital stays (**Figure 1C**).

### *The correlation between nesfatin-1 and clinical severity*

The serum concentration of nesfatin-1 was positively correlated with the Hunt-Hess grade ( $r = 0.417$ ,  $P < 0.01$  95% CI [0.237-0.569]) and a higher Hunt-Hess grade (**Figure 2B, 2C**). But serum nesfatin-1 failed to show any correlations with the GCS or Fisher grade. CRP, an important acute phase stress protein, has a significant correlation with the clinical progn-

osis of patients with aSAH [14]. Our preliminary analysis showed a significantly positive correlation between nesfatin-1 and CRP (**Figure 2A**).

### *Risk factors associated with poor outcome*

Univariate and multivariate analyses were used to analyze the risk factors associated with the poor outcomes of patients with aSAH. The results showed that GCS, Hunt-Hess III-V, DCI, and an elevated level of nesfatin-1 were significantly associated with poor outcomes. The results of the univariate and multivariate analyses are shown in **Table 2**.

### *ROC of nesfatin-1 in predicting poor outcome*

ROC was used to accessed the predictive power of nesfatin-1. The area under the curve (AUC) was 0.721, 0.748, 0.808, and 0.852 of the Fisher grade, GCS, Hunt-Hess and nesfatin-1, respectively. The sensitivity and specificity of nesfatin-1 in predicting a poor outcome were 0.760 and 0.806, respectively. The ROC curves of the variants are presented in **Figure 3**.

## **Discussion**

In this retrospective study, we found that patients with poor outcomes had higher serum levels of nesfatin-1 when compared with patients with good outcomes. What's more, nesfatin-1 was significantly elevated in patients who suffered DCI. The serum level of nesfatin-1 was positively correlated with Hunt-Hess grade and serum CRP level. Further multivariate regression analysis revealed Hunt-Hess III-IV, GCS, DCI, and sBP on admission, and elevated levels of nesfatin-1 were significantly associated with the poor outcomes of patients with aSAH. The predictive values of the variates were evaluated using ROC, and the results showed that nesfatin-1 was better than Hunt-Hess, GCS, and Fisher grade in predicting the poor outcomes of patients with aSAH.

Inflammatory response was one of the most important causes of secondary brain injury, cerebral vasospasm and DCI [15, 16]. Serum biomarkers, such as CRP and D-dimer had been reported to be independent risk factors associated with the poor outcomes of patients with aSAH in several studies [17, 18]. The role of nesfatin-1 was mainly investigated in patients with traumatic brain injury [13] and few studies were performed to demonstrate its role in hemorrhagic stroke. Previous studies found

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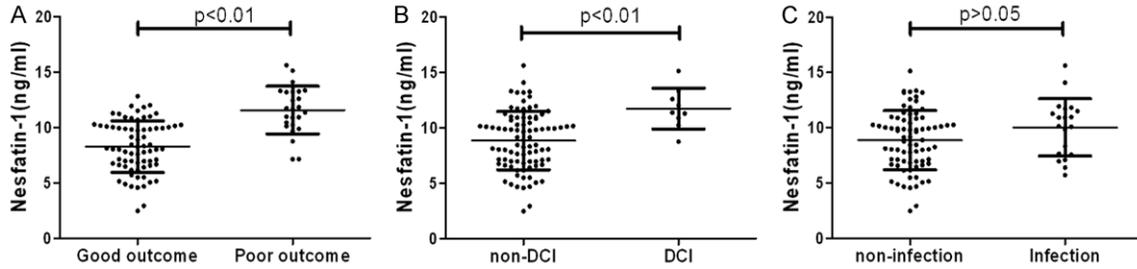


Figure 1. Serum levels of nesfatin-1 of patients in two different groups.

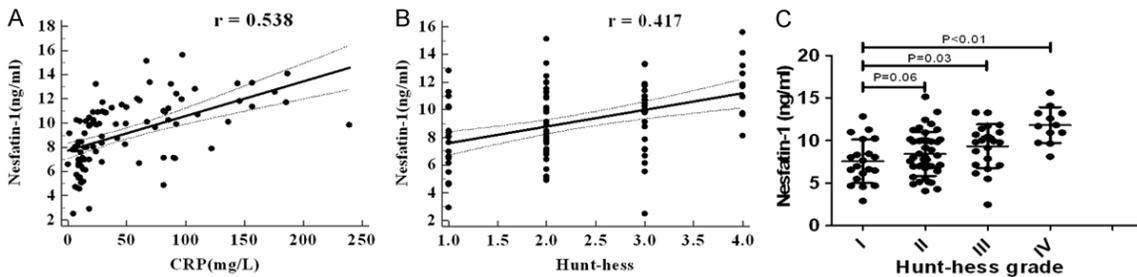


Figure 2. Correlation between nesfatin-1 and clinical severities.

Table 2. Risk factors associated with poor outcome

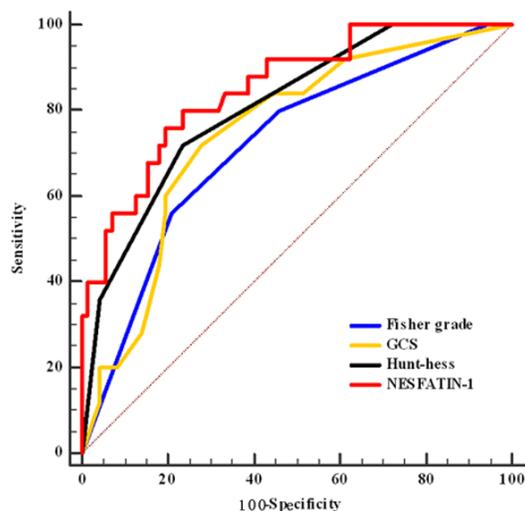
| Variables                   | Univariate analysis   |         | Multivariate analysis |         |
|-----------------------------|-----------------------|---------|-----------------------|---------|
|                             | OR (95% CI)           | P value | OR (95% CI)           | P value |
| Age                         | 1.035 (0.990-1.097)   | 0.113   | -                     | -       |
| Male                        | 0.640 (0.251-1.613)   | 0.337   | -                     | -       |
| Hypertension                | 1.221 (0.461-3.284)   | 0.694   | -                     | -       |
| Blood glucose (mmol/l)      | 0.911 (0.827-1.039)   | 0.961   | -                     | -       |
| Smoking                     | 2.607 (0.914-7.510)   | 0.078   | 4.454 (0.796-5.098)   | 0.093   |
| Intraventricular hemorrhage | 1.563 (0.741-2.879)   | 0.675   | -                     | -       |
| sBP                         | 0.977 (0.938-0.988)   | 0.012   | 0.980 (0.947-1.029)   | 0.577   |
| GCS                         | 0.789 (0.690-0.911)   | 0.008   | 0.767 (0.613-0.974)   | 0.034   |
| Hunt-Hess grade III-IV      | 5.240 (2.569-10.584)  | 0.008   | 2.537 (1.047-6.191)   | 0.041   |
| Fisher grade III-IV         | 2.668 (1.503-4.757)   | 0.006   | 1.761 (0.713-4.327)   | 0.219   |
| Aneurysmal location         | 1.062 (0.396-1.907)   | 0.914   | -                     | -       |
| Clipping                    | 1.794 (0.647-4.933)   | 0.258   | -                     | -       |
| DCI                         | 14.668 (2.586-32.640) | 0.003   | 7.35 (2.18-16.82)     | 0.028   |
| Nesfatin-1                  | 1.789 (0.651-4.928)   | 0.058   | 2.06 (1.33-3.17)      | < 0.001 |

that nesfatin-1 plays an important role in the process of inflammation, especially in central nervous system (CNS) diseases. Wistar albino rats were randomly divided into a saline-treated SAH group and a nesfatin-1-treated SAH group, and each group had 18 rats. 48 hours after SAH induction, the rats in the nesfatin-1 treated SAH group had better conditions of neurological impairment and oxidative brain injury [19]. What's more, pro-inflammatory cytokines in the peripheral blood were also depre-

ssed by treatment with nesfatin-1 [20]. Another study conducted by Chon-hui Tang et al. showed that 20 g/kg nesfatin-1 treatment could significantly suppress inflammation after traumatic brain injury in rats [19]. The above studies indicated that nesfatin-1 might play an important role in the inflammatory processes of central nervous system diseases, including aSAH.

Recently, Cakir et al. reported that aSAH patients in non-survival group had higher level of

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**Figure 3.** Values of variants in predicting poor outcome.

nesfatin-1 than patients in survival group, which indicated that nesfatin-1 was a useful biomarker in the management of aSAH [21]. Our study found that levels of nesfatin-1 in the blood of the poor outcome patients was higher than the good outcome patients and an obvious difference was observed between patients with DCI and non-DCI. Although nesfatin-1 was a vital protein in the inflammation-mediated process, it failed to show any advantages in the identification of systemic infections and non-infected aSAH patients. We usually use the Hunt-Hess grade, the Fisher grade, and the GCS score to assess the severity of aSAH. These indicators have been used clinically for a long time. In recent years, the assessment of the severity of aSAH by serum biochemical markers has gradually gained attention. A higher level of NSE was significantly associated with a high Hunt-Hess grade, the WFNS score, and GCS, which indicated that NSE was a promising tool in screening with a high risk of having a poor outcome after spontaneous SAH [22]. CRP was a highly sensitive inflammatory marker, and its serum level related to severity of aSAH. In this study, we found that serum nesfatin-1 positively correlated with CRP and the Hunt-Hess grade, but no correlation was observed between nesfatin-1 and the Fisher grade. Our results were not in line with previous studies. In a clinical study conducted by Cakir, the serum levels of nesfatin-1 were found to be significantly correlated with WFNS and the Fisher grade ( $r_2 = 0.521$ ,  $r_2 = 0.602$ , respec-

tively). But the relationship between the serum nesfatin-1 levels and CRP was consistent between these two studies.

There were some limitations in this retrospective study. This was a retrospective study and the number of patients was relatively small. We just evaluated the value of pre-operative nesfatin-1 in predicting poor outcomes, but the dynamic change of nesfatin-1 after aSAH is still unknown. Moreover, patients with aSAH might have received either clipping or coiling. Different surgical approaches might have different effects on nesfatin-1 elevation, because the inevitable brain tissue damage could be induced by microsurgery. So post-operative markers may be more reliable in predicting neurological complications and functional outcome. Finally, little is known about relationship between its peak level and poor outcome, so in our opinion, more clinical studies should be conducted to explore the role of serum nesfatin-1 in aSAH.

### Conclusion

Nesfatin-1 is significantly elevated in aSAH patients with poor outcomes. Nesfatin-1 is significantly associated with clinical severity, and it is an independent prognostic factor for poor outcome in patients with aSAH.

### Disclosure of conflict of interest

None.

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