SUBARACHNOID HEMORRHAGE DUE TO VASCULAR CAUSES AND GNATHOSTOMIASIS: CLINICAL FEATURES AND LABORATORY FINDINGS

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Abstract. Subarachnoid hemorrhage (SAH) is a serious neurological condition, commonly of vascular etiology. Gnathostomiasis is a common parasitic disease in Thailand and may also cause SAH. The purpose of this study was to find clinical differences between SAH due to these two causes. This was a retrospective study and collected data from medical charts of patients diagnosed with SAH at Srinagarind Hospital, Khon Kaen, during 2009 and 2011. SAH due to vascular causes was diagnosed by cerebral angiogram, while cerebral gnathostomiasis, in which cerebral angiograms were negative, was diagnosed immunologically. Differences in clinical features between the two groups were compared using descriptive statistics. Eighteen patients had SAH due to vascular causes and ten had gnathostomiasis. Most parameters were similar between the two groups. However, the cerebrospinal fluid glucose/plasma glucose ratio in the gnathostomiasis group was significantly higher than in the vascular group (80% *vs* 16.67%, respectively). In conclusion, cerebrospinal fluid glucose/plasma glucose ratio was significantly higher in SAH patients caused by gnathostomiasis than vascular group and may provide a diagnostic tool for distinguishing between these two etiologies.

Keywords: subarachnoid hemorrhage, aneurysm, arteriovenous malformation, gnathostomiasis

INTRODUCTION

Non-traumatic subarachnoid hemorrhage (SAH) is a serious neurological condition causing very severe headaches. Its prevalence is 6-9/100,000 person-years worldwide (van Gijn and Rinkel, 2001; de Rooij *et al*, 2007) and accounts for 5%-6% of all cases of stroke (van Gijn *et al*, 2007). The mortality at six months after diagnosis is 50% (Perry *et al*, 2011) and one-third of SAH patients are disabled by the condition (van Gijn and Rinkel, 2001).

Common causes of non-traumatic SAH are vascular abnormalities such as cerebral aneurysm or arteriovenous malformation. Only half of non-traumatic

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patients had definite vascular causes (Kitkhuandee *et al*, 2012). Gnathostomiasis, caused by *Gnathostoma spinigerum*, is endemic in Thailand, Japan, India, and Mexico. It is known to be a possible cause of non-traumatic SAH and intracerebral hemorrhage (Schmutzhard *et al*, 1988; Sawanyawisuth *et al*, 2009; Katchanov *et al*, 2011). A sensitive and specific serological test for gnathostomiasis is available and can be used to establish this as a cause of SAH when a cerebral angiogram does not demonstrate underlying vascular problems (Intapan *et al*, 2010). However, this test is not widely available. Here, we compared clinical factors in non-traumatic SAH due to these two causes: vascular abnormalities or gnathostomiasis.

MATERIALS AND METHODS

We retrospectively reviewed all non-traumatic SAH patients who were admitted to Srinagarind Hospital, Khon Kaen University, Thailand during the study period (January 2009 and December 2011). Included were all non-traumatic SAH patients who had undergone both cerebral angiogram and the serological test for *G. spinigerum*. We excluded patients who had a final diagnosis of other parasitic diseases such as cysticercosis or angiostrongyliasis.

The cerebral angiogram protocol was one of the following: CT angiography, MR angiography, or 3-dimensional digital subtraction cerebral angiography. These radiographic methods have been described elsewhere (Kitkhuandee *et al*, 2012). The serological test for *G. spinigerum* used immunoblotting for detection of antibodies against 21- or 24-kDa antigenic component of this parasite (Kitkhuandee *et al*, 2013). The sensitivity and specificity of the immunoblot analysis is 83.3%-91.7% and 100%, respectively (Intapan *et al*, 2010).

Clinical features were collected from medical charts of all eligible patients. Patients were divided into two groups: vascular SAH and gnathostomiasis. In vascular SAH patients, either cerebral aneurysm or vascular malformation had been detected by cerebral angiogram. Gnathostomiasis patients had negative cerebral angiogram and a positive serological test for *G. spinigerum*. Baseline and clinical characteristics of patients in both groups were compared using descriptive statistics. Wilcoxon rank-sum and Fisher's exact tests were applied to compare the differences in numbers and proportions between the two groups, respectively.

Univariate logistic regression analyses were applied to calculate the crude odds ratios of individual variables for non-traumatic SAH caused by gnathostomiasis. All clinically important variables or results of univariate analyses with a *p*-value <0.20 were included in subsequent multivariate logistic regression analyses. Analytical results were presented as crude odds ratios (OR), adjusted OR, and 95% confidence intervals (CI). All data analyses were performed with STATA software (College Station, TX). The study protocol was approved by the ethics committee in human research, Khon Kaen University (HE551056).

RESULTS

There were 28 eligible non-traumatic SAH patients during the study period. Of these, 18 patients had SAH due to underlying vascular causes and ten were diagnosed with gnathostomiasis. In the vascular group, 17 patients had a cerebral aneurysm and one patient had an arteriovenous malformation. The median age of all patients was 53 years (range 15-76 years) and 11 patients (28.9%) were male.

Table 1
Clinical features of non-traumatic subarachnoid hemorrhage patients categorized by cause (vascular or gnathostomiasis).

Variables	Vascular $n = 18$	Gnathostomiasis $n = 10$	p-value
Age, years	53 (15-76)	52 (33-71)	0.597[a]
Male, *n* (%)	6 (33.3)	5 (50)	0.444
Duration of headache, days	2 (0.04-20)	1 (0.1-3)	0.134[a]
Diabetes, *n* (%)	1 (5.6)	0	0.999
Hypertension, *n* (%)	6 (33.3)	4 (40.00)	0.999
Seizure, *n* (%)	1 (5.6)	1 (10.00)	0.999
Syncope, *n* (%)	0	1 (10.00)	0.357
Drowsiness, *n* (%)	0	1 (10.00)	0.357
Motor weakness, *n* (%)	4 (22.2)	3 (30.3)	0.674
Body weight, kg	56.5 (45-69)	58 (53-63)	0.999[a]
Height, cm	165 (152-165)	159 (153-165)	0.831[a]

Data shown as median (range) or numbers (percentage); p-value was calculated by Wilcoxon rank-sum test if indicated by superscript a: otherwise the Fisher exact test was used.

The median duration of headache was 1.5 days. There was no statistically significant difference in most clinical feature between the two groups in terms of age, duration of headache, co-morbidities, or symptoms of SAH (Table 1).

Regarding laboratory results, the gnathostomiasis group had higher (but not significantly so) blood eosinophils (35.4 cells *vs* 14.2 cells, p=0.320) and fewer cerebrospinal fluid (CSF) red blood cells (6,950 *vs* 41,750 cells/mm^3, p=0.806) than the vascular cause. The gnathostomiasis group had significantly higher CSF/plasma glucose ratios (80% *vs* 16.7%, p= 0.049). There was no overlap in the ranges of values between the two groups (Table 2). On multivariate logistic regression analyses, a CSF/plasma glucose ratio more than 40.2% perfectly associated with gnathostomiasis as the cause of non-traumatic SAH.

DISCUSSION

Gnathostoma spinigerum is a nematode that can infect humans who have consuming raw or under cooked freshwater shrimp or fish, or poultry (Nomura *et al*, 2000; Katchanov *et al*, 2011). In humans, which are a dead-end host, larva can migrate randomly in the body but are most common in subcutaneous tissue causing migratory swelling. Neurological involvement, such as radiculitis, myelitis, intracerebral hemorrhage, or SAH, is another common manifestation. Larvae often migrate to the subarachnoid space via the spinal canal causing bleeding in subarachnoid area (Schmutzhard *et al*, 1988). Recovery of larvae provides definitive diagnosis, but is rare in human gnathostomiasis (Nomura *et al*, 2000; Sangchan *et al*, 2006). Serological tests for *G. spinigerum* are therefore the main diagnostic tools.

Table 2
Laboratory results of non-traumatic subarachnoid hemorrhage patients categorized by vascular and gnathostomiasis cause.

Factors	Vascular cause $n = 18$	Gnathostomiasis $n = 10$	p-value
Hematocrit, %	39.4 (30.8-43.8)	37.65 (30.1-46.1)	0.962
White blood cells, cells/mm^3	13,460 (7,790-19,000)	12,315 (8,200-18,500)	0.811
PMN, %	82.7 (67.1-94.2)	87.9 (62.9-94.6)	0.598
Eosinophil count, cells	14.15 (0-245)	35.35 (0-1,117.6)	0.320
Platelet count	277,500 (96,000-430,000)	245,500 (150,000-474,000)	0.719
CSF open pressure, cmH_2O	21 (14-28)	26.5 (24-50)	0.643
CSF red blood cell	41,750 (100-257,000)	6,950 (4,250-60,000)	0.806
CSF white blood cell	85 (0-500)	25 (0-1,400)	0.798
CSF PMN, %	2 (0-79)	23.5 (0-64)	0.798
CSF eosinophils, %	0 (0-4)	0	0.179
CSF protein, mg/dl	61 (0-95)	41 (19-63)	0.327
CSF glucose, mg/dl	65 (17-95)	87.5 (64-180)	0.142
CSF /plasma glucose ratio, %	16.7 (15.6-40.2)	80 (58.18-111.1)	0.049
Plasma glucose, mg/dl	120 (109-174)	110 (90-162)	0.513

Data shown as median (range) or numbers (percentage); PMN, polymorphonuclear leukocytes; eosinophil count equals total white blood cells x percentage of eosinophils; CSF, cerebrospinal fluid; number of tests may not equal 18 in the vascular group and 10 in the gnathostomiasis group due to missing data; all p-values were calculated using the Wilcoxon rank-sum test.

In this study, most clinical features and laboratory findings were similar in non-traumatic SAH patients, whether the underlying cause was vascular or gnathostomiasis (Tables 1 and 2). As a response to the presence of parasites in tissues, the eosinophil count was slightly higher in the gnathostomiasis group, but only in blood and not in the CSF (Table 2). A high eosinophil count (the highest in this study was 1,117.6 cells) may be suggestive of *G. spinigerum* as the cause of SAH. Eosinophil levels are usually much elevated as a response to parasitic infection, particularly when due to angiostrongyliasis, strongyloidiasis, or gnathostomiasis (Nutman, 2007). The low eosinophil count in some of the patients might be due to the chronic nature of the infection of gnathostomiasis. Larvae of *G. spinigerum* can susvive in the body for years without causing symptoms.

Clinical features generally suggestive of gnathostomiasis include radicular pain or migratory swelling (Kanpittaya *et al*, 2012; Senthong *et al*, 2013). However, these clinical features were not mentioned on the medical charts of patients in this retrospective study. When taking the histories of patients, the physicians may not have been aware of the possibility of gnathostomiasis in SAH patients and hence did not ask appropriate questions or make the relevant physical examinations.

According to the multivariate logistic regression analysis, a high CSF/plasma

glucose ratio was the only factor suggesting gnathostomiasis rather than vascular abnormality as the cause of non-traumatic SAH. Severe SAH is known to be associated with higher plasma glucose levels (Naidech *et al*, 2010; Chen *et al*, 2014), which may result in a relatively low CSF/plasma glucose ratio. In this study, the median plasma glucose level was not significantly different in the two groups (Table 2). Low CSF/plasma glucose ratios may be found in several conditions such as bacterial meningitis or eosinophilic meningitis (Sawanyawisuth *et al*, 2012). The ratio is not specific in neurognathostomiasis such as myelitis or intracerebral hemorrhage; it can be normal or slightly low (Katchanov *et al*, 2011). In SAH caused by *G. spinigerum*, the ratio was more than 50% (Table 2). These findings indicate that non-traumatic SAH caused by vascular abnormalities may be more severe than gnathostomiasis.

In conclusion, CSF/plasma glucose ratios were substantially lower in patients with non-traumatic SAH of vascular origin than in those with SAH due to gnathostomiasis.

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REFERENCES

Chen S, Li Q, Wu H, Krafft PR, Wang Z, Zhang JH. The harmful effects of subarachnoid hemorrhage on extracerebral organs. *Biomed Res Int* 2014; 2014: 858496.

de Rooij NK, Linn FH, van der Plas JA, Algra A, Rinkel GJ. Incidence of subarachnoid haemorrhage: a systematic review with emphasis on region, age, gender and timetrends. *J Neurol Neurosurg Psychiatry* 2007; 78: 1365-72.

Intapan PM, Khotsri P, Kanpittaya J, Chotmongkol V, Sawanyawisuth K, Maleewong W. Immunoblot diagnostic test for neurognathostomiasis. *Am J Trop Med Hyg* 2010; 83: 927-9.

Kanpittaya J, Sawanyawisuth K, Intapan PM, Khotsri P, Chotmongkol V, Maleewong W. A comparative study of neuroimaging features between human neuro-gnathostomiasis and angiostrongyliasis. *Neurol Sci* 2012; 33: 893-8.

Katchanov J, Sawanyawisuth K, Chotmongkoi V, Nawa Y. Neurognathostomiasis, a neglected parasitosis of the central nervous system. *Emerg Infect Dis* 2011; 17: 1174-80.

Kitkhuandee A, Munkong W, Sawanyawisuth K, Janwan P, Maleewong W, Intapan PM. Detection of *Gnathostoma spinigerum* antibodies in sera of non-traumatic subarachnoid hemorrhage patients in Thailand. *Korean J Parasitol* 2013; 51: 755-7.

Kitkhuandee A, Thammaroj J, Munkong W, Duangthongpon P, Thanapaisal C. Cerebral angiographic findings in patients with non-traumatic subarachnoid hemorrhage. *J Med Assoc Thai* 2012; 95: S121-9.

Naidech AM, Levasseur K, Liebling S, *et al*. Moderate hypoglycemia is associated with vasospasm, cerebral infarction, and 3-month disability after subarachnoid hemorrhage. *Neurocrit Care* 2010; 12: 181-7.

Nomura Y, Nagakura K, Kagei N, Tsutsumi Y, Araki K, Sugawara M. Gnathostomiasis possibly caused by *Gnathostoma malaysiae*. *Tokai J Exp Clin Med* 2000; 25: 1-6.

Nutman TB. Evaluation and differential diagnosis of marked, persistent eosinophilia. *Immunol Allergy Clin North Am* 2007; 27: 529-49.

Perry JJ, Stiell IG, Sivilotti ML, *et al*. Sensitivity of computed tomography performed within six hours of onset of headache for diagnosis of subarachnoid haemorrhage. *BMJ* 2011; 343: d4277.

Sangchan A, Sawanyawisuth K, Intapan PM, Mahakkanukrauh A. Outward migration of *Gnathostoma spinigerum* in interferon alpha treated hepatitis C patient. *Parasitol Int* 2006; 55: 31-2.

Sawanyawisuth K, Chlebicki MP, Pratt E, Kanpittaya J, Intapan PM. Sequential imaging studies of cerebral gnathostomiasis with subdural hemorrhage as its complication. *Trans R Soc Trop Med Hyg* 2009; 103: 102-4.

Sawanyawisuth K, Sawanyawisuth K, Senthong V, *et al.* How can clinicians ensure the diagnosis of meningitic angiostrongyliasis? *Vector Borne Zoonotic Dis* 2012; 12: 73-5.

Senthong V, Chindaprasirt J, Sawanyawisuth K. Differential diagnosis of CNS angiostrongyliasis: a short review. *Hawaii J Med Public Health* 2013; 72: 52-4.

Schmutzhard E, Boongird P, Vejjajiva A. Eosinophilic meningitis and radiculomyelitis in Thailand, caused by CNS invasion of *Gnathostoma spinigerum* and *Angiostrongylus cantonensis*. *J Neurol Neurosurg Psychiatry* 1988; 51: 80-7.

van Gijn J, Rinkel GJ. Subarachnoid haemorrhage: diagnosis, causes and management. *Brain* 2001; 124: 249-78.

van Gijn J, Kerr RS, Rinkel GJ. Subarachnoid haemorrhage. *Lancet* 2007; 369: 306-18.