

Original Article

Correlation of the ferrum and VEGF with magnetic resonance imaging in chronic subdural hematomas: a prospective study

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Abstract: In this study, we measured the level of ferrum ion and VEGF from the hematoma cavity fluid in chronic subdural hematoma (CSDH), and analyzed the relationship between these values with the CSDH diagnosis by MRI. The cranial MRI appearance of each CSDH were classified as hypodense, isodense, hyperdense, or mixed density. The concentrations of ferrum ions in serum and hematoma cavity fluid were measured before and after operation, and the VEGF level in hematoma cavity was examined by ELISA. Totally, the ferrum ion concentration in hematoma cavity fluid was higher than that in periphery serum. In hypodense hematomas (n=5), ferrum and VEGF was 55.24 ± 12.57 $\mu\text{mol/L}$, 7254.40 ± 575.88 pg/ml, respectively. In isodense hematomas (n=6), ferrum and VEGF was 65.18 ± 8.63 $\mu\text{mol/L}$, 8194.17 ± 619.47 pg/ml, respectively. In hyperdense hematomas (n=7), ferrum and VEGF was 38.96 ± 6.37 $\mu\text{mol/L}$ and 6142.43 ± 549.87 pg/ml, respectively. In mixed-density hematomas (n=7), ferrum and VEGF was 45.99 ± 10.11 $\mu\text{mol/L}$, 6861.00 ± 686.89 pg/ml, respectively. There was significant difference of ferrum and VEGF between 4 classes of MRI appearance ($P < 0.05$). Meanwhile, earlier age and shorter onset days were found in hyperdense hematomas with lower ferrum and VEGF. The ferrum concentration and VEGF level was correlated with the MRI signal density in CSDH.

Keywords: Chronic subdural hematoma, ferrum ions, VEGF, MRI, diagnosis

Introduction

Chronic subdural hematoma (CSDH) is a common neurological disorders, and especially prevalent among the elderly individuals [1]. Minor head trauma preceding development of CSH is documented in 2/3 of patients; however, a CSDH may also occur without such an obvious insult [2]. Clinically, patients present with neurological impairment of varying intensity, which is classified using the scoring system suggested by Markwalder some 30 years ago [3]. The diagnosis of CSDH has evolved in the last 20-30 years together with the introduction of the CT scan, the MRI and the access of the patients to this type of explorations. There are studies concerning the CSDH, which also demonstrate the superiority of the MRI examination in comparison with the CT. The MRI examination better shows the location of the chronic subdural haematoma and evidences its

dimensions much clearer together with the mass effect of the adjacent structures. Moreover, it is more useful in cases of bilateral and isodense chronic subdural haematomas [4].

In order to optimize patient care and outcomes, early diagnosis and subtype the patient is important. The pathophysiology of CSDH is not yet clear [5], which is mainly caused by the mutual effects from the damage of the membrane and pro-inflammatory stimulating factor including IL-6 and IL-8, etc [6, 7]. In recent years, it was found that the increase of VEGF expression level were also one of main factors of CSDH. Recently, it was found that ferrum (Fe) concentration was correlated tightly with VEGF [8, 9]. Ferrum element was widely distributed in the brain and participated in Creb's cycle, electron transferring and protein synthesis. It was showed that overloading of Fe was closely relat-

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Table 1. Comparison of onset age and onset time

Groups	Age (years)	Onset time (days)
Isodense	67.00±8.63	36.32±3.47 ^{b,c,d}
Hypodense	70.83±6.40 ^c	44.87±4.31 ^{a,c,d}
Hyperdense	62.29±3.44 ^{b,d}	30.26±3.57 ^{a,b}
Mixed-dense	68.57±7.19 ^c	29.26±3.20 ^{a,b}
	P>0.05	P<0.05

^aCompared with Isodense group; ^bCompared with Hypodense group; ^cCompared with Hyperdense group; ^dCompared with Mixed-dense group.

ed with cerebral injury [10]. Iron chelator desferrioxamine could cure cerebral injury through reducing the formation of hydrocephalus. It is unknown that was there any relationship between Fe and CSDH and whether Fe could interfere the MRI signal. According to the classical classification system proposed by Park et al, CSDH images may appear as hypodense, isodense, hyperdense, or mixed density types [11]. Given the well-established permeability-promoting effect of VEGF and Fe, we evaluated our patient database and related the concentrations of VEGF and Fe in hematoma fluid to the MRI characteristics.

Patients and methods

Patient selection

Between January 2014 and January 2015, 25 consecutive patients (17 males and 8 females) had been admitted to Jinshan Hospital of Fudan University for surgical management of CSDH, and they provided written informed consent to participate in the study. All of these patients had light injury history before surgery and onset time was recorded. All of the chosen patients were performed MRI scanning before surgery, including T1 and T2 MRI. After MRI scanning, patients were performed routine drilling drainage in order to eliminate the possibility that the patients with vascular disease, tumor, anemia and blood coagulation dysfunction.

The inclusion criteria were as following: the patients were diagnosed with CSDH by using cranium CT scan and MRI, suffered from headache or activity obstacle, with a history of head trauma. All of the recruited patients were treated with drilling drainage.

The exclusion criteria were as following: the patients suffered severe trauma in recent 6

months, or cerebrovascular accident, or mental diseases, or severe liver and kidney dysfunction, or heart failure, or diabetes, or blood system diseases, or autoimmune diseases, or tumors, or patients with drilling drainage contraindication.

The study was approved by the ethics committee of our hospital, and the patients' families signed the informed consent.

Specimen collection

During the operation, the thickest layer of hematoma should be chosen as the drilling point. For the routine surgical drilling, syringe of 5 ml was used to collect hematoma cavity fluid before opening dura mater, and the corresponding periphery blood was collected to put in anticoagulation tubes. All samples were collected into siliconized vacuum tubes containing protamine sulfate and ethylenediamine tetraacetic acid and were immediately centrifuged at 3000 rpm for 10 minutes. The supernatants were stored in sealed polypropylene tubes at -70°C until analysis.

Measurement of VEGF and by ELISA

The levels of VEGF in hematoma fluid were measured using ELISA kit according to manufacturer's instruction. The VEGF ELISA kit was purchased from Shanghai ViAo biotechnology Co.Ltd. The ferrum of hematoma fluid and periphery blood were measured by ELISA kit (Roche Diagnosis Co.Ltd, Germany) and chromatometry was performed with HITACHI 7600 automatic biochemistry analyzer (Japan).

Statistical analysis

Statistical analysis was performed using SPSS 13.0 statistical software. All the data were expressed as the mean ± standard deviation. Concentration of VEGF and Fe in hematoma fluid was related to the MRI classes of appearance and to the mean exudation rates available. Differences of mean concentrations between groups were evaluated using one-way analysis of variance. Differences between means were assessed using Student's t-test, ANOVA, or X²-tests. Significant differences were determined by ANOVA followed by *pos hoc* comparisons with Fisher's protected least significant difference test. Data were considered statistically significant at P<0.05.

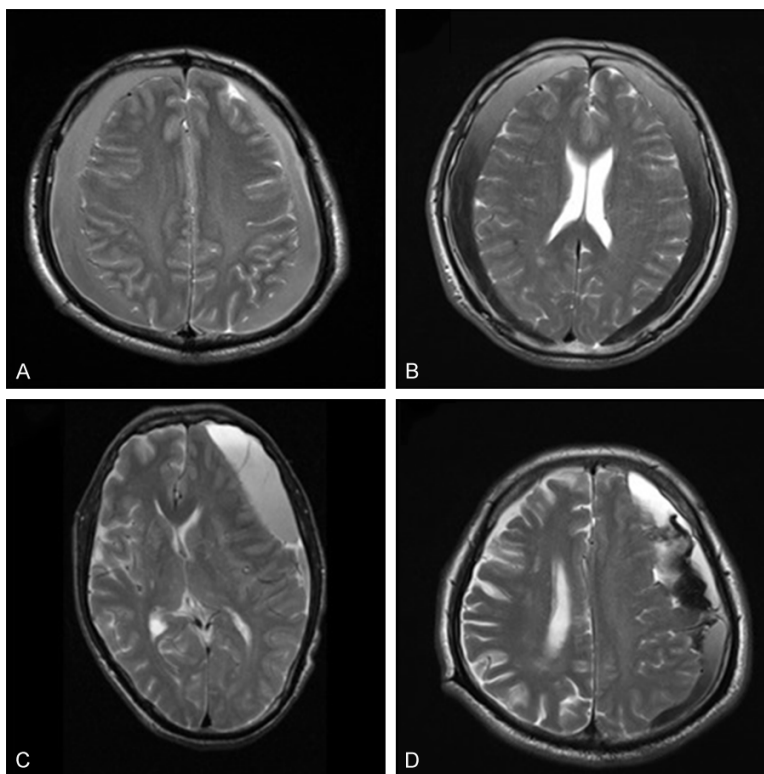


Figure 1. Axial T2-weighted magnetic resonance imaging showing an isodense type (A), hypodense type (B), hyperdense type (C) and mixed-dense type (D).

Table 2. The ferrum and VEGF levels in chronic subdural hematoma and peripheral blood by classification of MRI pattern

Groups	Ferrum in PB μmol/L	Ferrum in HCB μmol/L	VEGF pg/ml
Isodense	21.78±2.78 ^b	55.24±12.57 ^c	7254.40±575.88 ^{b,c}
Hypodense	18.32±2.91 ^{a,d}	65.18±8.63 ^{c,d}	8194.17±619.47 ^{a,c,d}
Hyperdense	19.43±1.98 ^d	38.96±6.37 ^{a,b}	6142.43±549.87 ^{a,b,d}
Mixed-dense	22.43±2.83 ^{b,c}	45.99±10.11 ^b	6861.00±686.89 ^{b,c}
F	3.427	9.329	12.489
P	0.036	0.000	0.000

PB: peripheral blood. HCB: hematoma cavity fluid. ^aCompared with iso, P<0.05; ^bCompared with low, P<0.05; ^cCompared with high, P<0.05; ^dCompared with mixed, P<0.05.

Results

Epidemiological and clinical data of the patients were presented in **Table 1**. There were 17 males and 8 females who fulfilled the inclusion criteria. According to the MRI T2 signal, the 25 patients were classified into 4 groups, hypodense hematoma (n=5), isodense hematoma (n=6), hyperdense hematoma (n=7) and mixed-dense hematoma (n=7) (**Figure 1**). The

differences of average age between groups were not significant (P>0.05). The differences of onset time between groups were significant (P<0.05). Briefly, the onset time of hyperdense or mixed-dense hematoma was much shorter than that of isodense or hypodense hematoma (P<0.05), and the onset time was longest in hypodense hematoma (44.87 days) and shortest in mixed-dense hematoma (29.26 days).

All concentrations of ferrum from the CSDHs were higher than those in the peripheral venous blood (P<0.05) (**Table 2**). For T2-WI MRI, the mean concentrations of ferrum and VEGF in hematoma cavity fluid for the hyperdense group were 38.96±6.37 μmol/L and 6142.43±549.87 pg/ml, respectively, which were significantly lower than those in non-hyperdense groups (P=0.000 and P=0.000). Meanwhile, the mean concentrations of ferrum and VEGF in hematoma cavity fluid for the hypodense group were 65.18±8.63 μmol/L and 8194.17±619.47 pg/ml, respectively, which were significantly higher than those in non-hypodense groups (P=0.000 and P=0.000). The concentrations of ferrum in different groups showed a similar rule as VEGF, that highest in hypodense group, followed by in isodense group, in mixed-

dense group and in hyperdense group (from high to low) (**Figure 2**). These results suggested that the concentrations of ferrum and VEGF in hematoma cavity fluid were closely related with the MRI intensity.

Discussion

CSDH is a common clinical disease widely found among elderly people, of which patho-

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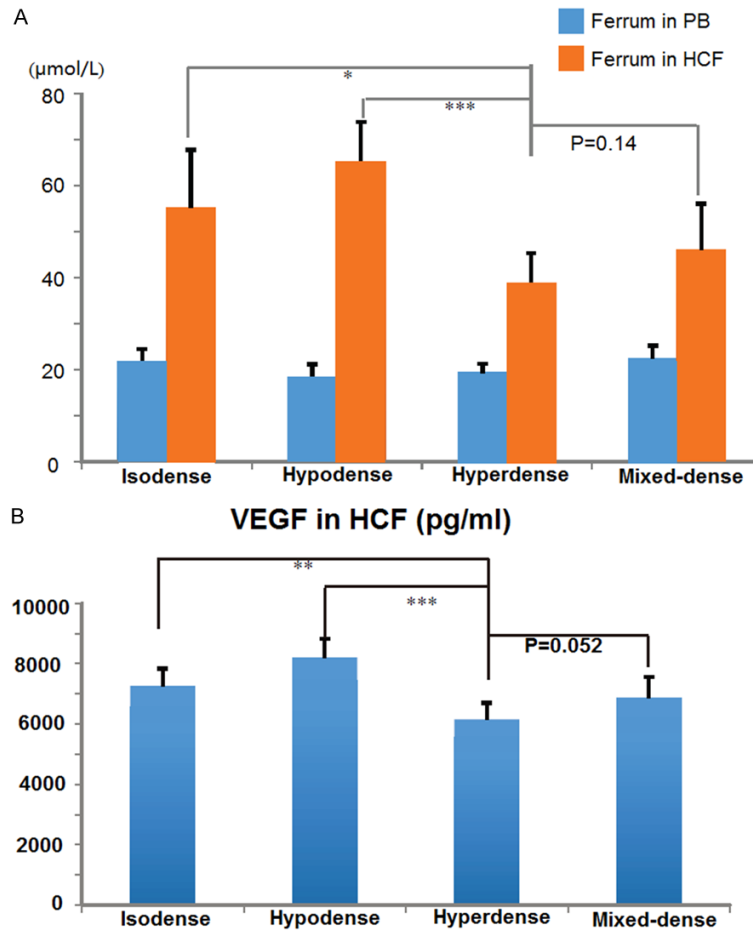


Figure 2. Ferrum concentration of PB (peripheral blood) and HCF (hematoma cavity fluid) in patients of each groups (A); VEGF concentration in HCF. VEGF concentration in HCF of the isodense group, hypodense group, hyperdense group and mixed-dense group (B).

geneses is remain unclear [12]. Surgery operation could reduce clinical progression of subdural effusion [13]. However, injury of blood vessel secreted angiogenic growth factors, stimulated the expression of inflammatory cytokine like IL-8, were considered as the main factor in the CSDH formation and development [14, 15]. In 2007, Tokmak found that the VEGF concentrations in hematoma cavity from CSDH patients closely related with the formation of hematoma in brain CT images [16]. The increase of VEGF level could stimulate the formation and progression of hematoma [10]. VEGF mainly took part in formation of new vessels, of which over-expression was related to many factors [8]. Otherwise, it was found that increase of Ferrum concentration could stimulate the formation of new vessel, which was mainly correlated with over-expression of VEGF [14]. Ferrum was wide-

ly distributed in the brain and located at the white matter. Ferrum played important roles in many physiological functions such as brain oxygen transport, electron transport, neurotransmitter and myelin synthesis [10]. Ferrum mainly existed in hemoglobin, occupying approximately 2/3 of total ferrum element in the human body. During the formation process of chronic hematoma, Fe concentration increase gradually as the hemoglobin increase in hematoma cavity. In our study, the **Table 2** showed that among the CSDH patients, Ferrum concentration showed significant difference between the hematoma cavity fluid and the peripheral blood ($P < 0.05$). In **Figure 1**, hematoma cavity ferrum ion concentration was also showed higher level than in the peripheral blood. Ferric ion could cause cascade reaction, stimulate the VEGF over-expression then cause the new vessels formation. The new vessels were different from normal vessels. The gap between epithelial cells of vessels became wider, so the transudations in vessels came into CSDH hematoma cavity. CSDH formation was a long process, which could range from several months to several years. The different stage showed different MRI signals. As a usual, the classical CSDH indicated high signal on T1 and T2 weighted images. It could also be showed with low, iso or mixed density signals because of the discovering time difference [17]. Hosoda performed a study with 20 CSDH patients [18] and patients with T1 image showed isodensity or low density signals occupy 30%. They speculated that that there was fresh hemorrhage in the hematoma cavity of above patients. For the patients with high density signals, researchers speculated that most of arterin had become methaemoglobin, indicated bleed several days ago. According to the changing process of hematoma of intracerebral hemorrhage, it

could be showed by MRI signals. In **Table 1**, onset time had significant difference among all groups ($P < 0.05$). If it was the only reason that caused CSDH enlargement by the repeatedly micro hemorrhage in new vessel of hematoma outer membrane. Most of CSDH should show low, iso or mixed density signal on T1 MRI. MRI indicated that brain Fe precipitation shortened the T2 time, while did not change the T1 time. The ferrum in cell had a high magnetic susceptibility, so the ferrum over precipitation in brain could cause hypo magnetic susceptibility in the cell and low magnetic susceptibility out of the cell. The uneven local magnetic field shortened T2 time and showed low density signals on T2 weighted images. Although ferrum existed in normal cells, it was not sufficient to exhibit the obvious low density signal on MRI, especially on low field MR facility. In the early stage of CSDH, cavity substance had different content. Gore existence caused the signal uneven. Different ferrum concentration caused magnetic resonance signal density uneven. In this research, it showed that there was significant difference on onset time ($P < 0.05$). If no haemorrhage in CSDH, it showed low density signals. If the patients were with bleeding, it would show mixed density signals because of varied haemorrhage times. In this study, through immunohistochemistry analysis on VEGF concentration, it was found that the patients with high Fe also had high VEGF level ($P < 0.05$). According to this results, the increase of Fe concentration further stimulated VEGF over-expression, while VEGF could further stimulate immature new vessels formation. It was reported that the enlarged gap of immature new vessels of CSDH hematoma, caused the higher concentration of VEGF in the fluid. It was considered that ferrum ion related to tissue toxicity, including apoptosis and oxidative damage, which aggravated the secondary brain injury. It was also found that the ferric ion was overloaded in the group of serious edema.

Conclusion

In conclusion, it was found that ferrum ion played certain effects in the process of hematomal formation, and confirmed that VEGF could stimulate the CSDH formation. Ferrum ion may stimulate CSDH hematomal formation through promoting the VEGF over-expression. The detailed mechanism would be investigated further.

Disclosure of conflict of interest

None.

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