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Original Article

Spontaneous intracerebral hemorrhage, initial computed tomography (CT) scan findings, clinical manifestations and possible risk factors

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Abstract: Intracerebral hemorrhage is one of the types of stroke in patients with risk factors. In this study, we aimed to evaluate the initial computed tomography (CT) scan findings, clinical manifestations and possible risk factors of patients with intracerebral hemorrhage. This is a cross-sectional study that was performed in 2015-2022 on 900 patients with definite diagnosis of intracerebral hemorrhage. Data of patients were evaluated for patient's age, gender, clinical manifestations, primary radiologic signs in CT scan and possible risks factors for stroke. Lobar hemorrhage was the most common site of involvement (324 patients, 36%) followed by lenticular (putamen) (294 patients, 32.7%) and thalamus (135 patients, 15%). Among patients, 543 patients (60.3%) had hypertension, 81 patients (9%) had histories of anticoagulant. Hemorrhages in putamen were significantly more common in patients with hypertension (P<0.001) and lobar hemorrhages were significantly more common in patients with the use of anticoagulant drugs (P=0.033). The most common presentation of hemorrhagic stroke was decreased consciousness level (428 patients, 47.5%) followed by headache (343 patients, 38.1%), coma (81 patients, 9%) and seizure (48 patients, 5.4%). Evaluation of the relationships between patient's main symptoms and sites of involvement showed that patients with decreased consciousness as their most common symptom had more frequently diagnosed with lobar hemorrhage (54%) and putamen hemorrhage (30.4%) (P<0.001). Hypertension was the most common past medical history that was significantly related to hemorrhage in basal nuclei. Hemorrhages in putamen were common in hypertensive patients and lobar hemorrhages were common in patients with anticoagulant use.

Keywords: Stroke, intracerebral hemorrhage, CT scan, risk factor

Introduction

Stroke is one of the most common causes of death (after cardiovascular disease and malignancy) [1]. The incidence of cerebral hemorrhage increases with age and the prognosis of patients with cerebral hemorrhage decreases and their mortality rate increases [2, 3]. About 80% of strokes are ischemic and 15-20% are hemorrhagic [4]. Hemorrhagic strokes are caused by rupture and rupture of cerebral arteries, leading to intracranial hemorrhage [5]. Another type of bleeding in this type of stroke is bleeding into the meninges or into the ventricles of the brain. This type of bleeding is divided into two categories: traumatic or non-traumatic (spontaneous) [6].

Spontaneous cerebral hemorrhages are twice as common as subarachnoid hemorrhages. It should be noted that most ischemic or hemorrhagic strokes into the subarachnoid space cause mortality [7, 8].

According to epidemiological studies, the prevalence of spontaneous intracerebral hemorrhage in the United States is about 9 per 100,000 people per year [9, 10]. It has also been shown that 70 to 90% of these patients suffer from hypertension [11]. Amyloid vascular disease of the brain is another common cause

of spontaneous intracerebral hemorrhage, especially in the elderly. Vascular malformations, ruptured aneurysms, blood coagulation disorders, taking anticoagulants and thrombolytic drugs are other causes of this disease [12, 13].

Cerebral hemorrhage can occur near the surface or in deep areas of the brain. Sometimes deep bleeding can spread into the ventricles (fluid-filled spaces in the center of the brain) [14]. Obstruction of the normal cerebrospinal fluid (CSF) circulation can enlarge the ventricles (hydrocephalus) and cause confusion, lethargy, and loss of consciousness. Common symptoms include: Headache, Nausea and vomiting, Lethargy or dizziness, Sudden weakness or numbness of the face, arms or legs, usually on one side, Loss of consciousness and Temporary loss of vision [15, 16].

Pre-contrast computed tomography (CT) scan is the imaging procedure of choice to evaluate intracerebral hemorrhage. Acute hematoma is seen by pre-contrast CT imaging as an area of high density [17]. CT can detect acute intracerebral blood as small as 2 mm, due to contrast between high density of blood and low density of surrounding brain [18].

It has also been shown that sometimes spontaneous intracerebral hemorrhages occur within brain tumors. Despite advances in the treatment and management of intracerebral hemorrhage, this disease is one of the most important diseases. Evaluation of patients' epidemiological findings, their brain imaging findings, and assessment of risk factors for spontaneous intracerebral hemorrhage can help diagnose and treat the disease in a timely manner. In the present study, we aimed to evaluate the spontaneous intracerebral hemorrhage, its initial CT scan findings, clinical manifestations and possible risk factors.

Methods and material

Study design

This is a cross-sectional study that was performed in 2015-2022 in Kashani and Al-Zahra hospitals affiliated to Isfahan University of Medical Sciences and Imam Khomeini hospital affiliated to Tehran University of Medical Sciences. The study protocol was approved by the Research Committee of Tehran University of Medical Sciences and the Ethics committee has confirmed it (Ethics code: IR.TUMS.MEDICINE.REC.1392.280).

Study population

The current study was conducted on documents of all patients with definite diagnosis of intracerebral hemorrhage. From 8381 cases admitted to neurology department of our medical centers with the diagnosis of stroke, 964 patients with intracerebral hemorrhage entered.

Inclusion and exclusion criteria

The inclusion criteria were age more than 18 years, definite diagnosis of hemorrhagic stroke based on CT scan, presence of intracerebral hemorrhage, confirmation of the diagnosis by electroencephalogram (EEG), complete patient's documents and signing the written informed consent to participate in this study. The exclusion criteria were intracerebral hemorrhage due to traumatic injuries, vascular anomalies, brain tumor, incomplete data, and patient's will to exit the study.

Data gathering

Data of patients were evaluated for patient's age, gender, clinical manifestations, primary radiologic signs in CT scan and possible risks factors for stroke. It should be mentioned that the initial imaging of the patients were evaluated by two expert neurologists with at least 10 years of experience in stroke. Data regarding primary radiologic signs and location of hemorrhages were collected from neurologist's reports.

CT scan findings

The imaging findings were divided based on the location of hemorrhages. The classifications were lobar (hemorrhage in frontal, parietal, temporal or occipital lobes), deep (hemorrhage in putamen, thalamus, caudate nucleus, internal capsule), and posterior fossa (hemorrhage in medulla oblongata, pons, midbrain and cerebellum). Other radiologic findings were decreased density around hematoma (edema), subarachnoid hemorrhage, intraventricular hemorrhage, and other effects of hematoma on the brain tissue. In this study, we evaluated CT

Table 1. Frequency distribution of hemorrhage location

Location	Number (%)
Lobar	324 (36%)
Putamen	294 (32.7%)
Thalamus	135 (15%)
Cerebellum	81 (9%)
Pons	18 (2%)
Caudate nucleus	15 (1.7%)
Intraventricular	21 (2.3%)
Multiple	12 (1.3%)
Total	900 (100%)

parameters and findings including bleeding into the ventricles, association with subarachnoid hemorrhage and brain edema among patients. We also assessed possible relationships between male gender and higher ages (>65 years) with different CT scan findings.

Statistical analysis

The obtained data were entered into the Statistical Package for Social Sciences (SPSS) (version 24, SPSS Inc., Chicago, IL). Quantitative data were reported as mean ± standard deviation and qualitative data as frequency distribution (percentage). Independent T-test and Pearson Chi-Square were used to analyze the data. *P*-value <0.05 was considered as a significance level.

Results

Study population

Totally, 964 patients with intracerebral hemorrhage were assessed for eligibility. During the study course, 64 of them were excluded due to traumatic causes (N=38), brain tumor (N=19), and incomplete data (N=7). Data of 900 patients were analyzed.

Population characteristics

The study population consisted of 480 males (53.4%) and 420 females (46.6%) with the mean age of 64.39±11.08 years. It was observed that lobar hemorrhage was the most common site of involvement (324 patients, 36%) followed by lenticular (putamen) (294 patients, 32.7%) and thalamus (135 patients, 15%). These data are shown in **Table 1**.

Hemorrhage location and past medical history

Evaluation of their past medical history showed that 543 patients (60.3%) had hypertension, 81 patients (9%) had histories of anticoagulant use and 276 patients (30.6%) had no past medical histories. Evaluation of past medical histories based on the sites of involvements demonstrated that hemorrhages in putamen were significantly more common in patients with hypertension (P<0.001) and lobar hemorrhages were significantly more common in patients with the use of anticoagulant drugs (P=0.033) (**Table 2**). It should be mentioned that in patients with hypertension, hemorrhages occurred mostly in deep brain structures (basal nuclei) (312 patients, 73.5%).

Patient's symptoms

The most common presentation of hemorrhagic stroke was decreased consciousness level (428 patients, 47.5%) followed by headache (343 patients, 38.1%), coma (81 patients, 9%) and seizure (48 patients, 5.4%). Evaluation of the relationships between patient's main symptoms and sites of involvement showed that patients with decreased consciousness as their most common symptom had more frequently diagnosed with lobar hemorrhage (54%) and putamen hemorrhage (30.4%) (P< 0.001). Patients with headache had mostly hemorrhage in the putamen (41.6%, P=0.031) and patients with seizure had most frequently, lobar hemorrhage (60%, P<0.001). These data are summarized in **Tables 3**, **4**.

Initial radiologic findings

It was observed that among patients with bleeding into ventricles, 42% had hemorrhage in putamen, among patients with subarachnoid hemorrhage, 71.4% had lobar hemorrhage and among patients with brain edema, 54.6% had lobar hemorrhage (P<0.001 for all). Based on our data, higher ages (>65 years) increased the risks of bleeding into the ventricles (OR=2.14, P<0.001) and association with subarachnoid hemorrhage (OR=1.63, P=0.02). There were no other relationships between radiologic findings and gender (**Tables 5**, **6**).

Discussion

After evaluating data of 900 patients with intracerebral hemorrhage, we observed that

Table 2. Evaluation of patient's characteristics based on sites of involvements

Variable	Lobar (N=324)	Putamen (294)	Thalamus (135)	Cerebellum (81)	Other (66)	*P*-value*
Hypertension (N=543)	144 (26.5%)	222 (40.9%)	90 (16.6%)	54 (9.9%)	33 (6.1%)	<0.001
Anticoagulant use (N=81)	54 (66.7%)	9 (11.1%)	3 (3.7%)	9 (11.1%)	6 (7.4%)	0.033
No specified cause (276)	126 (45.7%)	63 (22.8%)	42 (15.2%)	18 (6.5%)	27 (9.8%)	0.011

*using independent T-test.

Table 3. Comparison of patient's main symptoms based on location of the lesion

Variable	Lobar (N=324)	Putamen (294)	Thalamus (135)	Cerebellum (81)	Other (66)	*P*-value*
Decreased consciousness level (N=415)	224 (54%)	126 (30.4%)	33 (7.9%)	18 (4.3%)	14 (3.4%)	<0.001
Headache (N=329)	35 (10.6%)	137 (41.6%)	78 (23.7%)	35 (10.6%)	44 (13.5%)	0.031
Come (N=81)	20 (24.7%)	24 (29.6%)	15 (18.5%)	22 (27.2%)	0	0.633
Seizure (N=75)	45 (60%)	7 (9.3%)	9 (12%)	6 (8%)	8 (10.7%)	<0.001

*using independent T-test.

Table 4. Evaluation of initial radiologic findings of hemorrhage based on the location of the lesion

Variable	Lobar (N=324)	Putamen (294)	Thalamus (135)	Cerebellum (81)	Other (66)	*P*-value*
Bleeding into the ventricles (N=336)	63 (18.7%)	141 (42%)	78 (23.2%)	24 (7.1%)	30 (9%)	<0.001
Associated with subarachnoid hemorrhage (N=315)	225 (71.4%)	54 (17.1%)	6 (1.9%)	12 (3.8%)	18 (5.8%)	<0.001
Brain edema (N=489)	267 (54.6%)	78 (16%)	39 (8%)	60 (12.3%)	45 (9.1%)	<0.001

*using independent T-test.

Table 5. Correlation between radiologic findings and age

Condition	*P*-value*	Odds ratio (OR)	CI (95%)	
			Upper	Lower
Bleeding into the ventricles	<0.001	2.14	1.721	1.013
Associated with subarachnoid hemorrhage	0.02	1.63	1.715	1.0437
Brain edema	0.62	Not significant	-	-

*using Pearson Chi-Square.

Table 6. Correlation between radiologic findings and male gender

Condition	*P*-value*	Odds ratio (OR)	CI (95%)	
			Upper	Lower
Bleeding into the ventricles	0.141	Not significant	-	-
Associated with subarachnoid hemorrhage	0.318	Not significant	-	-
Brain edema	0.072	Not significant	-	-

*using Pearson Chi-Square.

hypertension was the most common past medical history that was significantly related to hemorrhage in basal nuclei. It was also found that hemorrhages in putamen were common in hypertensive patients and lobar hemorrhages were common in patients with anticoagulant use. Furthermore, we found that decreased consciousness and headache were the most common symptoms especially in patients with lobar hemorrhage and putamen hemorrhage respectively. We found that based on imaging evaluations, among patients with bleeding into ventricles, 42% had hemorrhage in putamen, among patients with subarachnoid hemorrh-

age, 71.4% had lobar hemorrhage and among patients with brain edema, 54.6% had lobar hemorrhage.

These data support the importance and relationships of intracerebral hemorrhage with different clinical and radiological findings. It could be determined that in patients with presentations of headache and history of hypertension, possible hemorrhages in putamen should be noticed. On the other hand, in patients with histories of anticoagulant use and presentation of decreased consciousness, lobar hemorrhages should be noticed in brain CT scan. Previous studies have also evaluated different relationships between intracerebral hemorrhages and clinical and imaging findings in special conditions and few studies have evaluated the general relationships.

In a study by Cordonnier and others in 2018, it was stated that the location, mass effect, and intracranial pressure of the underlying haematoma, as well as subsequent cerebral oedema from perihaematomal neurotoxicity or inflammation, and consequences from prolonged neurological dysfunction, all affect survival and recovery. Therefore, early diagnosis of these lesions and possible relationships with patient's characteristics have high clinical importance [19]. In 2021, Rajashekar and colleagues, showed that the radiologic findings of intracerebral hemorrhages could be associated with different presentations. Based on this study, patients with hypertension are most vulnerable to this disease and lobar hemorrhages are the most common radiologic findings. Furthermore, it was shown that among patients with bleeding into ventricles, hemorrhage in putamen were most common [20]. These data were in line with the findings of our study. We observed similar data by evaluating both radiologic and clinical findings in patients.

An important point of this study was that we evaluated a large population and conducted a multi-centric assessment. Previous surveys have assessed limited study population. It should also be noted that our findings could have high values in clinics in the way that in patients with a particular presentation and past medical history, a particular site of involvement could be determined. In 2020, Benger and colleagues assessed the clinical characteristics of intracerebral hemorrhage in patients with COVID-19 infection. They showed that lobar hemorrhage was the most common site of involvement and was mainly found in patients with hypertension [21]. In another study, Haller and others demonstrated that hypertension was the strongest risk factor for cerebral microbleeds and intracerebral hemorrhage and also showed that putamen hemorrages were most commonly associated with headache as the clinical presentation [22]. The findings of our study were also consistent with these data showing the relationship between clinical presentations and sites of involvements.

Other important points in this study were risk assessments and description of risk factors for patients. Based on our results, hypertension with a long history of anticoagulant use were the main past medical histories. Diabetes, coronary heart disease and ischemic cerebrovascular diseases were also important risk factors causing spontaneous intracerebral hemorrhage. Furthermore, it was observed that age more than 65 years was significantly associated with increased risks of bleeding into the ventricles and associations with subarachnoid hemorrhage but these data had no relationships with gender.

In 2016, Boulouis and others showed that most of the patients with lobar hemorrhages experience decreased consciousness and seizure as their clinical finding. This issue could be a particular presentation in patients with hypertension [23]. Our data were in line with the findings of these studies. As mentioned earlier, these previous reports have evaluated restricted study population that is mainly due to the low prevalence of intracerebral hemorrhage in general populations. But in this study, we evaluated data of 8381 strokes during 7 years and in 3 educational centers in 2 cities. Among these cases, data of 900 patients with intracerebral hemorrhage were evaluated.

These data could also have high clinical significance. It could be determined that hemorrhages in putamen were common in hypertensive patients and lobar hemorrhages were common in patients with anticoagulant use. As a result, patients with these known risk factors should be treated more carefully.

The shortcomings of this study were not comparing our results with other types of stroke and not evaluating the statistical relationships

between the variables. Another limitation was that hypertensive patients with a long history of anticoagulant use were not excluded from this study. However, it is believed that these data have high clinical importance and neurologists should consider possibility of intracerebral hemorrhage in particular locations in patients with previously known risk factors.

Conclusion

Hypertension was the most common past medical history that was significantly related to hemorrhage in basal nuclei. It was also found that hemorrhages in putamen were common in hypertensive patients and lobar hemorrhages were common in patients with anticoagulant use. Furthermore, we found that decreased consciousness and headache were the most common symptoms especially in patients with lobar hemorrhage and putamen hemorrhage respectively.

Disclosure of conflict of interest

None.

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References

[1] Campbell BCV, De Silva DA, Macleod MR, Coutts SB, Schwamm LH, Davis SM and Donnan GA. Ischaemic stroke. Nat Rev Dis Primers 2019; 5: 70.

[2] Logroscino G and Beghi E. Stroke epidemiology and COVID-19 pandemic. Curr Opin Neurol 2021; 34: 3-10.

[3] Roozpeykar S, Azizian M, Zamani Z, Farzan MR, Veshnavei HA, Tavoosi N, Toghyani A, Sadeghian A and Afzali M. Contrast-enhanced weighted-T1 and FLAIR sequences in MRI of meningeal lesions. Am J Nucl Med Mol Imaging 2022; 12: 63-70.

[4] Doria JW and Forgacs PB. Incidence, implications, and management of seizures following ischemic and hemorrhagic stroke. Curr Neurol Neurosci Rep 2019; 19: 37.

[5] Toyoda K, Yoshimura S, Nakai M, Koga M, Sasahara Y, Sonoda K, Kamiyama K, Yazawa Y, Kawada S, Sasaki M, Terasaki T, Miwa K, Koge J, Ishigami A, Wada S, Iwanaga Y, Miyamoto Y, Minematsu K and Kobayashi S; Japan Stroke Data Bank Investigators. Twenty-year change in severity and outcome of ischemic and hemorrhagic strokes. JAMA Neurol 2022; 79: 61-69.

[6] Benakis C, Llovera G and Liesz A. The meningeal and choroidal infiltration routes for leukocytes in stroke. Ther Adv Neurol Disord 2018; 11: 1756286418783708.

[7] de Oliveira Manoel AL. Surgery for spontaneous intracerebral hemorrhage. Crit Care 2020; 24: 45.

[8] Luzzi S, Elia A, Del Maestro M, Morotti A, Elbabaa SK, Cavallini A and Galzio R. Indication, timing, and surgical treatment of spontaneous intracerebral hemorrhage: systematic review and proposal of a management algorithm. World Neurosurg 2019; 124: S1878-8750(19)30105-6.

[9] Godoy DA, Núñez-Patiño RA, Zorrilla-Vaca A, Ziai WC and Hemphill JC 3rd. Intracranial hypertension after spontaneous intracerebral hemorrhage: a systematic review and meta-analysis of prevalence and mortality rate. Neurocrit Care 2019; 31: 176-187.

[10] Fakan B, Reisz Z, Zadori D, Vecsei L, Klivenyi P and Szalardy L. Predictors of localization, outcome, and etiology of spontaneous intracerebral hemorrhages: focus on cerebral amyloid angiopathy. J Neural Transm (Vienna) 2020; 127: 963-972.

[11] Divani AA, Liu X, Di Napoli M, Lattanzi S, Ziai W, James ML, Jafarli A, Jafari M, Saver JL, Hemphill JC, Vespa PM, Mayer SA and Petersen A. Blood pressure variability predicts poor in-hospital outcome in spontaneous intracerebral hemorrhage. Stroke 2019; 50: 2023-2029.

[12] Al-Shahi Salman R, Frantzias J, Lee RJ, Lyden PD, Battey TWK, Ayres AM, Goldstein JN, Mayer SA, Steiner T, Wang X, Arima H, Hasegawa H, Oishi M, Godoy DA, Masotti L, Dowlatshahi D, Rodriguez-Luna D, Molina CA, Jang DK, Davalos A, Castillo J, Yao X, Claassen J, Volbers B, Kazui S, Okada Y, Fujimoto S, Toyoda K, Li Q, Khoury J, Delgado P, Sabín JÁ, Hernández-Guillamon M, Prats-Sánchez L, Cai C, Kate MP, McCourt R, Venkatasubramanian C, Diringer MN, Ikeda Y, Worthmann H, Ziai WC, d'Esterre CD, Aviv RI, Raab P, Murai Y, Zazulia AR, Butcher KS, Seyedsaadat SM, Grotta JC, Martí-Fàbregas J, Montaner J, Broderick J, Yamamoto H, Staykov D, Connolly ES, Selim M, Leira R, Moon BH, Demchuk AM, Di Napoli M, Fujii Y, Anderson CS and Rosand J; VISTA-ICH Collaboration; ICH Growth Individual Patient Data Meta-analysis Collaborators. Absolute risk and predictors of the growth of acute spontaneous intracerebral haemorrhage: a systematic review and meta-analysis of individual patient data. Lancet Neurol 2018; 17: 885-894.

[13] Jolink WMT, Wiegertjes K, Rinkel GJE, Algra A, De Leeuw FE and Klijn CJM. Location-specific risk factors for intracerebral hemorrhage: systematic review and meta-analysis. Neurology 2020; 95: e1807-e1818.
[14] Magid-Bernstein J, Girard R, Polster S, Srinath A, Romanos S, Awad IA and Sansing LH. Cerebral hemorrhage: pathophysiology, treatment, and future directions. Circ Res 2022; 130: 1204-1229.
[15] Md Noh MSF. COVID-19 and cerebral hemorrhage: proposed mechanisms. J Neuroradiol 2021; 48: 125-126.
[16] Salloum A, Khoury V, Hemi F, Khaddam A and Omran A. Cerebral hemorrhage as the initial symptom of delayed diagnosis of childhood pheochromocytoma. J Pediatr Surg Case Rep 2022; 80: 102234.
[17] Naruto N, Itoh T and Noguchi K. Dual energy computed tomography for the head. Jpn J Radiol 2018; 36: 69-80.
[18] Li H, Xie YL, Wang X, Chen FX, Sun JQ and Jiang XL. Radiomics features on non-contrast computed tomography predict early enlargement of spontaneous intracerebral hemorrhage. Clin Neurol Neurosurg 2019; 185: 105491.
[19] Cordonnier C, Demchuk A, Ziai W and Anderson CS. Intracerebral haemorrhage: current approaches to acute management. Lancet 2018; 392: 1257-1268.
[20] Cheruiyot I, Sehmi P, Ominde B, Bundi P, Mislani M, Ngure B, Olabu B and Ogeng'o JA. Intracranial hemorrhage in coronavirus disease 2019 (COVID-19) patients. Neurol Sci 2021; 42: 25-33.
[21] Benger M, Williams O, Siddiqui J and Sztriha L. Intracerebral haemorrhage and COVID-19: clinical characteristics from a case series. Brain Behav Immun 2020; 88: 940-944.
[22] Das RN, Mandal B, Das M, Sil K, Mukherjee S and Chatterjee U. Perinatal and fetal autopsies in neuropathology: how I do it. Indian J Pathol Microbiol 2022; 65: S207-S217.
[23] Boulouis G, Charidimou A, Auriel E, Haley KE, van Etten ES, Fotiadis P, Reijmer Y, Ayres A, Schwab KM, Martinez-Ramirez S, Rosand J, Viswanathan A, Goldstein JN, Greenberg SM and Gurol ME. Intracranial atherosclerosis and cerebral small vessel disease in intracerebral hemorrhage patients. J Neurol Sci 2016; 369: 324-329.