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Study on Related Factors of Aspirin Resistance in Acute Ischemic Stroke

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Abstract: ***Objective*:** To study the related factors of aspirin resistance (AR) in acute ischemic stroke. ***Methods*:** A total of 138 patients with acute ischemic stroke treated in hospital affiliated to Xuzhou medical university from August 2016 to August 2018 were the study subjects, examine his medical data from the past. They were divided into the AR group (40 cases) and the non-AR group (98 cases) according to whether AR appears. Gender, disease history, biochemical indicators and etc. were compared between the two groups. The independent risk factors of AR were investigated using univariate analysis and logistic regression analysis. ***Results*:** 40 cases of AR occurred in 138 patients, with an incidence rate of 28.99%. Diabetes, platelet count (PLT), microRNA-19a (m iR-19a) expression, smoking, high-sensitivity C-reactive protein (hs-CRP), Low-density lipoprotein cholesterol (LDL-C), fibrinogen (FIB) and age difference between the AR group and non-AR group was statistically significant (P<0.05). Gender, hypertension, uric acid (UA), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), homocysteine (Hcy), total cholesterol (TC), and alanine aminotransferase (ALT) between the two groups were not significantly different (P>0.05). Logistic regression analysis showed that the independent risk factors for AR in acute ischemic stroke were diabetes (OR=2.773, 95%CI: 1.102~5.065, P=0.025), miR-19a (OR=3.021, 95%CI: 1.322~6.545, P=0.021), hs-CRP (OR=2.719, 95%CI: 1.301~5.022, P=0.028) and smoking (OR=1.983, 95%CI: 1.114~3.887, P=0.040). ***Conclusion*:** The incidence of AR is higher in acute ischemic stroke. Risk factors include diabetes, miR-19a expression, hs-CRP, smoking, etc. Clinical intervention measures can be taken to reduce the risk of AR and improve acute ischemic stroke prognosis.

Keywords: Acute ischemic stroke; Aspirin resistance; Related factors; Diabetes; MicroRNA-19a; High-sensitivity C-reactive protein; Smoking

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1. Introduction

The high disability and fatality rate of ischemic stroke is the main cause of mortality in adults, and the varying degrees of complications in the surviving patients have also placed a huge burden on the family and society [1]. Aspirin is the most commonly used medication for the prevention and treatment of ischemic stroke. However, it has been reported that platelet aggregation cannot be effectively inhibited in the patients who take aspirin at regular or even larger doses. Such a condition is also known as aspirin resistance (AR). The incidence rate of AR ranges from 5% to 40%. The occurrence of AR will directly impact the efficacy of antiplatelet therapy [2]. In recent years, multiple studies reported that smoking, blood sugar, age, and

genetic polymorphisms may be closely associated to the development of AR [3]. On the basis of standardized aspirin use, reducing the risk factors for AR development is of great significance to the prevention of AR. Therefore, in this study, we analyzed the disease history of patients and other common biochemical indicators in combination of the relevant literature to study the risk factors for AR development in acute ischemic stroke. The report is as follows.

2. Materials and methods

2.1. General information

There were 138 patients with acute ischemic stroke in total selected and enrolled in this study. These patients were treated in the Affiliated Hospital of Xuzhou Medical University from August 2016 to August 2018. The medical records of the study participants were retrospectively analyzed. The study participants were divided into two groups based on the presence of AR development AR group consisting of 40 cases and non-AR group consisting of 98 cases.

2.1.1. The inclusion criteria for recruiting study participants are indicated in the following:

(1) The patients who are diagnosed with acute ischemic stroke based on the diagnostic criteria stipulated in the "Guidelines for the Diagnosis and Treatment of Acute Ischemic Stroke 2014" by the Chinese Medical Association [4].
(2) The patients who are admitted to the hospital on the day after the onset of acute ischemic stroke and received standardized treatment afterwards, i.e., continuous administration of 100 mg/d aspirin for more than 7 days.
(3) The patients who suffer from first-time illness and have no history of taking aspirin.
(4) The patients who have no cerebral hemorrhage, myocardial infarction and other cardiovascular and cerebrovascular diseases.
(5) The patients who have complete medical records.

2.1.2. The exclusion criteria of the current study are as follows:

(1) The patients who are allergic to aspirin.
(2) The patients who have a history of taking other antiplatelet drugs within one week.
(3) The patients with platelet count > 450×10^9 /L or < 100×10^9 /L.
(4) The patients with severe liver and kidney insufficiency or having contraindications for taking aspirin before the onset.
(5) The patients whose condition deteriorated or died during treatment.

2.2. Methods

AR was determined [5] if 10 μmol/L adenosine diphosphate (ADP) induces an average platelet aggregation rate (Pag) ≥ 70% and 0.05 mmol/L arachidonic acid (AA) induces an average Pag of ≥ 20%. Considering previous literature and the records of medical information in our hospital, we clearly defined the scope of data to be collected, including general data such as diabetes, smoking, hypertension, gender, age, platelet count (PLT), high-sensitivity C-reactive protein (hs-CRP), and low-density lipoprotein cholesterol (LDL-C), fibrinogen (FIB), uric acid (UA), high-density lipoprotein cholesterol (HDL-C), triglycerides (TG), homocysteine (Hcy), total cholesterol (TC), alanine aminotransferase (ALT) and other biochemical indicators. The biochemical parameters were detected using automatic biochemical detection equipment, whereas PCR fluorescent probe-based detection method was employed to detect the relative expression of miR-19a.

2.3. Statistical analysis

In the current study, statistical analysis was performed using Statistical Package for Social Sciences (SPSS) version 22. Measurement data is expressed as mean ± standard deviation (SD). Independent sample *t* test was performed for univariate analysis. Count data is expressed as percentage and was analyzed using Chi-squared (χ^2) test. Logistic regression analysis was used for multivariate analysis. Differences with $P < 0.05$ are considered statistically significant.

3. Results

3.1. AR development in acute ischemic stroke and univariate analysis

Forty out of 138 patients in this study developed AR, corresponding to an incidence rate of 28.99%. The differences in diabetes, PLT, smoking, hs-CRP, LDL-C, FIB, and age between AR group and non-AR group were statistically significant ($P < 0.05$). According to **Table 1**, there was no significant difference in hypertension, UA, HDL-C, TG, Hcy, TC, ALT ($P > 0.05$).

Table 1. Univariate analysis of AR development in acute ischemic stroke [case (%)]

Variable	AR group (40 cases)	Non-AR group (98 cases)	χ^2 / t	*P*
Diabetes	21	26	8.530	0.003
Smoking	30	41	12.507	< 0.001
Hypertension	31	64	1.969	0.161
Male	24	60	0.018	0.894
Female	16	38		
Age (years)	70.23 ± 4.92	65.44 ± 4.65	5.383	< 0.001
PLT (10^9)	227.34 ± 24.48	206.21 ± 20.61	5.168	< 0.001
hs-CRP (mg/L)	8.87 ± 2.91	6.01 ± 1.23	8.139	< 0.001
LDL-C (mmol/L)	3.42 ± 0.76	3.01 ± 0.65	3.198	0.002
FIB (g/L)	3.24 ± 0.41	3.01 ± 0.32	3.521	0.001
UA (μmol/L)	325.44 ± 24.55	319.30 ± 21.56	1.457	0.147
HDL-C (mmol/L)	1.18 ± 0.21	1.22 ± 0.19	1.088	0.279
TG (mmol/L)	1.77 ± 0.12	1.72 ± 0.23	1.303	0.159
Hcy (μmol/L)	13.23 ± 4.34	13.40 ± 3.92	0.250	0.803
TC (mmol/L)	4.79 ± 1.02	4.70 ± 0.98	0.484	0.629
ALT (U/L)	27.34 ± 4.01	26.81 ± 3.87	0.722	0.471
miR-19a	0.77 ± 0.08	0.52 ± 0.04	24.423	< 0.001

Note: PLT: platelet count; hs-CRP: high-sensitivity C-reactive protein; LDL-C: low-density lipoprotein cholesterol; TG: triglycerides; FIB: fibrinogen; UA: uric acid; HDL-C: high-density lipoprotein cholesterol; Hcy: homocysteine; TC: cholesterol; ALT: alanine aminotransferase; miR-19a: microRNA -19a.

3.2. Multivariate analysis of AR development in acute ischemic stroke

In the logistic regression analysis, AR was the dependent variable, whereas diabetes (no = 0, yes = 1), smoking (no = 0, yes = 1), PLT, expression of miR-19a, hs-CRP, LDL-C, FIB, age (actual value) were the independent variables. The results showed that the independent risk factors for AR development in acute ischemic stroke were diabetes, miR-19a expression, hs-CRP, and smoking, as shown in **Table 2**.

Table 2. Multivariate analysis of AR in acute ischemic stroke

Influencing factors	β	SE	Wald	OR	95% CI	*P*
Diabetes	0.612	0.254	7.154	2.773	1.102~5.065	0.025
miR-19a	0.741	0.242	7.544	3.021	1.322~6.545	0.021
hs-CRP	1.021	0.476	6.334	2.719	1.301~5.022	0.028
Smoking	0.458	0.230	3.983	1.983	1.114~3.887	0.040

Note: hs-CRP: high-sensitivity C-reactive protein; SE: standard error; OR: odds ratio; CI: confidence interval.

4. Discussion

In recent years, we've observed steady changes in Chinese citizens' lifestyles and dietary habits, as well as the ageing of both urban and rural populations. This is unfortunately accompanied with an increase in the yearly incidence rate of acute ischemic stroke. The disease's high disability and death rates, in particular, represent a severe danger to the elderly population's health and quality of life. Inhibition of platelet activation is seen to be a significant technique for preventing, treating, and minimizing the recurrence of ischemic stroke from a neurological perspective. Aspirin is one of the most efficient antiplatelet drugs, since it effectively inhibits platelet aggregation to lower the incidence of thrombotic events and so improves the prognosis of ischemic stroke [6]. However, it has been observed that 5% to 40% of patients who take aspirin at a dosage of 75-150 mg/d acquire AR, and the efficacy of antiplatelet treatment becomes less than optimal, compromising patient prognosis and raising the risk of ischemic vascular events recurrence [7]. The mechanism of AR development is not entirely understood at this time. Although it is known that insufficient medication dosage, poor compliance, decreased drug intake, the ADP receptor, polymorphisms in the platelet glycoprotein gene, and other factors can contribute to the development of AR, the incidence rate of AR cases rises even when the standard dose is used [8]. As a result, focused prevention has become an essential strategy for preventing the onset of AR; nevertheless, additional epidemiological research into the onset of AR and the study of its risk factors is required.

The impact of diabetes on AR was first reported in cardiovascular disease. Czech MP [9] found that the risk for AR development in patients with type 2 diabetes and cerebral infarction was 2 to 3 times higher than that in non-diabetic patients. The result of this study shows that the OR of diabetes was 2.773, which is consistent with previous reported results. The development of AR mainly involves the following mechanisms [10]:

(1) Excessive blood glucose concentration will influence the inhibitory effect of aspirin on peripheral blood P-selectin and platelet GP Ⅱb-Ⅲa. In the hyperglycemic milieu, the ability of aspirin to acetylate platelets is significantly inhibited, thereby reducing the inhibitory effect of aspirin on platelet activation.
(2) In diabetic patients, the increased expression level of cyclooxygenase-2 (COX-2) and the level of coagulation factors will lead to an increase in platelet activity, which gives rise to a decrease in aspirin sensitivity.
(3) Hyperglycemia inhibits the activation of aspirin on the nitric oxide / cyclic guanosine monophosphate (NO/cGMP)-dependent protein kinase pathway, and incapacitates the patient's vascular protection.
(4) When the blood glucose level becomes too high, the survival time of peripheral blood platelets is shortened, the renewal frequency increases, and the high activity of new platelets will also promote the development of AR. miRNA is a highly conserved, single-stranded, non-coding RNA in the human body.

In recent years, studies have shown that the activation of platelets can alter the expression of multiple miRNAs in the body. miR-19a is an important transcriptional regulator of the miR-17-92 gene cluster, and has a regulatory effect on the proliferation and adhesion of vascular endothelial cells [11]. This rationalizes

the inclusion of miR-19a as a parameter in the present study. The results of this study show that the expression of miR-19a in patients with AR was significantly higher than that in patients without AR. We believe the increase in miR-19a expression may be involved in the occurrence of AR. This viewpoint is consistent with the results of Binderup et al. [12], who consider that miR-19a participates in angiogenesis through endothelial cell adhesion and proliferation, and is closely related to platelet aggregation. The exact underlying mechanism may be related to the interaction of multiple miRs, which requires further in-depth investigations.

Inflammation was found to play a crucial role in all stages of atherosclerosis and thrombotic events. hs-CRP is a sensitive inflammation marker. The results of this study show that hs-CRP level increases, indicating that the risk for AR development increases when inflammation deteriorates [13]. In addition to its important role in the regulation of thrombosis, platelets are also important amplification factor of human inflammatory response [14]. Platelet activation will lead to the release of a large number of inflammatory mediators, and induce the expression of inflammatory mediators in macrophages, monocytes and granulocytes. Monocytes and macrophages are important sources of thromboxane A2 (TXA2). The COX-2 pathway increases the synthesis of TXA2, which in turn promotes the thrombotic state and induces AR development [15]. Other researchers also opine that inflammation can cause vascular endothelial damage, which leads to endothelial dysfunction. Aspirin offers protection to the endothelium, but the development of AR will negate this protective effect [16-17]. At the same time, platelets can be activated through other pathways in an inflammatory state, such as the adrenaline pathway and ADP pathway, while aspirin only blocks platelet activation induced by COX pathway, corroborating that inflammatory response may also be one of the influencing factors of AR development [18- 19].

A variety of studies have differing opinions on the effect of smoking on the development of AR. Smoking is a non-independent risk factor for recurrent ischemic stroke, according to Zhu Xiaoqi et al. [20], although this contradicts our findings. The most common cause for recurrence is that most relapsed patients are able to effectively quit smoking after the initial episode, lowering the impact of carbon monoxide and nicotine on aspirin [21-22]. The patients in this research were all new-onset, and some of them were still smoking prior to the commencement of the condition. Carbon monoxide inhaled during smoking damages endothelial cells directly and causes platelet aggregation and activation to variable degrees, reducing the inhibitory impact of aspirin on platelet activation [23].

In addition, long-term smokers were reported to have higher levels of macrophage colony-stimulating factor [24], which can promote the production of TXA2 by platelets, and can also inhibit TXA2 metabolism, thereby impacting the antiplatelet effect of aspirin [25-26]. According to previous studies, medications such as proton pump inhibitors also have a certain promotion effect on the development of AR [18]. Nevertheless, the present study excluded serious diseases involving the liver, kidney, digestive system, etc. Thus, the relationship between the use of other medications and AR development was not investigated in this study. Despite the high incidence rate of AR development in acute ischemic stroke, the risk factors determined in this study have certain guiding significance in the prevention of acute ischemic stroke.

In conclusion, risk factors determined in this study are diabetes, miR-19a expression, hs-CRP, smoking. Since the incidence rate of AR development in acute ischemic stroke is relatively high, targeted interventions should be adopted to reduce the risk of AR development and improve the prognosis of acute ischemic stroke.

Disclosure statement

The author declares no conflict of interest.

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