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Comparison of Erythrocyte Morphological Markers in Patients with the First Ischemic Cerebrovascular Attack

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ABSTRACT

Background & Objective: Stroke is the third leading cause of death in the world. Preventive and therapeutic approaches in this field are weak and further understanding of molecular mechanisms helps to diagnose and treat more effectively.

Methodology: This case-control study was conducted on 55 patients with ischemic stroke and 55 healthy individuals as a control group. The control group was collected from the staff and patients admitted to Valiasr Hospital in other wards. Demographic variables of the two groups were homogenized and then venous blood samples were taken to measure biochemical levels and complete blood counts.

Results: The mean age of patients was 69.98 years. The mean serum cholesterol level in patients was 157.1 mg/dL and it was 121.5 mg/dL in the control group and there was a statistically significant difference between the two groups ($P < 0.05$). MCV was 88.5 in patients and 83.2 in the control group with a statistically significant difference. Systolic and diastolic blood pressure were also significantly higher in patients. In addition, serum ferritin and iron levels and hemoglobin were significantly associated with stroke severity ($P < 0.05$).

Conclusion: The results of this study showed the relationship between erythrocyte morphology and stroke and based on this, more prospective studies can be recommended to evaluate the relationship between erythrocyte morphological markers and practical ways to prevent stroke.

KEYWORDS

Ischemic Stroke, Red Blood Cells, Mean Corpuscular Volume.

Introduction

Stroke is the third leading cause of death in the world after heart disease and cancer, and is emerging as the most common and life-threatening neurological problem worldwide (1). On average, every 4 minutes, someone dies of stroke (2). The prevalence of stroke is generally 2.8% and it is 2.7% in Asia (3). The incidence of stroke in Iran, which is a middle-income country, is significantly higher than in Western countries (4).

Worldwide, approximately 70% of strokes and 87% of deaths from stroke and disability occur in low- and middle-income countries (5, 6). Over the past few decades, the incidence of stroke has multiplied in low-income and middle-income countries, while it has fallen by 42% in high-income countries (5).

To prevent stroke, examining the underlying factors of stroke and its treatment can play an important role in reducing mortality (7-9). There is no difference between the prevention of subsequent stroke in a patient with symptomatic or asymptomatic stroke and in a patient with a transient ischemic attack (9).

Findings have shown that preventive and therapeutic approaches in this field are weak and more understanding of molecular mechanisms and prognostic indicators helps to diagnose and treat more effectively (10) and the important issue is to identify prognostic indicators and risk factors (11, 12).

Known risk factors include hypertension, hyperlipidemia and smoking, diabetes, obesity, and a family history of stroke (13-15). In Iran, risk factors such as hypertension and overweight are strong independent predictors of stroke (16). However, cerebrovascular accidents can sometimes occur in people who do not have any of these risk factors, as a result, there are likely to be other risk factors (13, 14).

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Identifying prognostic indicators can help prevent cerebrovascular disease. Pathophysiologies such as atherosclerosis, endothelial dysfunction (17), ferritin, iron and homocysteine levels have been reported in stroke (18-22). Vitamins B9 and B12 are important regulators of homocysteine metabolism. The association of homocysteine with other blood factors in stroke has been proven (23, 24). In addition, it is important to pay attention to micronutrients and factors affecting blood factors because of their role in blood cell morphology (20, 24).

According to the above, the study aimed to identify the rapid and inexpensive predictor of ischemic cerebral attacks and possibly other risk factors by focusing on morphological markers of blood cells.

Materials and Methods

Study Design

This case-control study was conducted on patients with ischemic stroke admitted to the emergency department of Valiasr Hospital with the diagnosis of the first ischemic stroke during March 2016 to March 2017. Eligible patients were included in the study. One hundred and ten patients were divided into two groups of case and control and studies were carried out on them.

Inclusion and Exclusion Criteria

Inclusion criteria were the first ischemic stroke with diagnosis of the emergency medicine attend and confirmation by imaging methods and consent of patients or their companions. Exclusion criteria were individuals with chronic illness, insertion of any invasive device, endarterectomy, stent, thrombectomy or any intravascular treatment of the carotid artery in the last 30 days, general anesthesia or hospitalization equal to or more than 3 days during the last two weeks, receiving lipid-lowering medications, taking medications that affect homocysteine levels in the last 30 days (methotrexate, tamoxifen, levodopa, niacin), any megaloblastic anemia being treated, and a history of taking supplements and vitamins in the last six months.

Patient Selection

Patients were selected by convenience sampling and the sample size was calculated 55 people for each group based on $\alpha = 0.05$, a generalizable ratio to the population of 84% using the following formula. 55 patients with ischemic stroke and 55 healthy individuals were classified as the control group (110 in total).

$$n_1 = n_2 = \frac{2(Z_{1-\alpha/2} + Z_{1-\beta})^2 \left[P_1(1-P_1) + P_2(1-P_2)\right]}{(p_1 - p_2)^2} = 55 \qquad \alpha = 0.05 \quad p_1 = 0.84 \quad p2 = 0.5 \quad \beta = 0.2$$

Method Description

Patients with a possible diagnosis of stroke based on clinical signs were included in the study and were considered as the case group after following the usual diagnostic and therapeutic measures if the type of cerebrovascular accident was diagnosed as ischemic type, while examining the inclusion criteria if approved by an emergency medicine specialist. The control group was collected from the staff and patients admitted to Valiasr Hospital in other wards. Demographic variables of the two groups were homogenized and then venous blood samples were taken for biochemical measurement and complete blood count.

Data Collection

Basic information of each patient was entered in the questionnaire. and at the same time, MRS questionnaire (Modified Rankin Scale) and MMS questionnaire (Mini Mental State Exam) and nutritional status checklist (Mini Nutritional Assessment) and NIH stroke scale evaluation form of patients with inclusion criteria were completed. The study was continued until the sample size was equal to or greater than 55 and, at the same time, the control group was selected from other patients who were hospitalized for other reasons by homogenizing in terms of age, sex and risk factors, especially diabetes and dyslipidemia.

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Biochemical Study

At the time of admission to the neurology ward, 5 cc of fasting blood samples were taken to measure blood factors and levels of cr, TG, Chol. Level (HDL, total, LDL), complete blood cell count (CBC) and iron profile were obtained and sent to the reference laboratory.

In complete blood cell count (CBC), quantitative values of erythrocyte indices such as RBC count, hemoglobin concentration (Hb), hematocrit percentage (HCT), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cellular hemoglobin concentration (MCHC), red blood cell distribution width (RDW) as well as differential count and number of white blood cells and platelet indices were determined in the same way by cell counter device (Horiba, made in Japan).

Ethical Considerations

Written consent was completed by the patients and the confidentiality of all information obtained from the research units was guaranteed. The cost of iron profile tests was requested with the patients' consent and no additional costs were imposed on the patients.

Data Analysis

After entering the data into SPSS.v22, the data were analyzed by drawing tables and graphs and using Chi-square and t-test and logistic regression test. A P-value of less than 05.0 was considered significant.

Findings

The two groups were similar in terms of primary demographic characteristics. The mean age of the total patients was 69.98 ± 14.28 years (it was 69.92.86 ± 14.61 and 70.07 ± 13.65 in the control and case groups, respectively) (Table 1). Comparison of qualitative indices between the two groups based on the hypothesis of equality in the study showed that the two groups had the same distribution in terms of age, sex, level of education, smoking, history of cardiovascular disease and taking medications and they had no statistically significant difference ($p \geq 0.05$).

Table 1. Frequency distribution of some demographic characteristics in all samples

Variable	Parameter	Number/Mean	Percentage
Age	Total	69,98±14,28	
Body mass index	Total	27,5±4,08	
Sex	Female	67	60.9
	Male	43	39.1
Education	Primary	74	67.3
	Diploma	7	6.4
	Other	27	24.5
	Unknown	2	1.8
Underlying disease	Blood pressure	16	14.5
	Diabetes	18	16.4
	Peripheral vascular disease	2	1.8
	Carotid artery stenosis	1	.9
	Blood pressure and diabetes	28	25.5
	Several diseases	2	1.8
	None	43	39.1
Smoking	Non-smoker	100	90.9
	Occasionally	4	3.6
	Continuously	6	5.5

According to Table 2, comparison of laboratory findings showed that the mean systolic and diastolic blood pressures in patients was 151.16 and 86.32, respectively, and was 121.54 and 76.18 in healthy individuals,

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which indicates higher blood pressure in the case group than the control ($p < 0.001$). Cholesterol levels in the control group were 184.24 and were 151.58 mg/dL in the control group, which showed a significant difference between the two groups. Also, the mean level of LDL was 124 and the level of triglyceride was 126 mg/dl and all the three were significantly higher than the control group and HDL with a mean value of 37.6 mg/dl was lower than the control group ($p < 0.001$). The other variables in the two groups were not statistically significant ($p \geq 0.05$).

Table 2. Comparison of laboratory findings in the two groups

Index	Group	Mean	Std. Deviation	p
sBP	Case	151.16	27.70	.000
	Control	121.54	18.75	
DBP	Case	86.32	17.01	.000
	Control	76.18	9.32	
PR	Case	85.91	11.08	.71
	Control	85.23	7.57	
chol.level	Case	184.16	50.24	.000
	Control	151.58	34.80	
LDL	Case	124.30	47.06	.000
	Control	85.10	25.75	
HDL	Case	37.63	11.46	.000
	Control	48.19	7.23	
TG	Case	126.07	69.63	.000
	Control	86.70	27.67	
BS	Case	162.74	60.03370	.000
	Control	113.43	30.37	
Serum cr	Case	.85	0.20	.19
	Control	.81	.130	
GFR	Case	71.39	31.64	.37
	Control	76.24	25.27	

Comparison of the two groups in terms of morphology and complete blood count (CBC) indices showed that the level of MCV in the case group was 88.53 and was 83.24 µg/ml in the control group ($p < 0.001$) and serum ferritin levels in the case group were 189.43 and 168.25 µg/ml in the control group ($p=0.03$). The two groups had a significant difference regarding these variables. But there was no significant difference in terms of other variables ($p \geq 0.05$).

Table 3. Comparison of morphological indices and CBC indices in the two groups

Index	Group	Mean	Std. Deviation	P-value
RBC	Case	4.79	0.77	.06
	Control	5.01	0.40	
Plt	Case	257.27	65.00	.23
	Control	273.08	74.15	
Hct	Case	41.85	4.66	.30
	Control	42.03	4.37	
Hb	Case	13.62	1.80	.96
	Control	13.61	0.78	
MC	Case	88.53	8.32	.000
	Control	83.24	2.33	
MCH	Case	28.48	3.42	.72
	Control	28.27	2.96	
MCHC	Case	32.48	18.89	.32
	Control	37.72	39.39	
Serum iron	Case	95.90	18.31	.24
	Control	93.22	21.97	
[1]TIBC	Case	279.13	27.75	.16
	Control	256.03	32.51	
Serum ferritin	Case	189.43	38.70	.03
	Control	168.25	43.52	
Transferin saturation	Case	37.72	12.44	.34
	Control	33.16	14.91	

[1]Total iron binding capacity

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The effect of erythrocyte morphological indices with ischemic stroke showed that only mean corpuscular volume (MCV) levels were significantly associated with stroke (p=0.001).

Table 4. Logistic regression of erythrocyte morphological indices with ischemic stroke

Index	**β coefficient**	**p**	**odds ratio**
RBC	0.245	0.08	1.34
Plt	0.198	0.23	1.03
Hct	0.607	0.09	0.986
Hb	0.451	0.07	1.06
MCV	0.676	0.001	1.89
MCH	0.421	0.08	0.93
MCHC	0.175	0.32	0.83
Serum iron	0.189	0.62	0.103
TIBC	0.296	0.67	0.80
Serumferritin	0.193	0.23	0.89
Transferin saturation	0.288	0.09	1.11

In order to determine the effect of erythrocyte morphology on the severity of ischemic stroke based on MRS index showed that none of the RBC indices were associated with stroke severity (P≥0.05). However, based on the statistical test, serum iron level and ferritin level were associated with the severity of ischemic stroke (p <0.05) (Table 5).

Table 5. Analysis of variance of ischemic stroke severity based on MRS index and serum iron indices

Index	**Stroke severity**	**Number**	**Mean**	**Std. deviation**	**P-value**
Serum iron	no significant disability	7	4.5	.13	**0.005**
	slight disability	19	4.9	.76	
	moderate disability	4	4.7	.46	
	moderatly sever disability	1	5.1	.	
	severe disability	33	4.6	.76	
	dead	0			
TIBC	no significant disability	7	253.5	47.22	**0.69**
	slight disability	19	210.9	44.72	
	moderate disability	4	248.5	52.03	
	moderatly sever disability	1	290.0	.	
	severe disability	33	283.8	82.34	
	dead	0			
Serum ferritin	no significant disability	7	42.2	3.86	**0.01**
	slight disability	19	42.7	5.08	
	moderate disability	4	41.7	2.73	
	moderatly sever disability	1	41.7	.	
	severe disability	33	40.8	4.20	
	dead	0			
Transferin saturation	no significant disability	7	13.7	1.08	**0.65**
	slight disability	19	14.4	2.11	
	moderate disability	4	13.1	1.19	
	moderatly sever disability	1	13.3	.	
	severe disability	33	13.3	1.81	
	Dead	0			

According to Table 6, the severity of dependence in patients with stroke based on Barthel Index with erythrocyte morphology by analysis of variance showed that no significant difference was seen in any of the cases (P ≥0.05).

Table 6. Analysis of variance of erythrocyte morphology and severity of dependence based on BI index

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Index	Dependence severity	Number	Mean	Std. deviation	P-value
RBC	total dependent	19	4.953	.96	**0.875**
	severe dep	12	4.732	0.70	
	mod Dep	10	4.68	0.36	
	slight dep	4	4.678	0.19	
	independent	10	4.71	0.93	
plt	total dependent	19	263.57	60.96	**0,112**
	severe dep	12	304.83	69.02	
	mod Dep	10	254.70	58.06	
	slight dep	4	235.25	10.04	
	independent	10	269.60	40.93	
HCT	total dependent	19	42.04	5.64	**0,597**
	severe dep	12	41.050	4.58	
	mod Dep	10	40.610	2.58	
	slight dep	4	44.750	2.04	
	independent	10	42.530	5.08	
HB	total dependent	19	13.48	1.98	**0,449**
	severe dep	12	13.24	1.96	
	mod Dep	10	13.26	1.23	
	slight dep	4	14.67	0.12	
	independent	10	14.29	1.99	
MCV	total dependent	19	87.71	7.30	**0,464**
	severe dep	12	87.20	10.27	
	mod Dep	10	86.63	3.39	
	slight dep	4	92.99	6.56	
	independent	10	91.79	11.20	
MCH	total dependent	19	27.78	2.42	**0,164**
	severe dep	12	27.67	4.19	
	mod Dep	10	28.39	2.06	
	slight dep	4	28.47	5.36	
	independent	10	30.90	3.82	
MCHC	total dependent	19	32.08	2.07	**0,136**
	severe dep	12	31.84	2.01	
	mod Dep	10	32.56	1.47	
	slight dep	4	33.44	2.33	
	independent	10	33.56	1.20	

There was no significant difference in terms of cognitive changes according to MMSE criteria and using statistical test in erythrocyte morphology (Table 7).

Table 7. Analysis of variance of erythrocyte morphology and cognitive changes based on MMSE criteria

Index	Cognitive changes	Number	Mean	Std. deviation	P-value
RBC	No cognitive impairment	9	4.67	1.02	**0.565**
	Mild cognitive impairment	10	4.61	0.54	
	Severe cognitive impairment	36	4.87	0.76	
plt	No cognitive impairment	9	221.66	56.81	**0,115**
	Mild cognitive impairment	10	261.10	55.00	
	Severe cognitive impairment	36	270.11	63.52	
HCT	No cognitive impairment	9	41.40	6.02	**0,945**
	Mild cognitive impairment	10	42.11	3.44	
	Severe cognitive impairment	36	41.89	4.69	
[1]HB	No cognitive impairment	9	13.98	2.34	**0,777**
	Mild cognitive impairment	10	13.70	1.35	
	Severe cognitive impairment	36	13.51	1.79	
MCV	No cognitive impairment	9	90.24	11.58	**0,375**
	Mild cognitive impairment	10	91.10	8.63	
	Severe cognitive impairment	36	87.40	7.28	
MCH	No cognitive impairment	9	30.55	4.09	**0,113**
	Mild cognitive impairment	10	28.69	4.14	
	Severe cognitive impairment	36	27.91	2.88	
MCHC	No cognitive impairment	9	33.72	1.11	**0,054**
	Mild cognitive impairment	10	32.82	1.63	
	Severe cognitive impairment	36	32.08	1.99	

[1]hemoglobin

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In terms of stroke severity according to NIHSS criteria and using analysis of variance test according to the table below, a significant difference was seen in erythrocyte morphology only in hemoglobin level (P=003) (Table 8).

Table 8. Analysis of variance of erythrocyte morphology and stroke severity according to NIHSS criteria

Index	Stroke severity	Number	Mean	Std. deviation	P-value
RBC	NO STROK	7	4.433	.18	**0,131**
	MINOR STROK	18	4.764	.71	
	MODERATE STROK	12	4.693	.95	
	MODERATE TO SEVER STROK	9	5.373	.80	
	SEVER STROK	9	4.684	.68	
plt	NO STROK	7	254.714	49.06	**0,253**
	MINOR STROK	18	235.833	61.00	
	MODERATE STROK	12	254.250	67.97	
	MODERATE TO SEVER STROK	9	267.667	63.65	
	SEVER STROK	9	295.778	74.58	
HCT	NO STROK	7	42.029	4.05	**0,145**
	MINOR STROK	18	42.272	4.00	
	MODERATE STROK	12	39.783	5.73	
	MODERATE TO SEVER STROK	9	44.822	4.24	
	SEVER STROK	9	40.656	4.29	
HB	NO STROK	7	13.571	1.40	**0,003**
	MINOR STROK	18	14.139	1.65	
	MODERATE STROK	12	12.617	1.36	
	MODERATE TO SEVER STROK	9	15.078	1.92	
	SEVER STROK	9	12.533	1.58	
MCV	NO STROK	7	93.984	7.17	**0,285**
	MINOR STROK	18	89.139	7.99	
	MODERATE STROK	12	87.163	8.53	
	MODERATE TO SEVER STROK	9	85.022	7.27	
	SEVER STROK	9	88.460	9.82	
MCH	NO STROK	7	30.4100	2.74	**0,193**
	MINOR STROK	18	29.4039	3.86	
	MODERATE STROK	12	27.6233	3.57	
	MODERATE TO SEVER STROK	9	27.4644	1.56	
	SEVER STROK	9	27.3444	3.55	
MCHC	NO STROK	7	32.2071	1.42	**0,111**
	MINOR STROK	18	33.4867	1.52	
	MODERATE STROK	12	31.7475	2.19	
	MODERATE TO SEVER STROK	9	33.0433	1.98	
	SEVER STROK	9	31.1456	1.31	

The mean severity of malnutrition and nutritional status in the two groups based on the MNA questionnaire showed that the groups were different in terms of nutrition (Table 9). 6 patients (11%) had clinical malnutrition and 22 patients (40%) were exposed to it. In all three cases, the control group was significantly different from the control group ($p < 0.05$).

Table 9. Comparison of nutritional status in the two groups

Index	**Group**		**Number**	**Percentage**	**P-value**
Frequency	Case	Malnutrition	6	11	0,000
		Exposed to	22	40	
		Healthy	27	49	
	Control	Malnutrition	0	0,0	
		Exposed to	0	0,0	
		Healthy	55	0,0	
		Number	Mean	Std. deviation	
MNA assessment	Case	55	12.54	2.54	0,000
	Control	55	15.18	1.18	
MNA screening	Case	55	10.7	2.94	0,000
	Control	55	13	0.86	
MNA total assessment	Case	55	23.25	4.75	0,000
	Control	55	28.2	1.41	
Frequency	Case		Number	Percentage	0,000

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Description: Nutritional status checklist is related to anthropometric and nutritional parameters such as albumin, prealbumin, transferrin, cholesterol, retinol, cholecalciferol and zinc. It is also associated with hematologic changes such as hematocrit and hemoglobin

Discussion

Comparison of erythrocyte morphological markers in stroke patients can predict the occurrence of the disease in the individual (11, 20). Our study showed that hyperlipidemia and hypertension were two effective factors in stroke patients and the mean systolic blood pressure was 151 mm Hg and the mean diastolic blood pressure was 86 mm Hg and both were significantly higher than the control group, which is similar to the results of previous studies (7, 25). Similar studies by Aksoy et al. after recording the laboratory findings of patients based on MRSS showed that hypertension has the highest ranking in terms of risk factor (26). In our study, the levels of HDL, LDL and TG in the two groups were significantly different, which was consistent with the studies (13, 14, 16).

Our findings showed that 6 patients (11%) had clinical malnutrition and 22 patients (40%) were exposed to it. However, all subjects in the control group had normal nutritional status. Similar studies have shown that clinical malnutrition is more common in people with stroke than in healthy individuals (27-29). The status studied in MNA is also associated with micronutrient intake and energy intake of the patient (30). According to the above and the results of this study, it can be claimed that more than 50% of patients with ischemic stroke suffer from some kind of energy or micronutrient eating disorder, or in other words, it can be claimed that ischemic stroke can be associated with energy or micronutrient deficiencies.

Our study showed that among the erythrocyte morphology and indicators, only mean corpuscular volume (MCV) is associated with ischemic stroke (Table 4). Hemoglobin was also an effective and significant factor in increasing the severity of stroke (Table 8).

Studies have shown that iron overload contributes to the development of vascular disease by causing thrombosis after arterial injury. High serum ferritin on admission of acute stroke patients (within 24 to 48 hours after stroke onset) had poor prognosis implicating that increase in body iron stores before stroke onset can aggravate the brain ischemia cytotoxicity. Therefore, it has been suggested that high serum ferritin affects the prognosis of ischemic stroke and also acts as a risk factor for ischemic episodes by enhancing atherogenesis (31, 32).

Studies have shown that folate and iron are directly related. Increasing the intake of high-dose dietary folate as a prophylactic agent in ischemic stroke will be associated with a significant reduction in stroke. (33) Sohini Sengupta et al. have stated that primary treatment measures with vitamins can prevent ischemic stroke attacks due to hyperhomocysteinemia (34).

In our study, mean serum iron and ferritin were significantly associated with stroke severity (Table 5). A consistent study showed that the mean serum iron level was lower in the group of stroke patients and this factor played an important role in stroke (35). In other studies in 2021, the results showed a significant effect of serum iron and ferritin in people with stroke (36, 37). Statistical findings showed that old age, high MCV and low folate levels were associated with stroke prognosis (27).

In the present study, hemoglobin was significantly lower in patients with ischemic stroke attacks with healthy individuals. Recent studies in 2021 show that hemoglobin levels play an important role in stroke (38). However, in the study of Santos et al., it was shown that the level of hematocrit was significantly lower in patients with ischemic stroke attacks than the control group, but the hemoglobin level was not different in the two groups (39).

In the analysis of variance, none of the indicators and erythrocyte morphology were associated with stroke severity. However, with the increase in iron levels, the stroke severity increased and, conversely, with the decrease in ferritin levels, the stroke severity increased.

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Conclusion

Based on the evidence of the above studies and the association of malnutrition and MCV levels with stroke, it can be concluded that micronutrient malnutrition, especially folic acid and vitamin B12, is associated with ischemic stroke. the role of easy, quick, and low-cost measurement of CBC and observation of high MCV as a risk factor for ischemic stroke and possibly prophylactic measures can be considered in subsequent complementary studies.

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