

Inflammatory biomarkers in the young stroke population

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Abstract

Objective: Studies showed that cerebral ischemia due to arterial occlusion is related to local inflammation. Neutrophil to Lymphocyte Ratio (N/L) and Monocyte to high density lipoprotein Cholesterol Ratio (M/H) are two biomarkers that are shown to be increased in inflammation. These biomarkers can be used to determine the risk for atherosclerotic ischemic stroke. Increased Homocysteinemia (Hcy) is also a risk factor for ischemic stroke, especially in the young population. There is insufficient data in the literature about the correlation of these biomarkers in young stroke patients.

We aimed to show if these biomarkers can be related to atherothrombotic cerebrovascular disease in the young population to provide information for young people to stroke risk.

Study design and method: This retrospective study included 43 atherosclerotic ischemic young stroke patients, age between 18- 55 years and age/ gender matched 42 healthy control group. Control group were enrolled from patients who were admitted to our outpatient clinics with headaches, having normal examinations and no other diseases.

Results: The ratios of N/L and M/H were both higher in the stroke group ($p=0.008$ and $p=0.011$ respectively), but the gender subanalysis showed there was no significance in the males. When the patients were sub-grouped as having hyper Hcy or not; these ratios did not show any significance.

Conclusion: Inflammatory biomarkers should be interpreted carefully by concerning the age and the gender of the patients. Further studies with large sample groups are necessary for the biomarkers of young stroke population.

Keywords: stroke, young adult, gender, inflammation, biomarkers

Introduction

Stroke is one of the leading causes of death and is also a major cause of disability(1). In the literature the upper age of “young stroke” is very variable and is accepted in the range of 45-60 years in various studies(2), (3).Hypertension, diabetes, hyperlipidemia, cardiac embolism, cervical arterial dissection and hyperhomocysteinaemia are some of the known risk factors of the young stroke patients and in 30% of the patients the etiology is unknown(3)–(5). Since the disability in young people has a greater effect on both the patients and their families as well as on the economy it is important to provide primary protection by defining the risk factors.

Studies showed that cerebral ischemia due to arterial occlusion is related with local inflammation (6), (7). Neutrophil to Lymphocyte Ratio (N/LRatio) is a marker, which can be evaluated with a simple blood count, can show overall inflammatory status of the body (8).Celiktepe et al. found N/L Ratio higher in atherothrombotic acute cerebral ischemia and concluded that there is a positive association between N/L Ratio and clinical outcome in acute ischemic stroke (6).

Increased level of monocyte to high-density lipoprotein Cholesterol Ratio (M/HRatio) was also suggested to be a biomarker of inflammation(9), (10). However there is no defined universal normal ratio. Forget et al. studied the normal value of N/L Ratio and they suggested a mean N/L Ratio as 1.65(11).

Demethylation of methionine, an essential amino acid, is a form of the four-carbon aminoacid homocysteine (Hcy). Its normal range is between 5-15 $\mu\text{mol/L}$ and above 15 $\mu\text{mol/L}$ is accepted as hyperhomocysteinaemia, which may be due to deficiency of vitamin B12, B6, folate, smoking, impaired renal function, aging and C677T homozygote mutation of 5,10 methylenetetrahydrofolate reductase (MTHFR) (12). Atherosclerosis and atherothrombotic disease is associated with hyper Hcy. Increased Hcy is a risk factor for ischemic stroke and one study showed that it was an independent stroke risk factor in 30% of Malaysian Patients (13).

According to the literature, N/L ratio, M/H ratio, and Hyper Hcy play role in the atherosclerotic cerebral ischemia and these biomarkers may be used to determine the risk of stroke .

To our knowledge, there is insufficient data in the literature about the correlation of these biomarkers in young stroke patients. In this study, we aimed to show if these biomarkers can be related to atherothrombotic cerebrovascular disease in the young population in order to provide information to detect young people under-stroke risk by using simple blood tests and help clinicians to lead them for further evaluation for primary protection of stroke.

Material and Methods

This is a retrospective study that included patients between January 2019 and March 2020 at Prof Dr. Cemil Tascioglu City Hospital and Ozel Acibadem Taksim Hospital Neurology Clinics. A total of 43 young stroke patients, age between 18- 55 years and age and gender-matched 42 healthy group were enrolled. Stroke patients were consisted of atherosclerotic ischemic cerebral ischemia. The transient ischemic attack, cardioembolic stroke, venous ischemia, recurrent stroke, and hemorrhages were excluded. Stroke diagnosis was based on clinical evaluation (acute onset of neurological deficit) and acute infarct seen on diffusion Magnetic Resonance Imaging (MRI). Patients with underlying infectious diseases, rheumatoid diseases, malignancy, other neurological diseases, and renal insufficiency (glomerular filtration rate <90 ml/mn) were also excluded. All stroke patients were screened by biochemical analysis, cerebral MRI, carotid-vertebral arterial Doppler ultrasonography, 12-lead ECG (24 Hour ECG if necessary). Control group were enrolled from patients who admitted to our outpatient clinics with headache and had normal neurological examination and normal cranial MRI and had no other systemic diseases. Fasting blood samples were taken between 8-12 am. Patients were accepted as hypertensive if the blood pressure was over 140 mmHg systolic and/or over 90 mmHg diastolic. Positive story of diabetes was accepted if there was use of diabetic medicine or fasting blood glucose level is over 125 mg/dl or HbA1c level is greater than 6%. Hyper Hcy was defined as Hcy level greater than or equal to 13 μ mol/L, since this was the cut-off value of our laboratory. Cases with a body temperature over 37.5 degrees Celsius and/or high Sensitive C reactive protein (CRP) over 0.5 mg/dL were considered to have acute infection and they were not included to the study. All stroke patients were evaluated with electrocardiogram (ECG) and echocardiography in order to exclude a possible cardioembolic source. Carotid and vertebral arterial doppler evaluation were done to all stroke patients and so arterial dissection was also excluded.

Our study has the approval of the institutional ethical committee (315/14.7.2020).

Statistical Analysis: The statistical analysis has been performed with IBM SPSS for Windows version 22.0. Numerical variables were given as mean \pm standart deviation or as median (25.-75. Percent)values. Categorical variables were shown as numbers and percentages. Ki square test or Fisher's test were used to showing any difference between the categorical variables of the groups. Kolmogrov Smirnov test was used to see if the numerical variables showed a normal distribution and the homogeneity of the

variants was tested with the Levene test. T-test was used to show any numerical difference between two independent groups, if the parametric test conjectures were provided and if the conjectures were not provided then Mann Whitney U test was used. Statistical significance was accepted as $p < 0.05$.

Results

This study involved 43 young stroke patients and 42 headache patients with the normal neurological examination to form the control group. There was no statistical difference between the groups ($p=1$). Figure 1 shows the gender distribution of the groups.

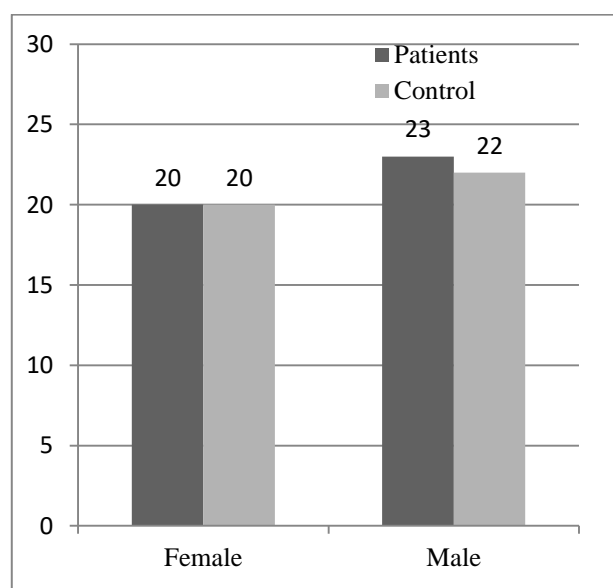


Figure1: Gender distribution of the groups

The ratios of Neutrophil to Lymphocyte (N/L) and monocyte to high-density lipoprotein Cholesterol (M/H) were both higher in the stroke group. This finding was statistically significant ($p=0.008$ and $p=0.011$ respectively). These differences of the N/L and M/H ratios between the groups were also analyzed according to the genders. There was no significance in the males but in the females, both ratios were higher in the stroke patients (Table 1 and 2).

Hyper Hcy was defined as a value equal to or greater than 13 μ mol/L. When the patients were sub grouped as having hyper Hcy or not; the N/L and M/H ratios did not show any significance (Table 3). Also, there was no significance within the genders.

Metabolic factors (B12, folate, and GFR levels) that can affect Hcy level were investigated in the patients' group; between the cases who had Hcy < 13 μ mol/L and \geq 13 μ mol/L. There were no significant differences of mean GFR and folate levels (Table 4), but the B12 level was significantly related to the higher Hcy level (Table 4).

Table 1: N/L and M/H comparison of the groups

GROUP		N/L	M/H
PATIENTS	N	43	43
	Average	2.5 ± 1.17	16.65 ± 11.03
	Minimum	0.87	5.42
	Maximum	5.81	72.73
CONTROL	N	42	42
	Average	1.86 ± 0.68	12.33 ± 5.19
	Minimum	0.99	4.78
	Maximum	3.63	23.23
p		0.008	0.011

Table 2: N/L and M/H comparison of the groups according to genders

GENDER		N/L	M/H	
FEMALE	PATIENTS	N	20	
		Average	2.65 ± 1.05	
		Minimum	1.03	
		Maximum	5.1	
	CONTROL	N	20	
		Average	1.93 ± 0.62	
		Minimum	0.99	
		Maximum	2.98	
	p		0.013	0.001
	MALE	PATIENTS	N	23
			Average	2.36 ± 1.27
			Minimum	0.87
Maximum			5.81	
CONTROL		N	22	
		Average	1.79 ± 0.74	
		Minimum	1.00	
		Maximum	3.63	
p		0.086	0.570	

Table 3: Comparison of N/L and M/H ratios according to Hcy levels.

GENDER		N/L	M/H	
FEMALE	Hcy < 13 µmol/L	N	3	
		Average	2.27 ± 0.13	
		Minimum	2.14	
		Maximum	2.41	
	Hcy ≥ 13 µmol/L	N	17	
		Average	2.71 ± 1.12	
		Minimum	1.03	
		Maximum	5.10	
	p		0.616	0.118
	MALE	Hcy < 13 µmol/L	N	11
			Average	2.55 ± 1.64
			Minimum	0.87
Maximum			5.81	
Hcy ≥ 13 µmol/L		N	12	
		Average	2.17 ± 0.85	
		Minimum	1.18	
		Maximum	4.33	
p		0.786	0.976	

Table 4: Comparison of metabolic factors between the cases having Hcy < 13 µmol/L and ≥ 13 µmol/L

Metabolic Factor	Hcy µmol/L	Hcy µmol/L			p
		N	Mean	Std. Deviation	
GFR	< 13	14	105.37	12.13	0.47
	≥ 13	29	101.51	18.26	
Folate	< 13	14	7.86	2.35	0.21
	≥ 13	29	6.68	3.13	
B12	< 13	14	237.21	89.98	0.01
	≥ 13	29	172.17	71.94	

Discussion

Atherosclerotic cerebral ischemia is related to local inflammation and it is shown that higher N/L ratio is associated with atherothrombotic acute cerebral ischemia(6). M/H ratio is also a biomarker of inflammation and in a recent study M/H ratio showed an association with the odds of having ischemic stroke(9), (10),(14).

In this study it is found that both ratios were higher in the stroke patients when compared to the control group. This finding is in line with previous studies. When we compared the ratios in the gender subgroup we found that these ratios were significant in the female; but not in the male gender. The reason for this finding may be the difference of inflammatory reaction in opposite genders.

It is shown that, in some regions, healthy females may have higher N/L ratio, especially in the young population (age under 50 years)(15)–(17). Estrogen decreases during menopause and this hormonal change affects neutrophils and inflammatory response(18). In a study, which has investigated the gender difference in the inflammatory response in stomach cancer patients; preoperative blood tests showed similar N/L ratios between males and females, but postoperative tests showed a higher N/L ratio in the females, because of increased neutrophils and decreased lymphocytes. It is concluded in the paper that the females showed more immune-compromised pattern of immune cells after gastric surgery (19). To the best of our knowledge, there are no studies of N/L and M/H ratios, concerning only young stroke patients and subgrouping genders. We conclude that gender difference and age are two factors that must be considered while interpreting N/L ratio in stroke patients and more studies with larger sample groups are needed.

Inflammation and lipid abnormalities are two factors that are considered in the pathophysiology of atherosclerosis (20), (21). Since monocytes increase during inflammation and HDL decreases in atherosclerosis, it is expected that M/H ratio would increase and in a recent paper, this ratio is studied in stroke patients. It is found that M/H ratio is an independent predictor of ischemic stroke (14).

In this study, it is found that M/H ratio was increased only in the female gender. One of the explanations of this gender difference is the difference of inflammatory response, similar with the N/L ratio in the females, as discussed before. Another explanation may be the difference between the HDL levels of the healthy cases and patients, so we also compared the HDL levels between the groups. When both genders were included in the analysis, the mean HDL level of the patients was 38.74 ± 8.16 and the controls' were 48.02 ± 12.63 ($p=0.0001$). This difference was greater in the female gender (37.45 ± 6.33 and 54.5 ± 14.09 ; $p=0.00001$) and no significant difference was present in the male gender (39.86 ± 9.46 and 42.13 ± 7.44 ; $p = 0.37$). This significantly lower HDL level in the patient group, especially in the female gender, is preventing us to come to a definite conclusion of the increased inflammatory response in the young stroke patients.

Future studies must be planned with sample groups having similar HDL levels in order to see the relation of M/H ratio and ischemic stroke in the young population.

Strong association is shown in a recent study between young stroke patients and hyper Hcy especially in male patients between 36-45 years of age (22). There is still a controversy in the literature about which stroke subtype is related with the hyper Hcy, but studies support a relationship between large artery atherosclerosis and increased levels of Hcy (22), (23).

We tried to investigate any relation between hyper Hcy and inflammatory biomarkers, N/L and M/H ratios. During the literature research, we found only one study investigating the relation between hyper Hcy and N/L ratio in hypertensive patients (5). In the study, the authors found a correlation between hyper Hcy and N/L ratio. However, we were unable to find the same correlation in our study in both genders. Metabolic factors (B12, folate, and GFR levels) that can affect Hcy level were investigated in the patients group; between the cases who had $Hcy < 13 \mu\text{mol/L}$ and $\geq 13 \mu\text{mol/L}$. There were no significant differences of mean GFR and folate levels, but the B12 level was significantly related with the higher Hcy level (Table 4). The literature does not have enough studies to come to a conclusion about the relationship of Hcy and inflammatory biomarkers, especially for the young stroke patients. More studies with larger sample groups may provide more certain results.

Conclusion

This study has several limitations such as the number of the cases included is not enough for a definite conclusion for the whole young stroke population. We don't know the exact smoking histories of the patients and the control groups since this data was not available for the most of the cases and smoking is a separate risk factor for both ischemic stroke and hyper Hcy. On the other hand this study contributes to the literature in the perspective of concerning only young stroke patients. There is very limited data of the inflammatory biomarkers for the young population and again there are few studies investigating the biomarkers according to the genders. We can conclude that N/L ratio should be interpreted carefully by concerning the age and the gender of the patients. Hcy seems not to play a role in the inflammatory biomarkers and M/H ratio should also be evaluated carefully with significantly lower HDL levels. Finally, we think further studies with large sample groups are necessary for the biomarkers of young stroke population.

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Ethical issues: All authors declare originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the authors responsibilities. The study was conducted under defined rules by the local ethics commission guidelines and audits.

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