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Correlation of Folic Intake and Internal Carotid Artery Intima-Media Thickness Changes In Post Ischemic Stroke Patients

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Abstract *– The thickness of the carotid artery intima media / intima-media thickness (IMT) is one of atherosclerosis markers. Atherosclerosis is one of the causes of ischemic stroke. Some studies suggest that low folate intake is predicted to affect the atherosclerotic process, but this remains controversial. Our objective is to analyze the relationship between folate intake with changes in the internal carotid artery IMT after ischemic stroke patients.The study is one group pretest posttest design with 72 post ischemic stroke subjects from neurology polyclinic of Kariadi Hospital, from June to December 2013. Folate intake was measured by Food Frequency Questionnaire and the internal carotid artery IMT by duplex ultrasonography. Measurements were taken at two periods with 6 months interval. Other factors that affect atherosclerosis consisting of age, obesity, hypertension, dyslipidemia, diabetes mellitus. The analysis in this study using Spearman correlation, chi-square and logistic regression. Resultwas significant if the p value were <0.05.*

There were 44 male subjects (61.1%) and 28 female subjects (38.9%). The mean age was 61.6 (SD = 7.99) years. The mean intake of folate was 178.10 (SD = 38.875) mg / day. Median serum folic acid level 8.43 (4.96 to 55.01) NML / L. The mean change in ICA IMT was 0.10 (SD = 0.156) mm. Folate intake was not correlated with serum levels of folic acid. Serum folic acid levels are not correlated with changes in the internal carotid artery IMT. There was correlation between the risk factors of age, BMI, hypertension, diabetes and dyslipidemia with changes in the internal carotid artery IMT.

Keywords – folate intake, thickness of carotid artery intima media, ischemic stroke.

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I. INTRODUCTION

Ischemic strokes are caused by intracranial atherosclerotic process.[1-3] Some studies show effect of serum folic acid on atherosclerosis.[4-7]Folic acid acts as a cofactor in the metabolism of cofactors homocysteine.[8] Inadequate cofactor effect on elevated homocysteine will increase the risk of atherosclerosis progression, stroke and cardiovascular diseases.[8-10]Negative folic acid status is caused by inadequate folate intake, aging, drugs that interfere with the absorption of folate, kidney disease and smoking.[8,11-19]. Although some research had widely showed relationship of folic acid status and stroke risks, albeit still controversial.[4,7,20-24] In this research linking folate intake status linked with progression of intima media thickness of the internal carotid artery in patients after ischemic stroke.

II. RESEARCH METHOD

Subject Recruitments

The study is one group pretest posttest design with 72 post ischemic stroke subjects from neurology polyclinic of Kariadi Hospital, from June to December 2013. The subject of this research is part of Risk Factors Influence on Atherosclerosis progression Patients Post Ischemic stroke in Kariadi hospital study. The patient had been briefed before the study, they approved and signed the informed consent. Exclusion criteria covered patients with impaired renal function, smoking, consumption of anticonvulsant drugs, methotrexate, and alcohol consumption interfering with the folic acid absorption or metabolism.[8,11-19]

Folate intake measurements

Measurement of intake using a food frequency questionnaire (FFQ) done 2 times with 6 months interval.

Subject folate intake is the average of measurements of first and second intake of folate. FFQ was formed based on the study of 20 subjects who underwent food recall for 3x24 hours. Data obtained from the most common foods containing folate consumed based on the food recall. Semiquantitative measure taken from household size referrence with cooked food ingredients available at home.

Blood samples and Laboratory Analysis

Blood sample of 3 cc taken after night fasting > 8 hours. Serum stored in -80° until used for serum folic acid measurement by ELISA.

The tunica intima media thickness measurement

Left and right carotid arteries of each subject was measured using ultrasonography siemens soniline Omnia FBE 0322. The tunica intima media carotid artery was examined from the transverse projection and carotid artery along the cervical area starting from the supraclavicular region toward the cranial area of mandible angle was probed. Tunica intima-media thickness of the internal carotid artery is the maximum thickness of 1 cm distal of bifurcation. Progression in the intima media thickness of the internal carotid artery is a difference of thickness between initial measurements and measurements of the next 6 months.

Statistical Analysis

Primary data were obtained from patients after ischemic stroke (gender, age, smoking history, intake of folate, serum folic acid levels and intima media thickness of the internal carotid artery). Data normality test using the Kolmogorov-Smirnov test. Statistical analysis of each factors affecting atherosclerosis using Spearman correlation, chi-square and logistic regression. Significant result if p value <0.05

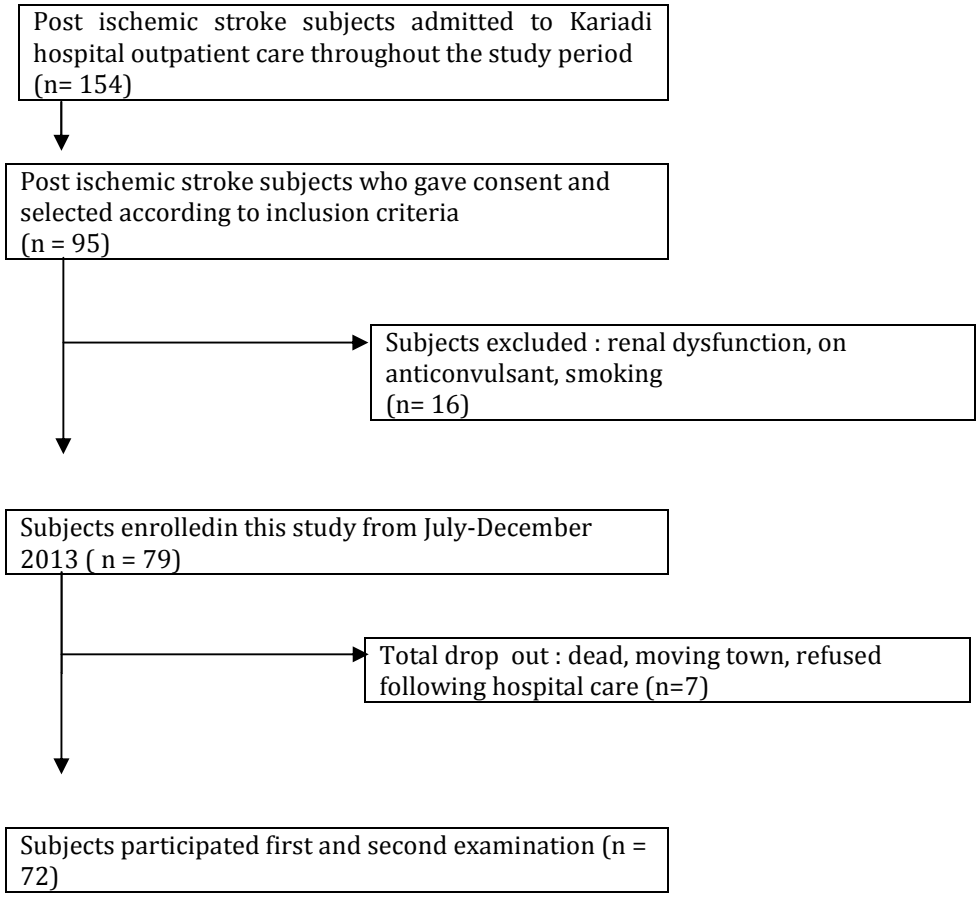


Figure 1. Participant flowchart in study subjects recruitment

III. RESULTS AND DISCUSSION

In this research, 72 subjects completed the study in 6-month period. Most subjects in this study were male, 44 (61.1%) and female, 28 (38.9%). The mean age of subjects 61.6 (SD = 7.99) years and the median age of the study subjects was 62 (44-81) years. Subject in the age group > 60 years amounted to 58.3%. In this study, the median intake of folate in the first examination was 119.50 (18 to 260.10) mg / day, the second median intake of folate was 219.8 (106.1 to 398.8) mg / day. The mean intake of folate first and second of each subject had a median value of 177.33 (114.55 to 292.05) mg / day. 50% subjects of this study fell in to the folate intake

group less than 177.33 g / day. Serum folic acid levels in the study subjects had a median value of 8.42 (4.96 to 55.01) nmol / L. The group of subjects with serum folic acid levels less than 8.42 nmol / L as much as 50%. At first ultrasound measurement, median right ICA IMT thickness was 0.60 (0.10 to 1.60) mm and the median thickness of the left ICA IMT was 0.60 (0.20 to 1.30) mm. In the second ultrasound measurement, median thickness of the right ICA IMT was 0.70 (0.2 to 1.40) mm and the median thickness of the left ICA IMT was 0.70 (0.12 to 1.6) mm. In this study, the median change in right ICA IMT (median delta right ICA IMT) was 0.09 (-1,08-0,8) mm and the median progression in left ICA IMT (median delta left ICA IMT) was 0.01 (-0 , 11 to 0.60) mm. The mean progression in left-right ICA IMT (mean delta ICA IMT) has a median was 0.05 (-0,54-0,6), the mean progression ICA IMT was 0.10 (SD = 0.156) mm. ICA IMT changes were grouped into two groups, namely the subject of progressive groups with a mean change in IMT of more than 0.1 mm ICA consisting of 24 subjects (33.3%) and non-progressive group with a mean change in ICA IMT is less than or equal to 0.1 mm consisting of 48 subjects (66.7%). In this study, folate intake was significantly correlated with serum levels of folic acid, a weak positive correlation (p = 0.008; r = 0.310). Folate intake with serum folic acid levels was not correlated in partial correlation test. In this study, folate intake was not correlated with progression in the intima media thickness of the internal carotid artery.

In this study, all subjects had lower folate intake according to folate nutritional requirements. RDA of folate in Indonesia is 400 µg/day which is equivalent to dietary folate equivalents (DFE) i.e 320 mg/day. Recommended folate intake in the adult population in some developing countries is 350 mg / day and 291 mg / day in European countries. In a previous study conducted in Germany on 178 subjects from elderly female population, around 80% showed dietary folate intake below minimum level.[34]

Serum folic acid level in this study had a mean of 9.85 (SD = 6.823) nmol / L. In this study of folate intake is correlated significantly yet weakly positively correlated with serum folic acid levels (P = 0.008 r = 0.310). Initial measurement of folate intake was not correlated with serum folate levels at first measurement (p = 0.982), this may be due to folic acid levels in serum fluctuating according to the last food consumed by the patient. FFQ is an intake questionnaire inquiring types of food containing folate often consumed by the patient and not the recent food consumed by patient. In a previous study, serum folic acid levels less than or equal to 9.2 nmol / L increases the risk of stroke by 1.37 times.[21] Other previous studies on 43.732 male subjects aged 40-75 years from the population of the health professionals in America free from cardiovascular disease and diabetes were followed for 14 years found a strong correlation between folate intake from the FFQ and serum folic acid levels (r = 0.63).20 In this study, the serum sample storage time of each subject differs so it will affect the serum folic acid level. Serum levels of folic acid with blood lysis increase due to erythrocyte folate levels.[8]

In this study there was no correlation between the progression in the thickness of the internal carotid artery intima with age and other risk factors of atherosclerosis, namely obesity, hypertension, diabetes mellitus and low folate intake. In this study, the number of subjects who experienced a change in intima media thickness progressive in men with a mean change in the intima media thickness of the internal carotid artery were progressively increased (> 0.1 mm) more than female subjects. A previous study in 1999 subjects of 425 males and 375 females from two cities who were included in the subject of the British Regional Heart Study and had risk factors for coronary heart disease, found that the common carotid artery IMT and bifurcation IMT correlated with stroke risk factors and prevalence of ischemic heart disease. Reports have shown that the relationship between the carotid artery intima thickness and atherosclerotic risk factors are inconsistent. This could be due to differences in carotid artery IMT measurement method, bifurcation mean, common carotid artery mean, internal carotid artery mean or a combination of these measurements. In IMT ultrasound measurements, there were also misinterpretation deviations of IMT measurement results, ranging about 0,05 mm to 0.1 mm compared to the actual length.[25,26] In a previous studies on 43.732 male subjects aged 40-75 years free from cardiovascular disease and diabetes, relating the intake of folate and vitamins B6 and B12 with the risk of ischemic stroke and hemorrhagic stroke. Folate intake was assessed every four years using a semi-quantitative food frequency questionnaire. The results of these studies indicated that increased folate intake is correlated with decreased ischemic stroke risk in men.[20]

In this study, age was not correlated with progression in the the internal carotid artery intima thickness. This can be due to the number of subjects included in the age group over 50 years is greater compared to the less than 50 years group. Although subjects of this study is of the ischemic stroke population, most subjects had normal baseline of internal carotid artery IMT i.e less than 0.72 mm.[29] However in the progressive IMT thickening group, total subjects over than 50 years of age are higher compared to group younger than 50 years of age. In this study, subjects with progressive IMT changes were more unbalanced compared to subjects with non-progressive IMT change. Age is one of the determinants of IMT, and may become confounding if not classified. The increase in IMT ranged from 0.01 mm to 0.02 mm per year, another evidence showed that IMT increased with age from about 0.01 to 0.3 mm per year.[25,26]

In this study there was no correlation between hypertension with changes in IMT thickness. This can be caused by single measurement of blood pressure. In this study, the number of subjects included in the hypertension group is greater than the non-hypertensive group. In the progressive intima media thickness change group, non-hypertensive subjects numbered more than the hypertensive subjects. In a previous study comparing changes in carotid artery intima media in subjects aged 18-45 years with hypertension grade 1 consisting of white coat hypertension patient and sustained

hypertension compared to normotensive subjects showed that there was no difference between white coat hypertension and sustained hypertension groups in mean maximum IMT and IMT values. Carotid artery IMT increased more rapidly in the white coat hypertension group compared to normotensive group, but there was no significant difference compared to sustained hypertension group.[27] In some interventional studies assessing the correlation of IMT changes as end points outcome of anti-hypertensive drugs and cholesterol lowering medicines, it was showed that IMT thickness decrease occurred after statin administration, but not after antihypertensive drugs administration.[25]

In this study, subjects classified in dyslipidemia with progressive changes in IMT thickness were less than dyslipidemia with non-progressive changes in IMT thickness group. A study on 175 patients with type 2 diabetes aged 35 to 70 years, showed that reduction of carotid artery IMT correlated with changes in postprandial blood sugar, but not correlated with hyperglycemia during fasting.[28] In this study, diabetes was not correlated with changes in internal carotid artery IMT. This can be due to diabetes mellitus classification based on all fasting blood glucose and postprandial blood sugar measurements.

In this study, serum folic acid levels were not correlated with carotid artery IMT thickness changes. First National Health and Nutrition Examination Survey Epidemiologic Follow-up Study involving 2006 subjects followed for 13 years found 98 ischemic stroke incidents. It showed that the serum folic acid levels less than or equal to 9.2 nmol / L increases the risk of stroke (RR 1.37; 95% CI, 0.82 to 2.29).[21]

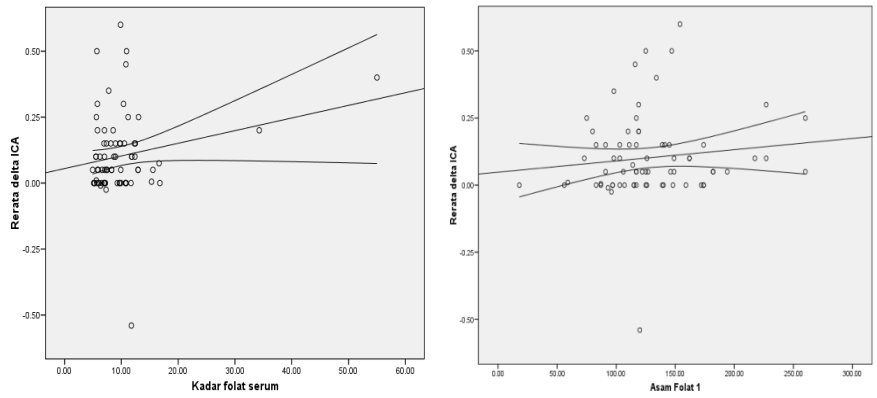
There was no correlation between folate intake with changes in internal carotid artery IMT in our study. 66.7% of the subjects had <0.1 mm changes in intima thickness. Tunica intima-media thickness of the carotid artery on the healthy population of METEOR study population was 0,79mm and RADIANCE I involving patients with hypercholesterolemia was 0,72mm.26,29 In previous studies, changes in the intima media thickness of the internal carotid artery by 0.1 mm increase after 6 months have 1,21 for HR.[35] Tthe National Health and Nutrition Examination Survey (NHANES I) in 9764 male subjects and women who participated in the National Health and Nutrition Examination Survey I Epidemiologic Follow-up Study (NHEFS) with baseline free of cardiovascular disease found that folate intake more than 300μg / day may reduce the risk of stroke by 20% while it was increased in the group of folate intake less than 136μg/day.[22] In a randomized double-blind placebo-controlled study on 819 men and post menopausal women with homocysteine levels > 13μmol / L in the Netherlands, subject received 800μg folic acid supplementation or placebo daily for 3 years. The results of these studies indicated that there is no significant difference between IMT change in folic acid group and placebo group.[29] Previous study on 506 subjects aged 40-89 years with initial homocysteine levels > 8,5μmol/ L with no diabetes and cardiovascular disease randomly received supplements (5 mg folic acid, 0.4 mg vitamin B12 + 50 mg Vitamin B6) or placebo for 3.1 years, primary and secondary outcome assessed by measuring carotid artery IMT through ultrasound and CT scan examination. The results of these studies showed that administration of high doses of vitamin B supplementation significantly decreased the progression of carotid IMT in healthy subjects with low cardiovascular risk factors and fasting homocysteine levels> 9.1 mol / L. The study was consisten with evidence that showed that the cardiovascular risk increased if the fasting plasma homocysteine levels reached 8-9μmol / L.30 In meta-analysis study of 10 researches involving 2052 subjects, folic acid and changes in carotid artery IMT were end points of the study. The results of the meta-analysis showed that folic acid supplementation is effective in lowering the progression of carotid artery IMT, especially in subjects with chronic kidney disease or high risk of cardiovascular disease in subjects with higher carotid artery IMT baseline and subjects with greater decrease in homocysteine levels.[31] In this study there was no correlation between folate intake with changes in the thickness of the internal carotid artery IMT. This can be caused due to under FDA standards folate intake in all study subjects and nutritional requirement in Indonesia was 400μg / day, folate intake was also influenced by aspirin which is one of the main post ischemic stroke therapy, which was not followed during research period research. Aspirin has anti-inflammatory effects that can inhibit the absorption of folate in the intestine. Aspirin is one of the treatment administered to patients after ischemic stroke. Aspirin as an tromboxan A2 inhibitor anti-platelet aggregation inhibits the process of hemostasis.[32]

In multivariate logistic regression analysis linking factors affecting changes in the intima media thickness of the carotid artery, such as age, folate intake, serum folic acid levels, BMI, hypertension, diabetes mellitus, and dyslipidemia. In this study it was showed that diabetes mellitus and dyslipidemia were most dominant in relation to changes in the intima media thickness of the internal carotid artery in patients after ischemic stroke among other factors. Vascular endothelial damage found in patients with diabetes mellitus will trigger atherosclerotic process. In patients with dyslipidemia, LDL excess will enter the artery and remain in the intima, especially arteries with injured endothels. Enzymes and free radicals will release inflamed lipid and cause endothelial cells susceptible to leukocytes adhesion. Modified LDL was eventually cleared by scavenger receptors eventually forming foam cells.[33]

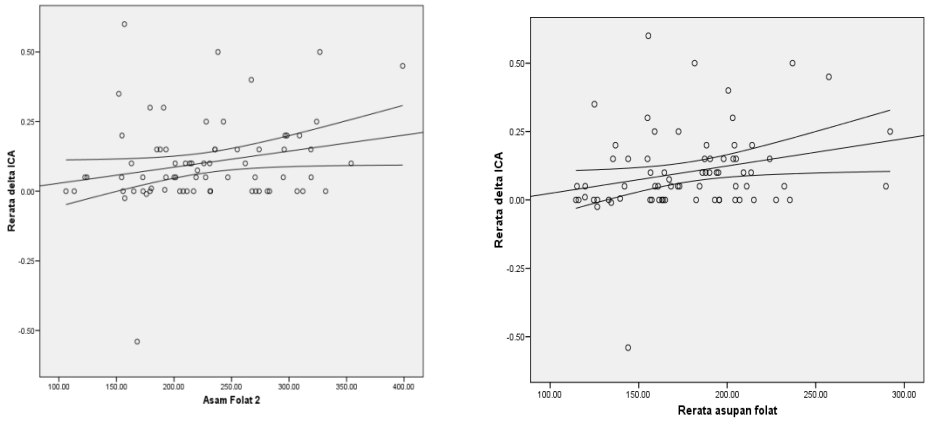
The hypothesis in this study can not be proven. This may result from ischemic stroke therapy factors affecting the absorption of folic acid and the progression of atherosclerosis of which had not been well controlled and followed in this study.

Table.1. Frequency Distribution ofStudy Subjects Characteristics

Variables	Frequencies N (%)
Demographic Factor	
Gender	
Male	44 (61,1)
Female	28 (38,9)
Age	
45-50	4 (5,6)
51-54	10 (13,9)
55-59	16 (22,2)
≥60	42 (58,3)
Atherosclerotic Risk Factor	
BMI	
Overweight	11 (15,3)
Normal	61 (84,7)
Hypertension	
Yes (systolic > 140 or> 90 mmHg)	24 (33,3)
No (systolic≤140 dan diastolic≤ 90 mmHg)	48 (66,7)
DM	
Yes	12 (16,7)
No	60 (83,3)
Dyslipidemia	
Yes	60 (83,3)
No	12 (16,7)
Folic Acid	
1st Folic Intake	
<320 µg / day	72 (100)
≥320 µg / day	0 (0)
2nd Folic Intake	
<320 µg / day	67 (93,1)
≥320 µg / day	5 (6,9)
Mean 1st & 2nd Folic Intake	
<320 µg / day	72 (100)
≥320 µg / day	0 (0)
Serum Folic Level	
≥9 nmol/L	40 (55,6)
<9 nmol/L	32 (44,4)
Intima Media Thickness (IMT)	
1st Right ICA IMT	
>0,7 mm	15 (20,8)
≤0,7mm	57 (79,2)
1st Left ICA IMT	
>0,7 mm	15 (20,8)
≤0,7 mm	57 (79,2)
2nd Right ICA IMT	
>0,7 mm	32 (44,4)
≤0,7mm	40 (55,6)
2nd Left ICA IMT	
>0,7 mm	32 (44,4)
≤0,7 mm	40 (55,6)
Right ICA IMT Delta	
>0,1 mm	18 (25)
≤0,1 mm	54 (75)
Right ICA IMT Delta	
>0,1 mm	23 (31,9)
≤0,1 mm	48 (68,1)
Mean ICA IMT Delta	
>0,1 mm	24 (33,3)
≤0,1 mm	48 (66,7)



P= 0,171; *r*=-0,163[£] *P*=0,915; ,013[£]



***P*= 0,074*; r*=-0,212[£] *P*=0,164*; r*=-0,166[£]**

Figure 2.Correlation of folic intake with internal carotid arteries intima media thickness progression in scatter plot. (IMT ICA).

Description : [£]Spearman's Correlation

Table 2. Logistic Regression of correlation of risk factors with carotid arteries intima media thickness changes

Variables	IMT ICA Progression		Bivariate		Multivariate	
	Progressive	Non- Progressive	Statistics	*p-value*	*p-value*	OR (95%CI)
	n = 24	n = 48				
Age (≥ 50 years)	23	45	Fisher=0,000	1,000[€]		
1st Folic Acid Intake (< 177 µg/day)	22	42	Fisher=0,018	0,710[€]		
2nd Folic Acid Intake (< 177 µg/day)	3	13	X^2=1,969	0,161[£]	0,705	0,708 (0,118 – 4,249)
Mean folic acid intake (< 177 µg/day)	9	27	X^2=2,250	0,134[£]	0,300	0,562 (0,189 – 1,669)
Serum Folic Level (< 8,425 nmol/L)	9	27	X^2=2,250	0,134[£]	0,604	0,735 (0,229 – 2,354)
BMI (Overweight)	2	9	Fisher=0,657	0,316[€]		
Hypertension (+)	6	18	X^2=1,125	0,289[£]		
Diabetes Mellitus (+)	7	5	Fisher=2,813	0,089[€]	0,077	3,273 (0,880 – 12,165)
Dyslipidemia (+)	23	37	Fisher=2,813	0,051[€]	0,090	6,329 (0,750 – 53,422)

Description : [£] Pearson Chi Square ; [€] Fisher Exact Test

CONCLUSIONS

There was no relationship between folate intake with serum folic acid levels in patients after ischemic stroke. There was no relationship between serum folic acid levels with changes in the intima media thickness of the internal carotid artery in post ischemic stroke patients. There was no relationship between folate intake with changes in internal carotid artery intima media in post ischemic stroke patients. There was no relationship between age, obesity, diabetes mellitus, dyslipidemia and hypertension with intima media changes the internal carotid artery in post ischemic stroke patients

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