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Abstract - Background and Objective : A number of studies have previously described elevated levels of homocysteine as an independent coronary heart disease and stroke risk factor. The results of above studies show different data for the methylenetetrahydrofolate reductase (MTHFR) genetic polymorphism and hyperhomocysteinemia, which is the cause of cerebrovascular accident. Purpose of the study was to determine whether there is a link between hyperhomocysteinemia and A1298CC, C677 genotype associated with acute cerebral infarction.

Methods : The prospective study included patients (n=102) with acute cerebral infarction, regardless of its genesis or transient ischemic attack, and patients with a history of cerebral infarction of any age, that correlates with the imaging techniques.

Keywords : cerebrovascular disease; homocysteine; mutation; risk factor; stroke.

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Increased Serum Homocysteine Level Association with Methylenetetrahydrofolate Reductase C677T and A1298C Mutations in Patients with Cerebral Infarction in the Latvian Population

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Summary - Background and Objective : A number of studies have previously described elevated levels of homocysteine as an independent coronary heart disease and stroke risk factor. The results of above studies show different data for the methylenetetrahydrofolate reductase (MTHFR) genetic polymorphism and hyperhomocysteinemia, which is the cause of cerebrovascular accident. Purpose of the study was to determine whether there is a link between hyperhomocysteinemia and A1298C, C677 genotype associated with acute cerebral infarction.

Methods : The prospective study included patients (n=102) with acute cerebral infarction, regardless of its genesis or transient ischemic attack, and patients with a history of cerebral infarction of any age, that correlates with the imaging techniques. The control group (n = 34) consisted of patients without a history of cerebrovascular disease, showing no indication of previous strokes according to imaging techniques. Homocysteine was determined using IMMULITE 2000 testing system.

Results : Comparing the both groups, increased homocysteine level association with the cerebrovascular event was not observed (p=0.4). By studying the genetic polymorphism of MTHFR, a statistically significant relationship of elevated homocysteine with C677TT (p=0.15), C677CT (p=0.61) and C677CC (p=0.90) was not detected. Similar results were obtained for A1298 genetic polymorphism.

Conclusion : This study showed that there is no link between hyperhomocysteinemia and MTHFR genetic polymorphism in the investigated population associated with risk of acute cerebral infarction. Taking into consideration the relationship of homocysteine with folic acid and vitamin B12 levels, the next phase of study will include the determination of these two parameters in addition.

Keywords : cerebrovascular disease; homocysteine; mutation; risk factor; stroke.

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I. INTRODUCTION

In Latvia mortality rate from ischemic and hemorrhagic stroke is 230/100 000 annually for people aged from 35 to 74 years (1); cerebrovascular diseases is the leading cause of severe disability (2). Healthcare costs due to cerebrovascular diseases in the year 2003 accounted for 21 billion euros in all 25 European Union countries alone (3).

Due to the trend of aging of the European Union's population the topicality of the problem is growing. Solution could be targeted identification of the ischemic stroke risk factors and their following modification. Multiple etiological moments of the cerebral infarction make the solution of these problems difficult. Atherothrombosis and atherosclerosis are important causes of stroke, the most common in the younger age group (4). Atherosclerosis is also indirectly involved in other stroke etiology and pathogenesis stages, for example, like coronary heart disease. A number of studies have previously described elevated level of homocysteine as an independent coronary heart disease (5) and stroke (6) risk factor, and there are indications of its possible special connection with cerebral infarction due to the large blood vessel atherothrombosis (7). However, the situation is not so unequivocal as there are also studies that do not detect such a relationship to cardiovascular diseases (8), (9). Moreover, taking into account the heterogeneity of etiology and pathogenesis of stroke, it is not completely clear how this relates to its sub-types. The situation is further complicated by the fact that right now most studies have not demonstrated the efficacy of folic acid in cerebrovascular and cardiovascular event reduction (10), (11), (12), there is only one meta - analysis, which indicates the contrary (13). Similar is the situation in the B group vitamins combination use in the cardio- and cerebrovascular disease risk reduction - several studies showed no reduction (14), (15), while others showed a positive effect (16), (17), but, for example, one study

showed that folic acid in combination with B vitamins possibly increases the risk of revascularization following percutaneous coronary intervention (18).

II. MATERIALS AND METHODS

The research was done in VSIA „Pauls Stradins Clinical Hospital” during the period from 01/01/2011 to 15/12/2011. The study was prospective in nature. The case study group included patients with acute cerebral infarction regardless of its genesis, or transient ischemic attack, as well as patients with a history of cerebral infarction of any age that correlated with the imaging techniques data. The control group consisted of patients without a history of cerebrovascular disease, or episode, excluding patients showing indications of prior cerebrovascular diseases according to the imaging techniques.

The work was received the Ethics Committee of Riga Stradiņš university approval.

Demographic, clinical and paraclinical data were taken from routine questionnaires, which are used in the stroke registry. For the control group was used in the same questionnaire as for the case group, only it was completed in the modified scale.

Homocysteine was determined using IMMULITE 2000 testing system, which uses solid phase hemiluminiscence enzyme immunoassay test for L-homocysteine quantitatedetermination in plasma and serum. It is based on two cycles – release of the bound homocysteine, and its subsequent conversion to S-adenosyl-L-homocysteine (SAH) and the immune response. Antibodies used in the test system are specific for homocysteine. Elevated homocysteine value was considered to be more than 14.25 mmol / l according to the American Stroke Association Guidelines (19). DNA was isolated from venous blood using standard phenol - chloroform method. Genotyping of C677T and A1298C polymorphisms was done by PCR and RFLP analysis (restrictases HinfI, MbolI). The study database was created in EpiData software. For statistically processing the STATA StataCorp (2007) Statistical Software was used: Release 11. College Station, TX: StataCorp. Both descriptive and analytical statistical methods were applied.

III. RESULTS

a) Demographic Data

Among 137 of the study subjects 43, 1% (59/137) were men and 56, 9% (78/137) women. Distribution by sex between cases and controls was not statistically significant ($p = 0.512$). In the control group, the age ranged between 45 and 87 years with median of 67 years and quartile boundaries between 59 and 72 years. In the cases group age ranged between 39 and 88 years with a median of 71 years and the quartile limits between 65 and 78 years. The both groups were

statistically significantly different by age, $p < 0.01$; the cases group was statistically significantly older.

b) MTHFR Genetic Polymorphism

C677 genetic polymorphism results for the cases and control groups are summarized in the 1th table.

A1298 genetic polymorphism represents the 2nd table, where A1298CC - homozygote, which is due to the labile form of MTHFR, A1298AA – a „normal” homozygote.

Homocysteine levels have been measured in 136 individuals, the average rate was 13.2 $\mu\text{mol} / \text{l}$, with a standard deviation of 4.7. In the cases group (102 persons), it ranged from 4.7 to 32.3 $\mu\text{mol} / \text{l}$, and the average level was 13.2 $\mu\text{mol} / \text{l}$ with a standard deviation of 4.85. In the control group (34 persons) homocysteine level ranged from 8.2 to 25.2 $\mu\text{mol} / \text{l}$, and the average rate was 13.4 $\mu\text{mol} / \text{l}$ with a standard deviation of 4.2. Between the two groups a statistically significant difference was not observed, $p = 0.4$. Relationship of an elevated homocysteine level with cerebrovascular event was not established, OR = 1.04, 95% confidence interval 0.42 to 2.66; $p = 0.9$.

c) MTHFR and Hyperhomocysteinaemia

Analyzing the association of an elevated homocysteine level with MTHFR genetic polymorphism no statistically significant relationship with C677TT mutations was observed, $p = 0.15$, such relationship was not found also with the C677CT genotypes, $p = 0.61$ and also with the C677CC – normal homozygote, $p = 0.90$. The 3rd table illustrates the tested relation of C677 genetic polymorphism to elevated serum homocysteine level.

Similar analysis was also done for A1298 genetic polymorphism, where there were found the same results, no statistically significant relationship between hyperhomocysteinemia and A1298CC genotype, $p = 0.09$, heterozygous variant, $p = 0.21$ and the normal homozygote, $p = 0.94$ was detected, that represent 4th table.

IV. DISCUSSION

The paper's findings have already been extensively studied and described before. From our neighbors in the north of Poland: their study has already found no link between the two investigated MTHFR polymorphism variants, in addition the genotype C677TT in the target group was found in 8% cases and A1298CC in 14% cases, which is close to our results, only in this study there was found the link of a variant of C677TT polymorphism with increased level of homocysteine in the serum (20). The study of our other neighbours in Sweden also found no relationship between the C677TT mutation, hyperhomocysteinemia,

acute cerebrovascular event, internal carotid artery stenosis and folic acid level (22). In this study the TT genotype was present in 10% of both control and cases groups. Another study from a country from our region - Denmark also did not show a direct relation of C677TT genotype with stroke, but did show relation of C677TT genotype with elevated homocysteine level; C677TT genotype was found in the 10.6% and 8.3% persons of the cases and control groups correspondingly (23). Another study, in which target group consisted of younger people with an acute stroke from Poland, also found no relationship between C677TT genotype in this age group, the TT genotype occurred in 12% persons in the stroke group and 10.9% in the control group (24). In Russia the genotype C677TT according to literature occurs in 8.4% of healthy population (25). Without taking into account the small size of the control group, but only considering the genotype frequencies of the target group, it can be concluded that there are no significant differences comparing with our neighbouring countries.

After the last great meta-analysis, at which was assessed the relationship between MTHFR genetic polymorphism, hyperhomocysteinaemia and dietary folic acid quantity, it was concluded that in areas with

increased use of folic acid or permanent food enrichment there is no benefit from reducing homocysteine with a purpose to prevent stroke. MTHFR 677C→T polymorphism association with stroke should be investigated in areas with a low folate level (21). In this phase of the study, neither vitamin B₁₂, nor folic acid were measured, this is planned to do in the next phase. The author failed to find large and high-quality population data on the vitamin B₁₂ and folic acid levels in the blood of Latvia's population as well; hopefully, we will be able to successfully reply to this question in our next study. In view of the above facts it is not possible to speak certainly on the lack of association between the two investigated genotypes and homocysteine level.

V. CONCLUSION

The incidence of MTHFR C677 T and C alleles was established, which will be used to calculate the size of the control and cases groups of the next phase of the study. The identical work was done with A1298 A and C alleles. It was found that there is no relationship between the hyperhomocysteinaemia and the studied MTHFR genetic polymorphism in the incorporated population.

VI. CONFLICTS OF INTEREST

The authors declare no conflicts of interests.

Table 1 : C677 genetic polymorphism in the control and cases groups. C677TT - homozygote that is associated with the MTHFR thermolabile form, C677CC - "normal" heterozygote

Group	Genotype			Total
	C677TT; N=13, 10.2 %	C677CT; N=47, 36.7 %	C677CC; N=68, 53.1 %	
Cases	10 (10.1)	33 (33.3)	56 (56.6)	99 (100.0)
Controls	3 (10.3)	14 (48.3)	12 (41.4)	29 (100.0)

Table 2 : A1298 genetic polymorphism in the cases and control groups

Group	Genotype			Total
	A1298CC; N=23, 17,4%	A1298AC; N=50, 37,8 %	A1298AA; N=59, 44,7 %	
Cases	17 (17.0)	40 (40.0)	43 (43.0)	100 (100,0)
Controls	6 (18.8)	10 (31.3)	16 (50.0)	32 (100.0)

Table 3 : C667 genetic polymorphism in relation to the serum elevated homocysteine level

Homocysteine level				
Genotype	Elevated, N (%)	Normal, N (%)	OR; 95% confidence interval	p value
C677TT (N=13, 10.1%)	11 (12.8)	2 (4.8)	0.33; [0.03 – 1.65]	0.15
C677CT (N=47, 36.7%)	17 (40.5)	30 (34.9)	1.22; [0.53 – 2.77]	0.61
C677CC (N=68, 53.1%)	23 (54.8)	45 (52.3)	1.05; [0.47 – 2.33]	0.90
Total (N=128, 100%)	42 (100%)	86 (100%)		

Table 4 : Relation of the A1298 genetic polymorphism to elevated serum homocysteine level.

Genotype	Homocysteine level		OR; 95% confidence interval	p value
	Elevated, N (%)	Normal, N (%)		
A1298CC (N=23; 17.4%)	11 (25.6)	12 (13.5)	2.21; [0.79 – 6.07]	0.09
A1298AC (N=50; 37.9%)	13 (30.2)	37 (41.6)	0.61; [0.26 – 1.40]	0.21
A1298AA ; (N=59; 44.7%)	19 (44.2)	40 (44.9)	0.97; [0.44 – 2.15]	0.94
Total (N=132; 100%)	43 (100%)	89 (100%)		

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