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Scientific Project

Homocysteinemia and Methylentetrahydrofolate Reductase Gene Mutation as a Risk Factor for Blood Vessel Disease

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Total homocysteine; immuno-enzyme method; reference values; arterial occlusive disease; thrombosis of deep veins; mutation; methylentetrahydropholat reductase.

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Abstract

The aim of the project was to investigate homocysteinemia and Methylentetrahydrofolate reductase (MTHFR) gene (C677T) mutation with vascular diseases. The investigation comprised a total number of 378 subjects divided into two main groups: 194 healthy individuals and 184 patients: 91 with arterial occlusive disease (AOD) and 93 with thrombosis of deep vein (TDV). Concentration of total homocysteine (tHcy) in serum was determined using the modified immuno-enzyme method. Mutation of *MTHFR C677T* was examined with polymerase chain reaction with CVD StripAssay (ViennaLab Labordiagnostika GmbH) as a segment of 12 mutations of the cardiovascular system. Concentration of tHcy in patients with AOD was 16.3 ± 8.4 μmol/L in men, 14.1 ± 3.3 μmol/L in women and it was statistically significantly increased as compared to healthy subjects from the same gender ($p<0.001$). Concentration of tHcy in men with TDV was 14.0 ± 3.5 μmol/L, 13.4 ± 3.0 μmol/L in women and it was significantly increased in comparison to healthy subjects ($p<0.001$). Concentration of homocysteine in serum of healthy subjects with different genotype of *MTHFR C677T* showed significantly higher values for *TT* when compared to *CC* genotype as well as to *CT* genotype. Comparison between healthy and sick individuals both separately, AOD and TDV, or as one entity, showed significantly higher values for the genotypes *CC* and *CT*, but not for the *TT* genotype. There was no correlation of *MTHFR C677T* gene mutations with AOD and TDV.

Ptroject Type : National.

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Project Objectives: The original aims of the project were**:** 1) to modify, elaborate and introduce an immuno-enzyme method for determination of total homocysteine concentration by Frantzen et al., examining the quality parameters of a certain method; 2) to determine reference values of total homocysteine (tHcy) in the Macedonian population; 3) to determine tHcy concentration in patients with arterial occlusive disease (AOD) and thrombosis of deep veins (TDV); 4) to determine the prevalence of *C677T* mutation of the enzyme methylentetrahydrofolate reductase (*MTHFR*) in healthy population, in patients with AOB and TDV and 5) to examine the correlation between *C677T* genotype and serum homocysteine values in the healthy population and in both groups of patients.

Project Results: Modification of immuno-enzyme method by Frantzen et al. consists of shortening the incubation period of immune reactions for 10 minutes and using smaller quantity of rabbit anti-mice antibodies in comparison with the original method. The BIAS of immuno-enzyme method is <7.2%, precision in series CV <6.1% , precision day by day CV <8.2%, recovery from 93 to 114%. The comparison of the modified immuno-enzyme method with the immuno-enzyme method with fluorescence polarization has shown high correlation (r=0.9862).

Reference tHcy values in men are 11.57 ± 1.97 μmol/L, whereas in women they are 8.63 ± 2.64 μmol/L, which means they depend on age.

Concentration of tHcy in patients with AOD was 16.3 ± 8.4 μmol/L in men, 14.1 ± 3.3 μmol/L in women and it was statistically significantly increased as compared to healthy subjects from the same gender ($p<0.001$). Concentration of tHcy in men with TDV was 14.0 ± 3.5 μmol/L, 13.4 ± 3.0 μmol/L in women and it was significantly increased in comparison to healthy subjects ($p<0.001$).

In the Macedonian healthy population, *C* allele was dominant with frequency of 0.645 in *MTHFR C677T* gene mutation, whereas *T* allele was found with frequency of 0.355. Frequencies of *T* and *C* alleles of the gene of *MTHFR C677T* were similar in patients with AOD and in patients with TDV. They were also similar in the analysis of the patients as a group with cardiovascular diseases (CAD). The greatest frequency in the Macedonian healthy population of *MTHFR C677T* gene mutations had the heterozygote gene *CT* with about 45%, followed by the homozygote for the wild genotype *CC* with about 42% and the lowest frequency had the genotype *TT* with 13%. The greatest frequency of *MTHFR C677T* gene mutations had the heterozygote genotype *CT* with about 52% in patients with AOD, TDV and CAD as a whole group. Homozygote for the wild genotype *CC* in all examined groups of patients (AOD, TDV and CAD) was present in 31-36%. The genotype *TT* had the lowest frequency (13-16%) in patients with AOD, TDV and CAD as a whole. There was no significant difference of *MTHFR C677T* gene mutations as risk factors for AOD TDV and CAD analyzed as a whole entity. The values of odds ratio in all examined groups ranged from 0.73 to 1.6, indicating that subjects with genotype *CT* or *TT* had approximately equal risk for onset of AOD or TDV.

Concentration of homocysteine in serum of healthy subjects with different genotype of *MTHFR C677T* showed significantly higher values for *TT* when compared to *CC* genotype as well as to *CT* genotype. Comparison between healthy and sick individuals both separately, AOD and TDV, or as one entity, showed significantly higher values for the genotypes *CC* and *CT*, but not for the *TT* genotype.

There was no correlation of *MTHFR C677T* gene mutations with AOD and TDV.

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