CLINICAL STUDY

Homocysteine Slovakia study: study design and occurrence of hyperhomocysteinaemia and other risk factors

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Abstract: *Background:* Slovakia belong to the European Union countries with the high incidence and prevalence of cardiovascular diseases in general and IHD in particular.
Method: Homocystein-Slovakia is crossectional population study realised in subjects in high risk age 35–75 years in two groups. The first consists of patients with verified stable ischemic heart disease (IHD) (M=152; F=167) aged 60.8±9.37 yrs (males) res. 63.1±7.56 years (females) (NS) who were randomly selected from two cardiological registrars. Second population was formed by general population who were dichotomised according their medical records into IHD patients (M= 31, F= 53) and apparently healthy controls (M=47; F=55), the later in significantly younger age as patients, but in same age for intergender comparison 49.6±10.3 vs 46.6±9.2 yrs (NS).
Results: We found very high prevalence of classic as well as newer risk factors and risk markers both in IHD patients and in controls.
Increased homocysteinen (Hcy >15 μmol/l for males and Hcy >13 μmol/l for females) was found even in 32.9 % of patients and 13.6 % of controls (p<0.001). Comparison of regulating vitamins levels between IHD patients and controls demonstrated similar prevalence.
Conclusion: Homocystein Slovakia study found very high prevalence of hyperhomocysteinemia in patients with stable ischemic heart disease. Even the prevalence in healthy controls correspond to data resported for MI patients in Western countries. Vitamins regulating metabolism of homocysteine also shown high prevalence, however, without differences between IHD pacients and controls *(Tab. 5, Fig. 1, Ref. 27).* Full Text in PDF *www.elis.sk.*
Key words: homocystein Slovakia, epidemiology, risk factors, vitamins B6, B12 and folic acid.

Increased plasmatic level of homocysteine (HCy) is associated with serious clinical manifestation of atherosclerosis such as myocardial infarction (MI), stroke or peripheral vascular disease (1, 2).

Population studies have documented a prevalence of hyperhomocysteinaemia at about 10 %, however, in a selected group of patients with apparent ischaemic heart disease (IHD) the prevalence reached up to 30–40 % (3, 4). Nested case-control studies showed that a 5-μmol/L increment in HCy results in a 20–30 % increase in cardiovascular risk (5), however despite the decrease in homocysteine, intervention did not decrease the cardiovascular or total mortality (6, 7).

Homocysteine is formed in the body from methionin. Methionin is metabolised via re-methylation and transsulphuration. These processes depend on vitamins B12, B6 and folic acid, thus regulated through nutrition.

Slovakia has a very high incidence and prevalence of cardiovascular diseases in general, and IHD in particular (8), so far however the prevalence of homocysteine and related vitamins has not been studied sufficiently.

The goal of the present study was to investigate the prevalence of hyperhomocysteinaemia and its association with risk factors of atherosclerosis in patients with ischaemic heart disease (IHD) and in the general population from selected rural and urban districts of Slovakia.

Materials and methods

We randomly selected both, male and female Caucasian patients between 35–75 years of age with known IHD from a mixed urban/rural district of Nové Zámky (standardised mortality: (SM) = 10.4), from the city of Bratislava (the capital of the Slovak Republic) (SM = 9.8) and we invited all residents from the village of Veľký Lom and its surrounding area (district Veľký Krtíš) (SM = 12.6) (9). The age range was chosen to estimate HCy levels in age groups with expected high risk of cardiovascular events. Patients suffering from megaloblastic anaemia, malabsorption or inflammatory bowel disease, serum-positive arthritis, drug addicts, toxicomania and alcoholism (Jelinek IV), chronic renal disease of stages III and IV according to K/DOQI, extensive trauma less than three months before the study, maniodepressive psychosis,

Tab. 1. Selection process of Homocystein Slovakia participants.

Nové Zámky, Bratislava		Veľký Lom	
Registars	6228	Population	325
Age (35-75 years)	3627	Age (35-75 years)	223
Proved IHD	1281		
Randomised	700		
Invited	300	Examined	186
Examined	247	Proved IHD	84
Participation	82.3 %	Participation	83.4 %

Abbreviations: IHD – ischemic heart disease

gastric surgery, immobility, acute MI or coronary intervention less than three months before study, on cancer chemotherapy or radiotherapy less than three months before study, on vegan diet, and polyvitamin substitution were excluded from the study. To obtain a high participation rate we published a series of articles in local newspapers explaining the goals of the study, and the patients were invited by telephone to participate. Local authorities supported the study by explaining its goals through local radio networks and by providing facilities to conduct the investigation.

IHD patients were selected from the register of two district cardiologists in Nové Zámky and one in Bratislava. IHD was defined as 1) MI requiring hospitalisation 2) and/or typical angina pectoris (AP) or 3) proven and treated silent IHD documented by medical report and ECG signs of ischaemia.

Two trained research assistants reviewed all medical records from the registers and included or excluded all subjects according to the set criteria. Subsequently the principal investigator examined all records and confirmed inclusion and exclusion criteria.

All adult residents from Veľký Lom and its surrounding area in age range of 35–75 years were invited to participate in the study. Their medical records were assessed in the offices of family physicians and the patients were excluded based on the principles described above. The results of the selection process according to the inclusion and exclusion criteria in individual districts are shown in Table 1.

At the end of the screening process, 130 patients in Nové Zámky, 117 in Bratislava, and 186 subjects in Veľký Lom were included in the study (Tab.1).

The selected patients and respondents had a brief medical examination and were interviewed by a physician using a standardised questionnaire. All physicians were pre-trained for filling in the standardised questionnaire. The questionnaire recorded personal and family history, nutritional habits assessing the basic pattern of meat, vegetable, fruit and salt intake, smoking status (current, former smoker, never smoked), physical activity, and risk factor evaluation. Socioeconomic status was evaluated by income and level of education (primary school, secondary school, university). Professional status was evaluated according to ILO standard classification of occupations (11). IHD part of the questionnaire was based on the Rose Protocol for Angina Pectoris (12). Blood pressure was measured in a separate quiet room after a five-minute rest. Three standard mercury sphygmomanometer measurements were taken from the right arm in a sitting position. Only the last two measurements were considered in the study.

We computed the absolute CHD risk according to recommendations of European Heart Society (13) using a special software developed for the study. Smoking index of risk assessment in current and former smokers was calculated (14).

Venous blood samples were collected into EDTA-Potasium tubes to obtain plasma and into standard tubes to obtain serum after overnight fasting without cubital compression. Blood for plasma was immediately centrifuged in a cooled centrifuge (4 °C) for 30 minutes; blood for serum was allowed to clot for 15 minutes at room temperature in range of 22–25 °C and underwent the same centrifugation procedure. Supernatants and sediment from plasma blood samples were removed and stored in cryovials. All three blood derivates were stored immediately after the procedure at -70 °C. Plasma levels of total cholesterol (TC) and triglycerides (TG) were measured enzymatically; ApoB and apoAI levels were measured by immunoturbidimetric method; HDL cholesterol (HDL-C) was determined directly by commercial kit (Genzyme) on autoanalyser (Hitachi 911, Roche, Switzerland). LDL-C was both, evaluated by direct method for LDL-C measurement (Randox, UK) and calculated using the Friedewald formula only if the triglyceride concentration was below 4.5 mmol/l (15). Glycaemia, Na, K, Ca^{2+}, Fe, Cl, Mg, creatinin, BUN, albumin, bilirubin, ALT, AST, and GMT were measured using standard methods. EDTA vacutainers for homocysteine measurement were kept on ice and centrifuged within 15 minutes after blood drawing in cooled centrifuge (3000 rpm/30 min/4 °C). The plasma was then stored at -80 °C until analysed by high-performance liquid chromatography (HPLC) using a standard kit (CHROMSYSTEMS Instruments & Chemicals GmbH, Munich). The interassay coefficient of variation of the method was 4.6 %. Moreover, in a subset of 100 samples, plasma Hcy levels were analysed using also ELISA method. Vitamin B_6 levels in serum were measured using HPLC method (CHROMSYSTEMS Instruments & Chemicals GmbH, Munich, Germany), serum vitamin B12 and folic acid levels were analysed by ELISA (Roche Diagnostics Corp., USA). The blood cells count and urinalysis were determined by standard methods. Twenty-four-hour urine was collected, actual volume, density and temperature were measured, and urine samples were stored at -80 °C.

Uric acid was measured with spectrophotometer at 456 nm (Hitachi 911, Roche, Switzerland), plasmatic total antioxidant status (TAS) was stated with colorimeter using original kit for TAS (Randox, Cat no NX 2332, Randox Laboratories, Cromlech, UK). Blood for total glutathione (tGSH) and oxidised glutathione (GSSG) measurement was deproteinised by 10 % sulphosalicylic acid (SSA) (400 μl 10 % SSA, 750 μl blood), centrifuged at 4 °C at 10,000 rpm for 12 minutes and the supernatant was stored in cryovials at –70 °C until spectrophotometrical analysis at 410 nm using microplate reader (TECAN Spectra Fluor, Austria) in kinetic-type of reaction (16). The Schiff base substance was measured with fluorometer using excitation wave length of 335 nm and emission wave length of 430 nm after repeated extraction in ethanolether mixture. IgG, IgM and IgE antibody to Clamydia trachomatis, C.pneumoniae and C.psittaci were measured from serum using ELISA and microimmunofluorescence test (Lab-

Tab. 2. Antropometric and laboratory characteristics of males.

	Males					
Parameter	IHD patients				Controls	
	Nové Zámky (N=60)	Bratislava (N=64)	Veľký Lom (N=31)	Sign.	Veľký Lom (N=47)	Sign.
Age [yrs]	61.2± 8.2	61.0±10.6	58.7±9.5	NS	49.6±10.3	0.001
BMI [kg/m2]	29.2± 3.9	28.7± 3.3	29.1±5.3	NS	100.0±11.3	0.06
Waist [cm]	105,9±9.8	102.2±8.8	106.0±11.7	NS	0.95±0.06	0.01
SBP [mmHg]	146.2±21.7	138.3±24.2	150.1±27.3	0.051	143.6±20.8	NS
DBP [mmHg]	88.0±11.5	86.1±12.3	95.9±12.3	0.003	93.4±13.5	0.05
TCH [mmol/l]	5.6±1.1	5.4±0.9	5.7±1.1	NS	6.2±1.1	0.001
LDL-C [mmol/l]	3.7±1.0	3.4±0.8	3.6±0.9	NS	4.1±1.0	0.002
HDL-C [mmol/l]	1.2±0.3	1.2±0.3	1.3±0.5	NS	1.4±0.3	0.001
TG [mmol/l]	1.9±1.5	1.8±1.0	1.6±0.9	NS	1.6±0.8	0.06
Apo-B [g/l]	1.1±0.3	1.0±0.2	1.1±0.2	NS	1.1±0.2	0.02
Apo-AI [g/l]	1.0±0.1	1.0±0.1	1.1±0.2	0.001	1.2±0.2	0.01
AI	5.1±1.3	4.9±1.2	4.6±1.2	NS	4.6±1.2	NS
Glycemia [mmol/l]	6.7±2.2	6.9±2.6	6.8±2.6	NS	6.1±2.4	NS
Hb [g/dl]	145±12.0	144±10.9	147±16.3	NS	154±14.3	0,001
Leu [*109/l]	6.6±1.6	6.8±3.2	6.9±1.9	NS	6.5±1.3	NS
Plt [*109/l]	189.7±53.6	191.4±59.1	222.1±79.7	NS	206.7±45.4	NS
Fe [mmol[l]	19.0±6.1	18.3±6.0	17.6±8.8	NS	17.9±6.4	NS
Bi [mmol[l]	12.5±7.9	10.3±5.2	12.1±6.0	NS	11.6±6.3	NS
GMT [μmo/l]	0.70±0.49	0.56±0.37	0.65±0.57	MS	0.64±0.56	NS
ALT [μkat/l]	0.45±0.24	0.44±0.25	0.44±0.38	NS	0.59±0.41	NS
ALP [μmo/l]	3.3±0.8	3.1±0.8	3.4±1.1	NS	3.2±0.7	NS
Creatinine [μmo/l]	102±15	107±20	92±12	0.001	90±11	0.001
UA [μmo/l]	393±83	389±94	358±104	NS – 0.07	360±88	NS
Hcy [μmo/l]	14.4±7.4	14.3±5.0	13.8±5.3	NS – 0.06	12.3±4.9	0.03
Vit. B6 [nmol/l]	0.80±0.33	1.16±0.52	1.06±0.51	0.001	1.11±0.58	NS
Vit. B12 [pmol/l]	276±104.1	282±117.9	249±75.3	NS	298±115.8	NS
Folic acid [nmol/l]	13.7±6.7	14.3±9.3	10.2±6.7	0.002	10.8±4.8	NS
hsCRP [mg/dl]	3.0±2.8	1.8±1.9	2.6±2.9	NS – 0.06	2.0±2.1	NS

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, TCH – total cholesterol, LDL – LDL cholesterol, HDL - HDL cholesterol, TG – triglycerides, ApoB- Apolipoprotein B, ApoA1 – Apolipoprotein A1, AI- atherogenic index, Hb – haemoglobin, Leu – leukocytes, Plt – platelets, Fe – iron, Bi – bilirubin, UA – uric acid, Hcy – homocystein, vit. B6 – vitamin B6, vit. B12 – vitamin B12, hsCRP – high sensitivity C-reactive protein, IHD – ischemic heart disease

systems, Finland). All the measurements done in the study were performed in certified laboratories.

Height, weight, waist and hip circumferences were measured according to standard protocols.

Ethics

The study was approved by the Ethics Committee of the Medical School of Comenius University in Bratislava, and all subjects signed the informed consent.

Results

Main characteristics of the groups by IHD status and geographical area are listed in Tables 2 and 3. There is a high occurrence of risk factors for atherosclerosis in all groups (Tabs 4 and 5).

In general, all groups were overweight, with majority of subjects with a BMI > 25 kg/m²; a substantial proportion of the subjects exceeded the recommended levels of total and LDL cholesterol and HDL cholesterol (with exception of both female and male control subjects from Veľký Lom). Moreover, in more than 50 % of IHD patients we detected increased systolic or diastolic blood pressure, namely over 140/90 mm Hg. Even in the control population, a high proportion of hypertensive subjects were revealed. There was a high-

er prevalence of smokers in both IHD and control subjects from the rural area in Veľký Lom, compared to IHD patients in Nové Zámky and Bratislava. We have found a high level of classic risk factors and other risk factors, as well as absolute risk for cardiac events, namely 61 % to 88 % of IHD patients (depending on region and gender) had an absolute 10 years risk ≥ 20 % compared to 15 % and 26 % of control females and males from Veľký Lom (Tabs 4 and 5).

When we compared personal history data obtained through the questionnaire filled in by patients with their medical records, we unexpectedly found a high number of IHD patients, both in Nové Zámky and Bratislava who were not aware of their diagnoses. In fact, 6.3 % of patients in Nové Zámky and 4.8 % in Bratislava were not aware of their diagnoses of AP, 6.3 % and 3.2 % of diagnoses of MI and 9.4 % and 6.6 % of diagnoses of hypertension. A similar situation was in males from Veľký Lom. These respondents considered themselves healthy even though they had been on disability benefit due to IHD or stroke (Fig. 1).

The mean plasma HCy level in men with IHD from Nové Zámky, Bratislava and Veľký Lom was 14.4±7.4, 14.3±5.0 and 13.8±5.3 μmol/l, and 12.3±4.9 μmol/l in control men from Veľký Lom, respectively. In women with IHD, plasma Hcy levels were 12.6±6.4, 11.0±3.0, 12.2±5.3 μmol/l in Nové Zámky, Bratislava and Veľký Lom groups, respectively, compared to 8.6±3.2 μmol/l

Tab. 3. Antropometric and laboratory characteristics of females.

Parameter	Females					
	IHD patients				Controls	
	Nové Zámky (N=70)	Bratislava (N=53)	Veľký Lom (N=53)	Sign.	Veľký Lom (N=55)	Sign.
Age [yrs]	62.5±6.6	66.1±7.6	60.2±8.5	0.001	46.6±9.2	0.001
BMI [kg/m2]	31.6±4.8	28.8±5.1	32.1±5.5	0.002	28.8±6.6	0.01
Waist [cm]	98.3±11.0	92.3±13.9	102.2±13.1	0.05	90.0±16.2	0.001
SBP [mmHg]	158.6±20.5	154.5±25.4	157.1±23.4	NS	138.7±29.4	0.001
DBP [mmHg]	94.0±8.5	91.2±11.4	96.8±12.1	NS	87.7±14.6	0.005
TCH [mmol/l]	6.0±1.0	6.2±1.4	6.6±1.1	0.03	6.2±1.1	NS
LDL-C [mmol/l]	4.0±0.9	4.2±1.2	4.4±1.0	0.03	4.1±1.0	NS
HDL-C [mmol/l]	1.3±0.3	1.4±0.3	1.5±0.3	NS- 0.07	1.5±0.3	NS – 0.06
TG [mmol/l]	1.8±0.9	1.4±0.7	1.6±0.8	NS	1.4±0.6	NS
Apo-B [g/l]	1.0±0.2	1.0±0.2	1.1±0.2	NS	1.0±0.2	NS
Apo-AI [g/l]	1.2±0.1	1.1±0.2	1.2±0.2	0.001	1.2±0.1	NS
AI	4.9±1.1	4.7±1.4	4.7±1.1	NS	4.4±1.2	NS
Glycémia [mmol/l]	7.2±3.1	6.1±1.6	6.5±2.2	0.05	5.8±1.3	0.008
Hb [g/dl]	140±10.2	136±11.8	139±12.6	NS	138±10.0	NS
Leu [*109/l]	6.1±1.9	6.3±1.8	6.9±1.6	0.01	7.3±1.6	0.004
Plt [*109/l]	255.5±54.4	277.7±96.3	244.4±66.7	NS	264.4±60.0	NS – 0.07
Bi [mmol[l]	13.3±4.8	12.6±3.8	12.7±4.3	NS	10.8±3.2	0.03
GMT [μmo/l]	0.31±0.20	0.33±0.30	????	NS	0.31±0.24	NS
ALT [μkat/l]	0.27±0.20	0.26±0.12	0.33±0.23	NS	0.27±0.21	NS
ALP [μmo/l]	3.2±0.9	3.2±0.9	3.4±1.0	NS	2.8±1.0	0.003
Creatinine [μmo/l]	89±13	90±15	84±19	0.003	79±9	0.001
UA [μmo/l]	308±80	313±74	313±102	0.02	261±82	0.001
Hcy [μmo/l]	12.6±6.4	11.0±3.0	12.2±5.3	0.003	8.6±3.2	0.001
Vit. B12 [pmol/l]	283±125.4	342±160.4	332±110.7	0.02	335±145.4	NS
Folic acid [nmol/l]	14.4±6.5	15.1±9.7	10.1±4.4	0.001	12.8±8.6	NS
u-CRP [mg/dl]	2.2±2.7	2.6±2.9	3.6±2.8	0.002	3.1±3.0	NS

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, TCH – total cholesterol, LDL – LDL cholesterol, HDL – HDL cholesterol, TG – triglycerides, ApoB – Apolipoprotein B, ApoA1 – Apolipoprotein A1, AI- atherogenic index, Hb – haemoglobin, Leu – leukocytes, Plt – platelets, Fe – iron, Bi – bilirubin, UA – uric acid, Hcy – homocystein, vit. B6 – vitamin B6, vit. B12 – vitamin B12, hsCRP – high sensitivity C-reactive protein, IHD – ischemic heart disease

Tab. 4. Prevalence of risk factors in males.

Parameter	Males					
	IHD patients				Controls	
	Nové Zámky (N=60)	Bratislava (N=64)	Veľký Lom (N=31)	Sign.	Veľký Lom (N=47)	
BMI [kg/m2] ≥25	90	81.3	74.2	0.02	72.3	NS
BMI [kg/m2] ≥30	40	33.3	38.7	NS	31.9	NS
SBP [mmHg] ≥140	65	50	71	NS	51.1	NS
DBP [mmHg] ≥90	50	33.5	71	0.001	64	0.02
TCH [mmol/l] ≥5.0	65.5	66.7	73.3	NS	85.1	0.001
LDL-C [mmol/l] ≥3.0	69.1	75.8	76.7	NS	89.1	0.002
HDL-C [mmol/l] ≤1.1	50	51.6	36.7	0.01	21.3	0.002
TG [mmol/l] ≥1.7	44.8	38.1	30.0	NS	29.8	0.058
Apo-B [g/l] ≥1.15	34.5	22.8	30	NS	43.5	0.01
Apo-AI [g/l] ≤1.19	93.1	96.5	80	NS	58.7	0.001
AI ≥5.0	46.7	43.2	50	NS	36.2	0.01
Smokers	15	9.4	32.0		46.8	0.001
AR 10 y ≥20 %	88.3	74.6	66.6	0.05	25.5	0,001
Income <average	13.3	34.4	16.1	0.001	23.4	NS
Education						
Elementary	15	7.8	38.7	0.001	31.9	NS
High school	71.7	37.5	38.7	0.001	59.6	NS
University	13.3	54.7	22.6	0.001	8.5	NS
Hcy [μmo/l] >15	30	37.5	32.3	NS	21.3	NS
Vitamin B6 [nmol/l] ≤0.6	26.7	15.4	20.8	NS	25.7	NS
Vitamin B12 [pmol/l] ≤178	18.3	15.3	26.7	NS	17.0	NS
Folic acid [nmol/l] ≤6.52	30	32.2	66.7	NS	44.7	NS
hsCRP [mg/dl] ≥0.3	36.2	14	26.7	0.01	22.7	NS

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, TCH – total cholesterol, LDL – LDL cholesterol, HDL – HDL cholesterol, TG – triglycerides, ApoB – Apolipoprotein B, ApoA1 – Apolipoprotein A1, AI – atherogenic index, AR – absolute risk, Hcy – homocystein, hsCRP – high sensitivity C-reactive protein, IHD – ischemic heart disease
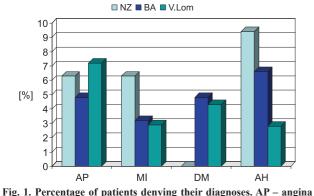
Fig. 1. Percentage of patients denying their diagnoses. AP – angina pectoris, MI – myocardial infarction, DM – diabetes mellitus, AH – arterial hypertension.

in control women from Veľký Lom. The proportion of male IHD subjects with HCy level >15 µmol/l was 30–38 %, and 21.3 % for male controls from Veľký Lom (Tabs 2 and 3). The proportion of female IHD patients with HCy level >13 µmol/l was highest in Nové Zámky, and 24.5 % for Bratislava and Veľký Lom. Similarly to men, female controls from Veľký Lom had a lowest proportion of subjects with increased Hcy levels (Tabs 4 and 5). Suprisingly, both IHD and control subjects from the rural area in Veľký Lom had a tendency to yield lower serum levels of folic acid.

Discussion

Coronary heart disease (CHD) constitutes a major public health burden worldwide (17) and remains one of the most challenging topics in disease prevention. In spite of positive trends in decreasing cardiovascular mortality in Central European countries (CE) there remains a deep gap between European Union (EU) and Central European countries (18, 19). This gap cannot be explained solely by a difference in major risk factors. In fact, prevalence of certain classical risk factors in Western countries was higher than in former communist countries (20, 21, 22) and it has been shown that over 10 years across the WHO MONICA Project populations, just 15% of coronary event rate trend variability in women and 40% in men could be explained by trends in major risk factors (21). The estimates are low, possibly due to imprecision in estimation of event rate trends; however, the data suggest that the remaining variance could be attributable to other factors. Mild hyperhomocysteinaemia was hypothesised to be an independent risk (1, 2) factor or marker (6, 23) for atherosclerosis and its complication. Nevertheless, the data on HCy levels distribution in Slovak population (24) are limited.

We therefore performed a study aimed at the description of prevalence of hyperhomocysteinaemia and major risk factors of atherosclerosis. We found a very high occurrence of risk factors regardless of geographic area (urban, urban/rural or rural) and health status of subjects (patients with IHD or general population). This finding is even more critical, when we consider that 6 out of 8 subgroups studied included patients in secondary prevention. The fact, that we have found a substantial part of patients to have an unfavourable profile of modifiable risk factors such as blood pressure, cholesterol levels, obesity and smoking suggests the limits of proper health care provided.

Our findings show the same trend with observations in previously published studies. In CINDI project in Central Slovakia,

Tab. 5. Prevalence of risk factors in females.

Parameter	Females					
	IHD patients				Controls	
	Nové Zámky (N=70)	Bratislava (N=53)	Veľký Lom (N=53)	Sign.	Veľký Lom (N=55)	Sign.
BMI [kg/m2] ≥25	92.9	75.5	90.6	0.02	65.5	0.001
BMI [kg/m2] ≥30	57.1	38.4	65	NS	43.6	NS
SBP [mmHg] ≥140	82.9	71.2	83	0.02	45.5	0.001
DBP [mmHg] ≥90	80	59.6	75.5	0.01	45.5	0.001
TCH [mmol/l] ≥5.2	89.4	84.0	96.2	0.03	88.9	NS
LDL-C [mmol/l] ≥3.5	92.4	90.0	92.5	0.03	92.6	NS
HDL-C [mmol/l] ≤1.3	43.9	41.5	26.4	0.001	25.9	0.02
TG [mmol/l] ≥2.0	39.1	26.0	30.2	NS	20.4	0.03
Apo-B [g/l] ≥1.15	32.9	26.4	26.5	NS	37.8	NS
Apo-AI [g/l] ≤1.19	60	75.5	36.5	0.001	51.9	NS
AI ≥5.0	35.7	37.3	57.7	0.02	29.6	0.03
Smokers	4.3	3.8	7.5	NS	36.4	0.001
AR 10 y ≥20 %	84.1	60.8	75.4	NS	15.4	0.001
Income <average	5.7	7.5	1.9	NS	9.1	0.002
Education						
Elementary	50	28.3	65.4	0,002	32.7	0.01
High school	45.7	56.6	30.8	NS	52.7	NS
University	2.9	15.1	6.8	NS	9.1	NS
Hcy [µmol/l] >13	37.1	24.5	24.5	NS	7.3	0.001
Vitamin B12 ≤178	14.3	15.1	1.9	NS	9.3	NS
Folic acid ≤6.52	22.9	28.3	50	NS	35.2	NS
hsCRP ≥0.3	30	34	50	NS	35.2	NS

BMI – body mass index, SBP – systolic blood pressure, DBP – diastolic blood pressure, TCH – total cholesterol, LDL – LDL cholesterol, HDL – HDL cholesterol, TG – triglycerides, ApoB – Apolipoprotein B, ApoA1 – Apolipoprotein A1, AI – atherogenic index, AR –absolute risk, Hcy – homocystein, hsCRP – high sensitivity C-reactive protein, IHD – ischemic heart disease

more than 2,000 randomly selected respondents in age range of 15–64 were screened for major risk factors of atherosclerosis. In a subgroup of males in age range of 25–64 there was a prevalence of 26.5 % in smokers, 66.2 % with BMI > 25, 66.3 % with total cholesterol more than 5.2 mmol/l, and 51.5 % had blood pressure more than 140 and/or 90 mmHg (25). With a small difference in lower prevalence of smokers, the results of the latter study corresponded with our findings. Similarly, in a secondary prevention study VITA LONGA (with more than 4,000 participants with total cholesterol > 5.2. mmol/l or treated with hypolipidaemic treatment), the mean systolic and diastolic blood pressure and BMI were similar to those in our study. However, the proportion of smokers in VITA LONGA study was only 9.3 % (26).

Both our study and other studies performed in Slovakia confirm high prevalence of classic and newer risk factors in our population. However, the lack of a systematic follow-up imposes a limit as to the predictive value of such information as well as to trends in risk factor changes over time. Therefore, it is important to perform a prospective study to obtain relevant data on such trends. This can form a base for a targeted population intervention focused on reversing the increasing trend of cardiovascular morbidity and mortality in Slovakia.

It has been suggested that low intake of vegetable and fruits can participate in the occurrence of mild hyperhomocysteinaemia in Slovakia (24, 27). However, there is only scarce data reporting plasma levels of HCY and modifying factors in Slovak population. In our study, we have found more than 30 % of IHD male patients and 21.3% of males from control population with plasma Hcy levels over 15 µmo/l. Similarly, in female IHD patients, the prevalence of Hcy levels >13 µmo/l was in the range of 24.5–37.1 % and 7.3 % only in control females. In previously published study of Slovak men who survived myocardial infarction (MI) by the age of 50 and in control men without a personal and family history of MI, the mean plasma Hcy levels exceeded 15 µmo/l (24). These results suggest that mild hyperhomocysteinaemia can contribute to high cardiovascular morbidity and mortality in Slovakia. Surprisingly, rural patients who exhibited lower mean levels of Hcy in general had also lower serum levels of folic acid. This fact may suggest that folic acid level is not a crucial factor determining the plasma Hcy levels in our population.

In conclusion, we found a high prevalence of major risk factors of atherosclerosis and hyperhomocysteinaemia in selected Slovak population. This fact should be considered when developing national policies for IHD prevention.

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