

Homocysteine and Risk of Premature Coronary Heart Disease

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Abstract

Background: Homocystinuria is a rare autosomal recessive disease complicated by early and aggressive occlusive arterial disease. This may be related to the grossly increased homocysteine concentrations seen in this disease. More recently, milder hyperhomocysteinemia has been proposed as an independent risk factor for coronary artery disease. Cardiovascular disease (CVD) is among the diseases with multiple contributing factors, hence making it difficult to pinpoint a particular factor alone. The main factor that is of relevance to this study is homocysteine. Coronary artery disease is the narrowing or blockage of the arteries and vessels that supply oxygen and nutrients to the heart (1, 2). CVD are the major cause of morbidity and mortality worldwide. Obesity, HTA, Diabetes mellitus, hypercholesterolemia and smoking have been recognized as major risk factors for CVD.

Aim: Aim of this paper is to examine concentrations of Hcyt and lipid profile in patients with CVD and positive personal history for CVD, comparing them with the control group composed from healthy individuals. Our study aimed to verify the role of Homocysteine as new independent risk factor on the onset of early atherosclerosis and atheromatous processes in coronary arteries in patients with CVD.

Materials and methods: The results obtained represent the average value earned once every three months in the 3 year period. 5ccm serum with a few heparin spots was sent to the Clinical Laboratory of the University Clinic of Skopje.

Results: The results obtained from patients with CVD and control group are presented in the following text, where a statistically significant difference was observed for $p < 0.0001$ between the parameters obtained by patients with CVD compared to the control group. In Concentrations of homocysteine and lipids in patients with CVD compared to the control group showed statistically significant difference with $p < 0.0001$, expected results and verified in many other multicentric studies. These facts show that raised Hcyt have more impact on the onset of CVD. When Hcy levels are in blood, the activity of cystathionine-synthetase enzyme is raised. It is believed that this enzyme plays vital role on the metabolism of Hcyt. Recent years a lot of studies have been made on the effect of hyperhomocysteinemia and its impact on the onset of coronary and all have verified that hyperhomocysteinemia is a significant parameter for the onset of early atherosclerosis of coronary and CVD(6,7,8).

Conclusions: In above mentioned cases it is recommended substitutive therapy with folic acid, pyridoxine, vitamin B12, vitamin E and other antioxidants which is found that have effect on prevention

of premature atherosclerosis in patients with CVD and raised Hcyt: acute myocardial infarction, CARB, angina pectoris. PTCA, Stenting and prevention of stroke.

Keywords: *premature coronary heart disease, total homocysteine*

Introduction

Both markedly and mildly elevated circulating homocysteine concentrations are associated with increased risk of vascular occlusion. Here we review possible mechanisms that mediate these effects. Inborn errors of homocysteine metabolism result in markedly elevated plasma homocysteine (200–300 $\mu\text{mol/L}$) and thromboembolic (mainly venous) disease: treatment to lower but not to normalize these concentrations prevents vascular events. Mild homocysteine elevation ($>15 \mu\text{mol/L}$) occurs in $\approx 20\text{--}30\%$ of patients with atherosclerotic disease.

A new class of emerging risk factor is elevated plasma homocysteine level. This study aims to determine whether there is any significant association between homocysteine lipid profile and other parameters in acute coronary artery disease. CVD still remain as main factor of invalidity, morbidity and mortality in developed and developing countries. Usually, this is easily normalized with oral folate and ongoing trials are assessing the effect of folate treatment on outcomes. Although there is evidence of endothelial dysfunction with both markedly and mildly elevated homocysteine concentrations, the elevated homocysteine concentration in atherosclerotic patients is also associated with most standard vascular risk factors, and importantly, with early decline in renal function, which is common in atherosclerosis. Atherosclerosis, and its most common manifestation, coronary artery disease (CAD), are rather common causes of morbidity and mortality worldwide. Recognition of its various risk factors is important to planning effective preventive measures. After the homocysteine theory was presented in 1969, attention has been directed toward the serum homocysteine level as a coronary artery disease risk factor. Correlation between raised Hcyt and CVD was disovered 25 years ago by Carson and Neil, who saw a defect of

Hcyt metabolism in a patient with raised Hcyt. In this cases is verified lack several enzymes which enable normal metabolism of Hcyt. Therefore as result of these metabolic disorders of Hcyt, clinical picture of raised Hcyt and its accumulation in blood-hyperhomocysteinemia appears. Several studies have verified that 15-30% of cases with CVD are result of hyperhomocysteinemia (3, 4, 5).

When hyperhomocysteinemia is correlated with lipid disorder (dyslipidemia, hypercholesterolemia) effects on cardiovascular system and CVD prevalence is higher. For this reason we decided in our study to include lipid panel also in patients with CVD. Homocysteine has been recognized as a risk factor as early as 1990s, for the presence of atherosclerotic vascular disease and hypercoagulability states. Subgroup analyses conducted in a study also showed that elevated homocysteine was associated with higher risk of coronary artery disease in patients with chronic renal dysfunction (9, 10). Cardiovascular diseases (CVD) as the name suggests, comprise of diseases of the heart and blood vessels. Cardiovascular disease is believed to account for one third of all deaths worldwide, and the prevalence is still on the rise (11, 12). CVD and their high mortality still remain as big problem and with high prevalence in general population. High concentrations of Hcyt in serum are considered as risk factor for CVD and can be associated with hypertension.

Although between hyperhomocysteinemia and CVD is found a significant correlation, still the role of homocysteine on cardiovascular manifestations remains unclear. It is verified that concentrations of homocysteine increases with smoking, aging and some diets with low folates, cyanocobalamin and pyridoxine. Many studies have verified a significant relationship between raised Hcyt and arterial hypertension and lipid disorders, compared with normotensive individuals. Lowering Hcyt con-

centrations can have some benefits in decreasing the risk of CVD in old age correlation between high levels of homocysteine(Hcy) and coronary artery diseases is discovered 25 years ago, when for first time was verified that patients with hyperhomocysteinuria are potential candidates for developing early atherosclerosis in puberty and before 20 years of age. In these cases is verified the lack of some enzymes responsible for metabolism of Hcyt, as result hyperhomocysteinemia occurs. Homocysteine was discovered in 1932, and chemical analysis showed similarity with cysteine. For this discovery Vincent du Vigneaud in 1955 was awarded Nobel Price in Chemistry for his work on sulfur components, especially for synthesis of polypeptide hormone (13).

Homocysteine is sulfidric amynocide as intermediary product of normal biosynthesis of methionine and cysteine (4). Hcy in serum is found in three forms: 1% is in free form, 70-80% as residual disulfides and 20- 30% in combined form with other thios. (14). Homocysteine is synthesized from essential aminoacid methionine. Cystathionine- β -synthetase is an enzyme, while pyridoxine (B6) is essential cofactor which converts homocysteine in cysteine. Hyperhomocysteinemia is a condition with raised homocysteine levels in blood above 15 $\mu\text{mol/L}$ (15,16, 17,18). CVD have high prevalence and still remain as main reason for high mortality and morbidity in world. Etiology of CVD is multifactorial and most often they are result of narrowed or occluded coronary arteries (19, 20). Researchers have long debated the extent to which Hcy should be considered as a risk factor for CVD, since according to some, only 50% of CVD can be explained by "classical" risk factors, and they say that "new" risk factors could significantly boost the CVD predictive power. But this has been widely criticized and there are other authors who show that up to three quarters of coronary heart disease (CHD) events, if not more, could be attributed to "classical" risk factors. For the purpose of use as a screening tool, a risk factor should be strongly and causally associated with the target disease, and many authors doubt whether such a relationship between homocysteine and CVD exists.

The Framingham risk score (FRS), known as an important instrument in predicting coronary artery disease in patients with traditional risk factors, such as dyslipidaemia, hypertension, diabetes mellitus (DM), and smoking, seems to have underestimated the coronary artery disease risk in individuals with high homocysteine plasma levels. Research has indicated towards a relationship between moderately elevated tHcy (Total homocysteine) levels and the risk of CVD (coronary, heart, cerebrovascular and peripheral artery diseases). The homozygous mutation of C677T can cause severe hyperhomocysteinemia where homocysteine concentration is up to 40-fold of the normal levels. This disease occurs in approximately 1 of 100,000 live births. Also Hcy levels correlated significantly with increasing severity of coronary artery disease ($p < 0.001$).

According to this paper, the most common and plausible mechanism for increased risk of CAD are endothelial dysfunction thought to occur primarily from changes in vascular endothelial compliance and platelet coagulation changes that promote CVD. In various in vitro studies, homocysteine was proved to trigger proliferation of vascular smooth muscle cells. It also has role in increasing the activity of HMG Co A reductase which in turn increases cholesterol synthesis (23). An increased cholesterol level promotes atherosclerosis and hence it is a risk factor for CAD. Serum levels of homocysteine were found to be significantly higher in CAD than in non CAD subjects. Increased serum Hcy levels positively correlated with severity of CAD. But the authors assert too that there is a correlation between homocysteine and coronary artery disease, despite the fact that every research, including this one, has its limitations. Carotid intima-media thickness (IMT) is a well-accepted non-invasive marker of subclinical atherosclerosis.

The role of homocysteine in endothelial dysfunction is thought to be mediated by mechanisms including oxidative stress, nuclear factor-kB (NF-kB) activation, inflammation, and inhibition of endothelial nitric oxide synthase (eNOS) (24). While several observational studies have reported weak positive associations between total homocysteine

ine concentration and carotid IMT in the non-diabetic population, few cross-sectional studies address this association in the context of diabetes mellitus. The possible mechanisms explaining the relationship between hyperhomocysteinemia and aortic stiffness are not yet fully well established. Main hypotheses based on this investigation are that homocysteine plays a potential role in remodelling of the arterial wall leading to vascular damage. There is also a strong evidence that oxidation is part of the mechanism attributed to increased homocysteine and atherosclerosis. Thus we see a common belief across many papers that an inflammatory response could be in play. In an experimental study on mini pigs, mild hyperhomocysteinemia was found to cause an arterial, site-dependent deterioration of the elastic structure involving metallo-proteinase-related elastolysis (25, 26).

Hyperhomocysteinemia is because of homozygote mutations of MTHFR-methylene tetrahydrofolate reductase. These individuals with MTHFR defects are exposed to early CVD. Homocysteine is independent risk factor for early atherosclerosis. Atherosclerosis is progressive inflammatory injury or arterial intimal layer, with increased permeability, lipidic deposition and calcification of intima. Correlation between hyperhomocysteinemia and atherosclerosis for first time was identified by McCully in 1969. Atherosclerosis is common pathological process which leads to CVD (myocardial infarction, atheromatous processes of carotid arteries, heart failure, stroke). Some of mechanisms of these effects are: endothelial dysfunction, oxidative injury, increased collagen production, damage of arterial wall and increased C reactive protein in vitro and in vivo (27, 28, 29). Concentrations of homocysteine in serum can be increased in different diseases which cause disturbance in the metabolism of folates, B6, B12, lipids, lipoproteins, inflammation etc.

Prevalence of hyperhomocysteinemia can vary between different populations and is tightly dependent on age, diet, genetic predisposition while in turn physical activity, moderate consumption of alcohol, folates and B12 are associated with lower levels of Hcyt. Many studies have found

that vegetarians can have higher risk for hyperhomocysteinemia compared with non vegetarians because of lower B12 levels in their diet. There are studies which have verified that in uremic patients increased Hcyt for 1 $\mu\text{mol/L}$ increases the risk for CVD 1% (31). Still unknown remains the impact and atherosclerotic effect of raised Hcyt, but it is believed it interferes with endothelial function, coagulation and platelets.

Studies have demonstrated significantly higher plasma homocysteine levels in patients with occlusive arterial disease than in controls. The causes are not clearly understood but may include deficiency of vitamin B6, vitamin B12, and folic acid and heterozygosity for cystathionine synthase deficiency. Vitamin supplementation can lower plasma homocysteine levels. These data show that hyperhomocysteinemia is related to CAD as an independent risk factor. In individuals without any risk factors a linear correlation between homocysteine level and numbers of coronary artery involvement was present. If this equation is confirmed prospectively in other studies, the level of plasma homocysteine may be used as a noninvasive way of predicting the number of diseased coronary arteries.

Material and method

As a working material, blood was taken from the patient's vein and the control group at 8 o'clock at room temperature of 19-24°C, in an extended position, after 12 hours of hunger. tHcy and lipid profiles were analyzed in 200 of which 120 were men of average age of 57.60 $\pm$ 10.00 years, while 80 were females of average age of 58.70 $\pm$ 12.00 years.

The control group consisted of 180 individuals, of whom 100 were males and 80 females with mean median age=58.70 $\pm$ 15.20. The results obtained represent the average value earned once every three months in the 3 year period. Serum with a few heparin spots was sent to the Clinical Laboratory of the University Clinic of Skopje.

Total pts N ^o =200	The average age ± SD	Control group N ^o =180 pts, ± SD
M=N ^o -120	57.60 ± 10.00	58.70 ±15.20
F=N ^o -80	58.70 ± 12.00 years	58.70 ±15.20

Table 1: Number of patients and control group by mean age and gender

With a family history for CVD	160(42.8%)
APNS	40 (9.50 %)
Status post Infarctum Myocardi	40 (9.50 %)
Smoker	140 (48 .50 %)
Control grup-180, The average age ± SD	58.70±15.20

Table 2: Tabelary presentation of patients by CVD

Concentrations of Hcyt were determined according to Miller's method of American Immunofluorescence with Immulite DPC machine, and normal ranges are =5-13 $\mu\text{mol/L}$, while lipid profile was determined by standard routinely methods.

Statistical analysis of the examined material

Statistical basic methods that were used are the arithmetic mean value and standard deviation $\bar{X} \pm \text{SD}$. Comparative statistics and tHcyLPL and lipid parameters between the two groups was analyzed by test called STUDENTOV and for examples of dependent or independent and non-parametric tests were used the tests: Mann-Whitney and Wilcoxon's test.

Statistically significant the differences between the Group of patients and control group obtained the values of lipid parameters and test tHcy analyzed the so-called, Anonova Two-Factor "with the amounts of domestic statistics for $p < 5\%$, Namely $p < \text{statistical } 0.0001$. The results of the lipid profile and Hcys presented in the form of graphicones, averages and proportional / $\bar{x}$, p /) were tested with accuracy higher than 95%, or rather, for $\text{mr.} > \text{SEM } 1.78$. The results of the lipid profile and Hcy are presented in the form of graph-cones, table and in the form of processed diagrams made with standard statistical program.

Results

	Tot.Pts.	ChT mmol/l	TG mmol/l	HDL-ch mmol/l	LDL-ch mmol/l	tHcy $\mu\text{mol/l}$
Patients with CVD, St.post MI, APNS	200	5.80 ± 2.80	3,40 ± 0.80↑	0.90 ± 0.20↑	4.80 ± 1.70↑	28.60 ± 10.40 ↑
Control group	180	4.80 ± 1.50	1.15 ± 0.70	1.40 ± 0.80	3.40 ± 0.90	6.80 ± 2.70
<i>p</i>		0.7600	0.0001	0.0001	0.0001	0.0001

Table 3: Obtained results from patients with CVD and control group for tHcy and lipid profile

*Pts-Patients. From the table itself, there is an increase in Hcy in patients with CAD (28.60 ± 10.40) compared to the control group for $p < 0.0001$.

Discussion

Homocysteine as risk factor for CVD, stroke, thrombotic processes, vascular hypercoagulability and atherosclerotic processes is known since early 1990. Scientists thought on homocysteine as risk factor on developing CVD still are controversial. Some scientists believe high concentrations of Hcyt are not a risk factor for CVD, while lot of research results show high positive correlation between raised Hcyt and CVD diseases. Genetic mutations of C and S homozygote can cause severe hyperhomocysteinemia where Hcy concentrations are 40 times higher than normal. This disease have incidence of 1:100.000. Another rare genetic cause of hyperhomocysteinemia is because of homozygote mutations of MTHFR-methylene tetra hydrofolate reductase. These individuals with MTHFR defects are exposed to early CVD. Cardiovascular disease is believed to account for one third of all deaths worldwide, and the prevalence is still on the rise. CVD is among the diseases with multiple contributing factors, hence making it difficult to pinpoint a particular factor alone. The main factor that is of relevance to this study is homocysteine. Coronary artery disease is the narrowing or blockage of the arteries and vessels that supply oxygen and nutrients to the heart (11, 13).

Homocysteine is independent risk factor for early atherosclerosis. Atherosclerosis is progressive inflammatory injury or arterial intimal layer, with increased permeability, lipidic deposition and calcification of intima. Correlation between hyperhomocysteinemia and atherosclerosis for first time was identified by McCully in 1969. Atherosclerosis is common pathological process which leads to CVD (myocardial infarction, atheromatous processes of carotid arteries, heart failure, stroke). Some of mechanisms of these effects are: endothelial dysfunction, oxidative injury, increased collagen production, damage of arterial wall and increased C reactive protein in vitro and in vivo (13,14). Homocysteine is an amino acid produced via demethylation of dietary methionine, which is abundant in animal protein. It is present in plasma in four different forms: around 1% circulates as free thiol, 70–80% remains disulphide-bound

to plasma proteins, mainly albumin and 20–30% combines with itself to form the dimer homocysteine or with other thiols. Homocysteine is a key determinant of the methylation cycle. It is methylated to methionine, which undergoes S-adenosylation and forms S-adenosylmethionine (SAM).

S-adenosylmethionine is the principal methyl donor for all methylation reactions in cells (29–32). Condensation of methionine with ATP, leads to the formation of SAM (S Adenosylmethionine). Vitamin B₆, B₁₂ and folic acid are essential cofactors in homocysteine-methionine metabolism.

Therefore low vitamin B availability (B₆, B₁₂ and folic acid) leads to impaired re-methylation of homocysteine to methionine and thus to homocysteine accumulation. Increased homocysteine levels were found to be associated with arteriosclerotic outcomes and risk of stroke in elderly individuals, and are considered as an independent risk marker for cardiovascular diseases. However, lowering homocysteine levels by B-vitamin supplementation failed to demonstrate beneficial effects in cardiovascular diseases and this has been proven to be true in many other research works. Hyperhomocysteinemia can be caused by deficiency of folate, vitamin B₆ and B₁₂ in food. An individual with deficiency of these above mentioned vitamins can develop raised levels of Hcyt and have risk from hyperhomocysteinemia.

Disorders of homocysteine metabolism and other sulfuric amino acids in patients with renal injury are described in 1980 by Willen et al. for first time, who saw that uremic patients treated with HD had raised cysteine and Hcyt. High levels of homocysteine were found in patients with chronic renal injury also, with increased urea, hypothyroidism, cancer, psoriasis, diabetes, excess alcohol, smoking, coffee, old age and menopause. Homocysteine is eliminated from the organism with kidneys therefore during renal injury when GFR is decreased the excretion of Hcy from organism is decreased, which causes moderate hyperhomocysteinemia. Concentrations of homocysteine in serum can be increased in different diseases which cause disturbance in the metabolism of folates, B₆, B₁₂, lipids, lipoproteins, inflammation etc. Prevalence of hyperhomocysteinemia can vary

between different populations and is tightly dependent on age, diet, genetic predisposition while in turn physical activity, moderate consumption of alcohol, folates and B12 are associated with lower levels of Hcyt. Many studies have found that vegetarians can have higher risk for hyperhomocysteinemia compared with non vegetarians because of lower B12 levels in their diet. A new multicentric study which included 80.000 female individuals, for 14 years, found that onset of CVD was lower in the group which during that time consumed substitutive therapy with vitamins or consumed with food higher concentrations of abovementioned vitamins compared with the group who haven't consumed enough of them. Authors Victor and Hebert in their studies concluded that low levels of folic acid are as result of decreased absorption of vitamin B12 which is tightly related with old age (33). It is verified that by lowering Hcyt in serum the risk for atherosclerosis, CVD and stroke in patients with homocysteinuria decreases also. Even after many studies regarding to hyperhomocysteinemia experts still cannot conclude and verify that by lowering high levels of homocysteine decreases the risk for CVD. Regarding to this, a 4 year study in 101 patients with CVD who every day consumed folic acid, pyridoxine and cyanocobalamin found a decrease in the size of their atheromatous plaques, even better results were obtained in those patients who before the study had higher levels of Hcyt. The cause of hyperhomocysteinemia in patients with chronic renal failure still is unknown, and an appropriate therapy for normalizing Hcy in these patients doesn't exist. Experts suggest that patients with CVD should analyse their Hcyt levels, and those with levels from 9-10 $\mu\text{mol/L}$ should be treated at least one month with substitutive therapy, this has shown positive effects. A recent study on positive effects of folic acid and vitamin B12 (combined or separately) on hyperhomocysteinemia has verified that by substituting B6 and B12, organism can easily correct Hcyt levels. In USA, Canada and Europe an study with 60.000 individuals, still ongoing, are studying the effect of raised Hcyt and onset of myocardial infarction, cerebrovascular embolia and possible ways of decreasing it (34). Many studies have veri-

fied that patients after undergoing stenting or angioplasty with normalised Hcy levels have lower incidence of re-occurring of atheromatous processes compared with those who have high Hcy.

A recent study, which has lasted 6 months, a time which in patients vitamin B6 and B12 was given found that cardiac events and need for revascularisation was 1/3 time lower compared with patients who haven't consumed above-mentioned therapy (35,36). High levels of Hcyt can be as result of cyanocobalamin deficiency which occurs because of vitamin B12 malabsorption as result of gastric atrophy, which is more often seen after age of 50. B12 deficiency causes anemia. If this deficiency is left untreated it causes damage to nervous system and early atherosclerosis. Individuals above 50 years of age are advised to consume folic acid and vitamin B12 because in this age most of them have gastric atrophy. A multicentric study concluded that females during menopause have raised homocysteine and an increase of coronary diseases, compared to females before menopause (37). Consulted literature and many studies have concluded that in the etiology of coronary atherosclerosis many factors are included: genetic predisposition, environment, life style, sedentary life, obesity etc.

There are facts that by supplementing vitamin B12 has decreased Hcyt concentrations with 17-30%. For decreasing Hcy and correcting dyslipidemia intravenous application of acetylcysteine is required. Many studies have shown that by applying folic acid, vitamin B12 and B6 can reduce Hcy levels for 35%. In a larger study, it was documented that patients with coronary disease who were treated with folic acid, after 2 year follow up homocysteine decrease for 18% occurred, but mortality didn't show significant reduction. Documented facts exist that folic acid, cyanocobalamin and acetylcysteine have positive effects on decreasing homocysteine in one side and improving blood vessel function in other side. Nevertheless to verify or to dismiss abovementioned facts more studies need to be made, with more patients and more countries, so the final conclusion can be taken on the effect of folic acid, cyanocobalamin and acetylcysteine on improving

endothelial function of blood vessels (38, 39, 40). Some studies have concluded that hyperhomocysteinemia is result of conversion of hydrogen peroxide in free oxygen radicals and conversion of oxidized Hcy in Homocysteine disulfide. Effect of oxidized Hcy which is increased by hydrogen peroxide explains the LDL raise. Hydrogen peroxide causes endothelial desquamation, with inhibitory effect on prostacyclins and prostaglandins who are antagonists of platelet adhesion (28, 29, 30, 31). Homocysteine has been positively associated with both diastolic and systolic blood pressure. In case of homocysteine concentration increase of 5 $\mu\text{mol/L}$ (about 1 SD), diastolic and systolic blood pressure in men increased by 0.5 and 0.7 mmHg, respectively. In case of women, the correlation of homocysteine and blood pressure was stronger, with 0.7 and 1.2 mmHg increase in diastolic and systolic blood pressure, respectively. As mentioned earlier, high levels of homocysteine and its derivatives add to the process of modification of LDL and HDL particles, inflammation, coagulation disorders as well as fibrinolysis. Hyperhomocysteinemia may lead to biochemical effects on endothelium and cause damage to endothelial cells, diastolic dysfunction of vessels and reduction of flexibility due to its influence on vascular wall remodelling. These mechanisms may lead to an increase in blood pressure and strengthen the development of hypertension and damage body organs in patients with this disease. The question therefore exists if homocysteine is a biomarker or a risk factor? Current guidelines have not classified homocysteine as cardiovascular disease risk stratification. Although lowering homocysteine levels in individuals with pre-existing cardiovascular disease has not shown any benefit, medications as part of a primary prevention strategy need to be evaluated further for confirmation. Therefore, it seems unfair to underestimate the utility of homocysteine in cardiovascular disease risk prediction solely because interventions to lower plasma homocysteine levels have not shown a favourable outcome regarding the risk of cardiovascular disease incidence. Yet there is always room for more research to validate homocysteine as a risk factor and this is absolutely necessary for the sake of solid evidence. High

concentrations of Hcy can be normalized by substituting 1 or 2 from above mentioned deficient vitamins. Homocystinuria is genetically transmitted disease. If a patient inherits 2 defective alleles the risk is much higher than in patients who inherit 1 allele. It is verified that in 100 individuals 1 person inherits 1 defective allele. Nair et al. in a study in Indian population verified genetic mutations of Methylene-tetra-hydrofolate-reductase, which is main cause of hyperhomocysteinemia in this population.

Authors Victor and Hebert in their studies concluded that low levels of folic acid are as result of decreased absorption of vitamin B12 which is tightly related with old age. It is verified that by lowering Hcyt in serum the risk for atherosclerosis, CVD and stroke in patients with homocysteinemia decreases also. Even after many studies regarding to hyperhomocysteinemia experts still cannot conclude and verify that by lowering high levels of homocysteine decreases the risk for CVD (41-44). First, it must be emphasized that the vascular disease in homocystinuria due to cystathionine β -synthase (CBS) deficiency, methylenetetrahydrofolate reductase (MTHFR) deficiency, or inborn errors in cobalamin metabolism bears little resemblance to the atherosclerotic and atherothrombotic vascular disease seen in the adult general population. Atherosclerosis is characterized by a thickening of the arterial wall due to smooth muscle cell proliferation, lipid deposits, and fibrosis (1). Rupture of the lipid-containing atherosclerotic plaques results in thrombosis (atherothrombosis) and leads to myocardial infarction and stroke (1). In contrast, homocystinuria seems to be associated with a primary thrombotic disorder that affects veins more often than arteries.

Conclusion

We can conclude that in our paper also high levels of Hcyt and lipid profile were recorded in patients with CVD. These results are in line with many other multicentric studies, on the role of Hcyt as new independent risk factor for early atherosclerosis. In above mentioned cases it is preferred substitutive therapy with folic acid, pyridox-

ine, cyanocobalamin, tocoferol, acetylsalicylates and other antioxidative agents, which clearly can prevent early atherosclerosis in CVD with: PTCA, CARB, AMI, APNS, Stenting and prevention of stroke.

The study reveals homocysteine, diabetes mellitus, hyperlipidemia, hypertension, obesity and smoking has definite role in the generation of coronary artery disease in the patients with cardiovascular disease. Though most research work suggests a relationship, yet there seems to be other evidence that still prevents its inclusion as a biomarker.

With every ten steps forward, we might have to face a step or two backward, but this should only further increase the enthusiasm of research in this field. This field definitely needs more research input until a definitive proof is available to cast off any shadow of doubt regarding the corre-

lation between homocysteine and cardiovascular disease. Nevertheless, the present review should provide some insight into the role of homocysteine in the development of cardiovascular disease summarizing both central and peripheral effects of homocysteine.

The authors feel that it is necessary to combat the ill effects of hyperhomocysteinemia as it has a pivotal influence on the pathology of the diseased process.

The published literature indicates that homocysteine is an independent cardiovascular disease risk factor modifiable by nutrition and exercise. Whether measuring plasma homocysteine levels in patients with coronary artery disease should be routine and whether treating hyperhomocysteinemia in these patients may reduce the risk of coronary events remains to be determined.

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