Homocysteine and Risk of Premature Coronary Heart Disease

Lutfi Zylbeari[1,2], Sihana Ahmeti Lika[2], Nasir Behxheti[2],Mirlind Behxheti[2], Zamira Bexheti[2], Jetmire Jakupi-Alimani[2], Hanife Ahmeti[2], Ferizate Dika Haxhirexha[2], Ledia Kaci[2], Kastriot Haxhirexha[1,2]

[1] Clinician and Professor at Department of Internal Medicine, Faculty of Medical Sciences, U. of Tetova, Macedonia
[2] Clinical Hospital, Tetova, Macedonia
*Corresponding author: Prof. Dr. Lutfi Zylbeari, lutfi.zylbeari@unite.edu.mk

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 indipendent risk factor on the onset of early atherosclerosis and atheromateous 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 patietns with CVD compared to the control group showed satisticaly significant difference with $p<0.0001$, expected results and verified in many other multicentric studies. These facts show that raised Hcyt have more immpact on the onset of CVD .When Hcy levels are in blood, the activity of cystathionine-synthethasae 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 artherosclerosis of coronary and CVD(6,7,8).

Conclusions: In above mentioned cases it is recom-mended substi-tuive therapy with folic acid, pyridoxine, vitamin B12, vitamin E and other antioxidants which is found that have effect on prevention

of premature artherosclerosis 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 µmol/L) and thromboembolic (mainly venous) disease: treatment to lower but not to normalize these concentrations prevents vascular events. Mild homocysteine elevation (>15 µmol/L) occurs in ≈20–30% 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.Corelation between raised Hcyt and CVD was dissevered 25 years ago by Carson and Neil, who saw a defect of Hcyt metabolism in a patient with rasied 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-hyperhomocysteinema appears. Several studies have verified taht 15-30% of cases with CVD are result of hyperhomocysteinemia (3, 4, 5).

When hyperhomocysteinemia is corelated 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 corelation, still the role of homocysteine on cardiova-scular manifestations remanis unclear. It is verified that concentrations of homocysteine increases with somoking, aging and some diets with low folates, cyanocobalamin and pyridoxine. Many studies have verified a signifficant relationship between raised Hcyt and arterial hypertension and lipid distorders, compared with normotensive individuals. Lowering Hcyt con-

centrations can have some benefits in decreasing the risk of CVD in old age corelation 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 artherosclerosis in puberty and before 20 years of age. In these cases is verified the lack of some enzymes responsible for metabolisation 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 compo-nents , especially for synthesis of polypeptide hormone (13).

Homocysteine is sulfudric 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 thilos. (14).Homocysteine is synthesized from essential aminoacid methionine. Cystathionine-β-synthe-tasae is an enzyme, while pyrido-xine (B6) is essential cofactor which converts homocy-steine in cysteine. Hyperhomocystei-nemia is a condition with raised homocysteine levels in blood above 15 µmol/L (15,16, 17,18). CVD have high prevalence and still remain as main reason for high mortality and morbidity in world. Ethilology of CVD is multifactorial and mos 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 predi-cting coronary artery disease in patients with traditional risk factors, such as dyslipidaemia, hyperten-sion, diabetes mellitus (DM), and smoking, seems to have underestimated the coronary artery disease risk in individuals with high homocysteine plasmatic 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 dise-ases). The homozygous mutation of C,S can cause severe hyperhomocysteinemia where homocysteine concentra-tion is up to 40-fold of the normal levels. This disease occurs in approximately 1 of 100,000 live births. Also Hcy levels correlated significa-ntly 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 homocyste-

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 hyperhomocystei-nemia 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 od homozygote mutations of MTHFR-methylene tetra hydrofolate reductasae.These individuals with MTHFR defects are exposed to early CVD. Homocysteine is indipendent risk factor for early arthrosclerosis. Atherosclerosis is progressive inflamatory injury or arterial intimal layer, with increased permeability, lipidic deposition and calcification of intima. Corelation between hyperhomocys-teinemia and atheros-clerosis for first time was identified by McCully in 1969. Atherosclerosis is common patholo-gical process which leads to CVD (myocardial infarction, atheromatous processes pf carotid arteries, heart failure, stroke). Some of mechanisms of these effects are: endothelial disfunction, oxidative injury, increased collagen production, damage of arterial wall and increased C reactive proteine in vitro and in vivo (27, 28, 29). Concentrations of homocysteine in serum can be increased different diseases which cause disturbance in the metabolism of folates, B6, B12, lipides, lipoproteins, inflamation etc.

Prevalence of hyperhomocysteinema can vary between different populations and is tightly dependet 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 hyperhomo-cysteinemia 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 µmol/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 paltelets.

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 he used as a noninvasive way of predicting the number of diseased coronary arteries.

Matherial 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 19-24^0 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±10.00 years, while 80 were females of average age of 58.70±12.00 years.

The control group consisted of 180 individuals, of whom 100 were males and 80 females with mean median age=58.70±15.20. 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.

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 µmol/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 devijacioni X ± SD. Comparative statistics and tHcyLPL and lipid parameters betwe-en the two groups was analyzed by test called STUDENTOV and for examples of dependent or independent and non-para-metric 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 <statistical 0.0001. The results of the lipid profile and Hcys presented in the form of graphicones, averages and proportional / x, p /) were tested with accuracy higher than 95%, or rather, for mr. > 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 sta dard statistical program.

Results

	Tot.Pts.	ChT mmol/l	TG mmol/l	HDL-ch mmol/l	LDL-ch mmol/l	tHcy µmol/
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
p		0.7600	0.0001	0.0001	0.0001	0.0 001

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.**

Discusion

Homocysteine as risk factor for CVD, stroke, thrombotic processes, vascular hypercoagulability and atherosclerotic processes is known sinci early 1990. Scientists thought on homocysteine as risk factor on developing CVD still are controversial. Some scientists believe high concen-trations of Hcyt are not a risk factor for CVD , while lot of research results show high positive corelation 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 hyperho-mocysteinemia is because od homozygote mutations of MTHFR-methylene tetra hydrofolate reductasae. 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 indipendent risk factor for early arthrosclerosis. Atherosclerosis is progre-ssive inflamatory injury or arterial intimal layer, with increased permeability, lipidic deposition and calcification of intima. Corelation betweenhyperho-mo-cysteinemia and atherosclerosis for first time was identified by McCully in 1969. Atherosclerosis is common pathological process which leads to CVD (myocardial infarction, atheromatous processes pf carotid arteries, heart failure, stroke). Some of mecha-nisms of these effects are: endothelial disfunction, oxidative injury, increased collagen produ-ction, damage of arterial wall and increased C reactive proteine in vitro and in vivo (13,14). Homocystein 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 B_6, B_{12} and folic acid are essential cofactors in homocysteine-methionine metabolism.

Therefore low vitamin B availability (B_6, B_{12} 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. I Hyperhomocysteinemia can be caused by deficiency of folate, vitamine B_6 and B_{12} in food.An individual with deficiency of these abovementioned vitamines can develop raised leveles of Hcyt and have risk from hyperho-mocysteinemia.

Disorders of homocy-steine metabolism and other sulfuric aminoacids 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, exess alcohol, smoking, cafee, 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 hyperho-mocysteinemia. Concentrations of homocysteine in serum can be increased different diseases which cause disturbance in the metabolism of folates, B6, B12, lipides, lipoproteins, inflamation etc. Prevalence of hyperhomocystei-nemia can vary

between different populations and is tightly dependet 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 subsitutive therapy with vitamines or consumed with food higher concentrations of abovementioned vitamines compared with the group who havent consumed enough of them. Authors Victor and Hebert in their studies concluded that low levels of folic acid are as result od decreased absorbtion 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 exeprts still canot conclude and verify that by lowering high levels of homocystein 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 hyperhomo-cysteinemia in patients with chronic renal failure still is unknown, and an appropriate therapy for normalizing Hcy in these patients doesnt exist.Experts suggest that patients with CVD should analyse their Hcyt levels, and those with levels from 9-10 µmol/L should be treated at least one month with substituive therapy, this has shown positive effects. An recent study on positive effects of folic acide and vitamine B12 (combined or separately) on hyperhomo-cysteinemia has verified that by substi-tuting 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, cerebrovas-cular embolia and possible ways of decreasing it (34) Many studies have verified that patients after undergoing stenting or angioplasty with normalised Hcy levels have lower incidence of re-occuring of atheromatous processes compared with those who have high Hcy.

A recent study, which has lastet 6 months, a time which in patients vitamine B6 and B12 was given found that cardiac events and need for revasularisation was 1/3 time lower compared with patients who havent consumed above-mentioned therapy (35,36).High levels of Hcyt can be as result of cyanocobalamine deficiency which occurs because of vitamine B12 malabsorbtion as result of gastric atrophy, which is more often seen after age of 50. B12 deficiency causes anemia. If this deficiency is let untreated it causes damage to nervous system and early atherosclerosis. Individuals above 50 years of age are advised to consume folic acid and vitamine B12 because in this age most of them have gastric atrophy. An 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 artheriosclerosis many factors are included: genetic predisposition, environment, life style, sedentary life, obesity etc.

There are facts that by suplementing vitamine 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 apllicating folic acid, vitamine 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% occured, but mortality didnt show significant reduction. Documented facts exist that folic acid, cyanocobalamin and acetilcysteine have positive effects on decreasing homocysteine in one side and improving blood vessel function in other side. Nevertheless to verifu 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, cyanocobalamine and acetylcysteine on improving

endothelial funciton of blood vessels (38, 39, 40). Some studies have concluded that hyperhomocysteinemia is result of convertion of hydrogen peroxide in free oxygen radicals and convertion of oxidated Hcy in Homocysteine disulfide. Efect of oxidated Hcy which is increased by hydrogen peroxide explains the LDL raise. Hydrogen peroxyde causes endothelial desquamation, with inhibitory effect on prostacyclines and prostaglandines 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 homocy-steine concentration increase of 5 µmol/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 bioche-mical 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 homo-cysteine is a biomarker or a risk factor? Current guidelines have not classified homocysteine as cardio-vascular 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 homocy-steine 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 concen-trations of Hcy can be normalized by substituting 1 or 2 from above mentioned deficient vitamines. Homocysteinuria is geneticaly transmited disease. If a patients inherits 2 defective aleles the risk is much higher that in patients who inehret 1 alele. It is verified that in 100 individuals 1 person inherets 1 defective alele. Nair et al. in a study in indian population verified genetic mutations of Methylen-tetra-hydrofolate-reductasae, 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 od decreased absorbtion 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 homcysteinemia decreases also. Even after many studies regarding to hyperhomo-cysteinemia exeprts still canot conclude and verify that by lowering high levels of homocystein 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 resem-blance 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 indipendent risk factor for early atherosclerosis . In above mentioned cases it is prefered substitutive therapy with folic acid, pyridox-

ine, cyanocobalamin, tocoferol, acetylsalicilates and other antioxidantive agents, which clearly can prevent ealry atherosclerosis in CVD with: PTCA, CARB, AMI, APNS, Stening and prevention of stroke.

The study reveals homocysteine,diabetes mellitus, hyperlipidemia, hyper-tension, 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 correlation between homocysteine and cardiovascular disease. Nevertheless, the present review should provide some insight into the role of homocy-steine 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 hyperhomo-cysteinemia 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.

Reference

1. Shenov V, Mehendale V, et. al. Correlation of serum homocysteine levels with the severity of coronary artery disease. Ind J Clin Biochem. 2014;29(3):339–44. doi: 10.1007/s12291-013-0373-5.
2. Carmel R, Jacobsen DW. In: Homocysteine in health and disease. Carmel R, Jacobsen DW, editors. Cambridge: Cambridge UP; 2001. pp. 183–93.
3. Moustapha A, et al. Prospective study of hyperho-mocysteinemia as an adverse cardiovascular risk factor in end-stage renal disease. *Circulation* 1998; 97: 138−141.
4. Veeranna V, et al. Homocysteine and reclassification of cardiovascular disease risk. *J Am Coll Cardiol*. 2011; 58:1025-33.
5. Schaffer A, Verdoia M, et al.Relationship between homocysteine and coronary artery disease. Results from a large prospective cohort study. *Thromb Res*. 2014; 134:288-93.
6. Kazemi et al . Homocysteine level and Coronary Artery Disease. *Angiology , 2006;(57):9-14*.
7. Ridker PM and others. Homocysteine and risk of cardiovascular disease among postmenopausal vomen. *JAMA 1999; 281 : 1817-1821*.
8. Veeranna V, et al. Homocysteine and reclassification of cardiovascular disease risk. *J Am Coll Cardiol*. 2011; 58:1025-33.
9. Baszczuk A, Kopczynski Z. Hyperhomo-cysteinemia in patients with cardio-vascular disease [Abstract] Postepy Hig Med Dosw. 2014;68:579. doi: 10. 5604/17322693.1102340.
10. Wuerthele SE, Yasuda RP, Freed WJ, Hoffer BJ. The effect of local application of homocysteine on neuronal activity in the central nervous system of the rat. Life Sci. 1982;31:2683–91. doi: 10.1016/0024-3205(82)90712-3.
11 Mangge H, Becker K, Fuchs D, Gostner JM. Antioxidants, inflammation and cardiovascular disease. World J Cardiol. 2014;6(6):462–77. doi: 10.4330/wjc.v6.i6.462.
12. Schaffer A, Verdoia M, Cassetti E, Marino P, Suryapranata H, Luca GD. Relationship between homocysteine and coronary artery disease. Results from a large prospective cohort study. Thromb Res. 2014;134:288–93. doi: 10.1016/j.thromres.2014.05.025.
13. The Nobel Prize in Chemistry. Nobelprize.org. Nobel Media AB 2013. 1955.
14. Faeh D, Chiolero A, Paccaud F. Homocy-steine as a risk factor for cardiovascular disease: should we (still) worry about it? *Swiss Med Wkly*. 2006; 136:745-56.
15. Venes D, Clarence WT. In: *Taber's Cyclopedic Medi-*

cal Dictionary. 1st ed. Venes D, editor. Philadelphia: F.A. Davis; 2005: p.1089.
16. Hankey GJ, Eikelboom JW. Homocysteine and vascular disease. *Lancet*. 1999; 354:407-430.
17. Loscalzo J, Handy DE. Epigenetic modifications: basic mechanisms and role in cardiovascular Disease (2013 Grover Conference Series). *Pulm Circ*. 2014; 4(2):169-74.
18. Harvey RA, Ferrier DR. In: *Lippincott's Illustrated Reviews, Biochemistry*. 5th ed. Rhyner S, editor. Philadelphia: Wolters Kluwer Health; 2011: p.264-5.
19. Shenov V, Mehendale V, et al. Antioxidants, inflammation and cardiovascular disease. *World J Cardiol*. 2014; 6(6):462-77.
20. Verhoef P and others. Plasma total homocisteine, B vitamins and risk of coronary atherosclerosis. *Artheriosclerosis, Thrombosis, and Vascular Biology 1999; 17:989-995*.
21. Kazemi et al . Homocysteine level and Coronary Artery Disease. *Angiology , 2006;(57):9-14*.
22. Worachat M,. Thunyachai S,. Piyamitr S. Serum Homocysteine, folate and B12 concentration with Coronary artery Disease in thai patients. J Med Assoc Thai 2004; 87(6)674-678
23. Neki NS,. Hyperhomocysteinemia –An independent risc factorS for cardiovascular diseasse. *JIACM 2003;(4):55-60.21*.
24. Okura T, Miyoshi K, et al.. Hyperhomocysteinemia is one of the risk factors associated with cerebrovascular stiffness in hypertensive patients, especially elderly males. *Nature.com Sci Rep*. 2014; 4:5663.
25. Pang X, Liu J, et al.. „Homocysteine induces the expression of C - reactive protein via NMDAr-ROS-MAPK-NF-ϰB signal pathway in rat vascular smooth muscle cells. *Atherosclerosis*. 2014; 236:73-81.
26. Curro M, Gugliandolo A, et al. Toxic effects of mildly elevated homocysteine concentrations in neuronal-like cells. *Neurochem Res*. 2014; 39:1485-95.
27. Zhang S, Yong-Yi B, Luo LM, Xiao WK, Wu HM, Ye P. Association between serum homocysteine and arterial stiff-ness in elderly: a community-based study. *J Geriatr Cardiol*. 2014; 11:32-8.
28. Rhoda Makoff, PhD, et al. Folic Acid, Pyridoxine, Cobalamin, and Homocysteine and Their Relationship to Cardiovascular Disease in End-Stage-Renal-Disease. *Journal of Renal Nutrition,1 january 1996; vol (6): No 1 ; pp 2-11*.
29. Harish Rao B. V.Govindaraju and C.N. Manjunath. Risc Prediction-Homocystein in Coronary Heart Disease. *In J of Clinical Biochemmistry. 2007/22/(1) s.18-*
29. Schaffer A, Verdoia M, Cassetti E, Marino P, Suryapranata H, Luca GD. Relationship between homocysteine and coronary artery disease. Results from a large prospective cohort study. Thromb Res. 2014;134:288–93. doi: 10.1016/j.thromres.2014.05.025.
30. Pang X, Liu J, Zhao J, Mao J, Zhang X, Feng L, et al. Homocysteine induces the expression of C - reactive protein via NMDAr-ROS-MAPK-NF-ϰB signal pathway in rat vascular smooth muscle cells. Atherosclerosis. 2014;236:73–81. doi: 10.1016/j.atherosclerosis.2014.06.021.
31. Okura T, Miyoshi K, Irita J, Enomoto D, Nagao T, Kukida M, et al. Hyperhomocysteinemia is one of the risk factors associated with cerebrovascular stiffness in hypertensive patients, especially elderly males. Nature.com Sci Rep. 2014;4:5663.
32. Zhang S, Yong-Yi B, Luo LM, Xiao WK, Wu HM, Ye P. Association between serum homocysteine and arterial stiffness in elderly: a community-based study. J Geriatr Cardiol. 2014;11:32–8.
33. Malinow MR and others. Homocyst(e) ine,diet,and cardiovascular diseases: A statement for healthcare professionals from the nuntrition committee. *American Heart Association. Circulation,1999; (99): 178-182*.
34. Lentz SR. Does homocystein promote atherosclerosis. *Arterioscler Thromb Vasc Biol. 2001; 21: 1385-1386*.
35. Perna AF, Ingrosso D, et al. Possible mechanisms of homocysteine toxicity. *Kidney Int Suppl*. 2003; 63: S137–S140.
36 . Schnyder G, Roffi M, Flammer Y, et al. Effect of homocysteine-lowering therapy with folic acid, vitamin B12, and vitamin B6 on clinical outcome after percutaneous coronary intervention: the Swiss Heart study: a randomized controlled trial. *JAMA*. 2002; 288: 973–979.
37. Massy ZA. Potential strategies to normalize the levels of homocysteine in chronic renal failure patients. *Kidney Int Suppl*. 2003; 62: S134–S138.
38. Liem A, Reynierse-Buitenwerf GH, Zwinderman AH, et al. Secondary prevention with folic acid: effects on clinical outcomes. *J Am Coll*

*Cardiol.*2003; 41: 2105–2113.

39. Tepel M, van der Giet M, Statz M, et al. The antioxidant acetylcysteine reduces cardiovascular events in patients with end-stage renal failure: a randomized, controlled trial. *Circulation.* 2003; 107: 992–995.
40. Killian C. Robinson, MD. Renal Disease, Homocysteine, and Cardiovascular Complications. *Circulation* .2004;109:294-295 doi:10.116.
41. Kang SS and others. Prospective study of serum homocysteine and risk for occlusive vascular disease. *Annual Review of Nutrition 1992;(12):279-298*. Rimm EB and others. Floate and Vitamin B6 from Diet Supplements in relation to risk of Coronary Heart Disease among Women. *JAMA 1998;(279):359-364*.
42. Malinow MR and others. Homocyst(e) ine,diet,and cardiovascular diseases: A statement for healthcare professionals from the nuntrition committee. *American Heart Association. Circulation,1999; (99): 178-182*.
43. Dudman NPB, Wilcken DEL, Stocker R: Circulating lipid hydroperoxide levels in human hypr homocysteinemia: Relevance to development of arteriosclerosis. *Arterioscler Thromb 1993; 13: 512-516*.
44 . Schnyder G, Roffi M, Flammer Y, et al. Effect of homocysteine-lowering therapy with folic acid, vitamin B12, and vitamin B6 on clinical outcome after percutaneous coronary intervention: the Swiss Heart study: a randomized controlled trial. *JAMA*. 2002; 288: 973–979.