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Professional paper

HOMOCYSTEINE AS A RISK FACTOR FOR CARDIOVASCULAR DISEASES IN PATIENTS WITH END-STAGE RENAL DISEASE

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Abstract

Cardiovascular diseases (CVD) still remain the main cause of mortality in patients with chronic renal failure in the final stage (chronic terminal renal insufficiency- ESRD), which appears as a consequence of premature atherosclerotic processes (atherosclerosis praecox). In addition to known factors such as: diabetes, arterial hypertension, sedentarity, uremic dyslipidemia, obesity, oxidative stress, MIA syndrome, increased proinflammatory cytokines (interleukin 6, Interleukin 18, Interleukin-1 beta (IL-1β) alpha necrosis factor (alpha tumor necrosis factor (IL-1α) [1] in recent years as an independent non-traditional risk factor for the occurrence of CVD in patients with chronic renal failure and those with ESRD (uraemic) treated with chronic hemodialysis (HD) is also homocysteine (Hcy) with its high concentrations - hyperhomocysteinemia (HHcy). In recent years, a large number of studies suggest that high levels of Hcy are an important biomarker for the risk of CVD, the increased level of Hcy significantly increases the impact on CVD and mortality in this population (2,3). Therefore, it is necessary to evaluate the level of Hcy in patients with ESRD from the initial stages in order to prevent the occurrence of premature atherosclerotic processes that lead to the risk of CVD.

Keywords: homocysteine, risk factor, cardiovascular disease, end-stage renal disease.

1. Introduction

CVD continues to be a major cause of disability and mortality in both developed and developing countries. Increased concentrations of Hcy (Hhcy) associated with hyperlipidemia are counted as a risk factor for the occurrence of premature atherosclerosis processes in patients with ESRD and uremic ones treated with chronic HD. Hyperhomocysteinemia (HHy) increases the risk of CVD in patients with CKD through several mechanisms: endothelial cell damage, reduced nitric oxide (NO) production, NO insufficiency affects blood vessel vasoconstriction and the formation of atherosclerotic plaques, increase in oxidative stress, increase in free radicals [4,5]. HHcy affects the activation of the Renin-Angiotensin-Aldosterone (RAAS) system by increasing vasoconstriction, sodium and water retention, which affect the increase in arterial pressure. Hcy levels are less elevated in patients with preterminal ESRD compared to patients with ESRD treated with HD. Although the increase in the level of Hcy in patients with chronic renal failure is not exactly known, it is assumed that HHcy occurs as a result of an increase in the rate of production of Hcy (ie, transmethylation, transsulfuration), decreased excretion as a result of reduced metabolic clearance of the kidneys, reduced glomerular filtration rate, as a result of any genetic defect in Hcyt metabolism (which affects the increase of level of Hcyt and increased activity of the cystathionine-synthetase enzyme, which has an important role in the metabolism of Hcy), disorder of the methionine cycle, catabolism in uremia, reduction of peritubular excretion [6,7], etc. Patients with very high levels of total Hcy (> 100 μmol/L) due to inborn metabolic failures affecting cystathionine-β-synthase are potential candidates for the development of premature atherosclerotic processes and thrombolytic disorders. [8].Homocysteine (Hcy) is a

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non-essential sulfur-containing amino acid that plays an important role in the Hcy-methionine cycle through its interaction with folic acid and vitamin B12. Interruption of this metabolic process can result in the accumulation of Hcy, which affects vascular tissue damage, coronary, cerebral and peripheral artery damage. In recent years, many studies have been done on the effect of HHcy and its impact on the occurrence of atherosclerosis. (Ath) premature coronary arteries and all have proven that HHcy is an important parameter for the early appearance of atherosclerotic processes in coronary arteries and CVD [9,10]. The treatment of HHcy can affect the prevention of occlusive-vascular damage. The harmful effects of HHcy are due to the production of oxidants (reactive oxygen species) created during the oxidation of Hcy and disulfides in the blood. A large number of studies have proved that the level of total Hcy (Hcyt) increases as a result of folate deficiency, cyanocobalamin, pyridoxine, smoking and old age. A large number of studies have verified that there is a high positive correlation between HHcyt, arterial hypertension and hypertriglyceridemia, while there is a high negative correlation between Hhcy and the degree of glomerular filtration (an increase in Hcy consists of a decrease in the degree of glomerular filtration) [11-14].

2. Purpose of the work

The aim of this research was to verify the concentrations of Hcy in patients with ESRD in the preterminal phase and ESRD treated with HD compared to the values obtained from the control group of healthy individuals (volunteer blood donors).

3. Material and methods

Blood from the vein of all examinees at 8 o'clock in the morning in a state of starvation was used as working material. In the study there were 60 patients (25 women with an average age of 56.40±8.40 years and 35 men with an average age of 57.30±9.00 years) with preterminal ESRD (in stage III a and b) and 70 patients (30 women with an age average age of 58.00±4.00 years and 40 men with an average age of 59.60-±5.40 years (with ESRD) treated with chronic HD and biocompatible polysulfonic membrane F6HPS and F5 HPS over 12 months with a frequency of three times a week for 4.5 hours. Blood taken for analysis (5 ccm of serum dissolved with a few drops of heparin) for analysis it was sent to the Institute of Clinical Biochemistry and the Clinical Laboratory at the Clinical University Center of the Faculty of Medicine in Skopje. In the study we also had a control group of 50 healthy individuals (volunteer donors), of which 20 were female and 30 were male with an identical mean age of 57.40±10.00 years. In addition to the standard laboratory parameters determined by the protocol nephrology (urea, creatinine, uric acid, lipid fractions), electrocardiogram (EKG) and echocardiography in order to follow the changes of the left ventricle and interventricular septum (Left ventricular posterior wall end diastole LVPWd- Interventricular septum thickness in diastole) IVSd) > 12 mm, in all examinees (both patients and the control group) the level of total homocysteine (Hcyt) and blood pressure were determined every 4 months within 12 months, which was also the aim of the study.
As a reference value for Hcy, a value of 5-13 μmol/L was taken. The glomerular filtration rate in the third stage patients (a and b) and the control group was determined with the MDRD formula (Modification of Diet in Renal Disease (MDRD-GFR in mL/min/1.73 m2=175 x SerumCr-1.154 x age- 0.203 x 1.212 (for black patients) x 0.742 (for female gender).

4. Statistical processing of the results

The results obtained from the patients examined with IRK, IRKT and the control group were statistically processed with arithmetic mean value, standard deviation X ± SD, with the student's "t" test, Mann-Whitney and Wilcoxon test. The results were processed with the SPSS V26 program.

5. Results

The results obtained represent the average values obtained within 12 months (measurement every four months). The results obtained from the patients and the control group are presented in the following text, where a statistically significant difference for $p<0.0001$ was observed between the parameters obtained from the ESRD and uremic patients treated with HD compared to the control group. Hcy levels were also increased in patients with stage III CKD, but higher were manifested in patients with CKD treated with HD. The relationship between Hcy levels and glomerular filtration rate (GFR) in patients with stage 3 CKD the third was with negative significance, which means that with the increase of Hcy there was a decrease in the glomerular filtration rate and an increase in arterial pressure. Hcy and total cholesterol were in negative correlation for $p<0.001$).

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

Gender	ESRD patients phase III(a+b)=60	The average age±DS	HD patients=70	The average age±DS	Control group=50	The average age±DS
Female	**25 (41.7%)**	**56.40± 8.40**	**30 (42.85%)**	**58.00±4.00**	**20 (40%)**	**57.40±10.00**
Male	**35 (58.3%)**	**57,30±9.00**	**40 (57.15%)**	**59.60-±5.40**	**30 (60%)**	**57.40±10.00**

Table number 2: Obtained values of the examined parameters from patients with ESRD stage III(a+b)

Examined parameters	Female	Male	Control group-F	Control group-M	*P*
Hcyt (µmol/L)	**20.30±4.50**	**24.00±3.60**	**7.00±4.50**	**8.20±3.00**	**0.001**
Urea(mmol/l)	**15.80±4.00**	**15.80±4.00**	**6.50±1.80**	**7,40±2.00**	**0.001**
Creatinine(µmol/l)	**195.00±14.80**	**210.00±6.50**	**82.40±4.10**	**87.50±2.15**	**0.001**
Uric acid (µmol/l)	**390.20±5.20**	**410.00±6.50**	**310±7.00**	**370.00±6.50**	**0.001**
GFR(ml/min/1.73m^2)	**34.30±2.10**	**37.40±4.10**	**115,00±3,80**	**118,00±4.60**	**0.001**
LT (g/l)	**7.50±1.20**	**8.10±1.00**	**6,80±2,14**	**7.50±1.40**	**0.001**
ChT(mmol/l)	**4.60±1.50**	**4,90±2.60**	**5.60±1.80**	**6.40±1.20**	**0.001**
LDL-ch(mmol/l)	**3.80±0.90**	**3.90±0.80**	**3.20±0.40**	**3.50±0.20**	**0.001**
HDL-ch(mmol/l)	**1.02±0.40**	**1.03±0.30**	**1.45±1.00**	**1.52±0.60**	**0.001**
TG(mmol/l)	**2.90±0.90**	**3.00±0.75**	**1.50±0.70**	**1.40±0.90**	**0.001**

From the table itself it can be seen that the levels of Hcyt, urea, creatinine, LDL-ch and TG were increased in both groups of ESRD and HD patients) and the correlation between them was positive for $p=0.001$ while the correlation between Hcyt and HDL-ch and GFR was negative. Between female and male ESRD patients a significant difference in Hcyt levels was observed for $p < 0.05$.

Table number 3: Values of laboratory parameters obtained from patients treated with HD and the control group.

Examined parameters	Female	Male	Control group-Female	Control group-male	*P*
Hcyt (µmol/l	32.00±3.15	38.70±4.20	7.00±4.50	8.20±3.00	0.001
Urea(mmol/l)	30.60±2.00	34.00±4.00	6.50±1.80	7,40±2.00	0.001
Creatinine(µmol/l)	380.00±24.00	410.00±16.00	82.40±4.10	87.50±2.15	0.001
Ac.urik(µmol/l)	450.00±12.00	468.00±10.50	310±7.00	370.00±6.50	0.001
GFR(ml/min/1.73m^2)	<10	< 8	115,00±3,80	118,00±4.60	0.001
LT (g/l)	8.50±1.00	9.20±0.80	6,80±2,14	7.50±1.40	0.001
ChT(mmol/l)	4.50±120	5.00±1.00	5.60±1.80	6.40±1.20	0.750
LDL-ch(mmol/l)	4.50±0.60	4.80±0.60	3.20±0.40	3.50±0.20	0.001
HDL-ch(mmol/l)	0.80±0.25	0.75±0.40	1.45±1.00	1.52±0.60	0.001
TG(mmol/l)	3.80±1.00	4.06±1.40	1.50±0.70	1.40±0.90	0.001

From table number 3 itself, it is observed that the results obtained (for Hcyt, urea, creatinine, uric acid, TG, LDL-ch, total lipids from the group of uremic patients treated with HD were filled with a positive statistical significance except between tHcy , glomerular filtration rate, total cholesterol and triglycerides that manifested a statistically significant negative correlation.

6. Discussion

Homocysteine (Hcy) is a sulfur-containing non-essential amino acid that plays a role in the Hcy-methionine cycle through its interaction with folic acid and vitamin B12. Disruption of this metabolic process can result in the accumulation of Hcy, which can be in CVD [15]. Cardiovascular diseases (CVD) are the main cause of death in 44-75% of victims with CKD, of which only 22% from acute coronary syndrome [16,17,18]. Life expectancy of individuals with CKD is significantly lower than those with normal kidney function, estimated to be less than 50% compared to the general population [19]. Hcy is considered an unconventional prognostic biomarker for CVD both in the general population and in individuals with ESRD. [20,21,22]. It has been verified that reducing Hcy concentrations in serum reduces the risk of premature atherosclerosis and CVD. The decrease in NO synthesis has a key role in the development of the atherosclerosis process [23]. Successful correction of plasma Hcy concentrations may lead to a reduction in CVD-related morbidity and mortality. However, it is important to note that in patients with ESRD, other factors may also influence the efficacy of Hcy-lowering medications, including genetic polymorphisms, consumption of fortified cereals, inflammatory processes, malnutrition, high levels of glucose, advanced age, long period (years) of treatment with HD, use of low flux and incompatible membranes [24,25,26]. The use of statins, folic acid (B9), vitamin B6, vit.B12, N-acetylcysteine (NAC) and omega-3 fatty acids in patients with ESRD gave those uremic treated with chronic HD have shown positive effects in the treatment of HHcy with which significantly reduces the appearance of SKV (27-33). Our study showed a negative correlation between Hcy, glomerular filtration rate and HDL-ch, while a positive correlation between urea, creatinine, TG, LDL-ch. This correlation can be explained as a result of disorders in the metabolism of Hcy and lipid fractions. in patients with preterminal ESRD with GFR $\leq$ 60 ml/min/1.73m^2 and terminal [34,35,36]. The treatment of HHcy in patients with preterminal ESRD and uremic ones treated with HD consists of supplementation with folic acid (vitamin B9) in a dose of 5-15 mg/day, vitamin B12 1000 µg/day, vitamin B6 2x20 mg/day as and various antioxidants (tocopherol, Omega 3, 6, 9) etc.

7. Conclusions

In conclusion, we may prefer that the examination of Hcyt concentrations in patients with ESRD be included in the protocols of nephrological examinations from the initial stages of the disease in order to prevent the appearance of premature atertosclerotic processes with its impact on CVD.

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