

# Homocystein as a Risk Factor for Developing Complications in Chronic Renal Failure

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## ABSTRACT

**Aim:** Cardiovascular diseases are leading cause of death in patients with chronic renal failure. The aim of our study was to establish connection between levels of homocysteine and traditional and nontraditional risk factors for developing cardiovascular diseases in dialysis and pre dialysis patients. **Methods:** We included 33 pre dialysis (23 in stage three and 10 in stage four of chronic kidney disease) and 43 patients receiving hemodialysis longer than six months. Besides standard laboratory parameters, levels of homocysteine and blood pressure were measured in all patients. Glomerular filtration rate was measured in pre dialysis patients and dialysis quality parameters in dialysis patients. **Results:** Homocysteine levels were elevated in all patients ( $19 \pm 5.42$  mmol/l). The connection between homocysteine levels and other cardiovascular diseases risk factors was not established in pre dialysis patients. In patients treated with hemodialysis we found negative correlation between homocysteine levels and patients' age ( $p < 0.05$ ) and positive correlation between homocysteine levels and length of dialysis ( $p < 0.01$ ) as well as between homocysteine and anemia parameters (erythrocytes, hemoglobin), ( $p < 0.01$ ). Homocysteine and LDL (and total cholesterol) were in negative correlation ( $p < 0.01$ ). **Conclusion:** Homocysteine, as one of nontraditional cardiovascular diseases risk factors, is elevated in all patients with chronic renal failure and it's positive correlation with some other risk factors was found.

**Key words:** homocysteine, hemodialysis, cardiovascular diseases.

## 1. INTRODUCTION

Cardiovascular diseases (CVD) are leading cause of death in patients with chronic kidney disease (CKD). CVD are considered to be the cause of death in 44% CKD patients, out of which 22% were caused by acute coronary syndrome alone (1-4). Hyperhomocysteinemia has been recently recognized as independent risk factor for developing CVD in CKD with prevalence of 85-90% (5-7). However, there are studies who showed little or no connection between homocysteine (Hcy) levels and cardiovascular events (8-10). Due to inconsistency of literature data, we aimed to:

Establish connection between Hcy levels and traditional (hypertension, hyperlipidemia) and nontraditional (anemia, glomerular filtration rate, age, gender) risk factors in pre dialysis and dialysis patients. Find out if there is difference in Hcy levels between pre dialysis and dialysis patients

## 2. MATERIALS AND METHODS

Study included two groups of patients, pre dialysis (CKD in third and fourth stadium) and dialysis (treated with regular hemodialysis). Included patients did not have congestive heart failure or diabetes mellitus. We complied with Helsinki declaration for medical research and informed consent was signed by each participant in the study. Pre dialysis group had 33 patients, 15 male and 18 female, from Nephrology department of University hospital Clinical Centre Banjaluka. Dialysis group had 43 patients, 25 male and 18 female, treated at International dialysis center Banjaluka with regular hemodialysis three times per week, longer than 6 months. Standard bicarbonate hemodialysis was performed using Fresenius 4008S with programmed ultrafiltration and with high flux polysulfone membranes HF60, HF70 and HF80 sterilized with steam water. During hemodialysis, each patient was continuously heparinized with 3000-5000 IJ of heparin. All patients remained hemodynamically stable

during treatment periods. Total homocysteine concentration was measured by FPIA method (Fluorescence Polarization Immunoassay) using AxSYM analyzer. Reference range for total homocysteine (Hcy) is 5-15  $\mu\text{mol/L}$ . Blood samples were taken after 12 hours fasting, before start of hemodialysis and heparin treatment. Adequacy of hemodialysis was asses using URR (Urea Reduction Ratio) and  $K_t/V_{sp}$  index. Data were analyzed using descriptive statistical methods:  $X_{\min}$ ,  $X_{\max}$ , mean, Sd, variation coefficient. For establishing connection between different parameters, Pearsons coefficient of linear correlation was used.  $P < 0.05$  were used as significance levels in the study.

### 3. RESULTS

#### Pre dialysis patients

##### Laboratory values

Table 1 shows laboratory parameters values in pre dialysis patients. Significant difference in Hcy levels was found between female and male patients ( $p < 0.05$ ).

##### Blood pressure

Values of systolic blood pressure (SBP), diastolic blood pressure (DBP) and mean arterial pressure (MAP) in pre dialyzed patients did not differ significantly between male and female patients. Correlation analysis did not show correlation between Hcy and blood pressure.

##### Homocystein, creatinine and GFR – correlation analysis

In correlation analysis we did not divide patients according to gender, since we have not found significant difference between them. Analysis showed statistically significant correlation be-

tween nontraditional risk factors, serum creatinine levels and GFR and Hcy levels. We also found statistically significant negative correlation between Hcy and GFR ( $p < 0.01$ ).

#### Dialysis patients

##### Laboratory values

Table 2 shows laboratory parameters values in dialysis patients. Male and female patients differed significantly in values of LDL, total cholesterol, post dialysis urea and serum creatinine ( $p < 0.05$ ). Female patients had higher values of LDL and total cholesterol while male patients had higher values of serum creatinine and post dialysis urea.

##### Homocystein and traditional risk factors–correlation analysis

We used correlation analysis to determine if there was connection between Hcy levels and traditional risk factors (hypertension and hyperlipidemia). We found statistically significant negative correlation between Hcy levels and SPB ( $p < 0.05$ ) and no correlation between Hcy levels and DBP and MAP.

Using correlation analysis we established statistically significant negative correlation between Hcy and LDL ( $p < 0.01$ ) (Figure 1) and between Hcy and total cholesterol ( $p < 0.05$ ) (Figure 2).

##### Homocystein and nontraditional risk factors – correlation analysis

We used correlation analysis to determine if there was connection between Hcy levels and nontraditional risk factors (age, gender, duration of dialysis, anemia, creatinine, quality of dialysis) in dialyzed patients. We found highly significant negative correlation between levels of Hcy and age of dialysis

Laboratory parameters	female (N = 18)	male (N = 15)	p	total(N=33)	
	X±SD	X±SD		min.-max.	X±SD
Homocysteine ( $\mu\text{mol/L}$ )	17.56 ± 4.76	21.98 ± 5.31	<0.05	11.96-30.53	19.57±5.42
Er	4.08 ± 0.35	4.31 ± 0.43	ns	3.5-5.1	4.18±0.40
Hgb (g/L)	120.11 ± 11.27	121.73 ± 14.93	ns	100-153	120.85±12.87
Fe (mmol/L)	12.21 ± 3.61	11.74 ± 3.56	ns	6.7-21	12.0±3.54
Creatinine ( $\mu\text{mol/L}$ )	162.94 ± 70.9	183 ± 57.34	ns	90-320	172.06±64.90
Holesterol (mmol/L)	5.74 ± 1.04	5.15 ± 1.25	ns	3.2-7.8	5.48±1.16
HDL (mmol/L)	1.14 ± 0.46	0.93 ± 0.22	ns	0.52-2.3	1.05±0.38
LDL (mmol/L)	3.82 ± 1.1	3.38 ± 1.31	ns	1.49-5.97	3.62±1.20
Triglycerides (mmol/L)	2.48 ± 2.04	2.8 ± 1.39	ns	0.73-9.96	2.63±1.76
eGF(ml/min/1.73m <sup>2</sup> )	35.48 ± 14.73	39.65 ± 16.78	ns	12.91-73.14	37.37±15.59

Table 1. Laboratory parameters values in predialysis patients. Notes: X – mean value; SD – standard deviation; min. – minimum value; max. – maximal value; ns – no statistically significance;  $p < 0.05$  – significantly

Laboratory parameters	female(N = 18)	male (N = 25)	p	total(N=43)	
	X±SD	X±SD		min.-max.	X±SD
Homocist.( $\mu\text{mol/L}$ )	22.71 ± 7.84	27.86 ± 11.1	ns	12.8-62.7	25.70±10.09
Er	2.94 ± 0.43	3.04 ± 0.35	ns	2.3-4.08	3.00±0.39
Hgb (mmol/L)	98.94 ± 13.26	102.88 ± 12.4	ns	74-128	101.23±12.76
Fe (mmol/L)	13.82 ± 5.4	14.1 ± 4.58	ns	6.7-27.8	13.98±4.88
HDL (mmol/L)	0.91 ± 0.25	0.78 ± 0.4	ns	0.3-2.3	0.83±0.35
LDL (mmol/L)	3.28 ± 1.02	2.56 ± 0.87	<0.05	1.12-6	2.86±0.99
Holesterol (mmol/L)	5.01 ± 1.18	4.24 ± 1	<0.05	2-8.3	4.56±1.13
Triglycerides (mmol/L)	1.99 ± 0.99	2 ± 1.17	ns	0.3-5.8	1.99±1.09
Urea(pre) (mmol/L)	24.81 ± 5.32	26.02 ± 3.91	ns	16.8-42.2	25.51±4.54
Urea(post.) (mmol/L)	6.54 ± 1.96	8.99 ± 1.26	<0.05	4.3-12.3	7.97±1.99
Creatinine ( $\mu\text{mol/L}$ )	909.89 ± 156.44	1181.3 ± 215.27	<0.05	674-1598	1067.7±234.0

Table 2. Laboratory parameters values in dialysis patients. Notes: X – mean value; SD – standard deviation; min. – minimum value; max. – maximal value; ns – no statistically significance;  $p < 0.05$  – significantly

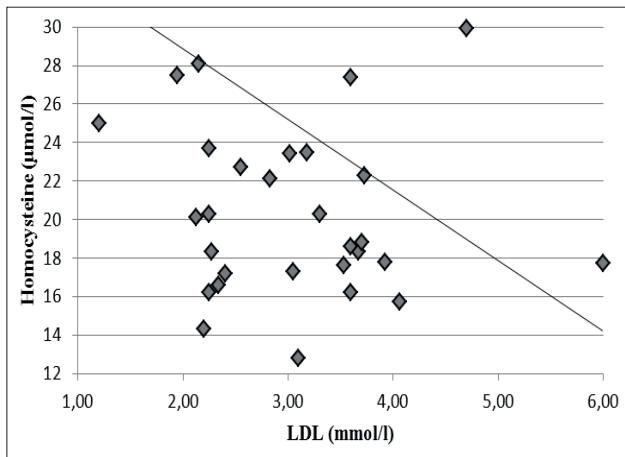


Figure 1. The relationship of homocysteine and LDL serum levels of dialysis patients

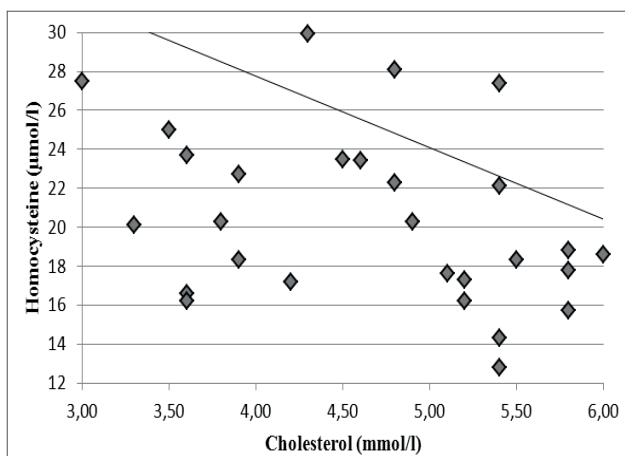


Figure 2. The relationship of homocysteine and total cholesterol serum levels in dialysis patients

patients ( $p < 0.01$ ), as well as between levels of Hcy and duration of dialysis ( $p < 0.01$ ).

#### Pre dialysis and dialysis patients – comparison of results

We investigated presence of statistically significant difference between pre dialysis and dialysis patients including relation between Hcy and tested parameters. We found statistically significant difference in age between pre dialysis and dialysis patients ( $p < 0.05$ ). Pre dialysis patients were significantly older.

It was confirmed highly significant difference between dialysis and pre dialysis patients in all tested parameters (Hcy, erythrocytes, hemoglobin, serum creatinine, total cholesterol, HDL and LDL).

#### 4. DISCUSSION

GFR was decreased in all pre dialysis patients (third and fourth stadium of CKD) with a mean value of 37,37 ml/min/1.73m<sup>2</sup>. Decreased GFR is not a CVD risk factor per se, but it causes metabolism changes and increased levels of certain parameters which then represent risk factors for CVD (total cholesterol, HDL, LDL, triglycerides, Hcy, calcium, phosphates, etc.) (11). Our study demonstrated high level of negative correlation between GFR and serum creatinine levels. There was a high level of negative correlation between GFR and Hcy; decrease in GFR leads to Hcy serum levels increase. We showed that serum creatinine levels and Hcy levels are in statistically significant positive correlation, which is in accordance to results

of other authors. This correlation can be explained by connection of metabolic pathways of these two substances. S-adenosil methionine is a methyl group donor for creatine, precursor of creatinine, and S-adenosil Hcy (Hcy) is created (12).

We haven't found statistically significant correlation between Hcy levels and HDL, LDL, triglycerides and total cholesterol levels in pre dialysis patients, although some authors had opposite results (13). CKD patients have altered metabolism of lipids in the earliest faze of disease which manifests as hypertriglyceridemia, decreased HLD and normal or decreased LDL and total cholesterol. Disturbance of lipid metabolism accelerates progression of CKD and in CKD patients it is usually present with  $GFR \leq 60$  ml/min/1.73m<sup>2</sup> (13). Some epidemiological studies showed negative correlation between HDL levels and future CDV events (14). Although CKD patients have usually have normal or slightly decreased levels of LDL, there is a serious disorder in distribution of its sub fractions. Low density particles, which are more aterogenic than high density ones, are predominant (15). Other CVD risk factors (anemia, lipid status, hypertension, age and gender) are not significantly correlated to Hcy. In dialysis patients we found high level of negative correlation between age and Hcy levels ( $p < 0.05$ ) and also between length of dialysis and Hcy levels ( $p = 0.01$ ). Older age and treatment with dialysis longer than 5 years are directly related to higher number of cardiovascular events. Older age is accompanied by muscle mass decrease and malnutrition, which can explain decrease in Hcy levels with age (3, 6, 14).

In our study, most dialysis patients had elevated levels of total cholesterol, and we established high level of negative correlation between levels of total cholesterol and Hcy which is similar to results of other authors (16). High levels of Hcy lead to increase in hepatic synthesis of cholesterol and triglycerides and decrease in levels of HDL and its most significant protein apo-A1 in serum. Low levels of total cholesterol in dialysis patients represent a sign of malnutrition or pronounced inflammation syndrome. Hcy induces endoplasmic reticulum stress which activates genes for coding enzymes in cholesterol and triglycerides biosynthesis (16). Increased synthesis and intracellular utilization and accumulation of cholesterol and triglycerides are the result of this hepatocyte activation. Lipid accumulation in tissues which are sensitive to endoplasmic reticulum stress might be explanation for apparently normal lipid profile in patients with hyperhomocysteinemia. Increased Hcy serum concentration leads to increase in cholesterol and triglycerides synthesis and accumulation in endothelial cells and this can be considered as link between hyperhomocysteinemia and development of atherosclerosis. Hyperhomocysteinemia accelerates utilization of LDL by macrophages which might explain normal or slightly decreased LDL levels in patients in our study (17, 18, 10). Some authors claim that (19) is not related to lipid status.

We showed in our study a high level of negative correlation between Hcy levels and MAP, and also between Hcy levels and DBP, while there was no statistically significant correlation between Hcy levels and SBP in dialysis patients. Hypertension is usually present in 50-90% of these patients (19-21). Each 10mmHg elevation in blood pressure increases CVD risk for 44%, while patients with left chamber hypertrophy and congestive heart failure are in even greater risk. Low blood pressure represents marker of severe heart failure, but it does not increase CVD mortality in dialysis patients (19).

Our study showed presence of hyperhomocysteinemia in 40 (93%) of dialysis patients. High Hcy level is considered to be the cause of endothelial dysfunction by blocking the enzyme diethyl-diamino-hydrolase (DDHA) in endothelial cells which biodegrades asymmetric dimethylarginine (ADMA) to L-citrulline and dimethylamine. ADMA is the most significant endogenous substance which blocks azote monoxide (NO) synthesis. Decreased synthesis of NO has key role in atherosclerosis process development (22). High level of Hcy indicates poor CVD outcome in hemodialysis patients (7, 23-26). However, some studies showed opposite results, by demonstrating very low or no connection between Hcy levels and cardiovascular events (8). Most authors agrees on treatment of hyperhomocysteinemia in dialysis patients with folic acid, 5-15mg OD per os, vitamin B1 1000 µg OD and vitamin B6 50 µg OD IV (27,28). There is still no unique opinion on whether decreasing Hcy levels leads to lower risk of cardiovascular events (29).

Comparing the results between pre dialysis and dialysis patients we found out that pre dialysis patients were older. It is well known that older age represents risk factor for cardiovascular events and mortality. All tested parameters – Hcy, erythrocytes, hemoglobin, serum creatinine, total cholesterol, HDL and LDL were higher in dialysis compared to pre dialysis patients. The only parameter which level did not differ significantly between these two groups of patients was serum iron. Hematologic parameters which are monitored in anemia (erythrocytes, hemoglobin, hematocrit) were lower in pre dialysis patients due to the fact that they haven't received erythropoietin which is used for correction of anemia in hemodialysis patients. Levels of serum creatinine and lipid status parameters were higher in dialysis patients due to higher stadium of CKD and more pronounced metabolic disorders.

## 5. CONCLUSION

Having in mind all disorders in CKD along with CVD risk factors specific for this group of patients it is of high importance to establish continuous monitoring and adequate treatment in order to prevent cardiovascular events and consequently decrease CVD morbidity and mortality. The limitation of this study is a small number of patients included which enabled generalization of results, but considering the impact of Hcy on blood vessels and process of atherosclerosis, it would be of high importance to monitor Hcy levels in all CKD patient, especially those treated with hemodialysis.

CONFLICT OF INTEREST: NONE DECLARED.

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