ORIGINAL ARTICLE

Homocystein and carotid atherosclerosis in chronic renal failure

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Abstract

Background: Since total homocystein (Hcy) is markedly elevated in patients with chronic renal failure (CRF), it has been presented as potential factor contributing to the high risk of cardiovascular disease (CVD) in CRF. The aim of the study was to examine the significance of elevated Hcy and other cardiovascular risk factors for carotid atherosclerosis in patients with CRF.

Material and Methdos: Fifty six patients 16-M, 40- F, average age 58±14.55, creatinine clearance 39.19±10.11 ml/min were examined. In addition, 20 control healthy subjects were examined. The association of Hcy levels and classic risk factors for atherosclerosis with common carotid intima-media thickness (IMT) was examined. B-mode ultrasound measurement of carotid IMT was performed in 56 hypertensive pts with CRF (glomerular filtration rate > 20 ml / min and < 90 ml/min), 44 hypertensive pts with normal renal function and 20 healthy volunteers. The mean duration of hypertension was 14±5.12 years.

Results: IMT in all examined hypertensive pts was increased above normal clinical value and significantly higher then in healthy controls (0.75± 0.006/ 0.60±0.1, $p<0.001$). The carotid IMT was similar between hypertensive pts with CRF and hypertensive pts with normal renal function (0.74 ±0.1/ 0.76 ±0.1, $p>0.05$). Significant predictors for IMT were age ($r=0.358$, $p<0.04$), duration of hypertension ($r=0.395$, $p=0.023$), diabetes duration ($r=0.343$, $p<0.02$), as well as duration of CRF ($r=0.324$, $p<0.006$). There was a negative correlation between IMT and glomerular filtration rate assessed by creatinine clearance ($r= -0.303$, $p<0.003$). Renal function, described by creatinine clearance was the strongest determinant for Hcy levels ($r =- 0.332$, $p<0.008$). Increased IMT was estimated in pts with CRF compared to healthy controls (0.74±0.10 vs 0,59±0.10, $p<0.001$). We found association between Hcy and carotid IMT ($r=0.344$, $p<0.015$). No consistent association was found between IMT and other specific for CRF cardiovascular risk factors.

Conclusion: The study suggests that patients with mild renal failure have increased IMT of the common carotid artery and that elevated plasma Hcy level in CRF is associated with carotid intima- media thickening. *Hippokratia 2007; 11 (4): 205*-209

Key words: *total homocystein, ultrasound examination, carotid artery, carotid intima- media thickness, chronic renal failure, cardiovascular risk factors*

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There are plenty of non-invasive techniques for examining and evaluating of the pathological changes in the vascular structure. The term "subclinical atherosclerosis" is more and more widely used[1,2]. Subclinical atherosclerosis is a stage at which atherosclerotic plaque hasn't appeared yet. Ultrasound measurement of the intima-media thickness (IMT) along with Pulse wave analysis of the vessels blood flow give valuable information about the vessel status.

The deviation from the normal IMT is referred to as precursor for atherosclerosis. The thickening enables lipid deposition in the vessel wall and by narrowing the vessel lumen changes the normal blood flow.

The thickening of intima-media predicts the risk of myocardial infarct and/or brain vessel incident. IMT over 0,8 mm is an independent prognostic value for increased risk of myocardial infarct and for brain stroke –over 0.75 mm. IMT increase of more than 0.0034 mm / year is also connected with a growing risk of vessel disease[3]. Classic risk factors directly affect IMT, which is proved by many epidemiological studies[3].

In contrast to the general population, for patients with chronic renal failure (CRF), facing high risk of cardiovascular disease, there are no many prospective studies on the assessment of IMT changes as well as in association of these changes with the classic and specific for CRF cardiovascular risk factors (CVRF). High homocystein (Hcy) levels have been found in patients with chronic renal failure[4,5].

Hcy levels in these patients correlated with serum creatinine and creatinine clearance. Hcy has been a disputable cardiovascular risk factor (CVRF) in recent years[4,5]. The aim of the study was to examine the significance of increased Hcy and other cardiovascular risk factors of carotid atherosclerosis in patients with CRF.

Materials and methods

IMT was examined in 100 patients 56 with CRF, 44 without CRF, as well as in 20 healthy volunteers. In the first group the patients with CRF had a creatinine clearance (CrCl) over 20 ml/min and below 90 ml/min. The second group included 44 pts with normal renal function, CrCl> 90 ml/min. All these patients had hypertension and 20/56 pts had diabetes. Twenty five over 44 pts of the group without CRF had diabetes type 2. Each group was further divided into two subgroups: with and without vascular disease (coronary, peripheral vascular or brain vascular). Table 1 summarizes the distribution of the patients into groups, subgroups according to gender, age and renal function. Twenty healthy persons with normal blood pressure (12F/8M, mean age 40.5±6.7, CrCl 117±18 ml/min) were used as a control group.

Table 1. *Distribution of the patients into groups and subgroups.*

Groups	**CRF** (n=56) 16M/40F 58.9 ±14.55; CrCl 39.19±		**Without CRF** (n=44) 17M/27F 55.7±15.22; CrCl 91.11 ±19ml/min	
Parameters	***with VD**** (n =22) 6M/16F	***wihtout VD**** (n=34) 10M/24F	***with VD **** (n=19) 6M/13F	***without VD**** (n=25) 11M/14F
Age	59.86±16.24	58.29 ±13.58	66.4 ±11.59	46.5 ±11.6
Serum creatinine µmol/l	222 ±70.05	234 ±74.16	91 ±10	82 ±20
Creatinine clearance (CrCl) ml/min	37.09 ±9.55	39.29 ±11.78	89 ±11.6	105 ±13.37

** VD: vascular disease (coronary, brain- vascular or peripheral-vascular disease. Patients with brain and peripheral vascular disease had coronary disease as well).*
CRF: chronic renal failure

The duration of arterial hypertension and diabetes, as well as BMI were given in all patients. BMI = kg/ m². The normal BMI was up to 24. All the measurements (hemoglobin, hematocrit, serum creatinin, C reactive protein) were done by routine laboratory methods. Total cholesterol was measured by an enzymatic method. The normal levels of the lipid fractions were according to the ATP III classification[6].

To evaluate renal function in all pts and healthy controls (n=120) creatinine clearance (CrCl) was calculated in ml/min according to the Cockcroft – Gault formula. The normal CrCl was (> 90 <160 ml/min.)

$$CrCl = \frac{(140\text{-age}) \times kg \times 0{,}814 \text{ (x 0,85 for female)}}{\text{Serum creatinine}}$$

IMT for either common carotid arteries was measured. IMT was determined by high resolution ultrasonography (ATL Phillips in B-mode regime), using 10 MHz linear transducer. The common carotid arteries were scanned in the longitudinal and transversal projections. The normal IMT is described as parallel double line whose thickness can be measured. The wall far from the transducer was measured because of its more distinctive acoustics characteristics. Each patients had undergone individual optimisation of the depth and gain adjustment. IMT was measured in an anterolatteral position 2 cm away from the bifurcation. The given values are the average of three consecutive measurements. The breach in the double- line parallelism in the wall layers and the IMT over 1.2 mm, were accepted as features of atherosclerotic plaque.

Specific laboratory methods

Intact parathyroid hormone (iPTH) was determined in 56 pts with chronic renal failure (CRF). The normal range of iPTH is from 1.18 to 8.43 pmol/l. The Hcy levels were determined in the same pts. The normal range of Hcy is up to 15 µmol/l. The laboratory data were received by the chemiluminescence assay - automatic system ACS: 180 PLUS Bayer Health Care.

The results were summarized as mean ± standard deviation. P value less than 0,05 were taken as statistically significant. Differences between groups were assessed by analysis of variance (ANOVA). To study the linear relationship between IMT and other variables Pearson's correlation test was used. All these analyses were performed using SPSS 13.0.1 software.

Results

Significant differences in the mean values of IMT for right and left carotid artery in all examined groups were not found. This is given in Tables 2, 3, 4.

Intima-media was thickened in pts with CRF, the mean value was over 0,75. The values in the CFR group were significantly higher than those in the healthy controls. In the latter IMT was normal. The difference of IMT between the two groups was of high degree of importance, $p < 0.001$ (Table 2).

Table 2. *Comparative analysis of IMT between patients with renal failure and healthy volunteers*

GROUP	CREATININE CLEARANCE	IMT* DEX	IMT *SIN
CRF (n=56)	39.19±10.11ml/min	0.74±0.10	0.76±0.06
Healthy controls (n=20)	117±18 ml/min	0.59±0.10	0.60±0.12
P	<0.001	<0.001	<0.001

** IMT: carotid intima- media thickness; CRF: chronic renal failure*

All pts with CRF had intima- media thickening whose mean value was 0.75mm.

The mean IMT in patients with CRF and vascular disease (VD) was significantly higher than that in pts with CFR without VD. The stage of significance was moderate ($p < 0.05$). The mentioned two groups were comparable according to age, gender and renal function (Table 3).

Table 3. *Comparative analysis of IMT between patients with renal failure with and without vascular disease*

GROUP	CREATININE CLEARANCE	IMT** DEX	IMT **SIN
CRF + VD* (n=22)	37.09±9.55 ml/min	0.78± 0.10	0.77±0.06
CRF without VD* (n=34)	39.29±11.78 ml/min	0.70±0.10	0.71±0.06
P	>0.01	<0.05	<0.05

** VD: vascular disease (coronary, brain- vascular or peripheral-vascular disease. Patients with brain and peripheral vascular disease had coronary disease as well).*
*** IMT: intima - media thickness*
CRF: chronic renal failure

When IMT in pts with VD and CRF (n= 22) was compared to that in pts with VD without CRF (n=19) it was seen that IMT had increased one- way, without significant differences in both groups (Table 4).

Table 4. *Comparative analysis of IMT in pts with VD with and without CRF*

GROUP	CREATININE CLEARANCE	IMT** DEX	IMT *SIN
VD*+ CRF (n=22)	37.09±9.55 ml/min	0.78± 0.10	0.77±0.06
VD* without CRF (n=19)	89±11.6 ml/min	0.77±0.06	0.80±0.09
P	<0.001	>0.1	>0.1

** VD: vascular disease (coronary, brain- vascular or peripheral-vascular disease. Patients with brain and peripheral vascular disease had coronary disease as well).*
*** IMT: intima - media thickness.*
CRF: chronic renal failure

To evaluate the importance of the study of IMT in the effort to precisely examine the early vessel changes in patients with CRF in predialysis stage an analysis was carried out to fined the correlation between the IMT and parameters of renal function, age, BMI and the duration of diabetes and hypertension. These results are given in table 5. A negative moderate correlation of IMT with CrCl was proved as well as the same degree of positive correlation of IMT with duration of CRF, hypertension, diabetes and age.

The mean Hcy value was 17.28±9.37 μmol/l. There was no difference between Hcy values in CRF groups with and without vascular disease 15.77±8.99/ 15.77±8.99, p>0.5. There was a moderate negative correlation between Hcy and renal function assessed by creatinine clearance (r= - 0.370, p=0.008, n=56). A moderate statistically significant positive correlation was found only between IMT and Hcy levels. Correlations with cholesterol, LDL, as well as with hemoglobin and hematocrit and iPTH were not proved. The results were summarized in table 6.

The relationship between IMT and several variables including Hcy, was investigated further by the use of multiple regression analysis (Table 7). IMT is significantly and independently influenced by age, presence of vascular disease, hypertension and total cholesterol. Hcy level and chronic renal failure were factors of borderline significance.

Discussion

The diagnostic value of the IMT measurement has been determined in prospective studies. The aim of EVA (Aging Vascular Study) was to find out the importance of the carotid artery wall thickening and the formation of atherosclerotic plaque. The results proved correlation between IMT and the appearance of an atherosclerotic plaque: the increase of IMT by 0,10 increases the relative risk of plaque appearance which grows with 1.23 for males and 1.27 for females[2]. The VHAS (Verapamil in Hypertension and Atherosclerosis Study) and ELSA (European Lacidipine Study of Atherosclerosis) data showed very high frequency of the structural changes in the carotid arteries, proven by IMT measurement. In the first study plaque was proved in 40% of the examined patients- IMT was 1.5 mm, while in the second - it was 82%- the IMT was over 1.3 mm and normal IMT values were registered in only 33%[7,8].

According to the literature IMT depends on the age, and directly on the hypertension and diabetes duration[2,9]. This correlation was proved by our results as well, without any differences between the patients with and those without CRF.

Different subtypes of lipoproteins- the high levels of LDL and triglycerides as well as the low levels of HDL correlate with the values of IMT. This dependence was particularly pronounced in diabetics[3]. In our study IMT correlation with the lipid fractions was not proved. In the

Table 5. *Correlation of IMT with renal function, duration of renal failure, hypertension and diabetes, BMI and age*

STATISTICS	PARAMETERS	CREATININE	CrCl *	DURATION OF HYPERTENSION	DIABETES DURATION	CRF ** DURATION	BMI	AGE
Quotient of correlation	IMT dex	0.095	-0.303	0.394	0.343	0.324	0.032	0,351
p	p	0.488	0.003	0.023	0.045	0.006	0.814	0,062
N	N	56	56	100	45	56	100	100
Quotient of correlation	IMT sin	0.108	-0.312	0.385	0.349	0.316	0.029	0,358
p	p	0.428	0.004	0.033	0.021	0.003	0.831	0,04
N	N	56	56	100	45	56	100	100

** CrCl: creatinine clearance; ** CRF: chronic renal failure*

Table 6. *Correlation of IMT with classical and specific of chronic renal failure cardiovascular risk factors*

STATISTICS	PARAMETERS	HB	HCT	HCY	PTH	CRP	CHOLESTEROL.	LDL
Quotient of correlation	IMT dex	0.035	0.006	0.344	0.111	0.052	0.061	0.035
p		0.798	0.963	0.015	0.414	0.703	0.653	0.798
N		56	56	56	56	56	56	56
Quotient of correlation	IMT sin	0.098	0.071	0.347	0.174	0.096	0.113	0.098
p		0.474	0.605	0.002	0.199	0.483	0.409	0.474
N		56	56	56	56	56	56	56

examined groups with CRF the levels of atherogenic lipids were slightly increased at the background of dietic restriction while in the patients without CRF there were data for statine administration during various periods before the exanimation, which makes the interpretation of the lipid deviations not quite precise.

The data about the correlation between IMT and renal function are few. There are studies of dialysis patient and single examinations of patients in predialysis stage. There are publications that in CRF patients the correction of classic CVRF does not decrease IMT, while in patients without CRF under the same condition IMT decrease. This supports the idea that CRF appears to be separately and complexly functioning "vasculopathic" state[10-12]. Tetsuo Shoji examined IMT in patients with CRF, dialysis patients and healthy controls. In the firs two groups IMT significantly higher than that in the healthy group and there is no difference between non-dialysis and dialysis patients. After multiple regression analysis it was proved that the presence of renal failure appeared to be an independent factor associated with the increase of IMT[13]. Leoncini examined 358 hypertensive patients with different levels of glomerular filtration (GF). The patients with deteriorated renal function had higher mean value of IMT- 0.79 mm, as compared to those with normal renal function, whose IMT was 0.65 mm. According to the author the presence of renal failure trice increased risk of appearance of left ventricular hypertrophy and carotid atherosclerotic plaques and IMT increase as well. After the correction of age, hypertension duration and severity it was proved that the risk of carotid atherosclerosis was increased by 43% for each 10ml/min clearance descent[14]. These results are very closed to our data about the IMT values in healthy subject, CRF patients and patients without CFR. Our multiple regression analysis indicated that CRF was a factor with borderline significance.

Table 7. *Multiple regression analysis of IMT*

Independent Variable	B	Std. Error	Beta	
Constant	0.635	0.049		<0.001
Age	0.001	0.001	0.191	0.090
Vascular disease	0.057	0.022	0.286	0.012
Total cholesterol	0.021	0.011	0.303	0.054
Hypertension	0.005	0.002	0.353	0.041

Leskinen coauthors examined the relation between Hcy levels in 135 pts with CRF and the severity of the vessel damage, evaluated by IMT. No significant dependence between the two values was proved[15]. At the beginning of 2006 year the results of HOPE- 2 (Heart Outcomes Prevention Evaluation) were published. The study included 5522 pts with proven vascular disease treated with folic acid and vitamin "B6". In the course of the 5 years' follow- up no decrease of the myocardial infarct frequency and brain vascular disease and cardiovascular death was found regardless of the fact that the Hcy levels decreased[16]. The NORVIT (2006) study proved the results from the HOPE study and the disputable importance of Hcy as a cardiovascular risk factor in the general population and in patients with CRF. Analyzing the results of the prospective studies date were found to dispute the independent role of Hcy as a cardiovascular risk factor. In the MRFIT study a small number of the patients with coronary disease had high Hcy levels[17]. The relation of the high Hcy with the repeated coronary incidents in patients who have had already suffered one was proved more definitely[18]. Therefore, the logical question whether the homocystein hypothesis of the atherosclerosis is right arises?

We have proven a relation between Hcy and IMT in patient with CRF, (about 50% of these pts. were with vascular disease) and that can be evidence to state that Hcy refers to atherosclerosis.

Despite the disputable data our results define homocystein like a CVRF but not with independent role (see regression analysis). Homocystein action is very likely not to be independent but connected with the other CVRF- hypelipidemia, smoking, hypertension. The interrelation between these factors stimulates the growth of the effect of homocystein.

Analysing the results, the following can be concluded:

1. Vascular wall thickening is proved even before the renal function deterioration under the action of classic CVRF.

2. IMT shows dependence on the presence of vascular disease being basically determined by the classical CVRF- hypertension, diabetes and age.

3. There is 2. IMT > 0.7 in patients with CRF that shows moderate dependence on the renal function and

weak relation with specific of CRF CVRF. Such relation is proved only with Hcy.

IMT can be used as a marker of vascular damage caused early in the course of the chronic kidney disease even before the start of a real renal function deterioration, in the zero stage of the chronic kidney disease when there is a risk of kidney damage.

References

1. Touboul PJ. Common carotid artery intima-media thickness and brain infarction : the Etude du Profil Genetique de l'Infarctus Cerebral (GENIC) case-control study. The GENIC Investigators. Circulation 2000; 102: 313-318
2. Adaikkappan M. Evaluation of Carotid Atherosclerosis by B'Mode Ultrasonographic Study in Hypertensive Patients Compared with Normotensive Patients. Ind J Radiol Imag 2002; 12: 365-368
3. Chambless LE. Risk factors for progression of common carotid atherosclerosis: the Atherosclerosis Risk in Communities Study, 1987-1998. Am J Epidemiol 2002; 155: 38-47
4. Francis ME. Association between serum homocysteine and markers of impaired kidney function in adults in the United States. Kidney Int 2004; 66: 303-312
5. Frantzen F. Enzyme conversion immunoassay for determining total homocysteine in plasma or serum. Clin Chem 1998, 44: 311-316
6. Stehouwer CD. Serum homocysteine and risk of coronary heart disease and cerebrovascular disease in elderly men: a 10-year follow-up. Arterioscler Thromb Vasc Biol 1998; 18: 1895-1901
7. Zanchetti A. The Verapamil in Hypertension and Atherosclerosis Study (VHAS): results of long-term randomized treatment with either verapamil or chlorthalidone on carotid intima-media thickness. J Hypertens 1998; 16: 1667-1676
8. Zanchetti A. Risk factors associated with alterations in carotid intima-media thickness in hypertension: baseline data from the European Lacidipine Study on Atherosclerosis. J Hypertens 1998; 16: 949-961
9. Ando F. Ultrasonic evaluation of common carotid intima-media thickness (IMT) - influence of local plaque on the relationship between IMT and age. J Epidemiol 2000; 10 (Suppl.1): S10-S17
10. Kissinger G, Hoppichler F. Prevalence of increasing carotid artery atherosclerosis in chronic renal failure: an ultrasonographic study. Hypertonie 2003; 12: 27-32
11. Bots ML. Common carotid intima-media thickness and risk of stroke and myocardial infarction: the Rotterdam Study. Circulation 1997; 96: 1432-1437
12. Konings C. Arterial wall properties in patients with renal failure. Am J Kidney Dis 2002; 39: 1206-1212
13. Shoji T. Advanced atherosclerosis in predialysis patients with chronic renal failure. Kidney Int 2002; 61: 2187-2192
14. Leoncini G. Mild renal dysfunction and subclinical cardiovascular damage in primary hypertension. Hypertension 2003; 42: 14-18
15. Leskinen Y. Homocysteine and carotid atherosclerosis in chronic renal failure – the confounding effect of renal function. Atherosclerosis 2004; 175: 315-323
16. Heart Outcomes Prevention Evaluation Study Investigators. Effects of ramipril on cardiovascular and microvascular outcomes in people with diabetes mellitus: results of the HOPE study and MICRO-HOPE substudy. Lancet 2000; 355: 253-259
17. Bostom AG, Culleton BF. Hyperhomocysteinemia in chronic renal disease. J Am Soc Nephrol 1999; 10: 891-900
18. Rahn KH. Vessel wall alterations in patients with renal failure. Hypertens Res 2000; 23: 3-6