The Role of Carotid Atherosclerosis in Prediction of Hemodialysis Patients Long-term Mortality

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Abstract

Background. The high prevalence of accelerated atherosclerosis (ascl) and vascular calcification in hemodialysis (HD) patients (pts) increase their cardiovascular (CV) morbidity and mortality. Common carotid artery intima-media thickness (CCA IMT) assessment is regarded as practical for non-invasive ascl imaging and some authors stress out CCA IMT as an independent predictor of HD patients CV mortality. The aims of the present study were to evaluate the association between some common and uremia-related CV risk factor and ascl evaluated by CCA IMT and to find out the role of CCA IMT in prediction of HD patients long-term CV mortality.

Methods. The prospective follow-up study of 82 pts (37 males, aged 54.0 ± 13.2 years) maintained by HD for 81.6 ± 57.6 months at our Institute was carried out for 60 months. CCA IMT and the presence of ascl plaques were evaluated by ultrasonography. Forty-six (53.7%) of the examined pts were supplemented with water soluble vitamins including folic acid and vitamin B complex pills or ampoules. Genetic analysis of the methyllentetrahydrofolate-reductase (MTHFR) thermolabile mutation was performed in all pts.

Results. A significant positive correlation was found between CCA IMT and age, but negative with creatinine and vitamins intake therapy. However, multivariate analysis indicated only the age (p= 0.000) as determinant of CCA IMT. During the follow up period 21 pts died out of which 15 (71.4%) pts because of fatal CV event. Deceased pts were significantly older, had significantly lower albumin, triglycerides, and creatinine serum concentration but higher C-reactive protein (CRP) than survived subjects. Also, deceased patients had significantly higher CCA IMT but insignificantly higher plaques score compared to survived patients. The multivariate Cox regression analysis of CV mortality revealed diabetes mellitus, CCA IMT and CRP protein as the most powerful positive predictors, while creatinine and vitamins intake therapy were negative predictors of CV mortality.

Conclusions. In conclusion, a measurement of CCA IMT could be clinically useful predictor of long-term CV mortality.

Keywords: hemodialyisis, CCA IMT, cardiovascular mortality

Introduction

Patients with the kidney disease stage 5 have much higher prevalence of atherosclerosis compared with age and gender-matched subjects from general population with normal kidney function. This high prevalence is associated with enormous increase in cardiovascular (CV) mortality and points to the fact that the CV disease is a major cause of hemodialysis patients morbidity and mortality (1,2).

Atherosclerosis is a chronic event which takes place in the intima-media layer of arterial wall. A different cellular elements and their host of compounds produced by and acting on them and their surroundings, are involved in this process (3). Also, numerous systemic factors, named traditional risk factors for CV disease play an important role in this process as well as hyperhomocysteinemia (hyperHcy), lipid disturbances, insulin resistance or oxidative stress. Additionally, in hemodialysis patients uremia-related risk factors such as chronic microinflammation, malnutrition, anaemia, as well as the disturbances of calcium-phosphate metabolism enhance the rate of atherosclerosis progression and contribute to the vascular calcification (4,5). Lim et al. (6) indicated the impact of polymorphism in methylenetetrahydrofolate reductase (MTHFR), gene on plasma Hcy levels and carotid atherosclerosis in hemodialysis patients. MTHFR is enzyme involved in Hcy metabolism.

Epidemiological studies in general population revealed that increases in common carotid arteries intima-media thickness (CCA IMT) were associated with risk of myocardial infarction and stroke (7) but the others demonstrated better correlation of plaque diameter measurement than the IMT with atherosclerotic event (8). In addition, in end stage renal disease (ESRD) population, some Italian (9) and Japanese (10) authors reported increased IMT as an independent predictor of hemodialysis CV events and mortality.

The aim of the present study was to evaluate the association between some traditional (age, diabetes mellitus, hypertension, smoking, lipid disturbance) and non-traditional risk factors (nutrition, inflammation, anemia, hyperhomo-cisteinemia and MTHFR gene polymorphism) for CV disease and the degree of atherosclerosis in hemodialysis patients. Additionally, we intended to find out the clinical importance of intima-media layer thickness, measured by B-mode ultrasonography of the bilateral common carotid arteries, for hemodialysis patients' long-term CV mortality.

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Patients and methods

The prospective follow-up study of 82 patients, as a part of larger prospective study of 113 patients, maintained by hemodialysis at the Institute of Urology and Nephrology, Clinical Center of Serbia, was carried out during 60 months from April 2001 to April 2006. The patients were dialyzed thrice weekly for 4-5 hours with bicarbonate hemodialysis using low-flux or high-flux membranes (1.3-1.6 m^2).

The causes of ESRD were: polycystic kidney disease (n = 15), chronic glomerulonephritis (n = 15), rapidoprogressive glomerulonephritis (n = 5), nephrosclerosis (n = 10), chronic pyelonephritis (n = 10), systemic disease (n = 7), diabetic nephropathy (n = 3), Balkan endemic nephropathy (n = 2) and other (n = 15).

Any history of hypertension, ischemic vascular disease (myocardial infarction, angina pectoris, cerebral stroke), heart failure, diabetes and smoking were obtained by interview, physical examination and analysis of medical records.

Forty-six of the 82 examined patients (53.7%) received water soluble vitamin supplements including oral folic acid (5-15 mg/day) plus either vitamin B complex pills (nicotinamide 25 mg, vitamin B_1 4 mg, vitamin B_6 2 mg, vitamin B_{12} 0.001 mg) three times per day or intravenous vitamin B complex (nicotinamide 100 mg, vitamin B_1 40 mg, vitamin B_6 8 mg, vitamin B_{12} 0.004 mg) three times a week plus 2500 μmg of vitamin B_{12} once weekly at least 6 months prior to the study and later. The patients receiving vitamin supplements were designated as the vitamins intake therapy group.

The control group consisted of 15 healthy persons, 8 females and 7 males, aged 45.3 ± 3.2 years.

Informed consent was obtained from each patient and healthy person.

Methods

Laboratory tests: Predialysis and postdialysis blood samples were obtained on a midweek day. At the outset of the study Kt/V, normalized protein catabolic rate (nPCR) and residual renal function were determined (mono compartmental urea kinetic models) (11).

Total Hcy (tHcy) level in serum was measured by high-performance liquid chromatography (HPLC) after reduction of the disulfide-bonds by dithiothreitol (normal range: 10-15 μmol/l).

Serum folate and vitamin B_{12} concentration were measured by radioimmunoassay (Abbot IMX kit). Normal values for folate and vitamin B_{12} were 7-28 nmol/l and 164-835 ρmol/l, respectively.

C-reactive protein (CRP) was determined by nephelometry (normal values <5 mg/l).

Genomic DNA was purified from peripheral white blood cells according to standard method. Rapid screening for genotypes for MTHFR polymorphism (the C677T mutation in the MTHFR gene) was determined using a PCR-based method followed by Hinf I digestion (12).

Blood pressure was measured before dialysis. The mean value of all recordings taken during the month before the study was considered as a representative of the BP of each patient. Arterial hypertension was diagnosed when the systolic blood pressure was ≥140 mmHg and/or the diastolic pressure was ≥90 mmHg, or if antihypertensive treatment was prescribed. The mean arterial blood pressure (MBP) was calculated as diastolic blood pressure plus 1/3 of the pulse pressure.

Body weight and height were measured and body mass index (BMI) was calculated according to the formula: weight (kg) /height ^{2} (m^2).

Evaluation of atherosclerotic changes. The right and left carotid arteries were examined using a 7.5 MHz linear array transducer with high-resolution B-mode ultrasonography (Toshiba PLE-705 S). All measurements were performed by the same researcher. The patients were in supine position. The wall of the common carotid artery, 0.5-1 cm proximal to the beginning of the carotid bulb, was used for the measurement of the CCA IMT and lumen diameter. The CCA IMT was defined as the distance between the leading edge of the lumen intima echo of the near wall and the leading edge of the media adventitia echo. The lumen diameter was defined as the distance between the near wall and the leading edge of the intima lumen echo of the far wall. The mean values of the CCA IMT and the lumen diameter were calculated from at least two measurements for each artery. The existence of carotid plaque, defined as localized echo structure encroaching a vessel which was thicker than the IMT of adjacent sites, was screened for in the common, internal and external carotid arteries. Plaques score indices were determined between 0-3 (0-without plaque, 1- minimal lumen stenosis <30%, 2-medium lumen stenosis 30-50%, and 3-extensive luminal stenosis > 50%).

Statistics: The data are presented as means with standard deviation (X ± SD) or as medians with inter-quartile range. Comparison between groups was made by *t*-test or the Mann-Whitney test, as appropriate, as well as with ANOVA. P-values less than 0.05 were considered statistically significant. Independent correlations of CCA IMT and plaque scores were identified by univariate and multivariate linear regression analysis. In order to analyze the risk of death, Kaplan-Meier curves and univariate survival analysis were obtained with the Cox proportional hazard model. The primary dependent variable was the time to death measured in months. Variables that were potential predictors of CV death in univariate analysis ($p < 0.01$) were tested in a multivariate Cox proportional hazard model. The calculations were made using the statistical program SPSS, release 10.

Results

The characteristics of the hemodialysis patients are shown in Table 1.

The mean value of CCA IMT was 0.91 ± 0.22 mm (0.6-1.9mm) which was significantly higher than in controls (0.72 ± 0.10 mm). Thirty-eight percent of the examined patients were without atherosclerotic plaques, 28% had plaque score index -1, 24% had plaque score index -2 and 10% had plaque score index -3.

The present study revealed a high prevalence of hyperHcy (89%) in the examined hemodialysis patients. The mean tHcy serum level in hemodialysis patients was 26.5 ± 8.9 μmol/l, which was significantly higher than in the healthy controls (13.0 ± 3.3 μmol/l).

Molecular analysis of the thermolabile MTHFR variants in the examined hemodialysis patients showed the following prevalence rates of the common mutations: 36% of the patients had normal genotypes (MTHFRCC), 50% were heterozygotes (MTHFRCT) and 14% were homozygotes with

MTHFRTT. Molecular analysis of DNA in the control group, revealed the similar prevalence rate. There was no significant difference in tHcy serum levels between the MTHFR genotypes but in all three genotypes the patients with vitamins intake therapy had significantly lower serum tHcy levels.

The univariate analysis showed that CCA IMT as well as plaque score index was directly related only to the age. Nevertheless, CCA IMT negatively correlated with the serum creatinine concentration and vitamins intake therapy (Table 2).

Table 1. Demographic, somatometric, clinical and biochemical characteristics of the hemodialysis patients (*n*=82)

Demographic and somatometric data	
- Age (years)	54.0 ±13.2
- Gender (M/F)	37/45
- Dialysis duration (months)	81.6 ± 57.6
- Body mass index (kg/m^2)	22.5 ± 3.7
- **Clinical data**	
-Hypertension	83.5%
-Mean arterial pressure-MAP (mmHg)	96.6± 8.2
-Myocardial infarction	8.1%
-Heart failure	11%
-Angina pectoris	8.1%
-Cerebral stroke	5%
-Diabetes	3.5%
-Smokers	23.5%
-On treatment with erythropoetin	12%
Biochemical data	
-Total serum homocysteine (µmol/L)	26.5±8.9 (10.4-52.7)
-Serum folate (nmol/L)	1179±9.2 (3.9-42.8)
-Serum B12 (ρmol/L)	614.3±386.88 (90-1476)
-Hemoglobin (g/L)	87.3 ± 16.1
-Serum albumin (g/L)	39.1 ± 3.7
-Serum total cholesterol (g/L)	4.8 ± 1.1
-Serum triglycerides (g/L)	2.4 ± 1.5
-Serum CRP (mg/L)	8.5± 10.5
-Serum creatinine (umol/l)	971.5 ± 188.1
-Kt/V	1.19±0.23
-nPCR (g/kg/day)	1.13±0.26
- CCA IMT (mm)	0.91±0.22
- Atherosclerotic PS index	1.15±1.0

Data given as mean ± standard deviation (inter-quartile rang), or percent frequency as appropriate; Abbrevations: CRP-C-reactive protein; Kt/V, fractional urea clearance. CCA IMT, Common carotid arteries intima media thicknes; PS plaques score

In multivariate analysis, age (coeff. ß =7263E-0.3, p=0.000 and coeff. ß =3.445E-0.2, p=0.000) was the only significant independent predictor of CCA IMT and plaque score index.

During the follow-up period of 60 months, four patients left the cohort, 21 patients died and the others are still on maintenance hemodialysis. The cause of death was a fatal cardiovascular event (heart failure, arrhythmia, myocardial infarction, thrombotic and hemorrhagic stroke) in 15 (71.4%) patients and other causes of death in the remaining 6 (28.6%) patients.

Deceased patients were significantly older, had significantly lower albumin, triglycerides, and creatinine serum concentration but higher CRP than survived subjects. Also, deceased patients had significantly higher CCA IMT but insignificantly higher plaques score compared to the survived patients (Table 3).

Table 2. Correlation coefficients between CCA IMT and plaque score index and some traditional and non-traditional cardiovascular risk factors

	Intima –media	**Thickness**	**Plaque score**	**Index**
Variables	R	p –value	R	p –value
CCA IMT	1	-	0.663	0.000
PS index	0.663	0.000	1	1
Age	0.536	0.000	0.486	0.000
HD duration	0.089	ns	0.102	ns
Diabetes mellitus	0.163	ns	0.135	ns
Smoking	0.143	ns	0.044	ns
BMI	0.149	ns	-0.052	ns
MAP	0.162	ns	0.210	0.065
Hemoglobin	-0.007	ns	-0.055	ns
Cholesterol	0.165	ns	0.165	ns
Triglycerides	-0.141	ns	-0.015	ns
Albumin	-0.198	ns	0.012	ns
Creatinine	-0.290	0.08	-0.161	ns
CRP	0.150	ns	0.021	ns
Homocysteine	0.023	ns	0.093	ns
MTHFRCC	-0.075	ns	-0.086	ns
MTHFRCT	0.052	ns	0.018	ns
MTHFRTT	0.029	ns	0.090	ns
Kt/V	-0.136	ns	-0.037	ns
nPCR	0.148	ns	0.117	ns
Vitamins intake th	-0.292	0.008	-0.271	0.016

BMI-body masse index, MAP-mean arterial pressure, CCA IMT – common arotid artery intima media thickness; PS Atherosclerotic plaques score, MTHFRCC-normal genotype, MTHFRCT – heterozygotes, MTHFR TT - homozygotes

Table 3. Initial clinical and laboratory parameters in surviving and expired hemodyalisis patients.

PARAMETERS	**Alive**	**Dead**
-Age (years)	51.0 ± 12.9	64.1 ± 7.6 *
-Hemodialysis duration (months)	81.5 ± 59.1	74.4 ± 47.9
- Body mass index (kg/m^2)	22.6 ± 3.4	22.6 ± 3.4
-Total serum homocysteine (µmol/L)	26.6 ± 9.1	24.9 ± 8.9
-Serum folate (nmol/L)	13.8 ± 10.8	7.4 ± 3.8
-Serum B12 (ρmol/L)	591.4 ± 349.6	750.0 ± 483.0
-Hemoglobin (g/L)	86.7 ± 15.8	84.9 ± 17.0
-Serum albumin (g/L)	39.6 ± 3.3	37.6 ± 4.1 *
-Serum total cholesterol (g/L)	4.8 ± 1.1	4.5 ± 1.0
-Serum triglycerides (g/L)	2.5 ± 1.6	1.9 ± 0.9 *
-Serum CRP (mg/L)	7.4 ± 9.5	13.9 ± 14.6 *
-Serum creatinine (umol/l)	1000.0 ± 188.6	858.1± 145.9 *
-Kt/V	1.19 ± 0.2	1.22 ± 0.2
-nPCR (g/kg/day)	1.1 ± 0.2	1.0 ± 0.2
- CCA IMT (mm)	0.86 ± 0.22	1.06 ± 0.53 *
- Atherosclerotic PS index	1.02 ± 0.94	1.6 ± 1.2

*p<0.05 Data are given as mean ± standard deviation (Student t-test and Mann-Whitney U test)

CCA IMT- common carotid artery intima media thickness, PS - plaques score.

Table 4. Predictors of CV mortality: univariate Cox proportional hazard model

Variable	Hazard ratio (CI)	P
Age	1.068 (1.034-1.141)	**0.001**
Gender	1.276 (0.464-3.11)	0.637
HD duration	0.998 (0.989-1.007)	0.366
BMI	1.040 (0.907-1.192)	0.575
Diabetes mellitus	12.378 (3.246-47.198)	**0.000**
MAP	1.027 (0.964-1.095)	0.406
Hemoglobin	0.992 (0.960-1.025)	0.620
Albumin	0.881 (0.767-1.013)	0.076
Cholesterol	0.722 (0.445-1.171)	0.187
Triglycerides	0.494 (0.249-0.981)	**0.044**
Creatinine	0.996 (0.992-0.999)	**0.009**
CRP	1.031 (1.000-1.064)	**0.053**
Hcy	0.988 (0.935-1.045)	0.681
Vitamin B12	1.001 (1.000-1.002)	0.167
Folic acid	0.811 (0.505-1.305)	0.388
Vitamins intake th	0.321 (0.114-0.904)	**0.032**
MTHFRCC	0.938 (0.235-3.207)	0.919
MTHFRCT	1.979 (0.576-6.763)	0.276
MTHFRTT	0.039 (0.000-49.160)	0.372
Kt/V	0.955 (0.115-7.927)	0.966
nPCR	0.210 (0.026-1.679)	0.141
CCA IMT	8.061 (1.824-35.627)	**0.006**
PS index	1.704 (1.025-2.831)	**0.040**

Hazard ratio –Exp (B), CI-95% confidence interval of Exp (B), p-significance of coeff.
BMI-body masse index, MAP-mean arterial pressure, CCA IMT – Common carotid arteries intima media thickness; PS Atherosclerotic plaques score

Potential predictors of CV mortality were selected by the univariate Cox proportional hazard model. Potential predictors of CV mortality were: age, triglycerides, CRP, creatinine as well as diabetes mellitus, vitamins intake therapy and both CCA IMT and plaque score index (Table 4). The multivariate analysis of CV mortality is shown in Table 5. Diabetes mellitus, CCA IMT and CRP remained the most powerful positive predictors, while creatinine, and vitamins intake therapy were negative predictors of the CV cause of patients' death.

Table 5. Predictors of CV mortality: multivariate Cox proportional hazard model

Variable	ß	Hazard ratio (CI)	P
Diabetes mellitus	3.200	24.533(4.639-129.743)	**0.000**
CCA IMT	5.645	282.780 (2.732-29270.575)	**0.017**
CRP	0.040	1.041 (1.001-1.082)	**0.045**
Creatinine	-0.006	0.994 (0.989-1.000)	**0.033**
Vitamins intake therapy	-1.776	0.169 (0.040-0.710)	**0.015**

Hazard ratio – Exp (B), CI-95% confidence interval of Exp (B), p-significance of coeff., CCA IMT – Common carotid arteries intima media thickness

The Kaplan-Meier estimate of survival in hemodialysis patients was divided according to the percentiles of intima media thickness (Figure 1). Patients with the lesser CCA IMT had a significantly more favorable survival rate (p=0.0012).

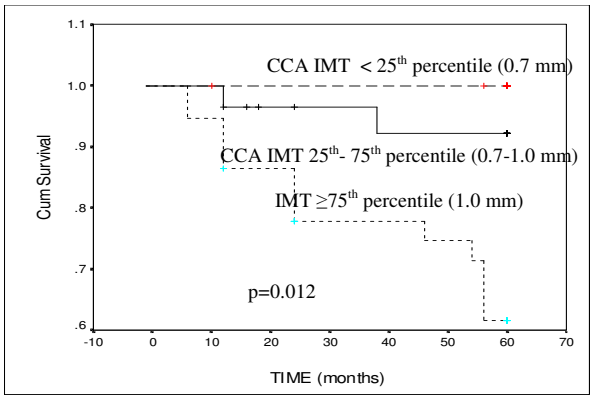

Figure 1. Kaplan-Meier estimate of survival in hemodialysis patients divided into groups in relationship to the intima media thickness

Discussion

The present study revealed significantly higher CCA IMT in examined hemaodialysis patients than in the healthy controls and also that the majority of patients (62%) had atherosclerotic plaques. These results are in accordance with the declared accelerated atherosclerotic process in ESRD patients which has been accepted by many investigators (13,14,15).

The variables most closely associated with CCA IMT and atherosclerotic plaque score index were only the age in a positive way but creatinine serum concentration and vitamins intake therapy in a negative. There is no doubt that the age is one of the most important traditional risk factors for atherosclerosis and CV disease in general population but also in patients with kidney function impairment (3,16,17). One half of our patients received long-term common vitamins supplementation and we recently concluded (18) that patients with vitamins intake therapy had better nutritional status (higher serum triglyceride and creatinine concentrations) compared to patients without vitamins supplementation. The higher degree of carotid atherosclerosis in patients without vitamins supplementation is in line with the numerous studies documenting the relationship between malnutrition and CV disease in ESRD patients (19, 20, 21).

We did not find any significant correlation between the carotid atherosclerosis and other traditional and uremia-related risk factors such are lipids, diabetes mellitus, hypertension, smoking, BMI, CRP protein, anemia nor with hyperHcy, as previously reported (14,15,17). Also, we did not confirm any significant correlation between CCA IMT and different MTHFR genotype as the Chinese authors did (6). None of the Chinese patients received vitamins supplementation and we already have mentioned that usual oral doses of folic acid together with oral or intravenous vitamin B complex and B_{12} therapy decrease the tHcy serum level in all MTHFR 677 genotype but the most being in the MTHFRTT genotype. Similar results were reported by Pastore et al (22) for this wild genotype.

During the follow-up period of 60 months, 21 patients died and the others are still on maintenance hemodialysis. In 15 (71.4%) patients the cause of death was a fatal cardiovascular event. One of the objectives of the present study was to estimate the relevance of the carotid atherosclerosis measured by CCA IMT for CV long-term mortality. Deceased patients had significantly higher CCA IMT but insignificantly higher

plaques score compared to survived patients. Also, deceased patients were significantly older, had significantly lower albumin, triglycerides, and creatinine serum concentration but higher CRP than survived subjects. An analysis of the predictors of CV mortality showed that CCA IMT along with diabetes mellitus and CRP as well as low creatinine serum concentration and no vitamins supplementation were the most powerful predictors of the CV death. At the beginning of 21st century Benedetto et al (9) first showed that CCA IMT was an independent predictor of end stage renal failure patients' deaths. The same results was confirmed by Nishisawa et al (10) in short-term and Kato et al (17) in a long-term prediction of hemodyalisis patients' all cause of CV mortality. So, our results are in accordance with these data.

We already have shown that hyperHcy was not established as a predictor of CV mortality. The multivariate Cox proportional hazard model showed that the majority of the CV mortality predictors were associated with a diminished nutritive status and higher inflammation presented by CRP. This accords with numerous studies documenting the relationship between malnutrition, inflammation and CV disease in the general population (23,24) and in ESRD (20,21, 25).

Conclusion

Estimation of different traditional and non-traditional risk factors revealed that only the age significantly correlated with the carotid atherosclerosis. The most important conclusion is that the increase of atherosclerosis degree, measured on common carotid arteries by non-invasive method, can be useful tool in prediction of long-term hemodialysis patients' cardiovascular mortality.

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