UNIVERSITY OF MEDICINE AND PHARMACY OF CRAIOVA
DOCTORAL SCHOOL

PhD THESIS

-ABSTRACT-

DYSLIPIDEMIA: CARDIOVASCULAR AND RENAL RISK FACTOR IN CHRONIC KIDNEY DISEASE

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SYNTHESIS OF MAIN PARTS OF THE THESIS
STATE OF KNOWLEDGE

Chronic kidney disease (CKD) is a significant health problem and its prevalence is increasing both in Romania and worldwide. It is estimated that the prevalence of CKD in the U.S. population during 1999-2004 was 15.3%. More recently, NHANES III study showed a prevalence of 11% (19.5 million people) in U.S. and AUSDIAB study showed a prevalence of 18.3% in Australia. In Western Europe the prevalence of CKD stages 3 to 5 is about 10% of the adult population. In Romania there is an explosion of dialysis patients from 5800 in 2003 to 9775 in 2009, leading to the consideration that 1 of 10 Romanians suffers from this disease.

Dyslipidemia (DLP) is a common complication of progressive kidney disease but not universal in this people. DLP is a renal and cardiovascular risk factor characterized by elevated triglyceride levels, low high-density lipoprotein cholesterol levels, and small, dense, low-density lipoprotein particles size.

The major determinants of DLP in CKD patients are glomerular filtration rate (GFR), nephrotic range proteinuria, concomitant diseases such as diabetes mellitus, hereditary disorders of lipid metabolism, immunosuppressive agents, modality of renal replacement therapy (hemodialysis, peritoneal dialysis or transplantation), comorbidity and nutritional status. A correct management of both non-traditional and traditional risk factors such as gender, age, race is a goal of great interest for the quality and duration of life for patients with chronic kidney disease.

Is it important DLP in patients with Chronic Kidney Disease? The answer is "yes" because: in patients with nephrotic syndrome and CKD stages 3-5, as well as in those on dialysis or transplantation there are a lot of defects in lipoprotein metabolism (Table1).

Furthermore, DLP may accelerate the progression of renal disease but most importantly DLP contributes to the high cardiovascular morbidity and mortality in CKD patients.

Table 1. Lipid abnormalities in CKD (Adapted from William F Keane.

	Nephrotic syndrome	Predialysis CKD (stages 3-4)	Hemodialysis	Peritoneal dialysis
Total Cholesterol	↑	↔	↔	↔ or ↑
LDL Cholesterol	↑	↔ or ↓	↔ or ↓	↑
Triglycerides	↔ or ↑	↑	↑	↑
HDL Cholesterol	↓ or ↔ or ↑	↓	↓	↓
Small dense LDL	↑	↑	↑	↑
Lipoprotein(a)	↑	↑	↑	↑
Lipase activity	↓	↓	↓	↓
Apolipoprotein B	↑	↑	↑	↑
Apolipoprotein A1	↑	↔ or ↓	↔ or ↓	↔ or ↓

↑= increase; ↓= decrease; ↔ = no change

The past 20 years have brought important certainties in this field but also new questions. What guidelines tell us? Can the target values proposed by the guidelines be achieved? Once achieved target values are there residual cardiovascular risks? Is sufficient lipid-lowering medication or is there a need of lifestyle changes? This doctoral thesis aims to answer all these questions but without becoming exhaustive.

Two studies were conducted:

-first study aims to evaluation the link between hypertension (HTA) assessed using ambulatory blood pressure monitoring (ABPM) and the DLP in a group of patients with CKD.

-the second study aims to assess whether there are significant differences in terms of renal function and cardiovascular risk among different groups of subjects with / without DLP and with / without CKD.

PERSONAL CONTRIBUTION

STUDY I. Role of ABPM in the management of patients with chronic kidney disease, hypertension and dyslipidemia

Background and aims

Hypertension (HTA) and dyslipidemia (DLP) constitute major public health problems as they increase the risk of cardiovascular diseases (CVD), especially in patients with chronic kidney disease (CKD). A non dipping pattern is very common in CKD. The aim of the study was to determine whether there is a difference between dipping/non dipping hypertension in subjects with CKD and DLP with or without lipid-lowering therapy (LLT).

Matherial and methods

We performed a retrospective study on 129 subjects from the Nephrology-Hypertension Out-patient Department of University Campus Bio-Medico, Rome from January 2011 to April 2013. The study was approved by the ethics committee of the University. For all the subjects included in the study, the following inclusion criteria were met: subjects who had more than 70% of successful readings of ABPM, subjects with an estimated glomerular filtration rate (eGFR) calculated by CKD-EPI formula ≥15 mL/min/1.73m^2, no recent history of acute diseases, no history of oncological diseases and subjects with normal liver function. ABPM and other clinical and paraclinical data were collected.

***ABPM recording*.** The subjects underwent 24 h ABPM with the SpaceLabs ABP UltraLite 90217. Its accuracy has been validated by official organizations in the US, UK, France and Germany. The diagnosis of hypertension was based on accepted ABPM criteria according to the reports in Japan, Europe and the United States. “Dipper” was defined as a decline in the nocturnal BP of >10%, whereas “non-dipper” was defined as a decline in the nocturnal BP of <10%.For each subject we collected the demographic data, laboratory data (serum levels of creatinine, cholesterol, TG, HDL-C, LDL-C, blood glucose, HbA1c, etc.), current therapy and medical history. eGFR was calculated by CKD-EPI formula.

Statistical Analysis was performed by the Biostatistics Department of the University of Medicine and Pharmacy of Craiova, Romania, using Microsoft Excel (Microsoft Corp., Redmond, WA, USA), together with the XLSTAT add-on for MS Excel

(Addinsoft SARL, Paris, France) and IBM SPSS Statistics 20.0 (IBM Corporation, Armonk, NY, USA) for processing the data.To test the normality of the data, we used the Anderson-Darling and Shapiro-Wilk tests. None of the numerical variables investigated had a normal distribution of data, globally or inside each studied group. Because the study involved a numerical comparison between 2 groups of patients that didn't have a normal (Gaussian) distribution of data, the nonparametric Mann-Whitney test was primarily used. For categorical data, in order to evaluate the significance of the association (contingency) we used Fisher test (chi-square test). P-values <0.05 were considered statistically significant.

Results

The most important characteristics of the patients in the study are shown in table 1.

We found statistically significant differences between the dipping and non-dipping pattern in subjects with CKD stages 1-2 versus stages 3-4 (p=0.018). When we analyzed the association between non-dipping status with DLP and type 2 diabetes (T2D), we did not find a statistically significant result.

In the dyslipidemic subjects, we found statistically significant differences between the dipping and non dipping pattern regarding the values of TG (p=0.019) and mean platelet volume (MPV) (p = 0,048).

We also compared subjects with dipping pattern and those with non dipping pattern in the group without dyslipidemia and we found statistically significant differences between the values of LDL-C (p=0.044) and atherogenic index of plasma (AIP) calculated using a logarithmic formula comprising TG and HDL cholesterol, log(TG/HDL-C) (p=0.049).

When we analyzed the differences between dipping and non dipping pattern in subjects with dyslipidemia and LLT, we found only one statistically significant differences for HbA1c (p=0,046).

Table 1. Baseline characteristics of the study population

Total subjects	129
Age (years)	62.75 ± 12.3
Man	68.99% (n=89)
Woman	31.01% (n=40)
Stage CKD	
-stage 1	19.38% (n=25)
-stage 2	37.21% (n=48)
-stage 3	37.98% (n=49)
-stage 4	5.43% (n=7)
eGFR/CKD–EPI ml/min/1.73m^2	65.14 ± 24.38
Dipper/non dipper pattern	
-dipper	43.41% (n=56)
-non dipper	56.59% (n=73)
Diabetes	
-with T2D	38.76% (n=50)
-without T2D	61.24% (n=79)
Dyslipidemia	
-with dyslipidemia	82.95% (n=107)
• With LLT	59.81% (n=64)
• Without LLT	40.19% (n=43)
-without dyslipidemia	17.05% (n=22)

Discussions

The results of our study proved that there is a statsitically significant difference between dipping and non dipping pattern in the subjects with CKD stages1-2 versus stages 3-4 (p=0.018). It is well known that nocturnal nondipping hypertension is very common in CKD patients and it is associated with the severity of the disease, increased proteinuria and inflammatory markers, such as C-reactive protein, but few studies have examined the exact relationship of nocturnal non dipping hypertension with DLP in CKD patients.

When interpreting the data presented above, we must also take into consideration that it was performed in subjects with hypertension and antihypertensive medication and many studies have confirmed that it is possible to achieve a dipping pattern and

improve the metabolic profile by administering antihypertensives in the evening. Also many studies have shown that antihypertensive medication is associated with a significant reduction in microalbuminuria. This could explain why we did not find statistically significant differences when we analyzed the values of proteinuria in any of the studied groups. We know that LLT, especially statins, have pleiotropic effects. In addition to lipid-lowering effect, statins acts also on endothelial dysfunction and may have the effect of lowering blood pressure. Also, statins have antiproteinuric effects and small studies have documented the improving dipping pattern when microalbuminuria was reduced.

Other studies have observed higher prevalence of DLP, T2D, or cardiovascular disease in non-dipping subjects. However, in our study we did not find statistically significant differences in the subjects with T2D but we supose that this is due to the small sample size of the study which prevents us from drawing strong conclusions. Another study conducted by Zeynep Tartan and al did not find singnificantly results when analyzing lipid parameters regarding dipping/non dipping pattern. Although they showed that metabolic syndrome is a predictor of non-dipping hypertension, when the authors report their results, they emphasize the fact that there was no significant difference regarding certain lipid parameters (total cholesterol, LDL-C and HDL-C).

Conclusions

1. Only CKD significantly influenced the dipping/non dipping pattern in the study group.
2. 24-hour ABPM is useful for the better management of hypertension in patients with CKD and for achieving the targets reccomandend by guidelines.
3. Furthermore, ABPM it is the only method for documenting dipper/non dipper pattern available so far.

STUDY II. Role of lipids parameters and apolipoproteins A and B in patients with chronic kidney disease and dyslipidemia

Background and aims

DLP is a common complication of CKD and may accelerate the progression of renal disease but most importantly DLP contributes to the high cardiovascular morbidity and mortality in CKD.

The association between lipid disorders and increased CV mortality in the general population is a well known fact and patients with CKD are at a higher risk of developing CVD than the general population.

Apolipoproteins A (Apo A) and B (Apo B) are the structural proteins of HDL-C and LDL-C, respectively. Levels of Apo B are better than LDL-C at reflecting the spectrum of proatherogenic lipid particles (VLDL, IDL and LDL). The antiatherogenic role played by Apo A appears to be more important than HDL-C. Circulating lipoproteins and their constituent proteins, apolipoproteins, are risk factors for CKD and CVD. Recent studies speak that apolipoproteins as better markers of CV risk, assessment than conventional lipids. Moreover Mortality Risk (Amoris) and INTERHEART showed that apoA/apoB ratiohas been shown to be strongly related to the risk of myocardial infarction, stroke and other CV events. Is it at all possible to simplify the CV risk assessment using apolipoproteins as markers of risk and as targets for lipid-lowering therapy?

Starting from these premises present study aims to:

- Evaluate a lipids disorders in patients with CKD with clinical, biological and echocardiography parameters;
- There is a correlation between lipid parameters and eGFR calculated by CKD-EPI formula.

Matherial and methods

We performed a study on 51 subjects from the Nephrology Department of Emergency Clinical County Hospital of Craiova, from November 2011 to July 2013. For all the subjects included in the study, the following inclusion criteria were met: subjects with DLP and CKD stages 1 to 5 predialysis, no recent history of acute diseases, no history of oncological diseases and subjects with normal liver function.

All patients signed an informed consent. For each subject we collected the demographic data, laboratory data such as: serum levels of creatinine, cholesterol, TG, HDL-C, LDL-C, blood glucose, CRP, apolipoproteine A1(apo A1) and apolipoprotein B (apo B), current therapy and medical history. Routine laboratory data except urea and serum creatinine (conducted in Emergency Clinical County Hospital of Craiova laboratory) were performed at the Laboratory Synevo Craiova. Statistical analysis was presented in the chapter matherial and methods of the first study.

Results

Table 2. Baseline characteristics of the study population

Total subjects	51
Age (years)	53.39 ± 13.75
Man	52.94% (n=27)
Woman	31.01% (n=40)
Dyslipidemia	
-with dyslipidemia	50.98% (n=26)
• With LLT	50% (n=13)
• Without LLT	50% (n=13)
-without dyslipidemia	59.01% (n=25)
Stage CKD	
-stage 1	49.02% (n=25)
-stage 2	11.76% (n=6)
-stage 3	17.64% (n=9)
-stage 4	11.76% (n=6)
-stage 5 predialysis	9.8% (n=5)
Diabetes	
-with T2D	23.52% (n=12)
-without T2D	76.47% (n=39)
Hypertension	
-with hypertension	54.90% (n=28)
-without hypertension	45.09% (n=23)

Statistical analysis of the entire group

A inverse linear correlation was observed between eGFR and:

- visceral adiposity index (VAI), (Spearman r= -0.311, p= 0.027);
- ratio Apo A/Apo B, (Spearman r= -0.410, p=0.003);

A direct linear corelation was observed between eGFR and:

- Apo A, (Spearman r= 0.284, p= 0.044);
- we did not find statistically significant corelation between eGFR with Apo B and eGFR with ratio Apo A/Apo B.

Was found a linear correlation between C-reactive protein (CRP) and:

- age, (Spearman r= 0.439, p=0.001);
- anthropometrical parameters: waist circumference (WC) (Spearman r=0.337, p=0.016) and body mass index (BMI) (Spearman r= 0.374, p=0.007);
- systolic blood pressure (SBP), (Spearman r= 0.281, p=0.046);
- blood glucose, (Spearman r= 0.388, p=0.005);
- eRFG, (Spearman r= -0.443, p=0.001);
- proteinuria/24h (Spearman r= 0.382, p=0.006);
- cholesterol (Spearman r= 0.310, p=0.027);
- TG (Spearman r= 0.416, p=0.003);
- Non HDL-C (Spearman r= 0.383, p=0.006);
- TC/HDL-C (Spearman r= 0.390, p=0.005);
- TG/HDL-C (Spearman r=0.383, p=0.006);
- VAI (Spearman r= 0.361, p=0.010);
- atherogenic index of plasma (AIP) (Spearman r=0.382, p=0.006);
- Apo B, (Spearman r= 0.453, p=0.001).

We found a invers linear correlation between ratio Apo A/Apo B and:

- age, (Spearman r= -0.423, p=0.002);
- WC, (Spearman r= -0.343, p=0.014);
- BMI, (Spearman r= -0.311, p=0.027);
- blood glucose, (Spearman r=0.311, p=0.027).

Statistical analysis between the 4 groups

We found satistically significant differences between the 4 groups (CKD and DLP, only CKD, only DLP and control group). The results are shown in table 3.

Table 3. The Dunn test and the differences between the 4 groups for all study parameters.

Parameter	CKD+ and DLP+ vs. CKD+	CKD+ and DLP+ vs. DLP+	CKD+ vs. DLP+	CKD+ and DLP+ vs. CONTROL	CKD+ vs. CONTROL	DLP+ vs. CONTROL
Age(years)	NS	NS	NS	**<0.0001**	**0.000**	NS
BMI(kg/m^2)	NS	NS	NS	**0.012**	NS	**0.046**
WC(cm)	NS	NS	NS	**0.012**	NS	**0.033**
SBP	NS	NS	NS	NS	NS	NS
DBP	NS	NS	NS	NS	NS	NS
Blood glucose (mg/dl)	NS	NS	NS	NS	NS	NS
eGFR (ml/min/1,73m^2)	NS	**<0.0001**	**<0.0001**	**<0.0001**	**<0.0001**	NS
Uric acid (mg/dl)	NS	NS	NS	**0.001**	**0.0001**	NS
Proteinuria/24h	NS	NS	**<0.0001**	**<0.0001**	**<0.0001**	NS
CRP (mg/l)	NS	**0.000**	**0.011**	**0.000**	**0.000**	**0.000**
TC (mg/dl)	NS	NS	**<0.0001**	**0.004**	NS	**<0.0001**
TG (mg/dl)	NS	NS	NS	**0,001**	NS	NS
LDL-C(mg/dl)	NS	NS	**<0.0001**	NS	NS	**<0.0001**
HDL-C(mg/dl)	NS	NS	NS	NS	NS	NS
Non HDL	NS	NS	**<0.001**	**0.000**	NS	**<0.0001**
TC/HDL-C	NS	NS	NS	**0.000**	NS	**0.001**
TG/HDL-C	NS	NS	NS	**0.002**	NS	NS
LDL-C/HDL-C	NS	NS	**0.000**	**0.002**	NS	**<0.0001**
AIP	NS	NS	NS	**0,000**	NS	NS
VAI	NS	NS	NS	**0.002**	NS	NS
Apo A (g/l)	NS	NS	NS	NS	NS	NS
Apo B (g/l)	NS	NS	NS	NS	NS	**0.001**
Apo A/Apo B	NS	NS	NS	NS	NS	**0.045**

NS – not significantly statistic

The differences between the 4 groups regarding cardiovascular risk

We used the Fisher test to show the differences between the 4 groups regarding CV risk and we found statistically significant differences ($p<0.0001$). The CV risk was estimated with CRP according American Heart Association/Centers for Disease Control and Prevention (AHA/CDC)(CRP<1mg/l low, CRP 1-3 mg/l average and CRP>3 mg/l high CV risk).

Discussions

The results of our study proved that there is direct correlation between eGFR and Apo A but we did not find statistically significant corelation between eGFR with Apo B.

Results of our study are strengthened by the results of two large studies ARIC (n = 10 292, 1996-1998) and NHANES III (n = 7023, 1988-1991). Those studies showed that elevated levels of Apo A is associated with a low rate of CKD , but the same thing can not be said about the relationship of eGFR and Apo B about in connection with which the authors of the two studies and we did not find a statistically significant correlation. It is true that in terms of the ratio Apo A / Apo B both ARIC and NHANES III studies have demonstrated a significant correlation with eGFR , but our study did not obtained statistically significant results .

Also, in our study we found statistically significant differences between the 4 groups of subjects (CKD and DLP , only CKD, only DLP and control group) not only in terms of lipid parameters which is somewhat predictable but I got differences between anthropometric parameters (BMI and waist circumference) or laboratory data (CRP and proteinuria / 24h) . DLP and chronic inflammation plays an important role in the pathogenesis and progression of atherosclerosis and although CRP is a nonspecific indicator of systemic inflammation is also an important marker of atherosclerosis and CV events . Elevated CRP were found in our study in patients with CKD and DLP compared to the 3 groups . Also we found in our study statistically significant correlations between CRP and Apo B and between CRP and Apo A/ Apo B ratio confirming once again the data in the literature. According to the classification AHA / CDC we wanted to see if there are differences between the 4 groups regarding CV risk estimated using CRP (CRP < 1mg / l low, CRP 1-3 mg / l average and CRP > 3 mg / high cardiovascular risk) and the results were highly statistically significant ($p < 0.0001$). When interpreting the results on CRP and CV risk assessment must take into account that I used for cardiovascular risk assessment CRP and not high -sensitive CRP (hsCRP). Therefore results in our study should not be interpreted singular but will add to those obtained for standard cardiovascular markers (total cholesterol , LDL cholesterol , non- HDL -C , etc.) thereby increasing their predictive value. In addition some authors says that statin therapy may be helpful in lowering CRP levels within in our group with CKD and DLP, the only with lipid lowering therapy, we found the highest levels of CRP.

Conclusions

1. There are significant differences between the 4 groups of patients (CKD and DLP, only CKD, only DLP and control group) both in terms of lipid parameters and other parameters studied.

2. eGFR was correlated with Apo A but not statistically significantly correlated with Apo B or Apo ratio A / Apo B.
3. CRP correlated with Apo B, the ratio Apo A / Apo B and the other lipid parameters.
4. Apo A / Apo Bratio inverse correlated with age, anthropometric parameters and glucose.
5. We found statistically significant differences between the subjects with CKD and DLP and subjects without DLP regarding levels of proteinuria/24h and eGFR.
6. We found statistically significant differences between the 4 groups regarding CV risk estimated by CRP.

FINAL CONLUSIONS

1. 24-hour ABPM is useful for the better management of hypertension in patients with CKD and for achieving the targets reccomandend by guidelines.
2. Furthermore, ABPM is the only method for documenting dipper/non dipper pattern available so far.
3. Lipid parameters and apolipoproteins are useful as markers to simplify CV risk assessment and as targets of lipid-lowering therapy in patients with CKD.
4. Patients with CKD and DLP showed higher values proteinuria/24h and lower eGFR values compared with those without DLP demonstrating that DLP is an important factor for progression of kidney disease.
5. Patients with CKD and DLP were in high cardiovascular risk patients compared with the 3 subgroups,showing the highest levels of CRP.
6. A correct management of hypertension and lipid disorders is a goal of great interest to increase the quality and length of life of patients with CKD.