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Original Scientific Paper

CONCENTRATION OF APOLIPOPROTEIN B-100 (β-LP, APO-B100, APO-LDL) IN UREMIC PATIENTS

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Abstract

Disorders of lipo/apoprotein metabolism in patients with chronic kidney disease (CKD) begin to appear in the early stages of the disease, before the start of treatment with chronic hemodialysis (HD) and they represent an essential risk factor for premature onset of atherogenic processes. Patients with CKD and who are uremic before starting HD (hemodialysis) which is manifested with a clinical picture of premature atherosclerosis (Ath). *Purpose of the work:* The work aimed to determine the concentrations of Apolipoprotein B_{100} (Apo-B_{100}), and lipid profile in uremic patients treated with long-term hemodialysis (HD) as well as their role in the etiology of premature Ath (Atherosclerosis).*Materials and methods:* In this the cross-sectional study included N^0=120 uremic patients (of whom 66 (55%) were males and 54 (45%) were females with an average age of 59,00±10,00) years old treated with long-term HD over 48 months with a frequency of three times per week with sessions lasting 4.5 hours. All patients were treated in the Clinic of Nephrology and HD at the Faculty of Medicine-Skopje. *Results:* The results obtained present an average value of three consecutive measurements under identical conditions and are presented in t**Error! Reference source not found.**-4.*Statistical processing:* The statistical methods used in this study were: mean arithmetic mean, standard deviation X±SD, Student's t-test and Mann Whitney U test. For statistical analysis we used the SPSS software package version 17. *Conclusion:* In conclusion, we can emphasize that the knowledge of due to etiology of lipo/apoproteins in patients with CKD and those with treated with long-term HD can contribute to the prevention of premature Ath, and to the undertaking of preventive measures that would reduce the high incidence of uremic dyslipidemia, thus slowing the development of Ath. It is a fact that multicenter and long-term studies are needed with a larger number of patients that will confirm or deny the role of Apo-B_{100} polymorphism in patients with CKD as a new independent factor for the development of premature Ath.

Keywords: Apolipoprotein B_{100}, uremia, Early atherosclerosis, Lipid profile

1. Introduction

Lipo/apoprotein disorders are manifested in the earliest stages of CKD (Chronic Kidney Disease), even before the beginning of HD treatment and they are a major factor in the onset of premature atherogenic processes and premature atherosclerosis (José M. Valdivielso et al. 2019,. Per-Ola A.et al.1999). Determination of lipid profile and apolipoproteins in patients with CKD in the early stages of the disease, can significantly help preventands low the progression of the underlying disease and the premature development of Ath, thus reducing the incidence of premature atherosclerosis in patients with CKD. Patients with CKD mostly present with type IV hyperlipidemia (Frederickson classification) in which high concentrations of polysaccharides predominate (hypertriglyceridemia with values of 40-95%, in the composition of the structure VLDL, IDL, LDL-ch and HDL-ch. The reduced presence of HDL-ch in uremic patients reduces the transport of cholesterol to the liver, enabling ideal conditions for the excess accumulation of cholesterol in extrahepatic tissues. These risk factors include inflammation, uremic toxins, oxidative stress, carboxylation, nitric oxide (NO), hyperhomocysteinemia, altered metabolism of lipids, calcium and phosphate. Endothelial dysfunction is a common feature of CKD that occurs in the early stages of the disease it is a predictor of Cardiovascular disease (CVD). Atherosclerosi is thought to be accelerated in patients with CKD due to the accumulation of oxidized atherogenic lipoproteins (Oxidized Cholesterol-LDLox),VLDL, along with lack of HDL, under the conditions provided by endothelial dysfunction, oxidative stress and inflammation (Allison B. Reiss, et al).

The replacement of normal, physiological apolipoproteins with pathological, along with their potent atherogenicity and the complementary influence of uremic toxins on the structure and function of apoproteins are not yet researched phenomena therefore many experimental and clinical studies are needed to confirm the predisposing atherogenic predispositions in this uremic environment. Numerous studies have documented and verified that in patients with ESRD the concentrations of Apo-B_{100} are considered as a new risk factor for the early onset of Ath. Decreased hepatic synthesis of apo-A_1, in patients with ESRD enhances the increase of Apo-B synthesis and production. Superimposed Ath in patients with ESRD treated with HD is considered to be due to the so-called small dense LDl-ch. The lipoprotein particles of LDL-ch in their composition have very large amounts of Apo-B, in the plasma of patients with ESRD. There are studies that show that GFR<60 ml/min positively corresponds to high concentrations of LDl-ch and IDL. The catabolism of LDL and IDL in patients with ESRD is extremely impaired but is masked by normal TG values. Prolonged circulation of IDL and LDL-ch particles in the body of patients with ESRD significantly proves the high risk of premature Ath and CVD.

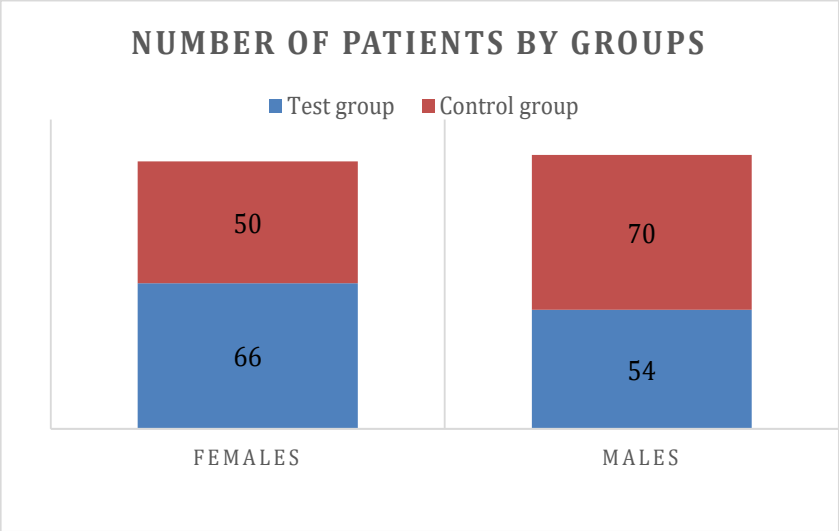
*Purpose of the study:*The work aimed to determine the concentrations of Apo-B_{100}, and the lipid profile (TCh, LDL-ch, TG, HDL-ch) in uremic patients treated with long-term HD as well as their role in the etiology of premature atheriosclerosis.

2. *Materials and methods*

During this time-prospective study, N^0=120 uremic patients were included (of whom 66 (55%) were males and 54 (45%) were females with an average age of 59,00±10,00 years old), treated with long-term HD over 48 months with frequency three times per week, with a session duration of 4.5 hours. The dialysis membrane parameters were biocompatible polysulfonic capillary membrane type F5HP with surface 1-1.3nm^2 sterilized with high steam pressure. All patients were treated at the Clinic of Nephrology and Hemodialysis at the Faculty of Medicine-Skopje. This study also included the control group of 120 healthy individuals (volunteer donors). Of the 120 healthy individuals 50 were females and 70 were males with an average age 58,00±9,50 years who served as a control group similar to the group of uremic patients by gender, age, and nationality. Blood for analysis was always taken at 08:00 in the morning, at room temperature from 19-24°C while patients were in a supine position in order to acquire all possible variations in the values of specific fractions of lipoproteins of 9-12%.The blood, taken with several drops of heparin (3 cm^3), was sent to the Institute of Clinical Biochemistry at the University Clinical Center in Skopje. Laboratory analyzes of lipid profile (TCh/mmol/l, TGmmol/l, LDLch/mmol/l, HDL-ch/mmol/l and Apo-B_{100}/g/l in every patient were determined once per month with three consecutive measurements over a period of two years. Concentrations of lipid fractions were examined according to laboratory standards at the Institute of Clinical Biochemistry at the Faculty of Medicine in Skopje, while the concentrations ofApo-B_{100} with the immune-turbidimetric method according to Rifai N. *(10. Rifai N. 1986)*

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

Total number 1	**120 (100%)**	**Average age ± DS**
Females	66 (45%)	59, 00 ± 10, 00
Males	54 (45%)	59, 00 ± 10, 00
Control group (F:50 + M:70)	120 (100%)	58, 00 ±9, 50

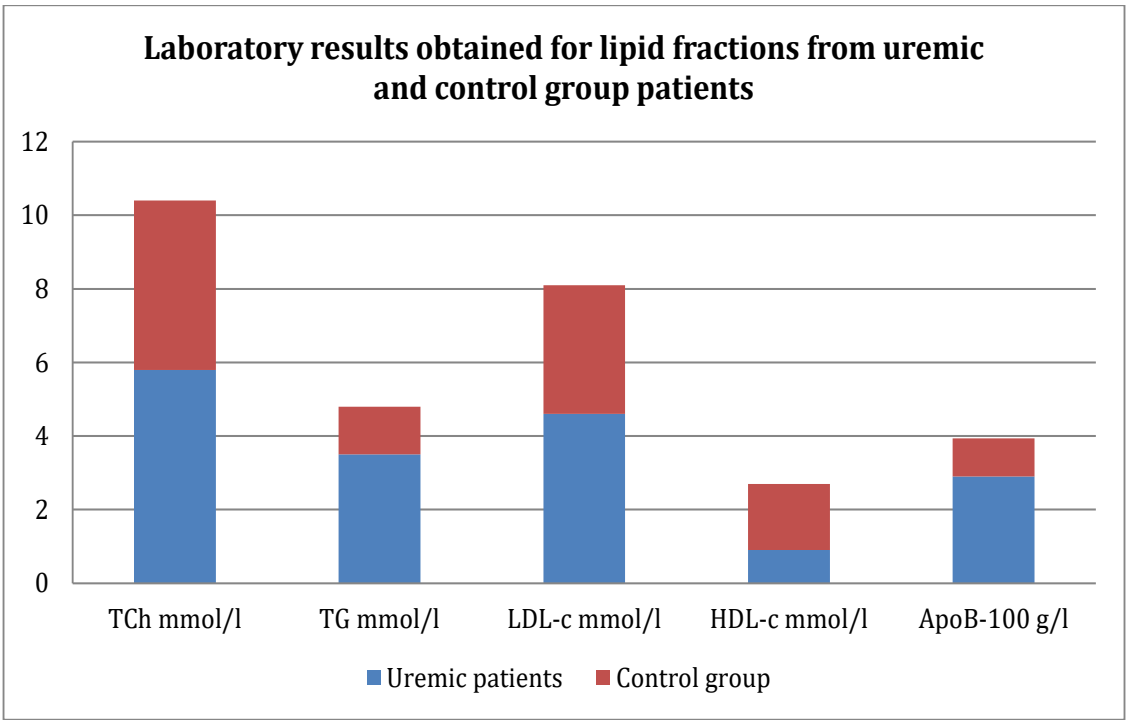


Statistical processing: The statistical methods utilized in this study were: mean arithmetic mean, standard deviation X ±SD, Student's t-test and Mann Whitney's U test and Wilcox in signed-rank test. For statistical analysis we used the SPSS software package (Statistical Packagefor the Social Sciences, version 17).The statistical significance of differences for the parameters examined between uremic patients and the control group was analyzed with "Anova Two Factor" with statistical value for $p < 0.0001$.

Results: The obtained results present the average value of three consecutive measurements under identical conditions and are presented in table number 2, 3.

Table 2: Laboratory results for parameters examined by uremic patients treated with HD

Patients	**TCh**	**TG**	**LDL-ch**	**HDL-ch**	**Apo-B$_{100}$**
Uremic=120	6,80 ± 1,70	3,90 ± 1,50	4,80 ±1,20	0,90 ± 0,40	3,10 ± 1,40
Control group- 120	4,50 ± 1,00	1,25 ± 0,40	3,20 ± 0,80	1,40 ± 0,20	1,10 ± 0,80
p	0,0001	0,0001	0,0001	0,0001	0,0001



Error! Reference source not found. shows a significant statistical change for all parameters examined (TCh=6,80 ± 1,70;Apo-B_{100}=3,10±1,40;TG=3,90±1,50;LDL-ch=4,80±1,20;HDL-ch=0,90 ± 0,40 for p <0,0001 compared to the values of acquired by the control group.

Table 3: Average values of control group

Patients	TCh mmol/l	TG mmol/l	LDL-ch mmol/l	HDL-ch mmol/l	Apo B_{100} g/l
Controlled group 120 (F+M)	4,50 ± 1,00	1,25 ± 0,40	3,20 ± 0,80	1,40 ± 0,20	1,10 ± 0,80
p	0,0001	0,0001	0,0001	0,0001	0,0001

Table 4: Correlation coefficient between the examined parameters

Ratio	Correlation coefficient	*p*
LDL-ch/HDl-ch	- 1, 28	0,17
Apo A_1/Apo-B_{100}	- 0, 26	0,02

The table itself shows significant statistical negative correlation between the values of ApoA_1/ApoB_{100} and between LDL-ch/HDL-ch

Table 5: Varian analysis between examined parameters

Parameters	SS	df	MS	SS	df	MS	F	*P*
TG	17. 11	3	5. 70	74. 50	116	0. 64	8. 87	0. 00024
TCh	0. 21	3	0. 07	278. 00	116	2. 39	0. 03	0. 99320
HDL-ch	8. 84	3	2. 95	43. 00	116	0. 37	7. 95	0. 00072
LDL-ch	8. 82	3	2. 94	94. 60	116	0. 81	3. 60	0. 01553
Apo-B_{100}	**0. 46**	**3**	**0. 15**	**51. 12**	**116**	**0. 44**	**0. 35**	**0. 79134**

According to the analysis of variance, the difference between the examined parameters of uremic patients is with a significant statistical difference of $p = 0.0001$.

Table 6: Mann-Whitney U test of the examined parameters

Parameter	U	Z	p-levev
TG	491, 50	-0, 01	0, 99
TCh	467, 00	0, 34	0, 73
HDL-ch	491, 00	-0, 01	0, 98
LDL-ch	459, 00	0, 45	0, 65
Apo-B_{100}	472, 00	0, 27	0, 78

According to Mann-Whitney U test the difference between the parameters examined in uremic patients is with a statistically significant difference for p = 0,0001.

Table 7: Average values of control group

Patients	TCh mmol/l	TG mmol/l	LDL-ch mmol/l	HDL-ch mmol/l	Apo B_{100} g/l
Controlled group 120 (F+M)	4,50 ± 1,00	1,25 ± 0,40	3,20 ± 0,80	1,40 ± 0,20	1,10 ± 0,80
p	0,0001	0,0001	0,0001	0,0001	0,0001

3. Discussion

The main risk factor for the development of premature Ath in patients with CKD and uremic patients treated with HD is the disorder and polymorphism of lipo/apoprotein metabolism. Genetic predictors of an early Ath predisposition are dysfunctional transport of HDL-cH and $ApoB_{100}$ and reduced conversion of VLDL to IDL to LDL-ch and to lesser extent chylomicrons (CM). In patients with ESRD dominates of hypertriglyceridemia (60-90% of patients) due to increased TG content in the composition of VLDL, IDL, LDL-ch. A large number of studies on the occurrence of lipo/apoprotein disorders in uremic patients have verified that in addition to Apo-A, Apo-C, Apo-E disorders, lipid fractions also have an increased concentration of Apo-B_{100} in the composition of VLDL. Concentrations of HDL-ch in uremic patients treated with HD have reduced reversal transport of Ch towards the liver, which creates ideal conditions for the increased accumulation of Cholesterol

in extrahepatic tissue. Low concentrations of HDL-ch in patients were verified at while increased concentrations were verified for Apo-B_{100}, TG and LDL-ch. Decreased Apo-A_1 synthesis in the liver is the primary reason for the low of Apo-A_1concentration in uremic patients with an extreme increase compensatory synthesis of Apo-B_{100}. Apo-B_{100} is a ligand for LDL receptors (B,E). Apo-B_{100} is synthesized in the liver. It has been proven that high levels of Apo-B_{100} are an early predictor of premature Ath and CVD. The high mortality rate in uremic patients is due to CVD compared to the general population (*Plus Ency. Apo-B100, Medline,2018.Foley RN, et al. Am J Kidney Dis, 1998,. Blankestijn PJ et al. J Am Soc Nephro; 1995).* The rapid onset of coronary lesions has caused an increase of observation of increased ratio intima-media thickness (IMT) vasculopathies, in patients with CKD since the early stages of the disease. Contributing factors of high mortality rate in uremic patients compared to the healthy population are: atherosclerotic plaques which grow more rapidly in the uremic environment, uremic toxins, MIA syndrome (Malnutrition Inflammation Atherosclerosis), oxidative stress, secondary anemia, advanced age, sedentary lifestyle, smoking, diabetes, hypertension, C-Reactive Protein (CRP) and uremic dyslipidemia. Atherosclerosis is associated with the development of Advanced Glycation end Products (AGEs) which is part of the aging process, is clearly progressive in uremia. Apo-B_{100} is a ligand of B, E, and for LDL receptors (Amann K, et al. 2003).The Apo-B_{100} contains 43% helices, 21% Beta connections, 16%. α-Coils and 20% case structure. The molecular weight of the Apo-B100 glycoprotein is 549 kD, and after reducing the glycoside sequence it decreases to 512.94 kD. The ApoB genes are located on the short arm of the second chromosome (2r23-r24).ApoB examination helps assess the risk of developing CVD and premature Ath. The disordered mechanism of Apo-B_{100} in uremic patients with CKD and treated with long-term HD still remains completely undiscovered although it is assumed that delayed catabolism of lipoprotein particles in the serum of uremic patients or a combination of reduced clearance and excess ApoB-100 have the main role. Apo-B is recognized by LDL receptors found on the surface of many of the body's cells. High concentration of Apo-B100 are observed in large number of diseases such as: inherited disorders of apolipoproteins, congenital defects of LPL (Lipoprotein Lipase),combined familial hyperlipidemia, type II hyperlipoproteinemia Type-II-b,Type-IV, diabetes mellitus, hypothyroidism, CKD, uremia, long-term HD treatment, during pregnancy, nephrotic syndrome, hepatic tract obstruction, tobacco consumption, excessive corticosteroid use, cyclos-porin, uncontrolled use of diuretics, androgens, uncontrolled use of beta-blockers, etc. However, low concentrations of Apo-B_{100} can also be harmful to the body causing: Reye's syndrome hyperthyroidism (which is a rare but serious condition that causes sudden swelling in the brain and liver), abeta lipoproteinemia, etc. Apo-B_{100}, LDL-ch and IDL show increased affinity for specific surface receptors of somatic cells, but much of their residual amount is deposited in the liver. Apo-B_{100} ensures the absorption of cholesterol by hepatic and extrahepatic tissues by binding to B/E receptors and enabling the removal of TG from the liver. Mutations (changes) in Apo-B_{100} can cause familial (inherited) hypercholesterolemia. The reference values of Apo-B_{100} are: 0.7-1.6g/l for males and 0.6-1.5 for females. It has been verified that the transfer of cholesterol (Reverse Cholesterol Transfer-RCT) from HDL-ch to VLDL/LDL is extremely compromised (reduced) in uremic patients compared to the control group; this is yet another evidence of the low effect of HDL-ch in patients. These RCT defect disorders can lead to accelerated atherosclerosis in patients with ESRD. In uremic patients, HTGL activity decreased by 33-40%, while LCAT activity decreased by 45-60% compared with the control group. Additional studies are required with a larger number of patients in order to accurately ascertain the metabolic disorders of Apo-B_{100} in patients with CKD. Analysis of the parameters examined in our patients showed that they had increased concentrations of TG, Apo-B_{100},LDL-ch and low Apo-A_1 and HDL-ch concentrations compared to the control group. Many studies such as Batista et al, Atmann et al,Per Ola et al (. etc. have confirmed the disorder of lipo/apoprotein status of patients with CRF and those with ESRD treated with long-term HD (Batista M.C. et al.,2004,.Abdellah Ali-,Phalisteen Sultan[2], et al.,2010. Llindner A, Charra B, et al. The increase in Apo-B_{100} concentrations is manifested by the increased accumulation of VLDL and IDL concentrations. In recent years it has been shown that the use of dialytic membranes (dialysis-artificial kidney) biocompatible coated with tocopherol and "High

Flux" (Goldberg IJ, Kaufman AM, *et al*. 1996; Wanner C, Bahner U, Mattern R, et al. 2004; significantly affect the faster elimination of atherogenic lipo-/apoproteins and improves the lipid profile, thus as a result we have a decrease in the concentrations of Apo-B_{100}(by as much as 30%) and AOPP (Advanced Oxidized Protein Products) in uremic patients]after the end of the HD session. In our study, higher concentrations of Apo-B_{100} compared to basal renal disease were observed in patients with chronic glomerulonephritis, followed by patients with uro-obstructive disease, diabetes, renal polycystic ovary disease. These results are consistent and further verify the many conclusions, which are cited in this study (Batista M. C., et al).

Conclussion: In conclusion, we emphasize that the knowledge of patho-genetic mechanisms of lipo-/apoproteins etiology in uremic patients with CKD treated with long-term HD can contribute to the prevention, and is also a measure which would reduce the high incidence of uremic dyslipidemia thus reducing the development of Ath. It is a fact that multicentric and long-term studies are needed that will confirm or deny the exact role of Apo-B_{100} polymorphism in the development of premature Ath in patients with CKD.

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