ORIGINAL ARTICLE

A 4 YEARS STUDY IN DIABETIC CHRONIC KIDNEY DISEASE PATIENTS ON KETOAMINOACIDS TREATMENT IN BUCHAREST. RESULTS AFTER 1-YEAR FOLLOW-UP

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Abstract

We initiated a 4-year prospective cohort study on 200 patients with type 1 and type 2 diabetes mellitus and stage 4 chronic renal disease (CKD) on ketodiet (very low-protein diet with 0.3 g protein/kg/day supplemented with ketoanalogues of essential aminoacids. We present the results of compliant and dietary non-compliant patients, for the first year, analysed at baseline, 6 and 12 months. The primary endpoint of the study was the reduction of renal function decline (decline in glomerular filtration rate estimated eGFR/year). The results of the first 20 patients completing one year of follow-up demonstrated that compliant patients had a slower decline of eGFR/year of 1.4 mL/min/1.73 m^2/year *vs.* 4.1 mL/min/1.73 m^2/year (p = 0.006), better serum bicarbonate (p = 0.006) and better parathyroid hormone levels (p = 0.04), while the nutritional status and glycaemic control remained unchanged. The ketodiet in compliant diabetic patients with CKD provides better outcomes, regarding nutritional safety parameters.

Rezumat

Am inițiat un studiu prospectiv de cohortă cu durată de 4 ani care urmărește 200 de pacienți cu diabet zaharat și boală renală cronică (BRC), stadiul 4, sub cetodietă (dieta extrem hipoproteică 0,3 g proteine/kg/zi suplimentată cu cetoanalogi ai aminoacizilor esențiali). Lucrarea prezintă rezultatele primului an de studiu, fiind analizate rezultatele pacienților complianți și necomplianți la dietă în momentul includerii în studiu, după 6 și 12 luni. Obiectivul primar al studiului a fost reducerea declinului funcției renale (declinul ratei de filtrare glomerulară estimată eRFG/an). Rezultatele primilor 20 de pacienți ce au completat 1 an de studiu au arătat că pacienții complianți au avut un declin mai lent al eRFG/an de 1,4 mL/min/1,73m^2/an *vs.* 4,1 mL/min/1,73m^2/an (p = 0,006), nivelul de bicarbonat seric mai bun (p = 0,006) și valori ale parathormonului mai bune (p = 0,04), în timp ce statusul nutrițional și controlul glicemic au rămas neschimbate. Cetodieta la pacienții diabetici cu BRC complianți oferă rezultate superioare, la parametri de siguranță nutrițională.

Keywords: ketodiet, diabetic kidney disease, ketoanalogues of essential aminoacids

Introduction

Since Cochrane's 2007 review concerning the disappointing outcomes of protein restriction for diabetics with renal disease [11], a great number of studies have been made to assess the effects of using mixtures of essential amino-acids and their ketoanalogues as substitutes for dietary protein during very low-protein diets (VLPD) with 0.3 g protein/kg/day in patients with chronic kidney disease (CKD) in order to prevent the proteic denutrition, to improve metabolic disturbances and delay the decline of glomerular filtration rate (GFR). Convincing data concerning the diabetic CKD patient are still lacking, because these patients are especially prone to develop malnutrition, which is one of the most important pejorative independent factor for their outcomes [3, 5, 8]. While efficacy of the VLPD remains questionable, the adherence to the diet is strongly considered the central point of interest, the key factor to any consistent outcome [9, 12, 14]. The main goal of our study was to demonstrate the benefits of VLPD supplemented with ketoanalogues in reduction of renal function decline and delaying dialysis initiation without risk of denutrition and a better inflammation-denutrition profiles.

Materials and Methods

Study design

Our prospective research developed in the nephrology unit of the National Institute of Diabetes "C. Paulescu", Bucharest, Romania aims to demonstrate the benefits of ketodiet beyond slowering progression of diabetic chronic kidney disease staged 4 by a stable and good nutritional status in the first 20 such patients completing a 1 year follow-up period. Type 1 and 2 diabetic patients (T1D, T2D) were included, on baseline having a treatment period of at least 3 months on angiotensin converting enzyme inhibitor or

angiotensin receptor blocker, stable antihypertensive and antidiabetic treatment, as well as a stable dose of epoetin in the anaemia correction therapy, if any. At baseline patients already on ketodiet were also accepted and their serum creatinine and GFR were evaluated using Modification of Diet in Renal Disease (MDRD) study equation (eGFR) at the diagnosis moment of their stage 4 CKD; the duration on ketodiet before baseline moment were also registered.

Two cohorts were compared the ketodiet - the compliant (n = 11, 3 patients with T1D, 8 with T2D) *versus* the non-compliant (n = 9, 1 patient with T1D, 8 with T2D) patients in their main outcome - the reduction on decline of eGFR at the end of the study and secondary to compare their metabolic outcomes concerning acidosis control (pH, serum bicarbonate) and glycaemic control (glycaemia, glycosylated haemoglobin 1C - HbA1c), calcium-phosphorus equilibrium (serum total calcium, phosphate and para-thyroid hormone) and nutritional status. The reduction on decline of eGFR was calculated using ΔeGRF decline/year (initial – 12 month) (eGFR at diagnosis of CKD stage 4 – eGFR at 12 months or at start of dialysis)/R survival x 12 (months from diagnosis of CKD stage 4 to final (12 months of study or dialysis)). The reduction on decline of eGFR on ketodiet was calculated using eGFR at baseline – eGFR at 12 month R survival x 12 (months from diagnosis of CKD stage 4 to final (12 months of study or dialysis).

Ethics. This study was conducted under the approval of the Ethics Committee of our institute, respecting the European Union Council Directive 86/609/EEC. All patients provided written and signed informed consent prior to enrolment.

Each patient who accepted to be included in the study was evaluated for nutritional status at baseline and at end of the 12 months by measurements of subjective global assessment score (SGA), mid-arm muscle circumference, hand-grip test, oedema-free weight, body mass index (BMI), serum albumin and protein, C reactive protein (CRP) and serum ferritin. Patients had psychological and nutritional guidance at baseline, at 3, 6 and 12 months controls.

Compliance of the patients was assessed by the diet questioner, diet recall and calculation of the protein equivalent of nitrogen appearance at 12 months.

Non-compliance was defined by any of the following 3 criteria: less ketoanalogues administred; proteic dietary income > 0.6 g/kg/day at least once; caloric intake < 30 kcal/kg/day at least once.

Patients characteristics at baseline were also registered: with/without partner, professional activity, children, persons to care about and sustain, ability to self-care, ability to hear or to see, their grade of instruction, smoker/non-smoker status, comorbidities (cardio-vascular, pulmonary, gastro-intestinal, hepatic, thyroidian, retinopathy and neuropathy). These characteristics were analysed in the diet-compliant patients in order to find a possible profile of compliant diabetic CKD patient.

Biochemical analyses

The biological parameters mentioned above were determined in our Institute's laboratory-the Gral Medical Movila Laboratory Bucharest, with legal approved and ISO controlled and annually verified equipment. The serum biochemistry: creatinine (mg/dL), urea (mg/dL), plasma glucose (mg/dL), cholesterol (mg/dL), HDL-cholesterol (mg/dL), triglycerides (mg/dL), albumin (g/dL), total proteins (g/dL), total calcium (mg/dL), phosphorus (mg/dL), were performed with commercially available kits from Roche-Hitachi Systems, and were analysed on a Hitachi 917 auto-analyzer. For the glycated haemoglobin HbA1c%, estimations were made with the ionic-exchange high-performance liquid chromatography (HPLC). Total haemoglobin determinations were made by the use of sodium lauryl-suphate method (DLS), on the XT4000i apparatus (Sysmex-Tokyo, Japan). Seric pH and bicarbonate (mmol/L) were assessed with the potentiometric method on the B 121 Cobas apparatus, using reagents and calibrators from Roche Diagnostics.

High-sensitivity C-reactive protein (mg/L) was measured using the immunoturbidimetric assays using a 1470 Wizard gamma-counter (Perkin-Elmer, Turku, Finland). Special seric determinations using electrochemoluminescence immunocomparable assay (ECLIA) were performed for the intact parathormone measurements i-PTH(pg/mL) and for ferritin (ng/dL) determinations with Roche Diagnostics reagent-kits and 6000-E601 Cobas analyser.

For the urinary calculation of albumin/creatinine ratio, the colorimetric kinetics method was performed on C311 Cobas analyser.

Statistical analyses. SPSS 19 ANOVA and the paired difference test were used in the statistical analysis of our data. It was analysed eGFR decline from the initial moment of stage 4 diagnosis to the 12 months endpoint (mL/min/1.73 m^2/year); eGFR decline/year on ketodiet from baseline to the 12 months endpoint (mL/min/1.73 m^2); eGFR variation from baseline to the 12 months endpoint (mL/min/1.73 m^2); eGFR from initial moment of stage 4 diagnosis to the 12 months endpoint (mL/min/1.73 m^2); urine albumin to creatinine ratio - ACR (mg/g); serum bicarbonate - HCO^3 (mmol/L); C serum reactive protein - CRP (mg/L); serum ferritin (ng/mL); pH; body weight without oedema - BW (kg); tri-glycerides (mg/dL); serum total calcium (mg/dL); intact-parathormone i-PTH (pg/mL); because most of data were skewed distribution we report results as median and interquartile range [7] Type I collagen of bovine origin was extracted by the currently

Results and Discussion

Analysing the compliant group (n = 11) of patients *versus* non-compliants (n = 9), at baseline there were no statistical differences noted for: gender (seven women and four men in compliant group and six women and three men in non-compliant group), serum albumin, ACR, HBA1c, serum cholesterol, HDL-cholesterol, triglycerides, serum calcium, phosphorus, ferritin, CRP, eGFR (Table I). No statistical significant differences was observed as concerning the degree of instruction, professional activity, partners, self-care ability, visual and auditive ability, number of children, type of diabetes, dys-lipidaemia, cardiovascular diseases, pulmonary diseases, gastro-intestinal, hepatic and thyroid diseases, retinopathy or neuropathy, SGA, handgrip results, mid-arm muscle circumference, insulin dose (data was not present). People who had to care about someone else were only in the compliant group (n = 4, 100%, p = 0.043) and obesity was more frequent in the non-compliant group (n = 7, 77.8% *vs.* complaints' n = 2, 18.2%, p = 0.008).

At baseline there were also some differences between the compliant *vs.* the non-compliant patients groups: compliants had a longer evolution from initial moment of stage 4 diagnosis (p = 0.014); compliants had a longer anterior evolution in months on keto-diet (p = 0.022); weight without oedema was higher in non-compliants (p = 0.035); BMI was greater in non-compliants (p = 0.041); glycaemic values were greater in non-compliants (p = 0.016) (Table I).

Table I

Biochemical characteristics of patients at baseline

Variables	Compliant (n = 11)		Non-compliant (n = 9)		Total (n = 20)		
	Median	IQR	Median	IQR	Median	IQR	p-value
Age (years)	63	21	65	9	64.5	13	0.255
Duration of diabetes (years)	14	14	16	20	16	16	0.556
Duration of CKD (years)	5	3	3	2	4	2	0.116
Duration of CKD stage 4 (months)	20	33	0	11	8.5	27	0.014
Month on ketodiet (month)	20	36	0	11	8	27	0.022
Body weight (kg)	72	17	95	33.3	78	29	0.035
BMI (kg/m^2)	26.98	7.32	34.92	11.07	29.01	12.78	0.041
SBP (mm Hg)	140	30	140	23	140	20	0.471
Diuresis (mL/24 h)	2000	900	2000	500	2000	650	0.902
Creatinine (mg/dL)	2.3	0.38	2.16	1.23	2.28	0.91	0.422
Urea (mg/dL)	113.57	49.11	105.57	31.63	106.14	35.32	0.323
eGFR at diagnosis of CKD stage 4 ($mL/min/1.73\ m^2$)	28	6.1	25	8.16	27.24	7.33	0.37
eGFR at baseline ($mL/min/1.73\ m^2$)	27.16	7.75	26	11.52	26.58	8.25	0.532
Uric acid (mg/dL)	6	6.12	5.78	5.96	5.94	1.46	0.619
FPG (mg/dL)	98	37	165	95.44	112	81.33	0.016
HbA1c (%)	7.3	1.5	7.5	2.5	7.4	1.57	0.924
Albumin (g/dL)	4.22	0.38	4.25	0.7	4.24	0.44	0.735
ACR (mg/g)	913	2580	1536	3801.94	1328.94	2631.7	0.244
Cholesterol (mg/dL)	164	82	172	57.67	168	67.51	0.857
HDL-C (mg/dL)	45.66	11	45	19.74	45.33	13	0.522
Triglycerides (mg/dL)	100.53	99.53	133.21	125.4	108.5	99.5	0.72
Ferritin (ng/mL)	105	80	233	279.91	165.5	209.59	0.12
CRP (mg/L)	1	2	0.8	4.5	1	2.6	0.343
Haemoglobin (g/dL)	10.9	3	11.5	2.6	11.2	2.65	0.577
HCO_3^- (mmol/L)	22.8	4.1	24.5	6	23.2	4.85	0.417
pH	7.29	0.09	7.29	0.1	7.29	0.09	0.799
Total calcium (mg/dL)	9.18	0.41	9.48	0.77	9.23	0.71	0.492
Phosphorus (mg/dL)	4	0.76	4.41	1.11	4.03	0.98	0.574
Intact parathyroid hormone (pg/mL)	113	156	104	141	108.5	139.45	0.336

CKD = chronic kidney diseases; BMI = body mass index; SBP = systolic blood pressure; Egfr = estimated glomerular filtration rate; FPG = fasting plasma glucose; ACR = urine albumine to cretinine ratio; HDL-C = high-density lipoprotein cholesterol, CRP = C-reactive protein; HCO_3^- = serum bicarbonate.

When analysing the 0 - 12 months variations of the parameters in the compliant *vs*. non-compliant cohorts a statistic significant difference was noted for: the decline in eGFR from initial moment of stage 4 diagnosis to the 12 months endpoint in favour of the compliant group; the improvement of serum bicarbonate (HCO_3^-) in favour of the compliant group; the improvement of i-PTH in favour of the compliant (Table II).

Table II

Compliance to ketodiet when analysing baseline - 12months variation

Variables	Compliants (n = 11)		Non-compliants (n = 9)		Total (n=20)		p value
	Median	IQR	Median	IQR	Median	IQR	
ΔeGRF decline/year (initial-12 month)	1.4	2.02	4.1	5.2	2.2	2.91	0.006
ΔeGFR decline/year on ketodiet (0 - 12 month)	3.75	7.4	4.1	5.6	3.89	6.18	0.37
ΔeGFR (mL/min/1.73 m^2) (0 - 12 month)	3.57	5.42	4.1	6.02	3.75	5.37	0.39
ΔeGFR (mL/min/1.73 m^2) (initial-12 month)	3.39	7.02	4.1	5.15	4.04	6.03	0.82
ΔACR (mg/g)	-8.88	872.93	584.87	1760.15	11	941.91	0.19
ΔHCO_3 (mmol/L)	-1.05	5.7	2	5.8	M1	5.3	0.006
ΔCRP (mg/L)	0	3.05	-0.06	2.2	0	1.62	0.15
Δ Ferritin (ng/mL)	-100.56	111.5	102.9	197	-46.1	189.15	0.14
Δ Ph	-0.04	0.11	-0.01	0.04	-0.02	0.08	0.34
Δ BW (body weight) (kg)	1.75	4.5	1.5	7	1.5	5.5	0.44
Δ Triglycerides (mg/dL)	13.59	45.34	73.79	135.68	20	95.84	0.07
Δ Total calcium (mg/dL)	-0.36	0.61	0.07	56	-0.07	0.77	0.1
Δ i-PTH (pg/mL)	13.95	56.83	-10.8	6.02	0.6	34.25	0.04

Dialysis was initiated in 18.2% (2 of 11) patients of the compliant group and 33.3% (3 of 9) patients in the non-compliant group (p = 0.298).

The annual decline of glomerular filtration rate expressed in mL/min/1.73 m^2/year from initial moment of stage 4 diagnosis to the 12 month endpoint had a median value of 4.1 in the non-compliant group *versus* 1.4 in the compliants (p = 0.006). Moreover Figure 1 shows similar decline (median) in the compliant group, dialyzed as well as non- dialysed patients (under 2 mL/min/1.73 m^2/year) and in the non-compliant group for the non-dialyzed patients (up to 2.5 mL/min/1.73 m^2). As for the non-compliants dialyzed at the end of 12 month monitoring, the decline was rapid with over 6 mL/min/1.73 m^2/year. Also very important to mention is the fact that in the compliant group, due to the mild decline of GFR the entrance in any renal substitution method can be done in a planned manner, with minimal complications of the acute vascular/abdominal access as compared to the non-compliant group, where most of the patients have had no time for preparing any dialysis access, due to rapid GFR decline (Figure 1).

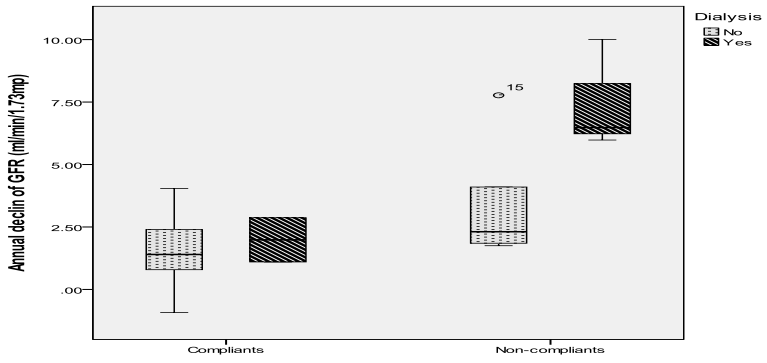

Figure 1.
Outcomes in annual GFR decline - compliants *vs.* non-compliants

There were no significant differences between the nutritional parameters at baseline and after 12 months, so the nutritional profile may be considered as safe in the both compliant and non-compliant groups. Anyway the follow-up period was relatively short in order to assess proteic malnutrition. Longer time-span is necessary to evaluate a significant impact of ketodiet on the nutritional status. As it came up during the whole 1 year period of follow-up, the psychological and dietetic support have been essential and they have been offered monthly, as well as any time requested by the patients, by using a telephonic scheduled programme of consultations.

Compliants representing 55% (n = 11) of the analysed patients seemed to have a lower weight, BMI and glycaemic level and they better care themselves.

Most of the compliants were women (63.6%, n = 7) and were mostly type 2 diabetic (72.7%, n = 8).
Concerning the compliant patients group there may not be so far any clearly defined portrait of the "successful" patients, but this might be due to the small number of patients enrolled.
The "real" diet, evaluated on diet questionnaires, diet recall and e-PNA (protein equivalent of total nitrogen appearance) calculations was estimated: for the compliants, a diet with 0.44 ± 0.11 g proteins/kg/day and 32.2 ± 1.7 kcal/kg/day and for the non-compliants, a diet with 0.67 ± 0.21 g proteins/kg/day and 33.1 ± 2.14 kcal/kg/day.
In the literature, without treatment, the progression rate has been reported to be 9 - 14 mL/min/year in type 1 diabetes mellitus with proteinuria [9, 12, 14], but slower in patients with type 2 diabetes mellitus and nephropathy around 6 mL/min/year [4].
Many studies have examined the effects of dietary protein restriction in diabetic patients [16]. Nearly all trials demonstrated the fact that protein restriction slows the decline of renal function [1, 10, 17]. In the low-protein diet (LPD) sample of type 1 diabetes studies, with 0.8 g proteins/kg/day, the average decline was 11 mL/min/year, less than in usual protein diets [6, 13, 15]. Since the usual decline in GFR is about 10 mL/min/year, a patient on LPD may delay the onset of dialysis about 10 months to 1 year [16]. The study by Pijls *et al* in type 2 diabetes showed disappointing results because the patients didn`t comply with the diet [10].
CKD patients are able to adapt to marked dietary restriction with simultaneous substitution of essential amino-acids and their ketoanalogues; the neutral nitrogen balance is achieved by a marked suppression of the aminoacids oxidation and postprandial inhibition of protein degradation, so that the dual-energy X-ray absorptiometry (DEXA) evaluation of body composition confirms the long-term safety of VLPD supplemented with ketoanalogues and indirect data suggest that the mechanism by which protein turnover adapts to a LPD can be impaired in the presence of metabolic acidosis [7]. Thus the protein metabolism and nitrogen balance adapt successfully in patients who are compliant to a VLPD supplemented with ketoanalogues and essential amino-acids in non-acidotic CKD patients.
Any profile of patient can be compliant with a convincing argumentation at the very first moment when ketodiet is proposed, no matter of comorbidity or social position or education [2].
Still there isn`t clearly demonstrated if ketodiet compliance is long-term satisfactory in both type 1 and 2 diabetes mellitus, if malnutrition can be avoided, with a stable metabolic and glycaemic control, on the use of stable doses of antidiabetic drugs or insulin, having in the end a better GFR outcome than patients on usual LPDs.

Conclusions

Ketodiet in compliant patients with diabetic CKD offers better outcomes on GFR, a slower deterioration of renal function, better acidosis control, better i-PTH, and better inflammation-denutrition parameters. Obese patients and improper controlled diabetes, with high glycaemic values are more prone to be non-compliant. Thus ketodiet is a diet to carry on for all diabetic CKD patients staged 4 in order to prospone renal substitution. Minocycline-loaded collagen matrices, uncross-linked

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