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Original article

Tricarboxylic Cycle Intermediates in Combination with Calcium Phosphate Chelators and Sodium Bicarbonate Increases eGFR in Patients with Stages 3b, 4 and 5 CKD: A Retrospective Observational Study

Jorge Antonio Hernández-Miramontes[1], Antonio Méndez-Durán[2] and Jorge A. Hernández-Villanueva[3]

[1]Private practice, Puerto Vallarta (Jalisco), México
[2]Instituto Mexicano del Seguro Social, Ciudad de México, México
[3]Hospital Regional de Occidente, Instituto Mexicano del Seguro Social, Guadalajara, México

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Abstract

Introduction: Currently, the conservative management of chronic kidney disease (CKD) involves treating the primary disease and its consequences, as well as improving altered biochemical markers. Evidence suggests that the key management strategy for slowing down the progression of the disease is nephroprotection.

Objective: This study aimed to evaluate the effect of the supply of TCA cycle intermediates in combination with calcium carbonate, calcium lactate, and sodium bicarbonate in patients with CKD.

Methodology: A retrospective observational study was undertaken in nephrology and internal medicine clinics in Mexico. The study enrolled patients aged over 18 with stages 3b, 4, and 5 chronic kidney disease (CKD) who were not undergoing renal replacement therapy (RRT) and had received treatment based on TCA therapy.

Keywords: Chronic kidney disease, CKD-EPI, creatinine, estimated glomerular filtration rate, TCA, nephroprotection.

Correspondencia: Jorge A. Hernández Miramontes. Plaza Genovesa L14-15, Zona Hotelera Las Glorias, Puerto Vallarta (Jalisco), México. E-mail: jhmiramontes@hotmail.com

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Results: The study included a total of 55 patients with CKD. The results showed an increase in eGFR from a baseline average of 16.73 ± 1,374 mL/min to a final average of 19.18 ± 1,516 mL/min and a decrease in creatinine values from a baseline average of 4.26 ± 2.44 mg/dL to a final average of 3.77 ± 2.23 mg/dL. These changes had a statistical significance P <0.05.

Conclusions: The observed benefits of TCA in combination with sodium bicarbonate, calcium carbonate, and calcium lactate include: 1) Increased eGFR, 2) Decreased serum creatinine, 3) Decreased serum urea, 4) Decreased serum phosphorus, 5) Increased serum hemoglobin, and 6) Maintenance of albumin levels within normal ranges. Adjuvant therapy with the combination of TCA could be a useful tool as a new therapeutic option in patients with CKD.

Intermediarios del ciclo de Krebs en combinación con quelantes cálcicos de fósforo y bicarbonato de sodio aumentan la TFGe en pacientes con ERC en estadios 3b, 4 y 5: un estudio retrospectivo observacional

Resumen

Introducción: en la actualidad, el tratamiento de la enfermedad renal crónica (ERC) consiste en tratar la enfermedad primaria y sus consecuencias, así como mejorar los marcadores bioquímicos alterados. Sin embargo, la nefroprotección es clave para retrasar la progresión de la enfermedad.
Objetivo: este estudio evalúa el efecto del suministro de intermediarios del ciclo de Krebs en combinación con carbonato de calcio, lactato de calcio y bicarbonato de sodio en pacientes con ERC.
Métodos: se realizó un estudio observacional retrospectivo en clínicas de nefrología y medicina interna de México. Se incluyeron pacientes mayores de 18 años con enfermedad renal crónica (ERC) estadios 3b, 4 y 5 que no estaban en tratamiento sustitutivo renal (TSR) y que han sido tratados con terapia basada en intermediarios del ciclo de Krebs.
Resultados: el estudio incluyó un total de 55 pacientes con ERC. Los resultados mostraron un aumento del TFGe de una media basal de 16,73 ± 1.374 mL/min a una media final de 19,18 ± 1.516 mL/min y una disminución de los valores de creatinina de una media basal de 4,26 ± 2,44 mg/dL a una media final de 3,77 ± 2,23 mg/dL, estos cambios tuvieron una significación estadística p<0,05.
Conclusiones: los beneficios observados de la mezcla evaluada incluyen: 1) Aumento de la TFGe, 2) Disminución de la creatinina sérica, 3) Disminución de la urea sérica, 4) Disminución del fósforo sérico, 5) Aumento de la hemoglobina sérica y 6) Mantenimiento de los niveles de albúmina dentro de rangos normales. La terapia adyuvante con la combinación de ATC podría ser una herramienta útil como nueva opción terapéutica en pacientes con ERC.

Palabras clave: enfermedad renal crónica, CKD-EPI, creatinina, tasa de filtración glomerular estimada, intermediarios del ciclo de Krebs, nefroprotección.

Introduction

Chronic kidney disease (CKD) is associated with many biochemical and physiological abnormalities, such as increased risk of cardiovascular events, end-stage renal disease involving renal replacement therapy to sustain life, and an increase in overall mortality [1]. Serum creatinine levels, glomerular filtration rate (GFR), urea, metabolic acidosis, malnutrition, and hyperphosphatemia are directly related to the symptomatology of CKD and progression to end-stage chronic kidney disease [2].

Currently, the conservative management of CKD involves treating the primary disease and its consequences, as well as improving altered biochemical markers. Evidence suggests that the key management strategy for slowing down the progression of the disease is nephroprotection [3]. Therefore, reducing uremic toxin production and correcting metabolic acidosis are crucial for slowing the progression of CKD and preserving kidney function. Treatment of metabolic acidosis with sodium bicarbonate supplementation slows the rate of decline of renal function [4, 5]. Conversely, various strategies have been used to decrease or eliminate the production of nitrogenous substances, including the reduction of protein in the diet, prebiotics, probiotics and ketoacid analogues (KA). However, effective treatment options remain limited.

Recent evidence has shown that metabolites of the tricarboxylic acid (TCA) cycle are related to various pathologies, including kidney diseases, with alterations in the levels of these metabolites and of the enzymes involved in their synthesis, which influences renal function [6]. TCA cycle is a mitochondrial process and increasing evidence highlights mitochondrial dysfunction as an important pathological mediator of CKD both in cases related to diabetic and non-diabetic nephropathy [7–9]. Furthermore, it has been shown that in patients with non-diabetic CKD, urinary excretion of TCA cycle metabolites and renal expression of genes regulating these metabolites are reduced, suggesting that diminished TCA activity in these patients may be due in part to reduced citric acid cycle substrate availability [9]. Therefore, it is possible that the administration of TCA cycle intermediates would benefit patients with CKD. Our hypothesis posits that by supplying TCA intermediates - citric, succinic, fumaric, and malic acid- we can replenish the cycle, generating two terminal molecules where transamination is possible: alpha-ketoglutarate and oxaloacetate, and using circulating amino groups for this process, similar to KA of essential amino acids that are used as supplements in the low-protein diets (LPDs) of CKD patients [10]. Alpha-ketoglutarate has the capability to seize an amino group, resulting in the formation of glutamic acid. Subsequently, glutamic acid can further acquire another amino group to synthesize glutamine,

along with various other non-essential amino acids. Similarly, oxaloacetate can generate aspartic acid via transamination, and this in turn may form asparagine and other related non-essential amino acids by capturing an additional amino group. In both cases, reused ammonium prevents its conversion into urea and promotes a better nutritional status by synthesizing non-essential amino acids.

The objective of this study was to determine the eGFR in patients with CKD in stages 3b, 4 and 5 that received the supply of TCA intermediates in combination with calcium phosphorus chelators and sodium bicarbonate and evaluate its evolution throughout the assessed period.

Material and methods

Study design

During the period from February 1rst, 2017, to August 1rst, 2019, an observational, retrospective, and descriptive study was conducted across various nephrology and internal medicine clinics in Mexico. These clinics included Eva Clinic in Puerto Vallarta, Jalisco; Fátima Hospital in Los Mochis, Sinaloa; Plenita Clinic in Guadalajara, Jalisco; Ángeles Hospital Querétaro in Querétaro; Sinai Clinic in Oaxaca, Oaxaca; and Medical Clinic London in Toluca, Mexico. Information retrieval was performed by examining the clinical records of patients with CKD who met the inclusion criteria and none of the exclusion criteria.

Selection of participants

Inclusion criteria: Patients with CKD stages 3b, 4, and 5, >18 years without RRT who have been treated with therapy based on TCA cycle intermediates who had at least two baseline biochemical determinations and then at least one month of treatment with TCA.

Exclusion and elimination criteria: Chronic liver disease, intake of alpha keto-analogues of amino acids, prebiotics, or probiotics, critically ill patients requiring initiation of RRT and clinical records with incomplete information.

Data collection

The nephrology and internal medicine records of the corresponding clinics were reviewed and patients who met the inclusion criteria and none of the exclusion criteria of the research protocol were included. The patients' clinical data were obtained from their medical records and laboratory test reports. Notably, as part of the treatment regimen for the enrolled patients, the clinical records documented the administration of a compound comprising TCA,

in combination with calcium carbonate, calcium lactate and sodium bicarbonate. During the assessed period, at least four measurements (t1, t2, t3 y t4) of the clinical parameters evaluated in each medical consultation were obtained and the approximate interval between visits was between 30 and 60 days. Additionally, patients underwent laboratory tests both prior to initiating therapy (t1) and during the course of treatment (t2-t4).

Product under investigation

This formulation is a mixture of citric, malic, fumaric, and succinic acid, in combination with calcium carbonate, calcium lactate, and sodium bicarbonate presented in an effervescent powder and manufactured by Virtus Humanitatis S. de R.L. de C.V. The dose used for each of the patients ranged from 20 to 60 grams per day in a dilution of 240 mL of water, divided into one to three doses a day to be ingested with food. The dose of the studied combination was calculated based on the value of the eGFR. For patients at stage 3b, a dosage of 20 grams per day was recommended to be taken with meals. For patients at stages 4 and 5, a dosage of 60 grams divided into three doses per day, also to be taken with meals, was advised. Notably, the 60 gram formulation contains 293 milliequivalents of TCA intermediates capable of binding with 293 milliequivalents of NH2 groups.

Statistical analysis

In this exploratory study, the Shapiro-Wilk test was employed to assess data distribution, revealing a normal distribution. Subsequently, a descriptive analysis of variables across the total sample was carried out. The mean and standard deviation were obtained in all cases, and the significance level used was $\alpha = 0{,}05$. The eGFR values for each participant were calculated from serum creatinine level, sex and age using the CKD-EPI formula [11], and eGFR gain or loss was calculated for one year. The change of the initial eGFR with respect to the final eGFR (final GFR – initial GFR) was taken as a basis to calculate the change over the evaluated period of each patient, and then adjusted to 12 months to calculate the annual change. The t-Student statistical test was performed for groups related to the variables of interest to analyze the baseline values vs. those obtained at the end of the treatment period. Pa tients' creatinine and urea data were categorized by disease stage for exploratory analysis, and baseline vs. last measurement was compared for each group: 3b, 4, and 5. Statistical analysis was performed in Jupyter Notebook with the Anaconda distribution for Python 3.7.13 [12] using the libraries: pandas 1.3.5 [13], numpy 1.21.6 [14], matplotlib 3.2.2 [15], scipy 1.7.3 [16] and seaborn 0.11.2 [17].

Results

Baseline Characteristics

The study included 55 patients with CKD: 27 women (49.1 %) and 28 men (50.9 %). The average age was 67.2 years (±13.2). The age range observed in the study spanned from a minimum of 31 years to a maximum of 88 years. Notably, over half of the patients (50.1 %) were aged over 70 years, underscoring the presence of a vulnerable population within the cohort. Regarding the etiology of CKD, among the most frequent etiologies, 69.1 % of patients had a history of type 2 diabetes mellitus and 23.6 % of systemic arterial hypertension. In relation to the stage of CKD, six patients had stage 3b (10.9 %), while 20 patients (36.36 %) had stage 4 and finally, 29 patients (52.73 %) were classified as stage 5 (Table 1). The patients, on average, underwent treatment for a duration of 11 months throughout the study period.

Table 1. Demographic information (Study Population, n=55)

Variables	**n (%)**
DEMOGRAPHIC	
Gender	
Male (%)	28 (50.91 %)
Female (%)	27 (49.09 %)
Age (Years)	67.24 ± 13.2*
AETIOLOGY	
Diabetes mellitus	38 (69.09 %)**
High blood pressure	13 (23.64 %)**
Lupus	4 (7.27 %)**
Tuberculosis	1 (1.82 %)**
Glomerulonephritis	1 (1.82 %)**
Wegener's disease	1 (1.82 %)**
STAGE	
Stage 3b Chronic kidney disease	6 (10.91 %)
Stage 4 Chronic renal failure	20 (36.36 %)
Stage 5 End-stage chronic renal failure	29 (52.73 %)

Note. *Data are represented as mean ± SD, **Data represent the percentage of patients who present each etiology separately.
Some patients have more than one concomitant disease.
Source: Own elaboration.

Estimated Glomerular Filtration Rate (eGFR) by CKD-EPI

The eGFR was estimated by the CKD-EPI equation and was evaluated throughout the assessed period. The results showed an increase in eGFR from a baseline of 16.73 ± 1.374 mL/min to a final mean of 19.18 ± 1.516 mL/min. Upon comparative analysis of these values, the final

mean of the eGFR showed an increase, which was statistically significant, reflected in a mean change of 2.455 (±7.74), P<0.05. This result was expected since 38 patients (69.1 %) reported a reduction in creatinine levels, while only 17 patients (30.1 %) registered a creatinine increase.

Additionally, the eGFR change (Δ) per year was estimated. As observed in Fig. 1, the mean Δ value of eGFR per year was 3.1 (±9.78) mL/min per 1.73 m^2.

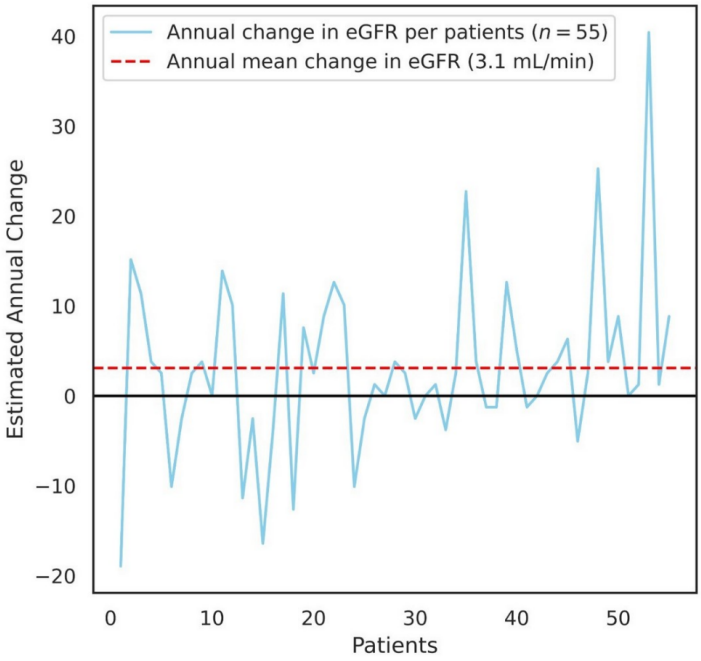

Figure 1. Annual change of the eGFR

Note. The image shows the annual change of eGFR calculated for each patient evaluated. The changes in the blue line represent an individual patient value. The dotted line indicates the mean change in eGFR (3.1 mL/min) of the total sample.
Source: Own elaboration.

Serum creatinine

Serum creatinine levels were also characterized throughout the evaluated period. The results showed a statistically significant decrease in serum creatinine (Table 2) from a baseline mean of 4.26 ± 2.44 mg/dL to a final mean of 3.77 ± 2.23 mg/dL, this is reflected in a reduction from baseline values of 0.49 (±1.75) mg/dL, P<0.05 (Fig. 2a).

Additionally, to explore the evolution of creatinine by stage of the disease, we divided the group of patients into stages 3b, 4, or 5. Figure 2b shows the trend of creatinine levels in each stage of CKD, revealing a marked reduction primarily in stage 5 patients with an average reduction close to the unit (0.9228 ± 2.2705 mg / dL).

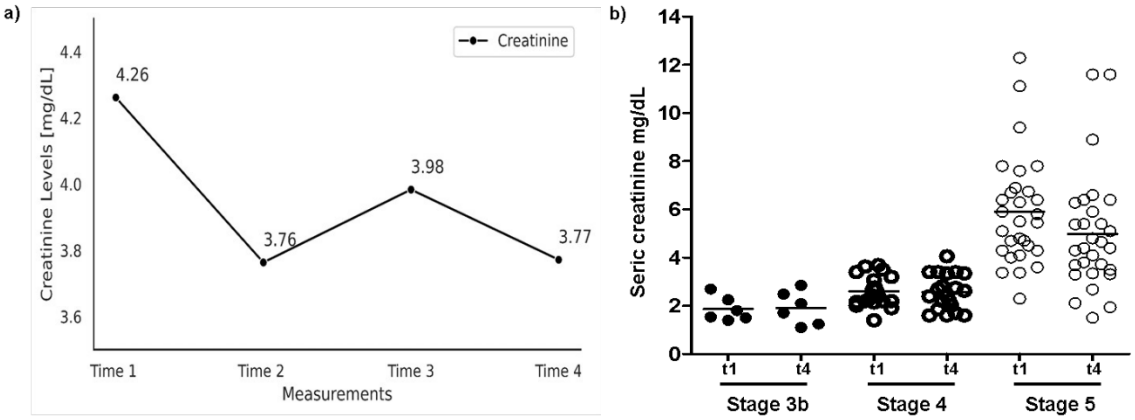

Figure 2. Creatinine values by measurement time and by stage of the kidney disease

Note. (a) Plasma concentrations of creatinine in the different evaluation times; t1 represent the basal levels pre-intervention, and t2-t4, represent levels post-intervention; data represent mean values (n=55).
(b) Comparison of baseline (t1: before starting test treatment) and final (t4: third measurement post-treatment) creatinine plasma concentrations in the different stages of CKD evaluated.
Source: Own elaboration.

Table 2. Clinical parameters evaluated

Variable	t	N	Mean	Std. deviation	Minimal	Median	Maximum	Basal vs. post basal change (SD)	P value*
CREATININE	1	55	4.26	2.44	1.4	3.6	12.3		
	2	55	3.76	2.14	1.4	3.28	13.18	0.498 (1.60)	25
	3	55	3.98	2.02	1.4	3.6	11.5	0.278 (1.86)	272
	4	55	3.77	2.23	1.1	3.35	11.6	0.490 (1.75)	43
UREA	1	55	136.92	50.68	38	131.7	299		
	2	55	118.82	43.12	42	120	258	18.09 (45.59)	5
	3	55	120.3	42.07	42	114	261	16.61 (49.82)	17
	4	55	113.07	44.95	53	109.05	265.36	23.84 (56.06)	3
PHOSPHORUS	1	55	5.04	1.23	3.4	4.8	10.4		
	2	55	4.53	1.25	1.7	4.3	10.9	0.50982 (1.10320)	1
	3	55	4.24	0.87	2.8	4.1	9.2	0.80491 (1.40837)	<0.001
	4	55	3.87	1.33	1.2	3.77	12.5	1.17182 (1.71013)	<0.001
HEMOGLOBIN	1	55	11.16	1.64	6.5	11.4	15.4		
	2	55	10.95	1.35	7.4	11	15.4	0.21782 (1.51522)	291
	3	55	10.9	1.37	7.1	10.9	14.9	0.26436 (1.91394)	310
	4	55	11.68	1.43	7.6	11.9	16.3	-0.51509 (1.37974)	8
ALBUMIN	1	55	3.85	0.41	2.4	3.9	4.8		
	2	55	3.8	0.36	2.6	3.8	4.8	.05800 (0.29331)	148
	3	55	4.0	0.33	3.0	4.0	5.8	-.14727 (0.44408)	17
	4	55	3.77	0.41	3.1	3.6	5.3	.08909 (0.46765)	163

Note. *Student t-test for related samples.
Source: Own elaboration.

Urea, phosphorus, hemoglobin and albumin

Another clinically significant parameter for assessing patients with CKD is urea levels, which we also evaluated during our study. Mean baseline urea levels were 136.92 mg/dL, while the postintervention levels were 118.82 mg/dL, 120.3 mg/dL, and 113.07 mg/dL, resulting in a statistically significant decrease P<0.05 (Fig. 3a). It is worth mentioning that the most pronounced reduction observed was 23.84 mg/dL, which was observed at the fourth measurement (Table 2).

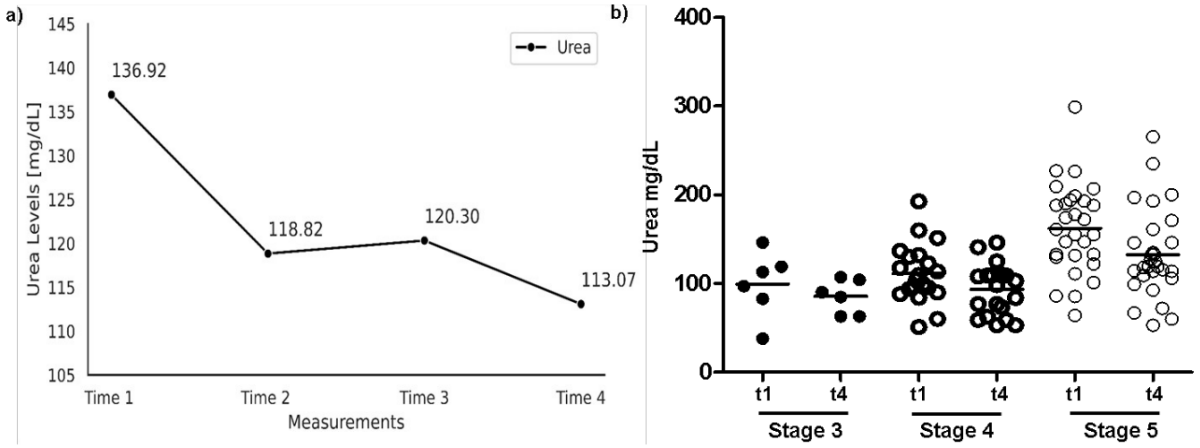

Figure 3. Urea values by measurement time and by stage of the kidney disease

Note. (a) Plasma concentrations of urea in the different evaluation times; t1 represent the basal levels pre-intervention, and t2-t4, represent levels post-intervention; data represent mean values (n=55). (b) Comparison of plasma concentrations of baseline and final urea in the different stages of CKD evaluated.
Source: Own elaboration.

Additionally, we also explored the evolution of urea by disease stage. Fig. 3b shows a decrease in urea levels across all three stages, nevertheless, patients in stage 5 exhibited a more significant reduction in urea, with a value of 29.85 mg / dL.

Regarding phosphorus, a significant decrease in each measurement was observed, as depicted in Fig. 4a, going from a baseline of 5.04 mg / dL to a final mean of 3.87 mg / dL. A mean change of 1.17 (±1.7) mg/dL, P<0.05, was verified.

Changes in hemoglobin were also evaluated. Fig. 4b shows the mean levels per measurement going from a baseline of 11.16 g/dL (±1.64) to a final mean of 11.68 g/dL (±1.43). The increase was concluded statistically significant, P<0.05 (Fig. 4b).

Regarding albumin values, no statistically significant change was observed at the end of the evaluated period. However, the values remained within the normal range (Fig. 4c).

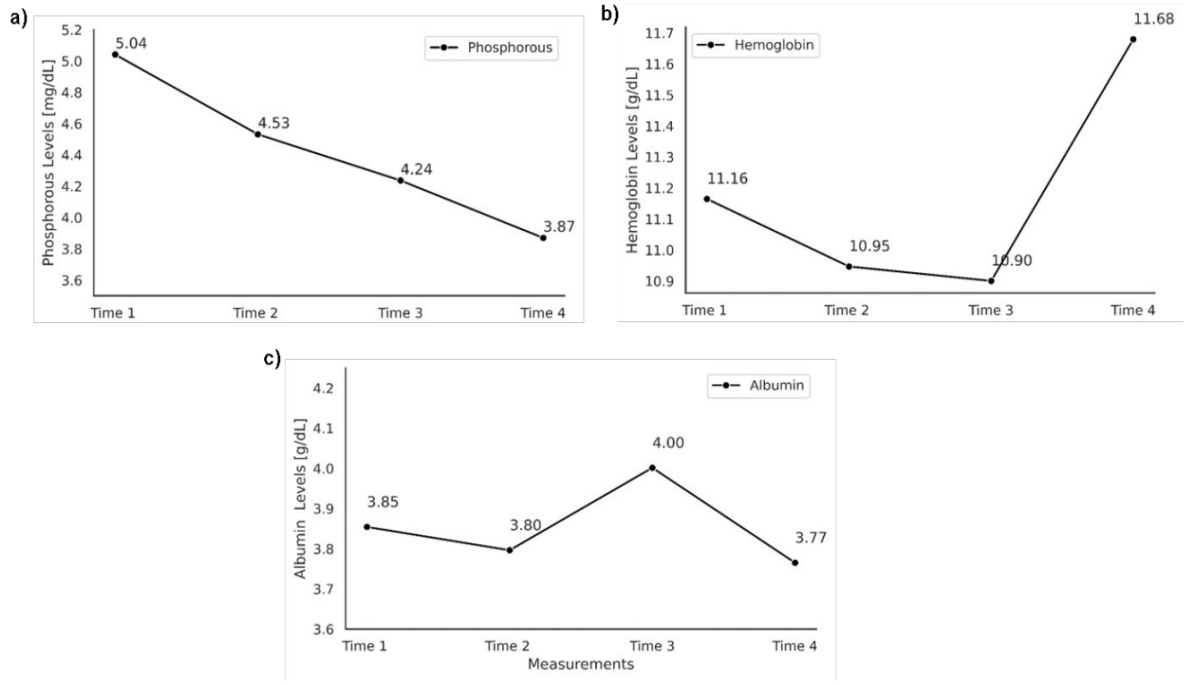

Figure 4. Phosphorus, hemoglobin and albumin Levels

Note. (a) Plasma concentrations of phosphorus at different evaluation times.
(b) Plasma concentrations of hemoglobin at different evaluation times.
(c) Plasma concentrations of albumin in the different evaluation times. t1 represent the basal levels pre-intervention, and t2-t4, represent levels post-intervention; data represent mean values (n=55).
Source: Own elaboration.

Discussion

The present composition of carboxylic acids managed to decrease creatinine with a statistically significant reduction of 0.49±1.75 mg/dL, and a significant increase of eGFR (a mean change of 2.455±7.74 mL/min). In addition, our observations revealed a reduction in urea and phosphorus levels, consistent with findings from our previous study [18]; an increase in hemoglobin, and a state of normo-albuminemia despite hypoproteic diets and the inherent inflammatory state of CKD. It is important to note that 50.1 % of the patients were over 70 years of age, and that 89.1 % were in stages 4 and 5 of CKD, which makes the study interesting for the type of labile population.

Several studies have demonstrated that multidisciplinary pre-dialysis education and team care confer significant benefits to patients with CKD extending survival rates when compared to those undergoing standard therapy. In particular, the study conducted by Yu-Ren Chen *et al.* [19], recognized as a benchmark study for nephrologists [20], demonstrated that CKD patients participating in multidisciplinary team care (MDC) benefited from slower renal function declines in advanced stage CKD with a difference per year of -5.1 in MDC group vs. -7.3 ml/min in control group (P = 0.01). However, despite these findings, the change remained negative. In contrast, our study yielded much more favorable outcomes, with a positive eGFR. On the other hand, various strategies have been used to reduce or eliminate uremic toxins production, including the reduction of protein in the diet, prebiotics, probiotics and KA. Numerous studies aiming to evaluate dietary interventions comprising prebiotics and probiotics have been performed. Nonetheless, there are no conclusive results regarding the beneficial effects of these compounds. A study assessing the probiotic Renadyl® in 28 patients at stages 3 and 4 of CKD noted a trend towards reduced urea levels, although this trend did not reach statistical significance [21]. Additionally, KA have been used for more than 40 years to supplement LPDs and very low protein diets (VLPDs) for patients with CKD [10, 22]. While there is evidence indicating potential benefits on renal outcomes, the observed effects are minor. The Modification of Diet in Renal Disease (MDRD) Study is the largest randomized prospective trial to date designed to assess whether LPDs could retard CKD progression. In Study 2 of this trial, 255 patients were assigned randomly to an LPD or VLPD supplemented with KA [23]. The trial findings suggested a slower rate of GFR loss with the supplemented VLPD compared to the LPD; however, this difference was not statistically significant. The formulation examined in the present study is based on a novel mechanism of action in medical therapeutics [24]. By leveraging intermediates of the TCA cycle and facilitating transamination, it potentially enhances the synthesis of non-essential amino acids and thus the capture of amino groups, thus preventing the conversion of excess ammonium to urea [25]. The addition of sodium bicarbonate and calcium-phosphorus chelators also allows the treatment of metabolic acidosis, preventing further catabolism, improving nutritional status, and delaying renal damage.

The application of TCA (citric, succinic, fumaric, and malic acid) relies on the replacement of intermediates of the cycle. Our hypothesis addresses the problem of urea toxicity in CKD patients and the proposed mechanism is related to an increment of ketoglutarate and oxaloacetate, which can incorporate NH_2 groups through the action of transaminases, thus reducing the amount of ammonium/ammonia. In our results, this would explain the decrease in urea. Pathologically increased urea levels in CKD patients are known to increase oxidative stress which can exacerbate the progression of CKD [26, 27]. For instance, it has been proved

that oxidative stress plays an important role in the development and progression of sclerosis and fibrosis in the remnant kidney model of chronic renal failure [28]. Hence, the reduction in urea levels could lead to an amelioration of CKD, as evidenced by our findings. Furthermore, TCA cycle intermediates can also perform other functions. There is evidence involving citrate and fumarate with a protective effect on the kidney. Citrate has been used in the treatment of kidney diseases such as renal lithiasis, acute kidney damage, and CKD [29–31]. Regarding acute kidney damage, administration of citrate reduced plasma creatinine levels. This reduction was reflected in an improvement in renal function [32], in line with our results. Citrate has also been associated with immunomodulatory effects by decreasing the levels of proinflammatory cytokines and increasing anti-inflammatory cytokines, both in patients with acute kidney damage and in animal models of CKD [33, 34]. Regarding fumarate, its administration in animal models of drug-induced renal damage reduces kidney injury by increasing the antioxidant response of the transcription factor Nrf2 [35, 36]. In addition, it is also used for its immunomodulatory capacity in autoimmune diseases [37].

Inflammation is directly related to the glomerular filtration rate (GFR) in dialysis patients [38]. Uncontrolled inflammatory infiltration can lead to the accumulation of inflammatory cells in the tubular interstitium, producing massive amounts of proinflammatory molecules, such as TNF-α, IL-1β, and IL-6, further exacerbating renal injury [39, 40]. Hence, the immunomodulatory and antioxidant properties of citrate and fumarate may potentially contribute to the amelioration of CKD, as observed in our study.

Finally, succinate and its receptor SUCRN1 have been described as local stress detectors, suggesting a key role in the development of hypertension and the complications of diabetes mellitus [41]. Furthermore, in mice with diabetic nephropathy, hyperglycemia induces renin release through SUCNR1 [42]. In CKD, there are alterations in the reninangiotensin-aldosterone axis; however, it is currently unknown if succinate and its receptor are involved. More studies are necessary to determine the exact mechanism of TCA intermediates slowing CKD progression.

The use of TCA cycle intermediates in combination with calcium chelators of phosphorus and sodium bicarbonate in patients with CKD stages 3b, 4, and 5 offers a new mechanism of action with effect on different axes of the disease and a therapeutic tool different from any other worldwide. However, our study has certain limitations: 1) Because of the observational retrospective design, it is necessary to confirm the results of this study in a clinical trial and with a higher number of patients; 2) The relatively short duration of patient evaluation com-

pared to other studies might not fully capture the long-term trajectory of the variables under investigation.

Conclusion

Overall, adjuvant therapy with the combination of TCA with calcium phosphorus chelators and sodium bicarbonate could be a useful tool in patients with CKD, in which the desired goal is to safeguard renal function and delay the use of renal replacement therapy.

Ethical statement

Study without risk according to the research standards established by the World Health Organization and the current Helsinki Law.

Conflicts of interest

J. Hernández Miramontes and J. Hernández Villanueva declare to be holders of the Mexican patent of the formulation studied.

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Authors' contribution

Jorge Antonio Hernández-Miramontes: conceptualization, methodology, project management, supervision, formal analysis, writing-original draft; Jorge A. Hernández-Villanueva: conceptualization, methodology, project management, supervision, formal analysis, and writing-original draft; Antonio Méndez-Durán: conceptualization, methodology, writing, review and editing.

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