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Effect of Serum and Urinary Magnesium Levels on the Progression of Renal and Atherosclerotic Disease in Patients with Non-Dialysis-Dependent Chronic Kidney Disease (MAG-PROGRESS STUDY): Methodological Design of the Cohort Study

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ABSTRACT

Background: Several evidences suggest that low serum magnesium (Mg) might be a risk factor for developing kidney disease. In addition, Mg deficiency is associated with an increased risk of cardiovascular events and vascular calcification. ***Methods***: A cohort study will be conducted in a sample of 152 patients with eGFR of 15-59 mL/min/1.73m², at the University Hospital of the Federal University of Maranhao. Patients will be evaluated at three different time's points: t1 (enrolment), t2 (12 months), and t3 (24 months), for the clinical, laboratory, and imaging findings. The following primary outcomes will be assessed: 1) decrease in eGFR > 5 mL/min/1.73m² per year or need for renal replacement therapy; 2) increase in the carotid intima-media thickness (cIMT) or increase in the coronary calcium score (CCS); 3) increase in albuminuria. Other outcomes such as cardiovascular events and mortality will also be evaluated. ***Results***: The study included 152 patients with CKD, with a mean age of 60.2 (11.9) years and an equal proportion between genders, 57.2% had albuminuria> 30mg/24h and 42.1% had FEMg> 6.1%. Were identified 38.8% of patients with cIMT> 0.9 mm and 65.1% with coronary calcification. It was observed that the highest tertiles of FEMg were related to lower eGFR (p <0.01) and to the highest levels of albuminuria (p = 0.02).***Conclusion***: The study is expected to provide information about the prevalence of Mg disorders and their repercussions on the progression of chronic kidney and atherosclerosis in CKD patients on conservative treatment followed for 24 months.

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INTRODUCTION:

Magnesium (Mg) is the fourth most abundant cation in the body and the second most abundant in the intracellular environment. It plays a prominent role in many cellular functions including transfer, storage, and use of energy; metabolism of proteins, carbohydrates, and fats; maintenance of normal function of the cell membrane; regulation of parathyroid hormone secretion; and decrease in systemic blood pressure and peripheral vascular resistance [1].

Low Mg intake, associated with changes in its internal distribution and excretion, leads to Mg deficiency, the incidence of which is approximately 2% in the general population [2]. Reduced Mg levels are related to oxidative stress, pro-inflammatory status, endothelial dysfunction, platelet aggregation, insulin resistance, hyperglycemia, atherosclerosis, and progression of renal disease [3].

The importance of Mg is well-known; however, it has not yet received the necessary attention in the medical literature and clinical practice, compared to other electrolytes such as sodium, potassium, and calcium. Although its association with potentially serious clinical complications is increasingly reported [4-6], serum and urinary levels of Mg are not routinely evaluated, especially in patients with chronic kidney disease (CKD). Most available studies only show the relationship between serum Mg levels and long-term renal and cardiovascular outcomes [7-10]; however, it is also necessary to know the effect of urinary Mg levels on the outcomes of these diseases.

MATERIALS AND METHODS:

Study design

This is a prospective cohort study to investigate the effects of serum and urinary Mg levels on the progression of renal and atherosclerotic disease in patients with non-dialysis-dependent CKD. The sample will comprise patients visiting the outpatient follow-up department at the Kidney Disease Prevention Center of the University Hospital of the Federal University of Maranhão (HUUFMA). The study has been approved by the Research Ethics Committee of HUUFMA and will be conducted by the principles established by the Declaration of Helsinki (1964).

To determine the sample size, non-probabilistic sampling was performed considering the calculation at a significance level of 5%, a test power of 0.80, and a correlation between serum/urinary Mg levels and estimated glomerular filtration rate (eGFR) of at least 0.25. The calculation showed that a sample size of 124 patients was necessary, and foreseeing the probable losses in the follow-up period, it was decided to increase the sample by at least 20%. At the end, a sample size of 152 patients was decided. The following inclusion and exclusion criteria were considered for recruitment:

Inclusion criteria: patients with stages 3A, 3B, and 4 CKD (eGFR between 15 and 59 mL/min/1.73 m²); age 18 years or older; regular follow-up with a nephrologist of at least 1 year before the initiation of the study;

Exclusion criteria: chronic consumptive diseases; pregnant women; urinary infection; autoimmune diseases; patients with a coronary stent.

Data Collection

Data will be collected through specific tests at different time points: t1 (enrolment), t2 (12 months), t3 (24 months).

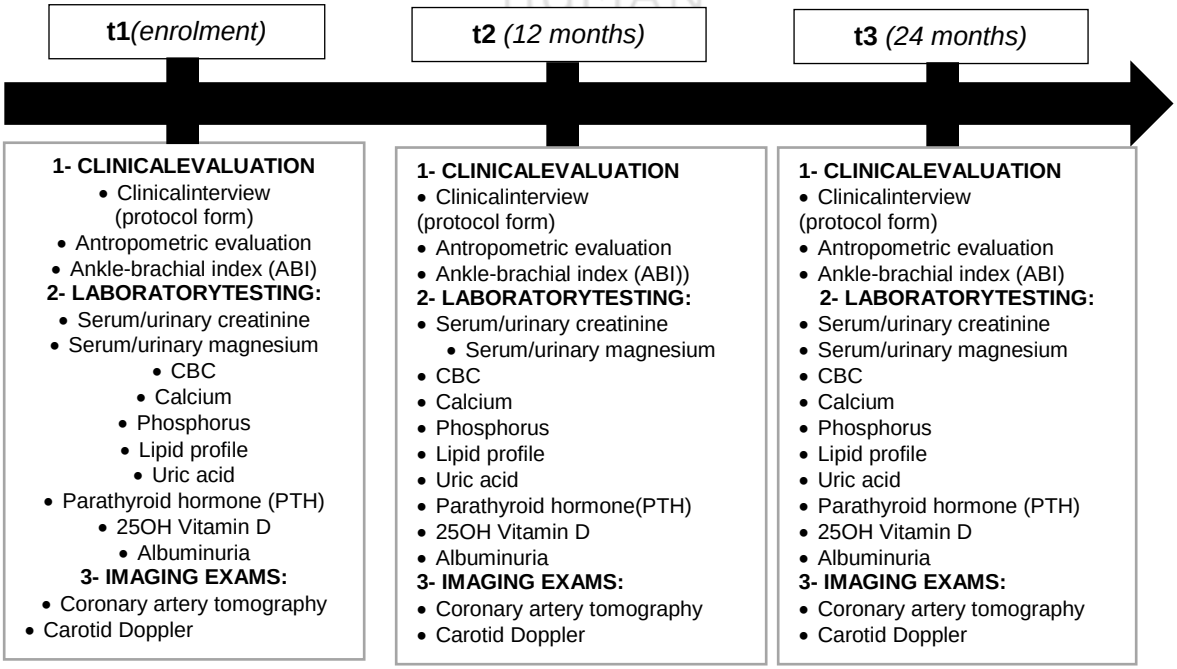

Figure 1 - Patient assessment routine

The period of enrolment which corresponds to the first stage of the study (t1), was between the months of August 2018 and January 2019. During enrolment, the patients were subjected to an interview to obtain demographic, socioeconomic, and clinical information. In addition, nutritional assessment, laboratory tests, and imaging exams were scheduled to be performed simultaneously with the clinical assessment. The patients will also be evaluated at t2 and t3 periods to observe the progression of chronic kidney disease and atherosclerosis, according to the assessment routine described in **Figure No. 1**.

Demographic and clinical variables

The demographic variables used in this study are as follows: sex, age, ethnicity, education, and income. To define CKD, two previous evaluations of renal function were considered with a minimum interval of 3 months, according to the KDIGO[11] guidelines. Patients will be deemed to have high blood pressure (HBP) and diabetes mellitus (DM) if they were previously diagnosed and are taking specific medication for these conditions. In addition, a history of stroke and coronary events (acute myocardial infarction or angina) will be documented. Smoking will be defined as current consumption of ≥ 1 cigarette/day and alcohol consumption as alcohol intake ≥ once/week. The diagnosis of metabolic syndrome will be made using the parameters considered by the International Diabetes Federation [12].

Anthropometric evaluation

The anthropometric evaluation will be performed by measuring body weight and height. Body weight will be determined using a calibrated scale (Filizola®, Brazil) with a maximum capacity of 150 kg, in increments of 100 g. Height will be measured using a portable stadiometer (Alturexata®, Brazil) with a range of 0-220 cm and a precision of 0.1 cm. Weight (kg) and height (m) will be measured to calculate the body mass index (BMI, in kg/m²) [13]. Data of the waist circumference (WC), hip circumference (HC), neck circumference (NC) will also be documented.

Blood pressure assessment and ankle-brachial index (ABI)

Blood pressure will be measured on all four limbs during the standard clinical examination with two validated automated oscillometric sphygmomanometers (Omron 705-IT). The following formula will be used to calculate the ankle-brachial index:

$$ABI = (P_{ankle}/P_{arm})$$

Where: P_{ankle} = Systolic blood pressure at the ankle; P_{arm} = Systolic blood pressure at the arm.

ABI between 0.90 and 1.30 will be considered normal [14].

Laboratory testing

Venous blood samples will be collected after a maximum of 12-hour fasting to measure the following: CBC, glucose, creatinine, Mg, calcium, phosphorus, parathyroid hormone (PTH), alkaline phosphatase, 25-OH vitamin D, lipid profile, high-sensitivity C-reactive protein(hs-CRP), and uric acid.

Urinary levels of Mg, creatinine, and albumin will be determined from 24-hour urine samples. The fractional excretion of magnesium (FEMg) will be calculated using the following formula:

$$FEMg= [(MgU \times CrS)/0.7 \times (MgS \times CrU)] \times 100$$

Where: MgU = urinary magnesium; CrS = serum creatinine; MgS = serum magnesium; CrU = urinary creatinine

Values above 6.1% will be considered elevated [15].

24-hour urine samples with a volume of less than 400 mL or with urine creatinine <15 mL/kg/24h (men) and <10 mL/kg/24h (women) will be disregarded due to the possibility of collection error.

Estimation of glomerular filtration rate (eGFR)

eGFR will be estimated using the formula derived from the Chronic Kidney Disease Epidemiology (CKD-EPI) [16] study, using creatinine as a reference for the calculation, as described below. The results will allow confirmation of the stage of CKD.

Creatinine CKD-EPI equation: GFR (ml/min/1.73 m²) = 141 × min (serum creatinine/κ, $1^{)\alpha}$ × max (serum creatinine/κ, 1)$^{-1.209}$ x 0.993I age × [1.018 (if female)] x [1.159 (if black)]

Where: κ = 0.7 for women and 0.9 for men; α = - 0.329 for women and - 0.411 for men; min indicates minimum serum creatinine or 1; and max indicates maximum serum creatinine or 1.

Imaging exams

a) Two-dimensional ultrasonography of the carotid arteries:

The *Vingmed* GE Ultrasound apparatus, model Vivid3 (Horten, Norway) will be used to measure the carotid intima-media thickness (cIMT). Carotid ultrasonography will be performed by a single experienced examiner blinded to the clinical and laboratory data. cIMT will be measured on the distal wall (furthest from the transducer) of the common carotid artery, 1 cm proximal to its bifurcation, according to the current recommendations. The measure indicates the distance between two echogenic lines represented by the lumen-intima and mid-adventitia interfaces of the arterial wall, and values are considered altered if cIMT> 0.9 mm [17,18].

b) Computed tomography of coronary arteries:

The images will be obtained using a 64-detector computed tomography (CT) scanner (Aquilion 64, Toshiba Medical Systems, Tochigi, Japan) without the use of contrast. The patients will be in the dorsal decubitus position on the machine and 3.0 mm-thick images (on average) will be obtained without gaps between them. The presence of calcium will be defined as a density of more than 130 Hounsfield units (HU) in at least three continuous pixels (>1 mm²) in the same artery. The coronary calcium score (CCS) will be calculated as the sum of the scores of the right and left coronary arteries. The result will be considered positive when the calcium score (Agatston score) is greater than zero [19]. The report will be provided by a single experienced examiner, blinded to the clinical and laboratory data.

Assessment of outcomes

At the end of the research protocol the relationship between both, serum and urinary magnesium levels and the following outcomes will be evaluated:

(a) Primary outcomes:

- Decrease in glomerular filtration > 5 mL/min/1.73m² per year or need for renal replacement therapy;
- Increase in carotid intima-media thickness or coronary calcium score;
- Increase in albuminuria.

(b) Secondary outcomes:

- Hospitalization due to cardiovascular or cerebrovascular events (acute myocardial infarction, angina pectoris, stroke);
- All-cause and cardiovascular mortality.

Statistical analysis

A descriptive analysis of the variables will be performed initially. The numerical variables will be expressed as means and standard deviation, and the categorical variables as frequencies and percentages. The Shapiro-Wilk test will be used to evaluate the normality of the numerical variables. The Kruskal-Wallis test was used to assess the association between serum magnesium and FEMg with the stages of CKD; the same test was used to assess the association between renal and atherosclerosis disease markers with the serum magnesium tertiles and FEMg tertiles. The level of significance established for these analyzes was 5%. In the following phases, given the objective of evaluating the levels of magnesium and other predictors of cardiovascular lesions, and progression of renal disease, univariate and multivariate analyses using logistic regression will be performed. Pearson's linear correlation coefficient (or Spearman's for the asymmetrically distributed variables) will be used to evaluate the degree of relationship between two quantitative variables. Survival analysis will be performed by Cox regression and Kaplan

Meyer curves. The criterion for including a variable in the multivariate model will be a p-value < 0.20 in the univariate analysis, and only those with p-value< 0.05 will remain in the final model. The data will be analyzed using the Stata 14.0 software.

RESULTS AND DISCUSSION:

In the first phase of the study, the clinical, laboratory and imaging characteristics of the 152 patients with CKD (eGFR 15-59mL/min/1,73m²) were identified. The average age of patients is 60.2 (11.9) years, with an equal proportion between men and women. More than 2/3 of the sample is represented by people of African descent. Only 7.2% have more than 8 years of study and about 75% have a family income of US $ 400 or less. Smokers and alcohol users are 6.6% and 16.5%, respectively. Sociodemographic characteristics are presented in detail in Table 1.

Table No. 1: Sociodemographic variables of the 152 patients with CKD included in the study. Kidney Disease Prevention Center-HUUFMA, São Luís-MA, 2018.

Variables	**n (%)**
Age [average=60,2(11,9) anos]	
< 40 years-old	12 (7,9%)
40-59 years-old	48 (31,6%)
≥ 60 years-old	92 (60,5%)
Gender	
Male	76 (50,0%)
Female	76 (50,0%)
Skin color	
White	33 (21,7%)
Brown	88 (57,9%)
Black	25 (16,5%)
Others	6 (3,9%)
Years of study	
None	14 (9,2%)
1-4 years	50 (33,1%)

5-8 years	77 (50,5%)
> 8 years	11 (7,2%)
Income	
No regular income	12 (7,9%)
< US$ 200	36 (23,7%)
US$ 200-400	68 (44,7%)
> US$ 400-600	25 (16,4%)
> US$ 600-1000	6 (3,9%)
> US$ 1000	5 (3,4%)
Smoking	
No	96 (63,1%)
Yes	10 (6,6%)
Stopped	46 (30,3%)
Alcoholism	
No	75 (49,3%)
Yes	25 (16,5%)
Stopped	52 (34,2%)

US$=American dollar

Were identified 90.1% of hypertensive and 44.7% of diabetics. Regarding the cardiovascular history, there was a report of 9.9% of heart failure, 7.9% of stroke, 7.2% of coronary disease, 5.3% of amputations and 1.3% of deep venous thrombosis. Sedentary lifestyle was observed in 48.0%. More than 3/4 of the patients met criteria for metabolic syndrome. Only 25.0% had a normal BMI. The mean ABI was 1.1 ± 0.1, with 8.5% being outside the normal range. Regarding the staging of CKD, most patients belonged to stage 3B (39.5%). Details are shown in Table 2.

Table No. 2: Clinical variables of the 152 patients with CKD included in the study. Kidney Disease Prevention Center-HUUFMA, São Luís-MA, 2018.

Variables	**n (%)**
Hypertension	
Yes	137 (90,1%)
No	15 (9,9%)
Diabetes mellitus	
Yes	68 (44,7%)
No	84 (55,3%)
Heart failure	
Yes	15 (9,9%)
No	137 (90,1%)
Coronary disease	
Yes	11 (7,2%)
No	141 (92,8%)
Stroke	
Yes	12 (7,9%)
No	140 (92,1%)
Deep venous thrombosis	
Yes	2 (1,3%)
No	150 (98,7%)
Amputations	
Yes	8 (5,3%)
No	144 (94,7%)
Sedentary lifestyle	
Yes	73 (48,0%)
No	79 (52,0%)
Metabolic syndrome	
Yes	120 (78,9%)
No	32 (21,1%)

Ankle-brachial index **[**mean=1,1 (0,1)]	
≤0,9	12 (7,9%)
0,91-1,30	139 (91,5%)
>1,30	1 (0,6%)
Body mass index [mean=27,8 (4,7) Kg/m²]	
<18,5	2 (1,3%)
18,5-24,9	38 (25,0%)
25-29,9	67 (44,1%)
≥30	45 (29,6%)
Stages of CKD	
3A	44 (28,9%)
3B	60 (39,5%)
4	48 (31,6%)

CKD: Chronic kidney disease

Regarding laboratory variables, it is noteworthy that 61.8% had fasting blood glucose ≥ 100mg/dL, 61.2% had LDL above 70mg/dL, 57.2% had albuminuria> 30mg / 24h, 48.0% with PTH > 65pg/dL and 42.1% had FEMg> 6.1%. The other laboratory variables are described in Table 3.

Table No. 3: Laboratory variables of the 152 patients with CKD included in the study. Kidney Disease Prevention Center-HUUFMA, São Luís-MA, 2018.

Variables	**n (%)**
Hemoglobin [mean= *12,8 (1,8)g/dL]*	
< 10g/dL	8 (5,3%)
Calcium [*median= 9,5 (7,7-10,8)mg/dL]*	
< 8,3mg/dL	3 (1,9%)
Phosphorus [mean= *3,5 (0,6) mg/dL]*	
> 5,5mg/dL	0 (0,0%)
Alkaline phosphatase [*median= 76 (29-152)U/L]*	

> 100 U/L	30 (19,7%)
Total cholesterol [*median= 158,5 (84-373)mg/dL]*	
> 190mg/dL	39 (25,7%)
HDL [*median= 44 (15-109) mg/dL]*	
≤ 40mg/dL	60 (39,5%)
LDL [*mediana= 81 (23-276) mg/dL]*	
> 70mg/dL	93 (61,2%)
Triglycerides [*median= 139,5 (47-1367) mg/dL]*	
> 150mg/dL	69 (45,4%)
Uric acid [mean= *7,0 (1,6) mg/dL]*	
> 7mg/dL (male)	40 (26,3%)
> 6mg/dL (female)	49 (32,2%)
PTH [*median= 58,5 (23,6-365,6) pg/dL]*	
> 65pg/dL	73 (48,0%)
25OH Vitamin D [*median= 34,5 (11,0-79,6) ng/dL)*	
< 30ng/dL	46 (30,3%)
Albumin [*median= 4,5 (2,3-5,2) g/L]*	
< 3,5g/L	3 (1,9%)
hs-CRP [*median= 0,2 (0-24,8)mg/dL]*	
> 0,30mg/dL	59 (38,8%)
Fasting blood glucose [*median= 100 (56-329) mg/dL]*	
≥ 100mg/dL	94 (61,8%)
Serum Mg[*mean=2,0 (0,2) mg/dL]*	
< 1,6 or > 2,5mg/dL	9 (5,9%)
FEMg [median= 5,6 (0,5-19,1)%]	
> 6,1%	64 (42,1%)
Albuminuria [*median= 27,8 (0,1-5552,7) mg/24h]*	
> 30mg/24h	87 (57,2%)

HDL: High Density Lipoproteins; LDL: Low Density Lipoproteins; PTH: parathyroid hormone; hs-CRP: high-sensitivity C-reactive protein; Mg: magnesium; FEMg: fractional excretion of magnesium

Were identified 38.8% of patients with cIMT> 0.9 mm and 65.1% with coronary calcification, 12.5% with a calcium score> 400, as seen in Figure 2.

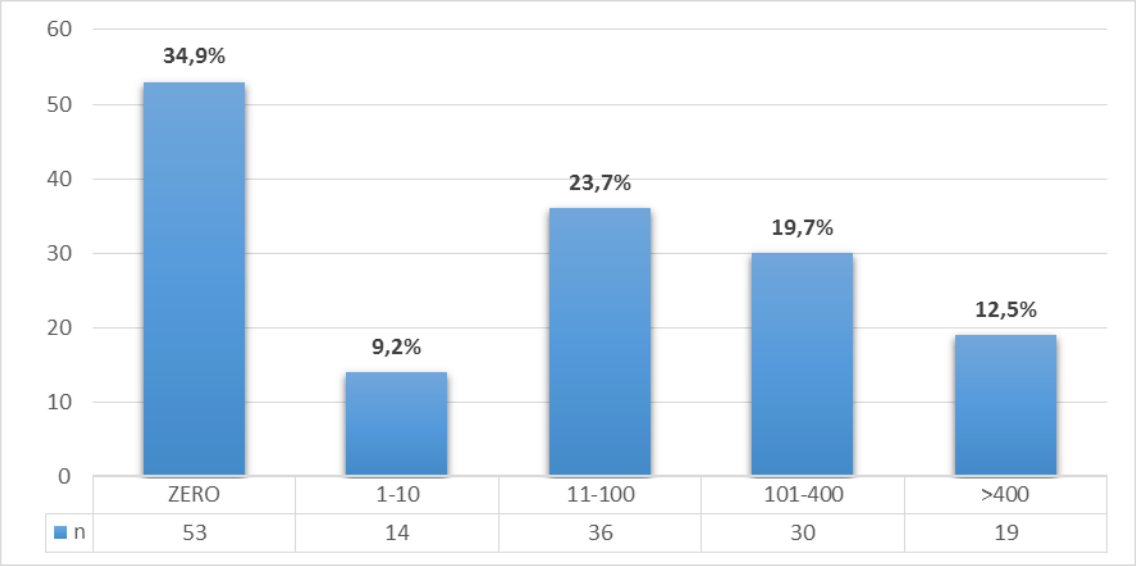

Figure No. 2: Distribution of 152 patients according to coronary calcification score. Kidney Disease Prevention Center-HUUFMA, São Luís-MA, 2018.

Figures 3 and 4 show the values of magnesium in 24-hour urine, FEMg and serum magnesium distributed by the stages of CKD, respectively. It is noteworthy that a statistically significant difference was observed only between the values of the FEMg in stages 3A, 3B and 4, with increased excretion associated with a worse stage of CKD.

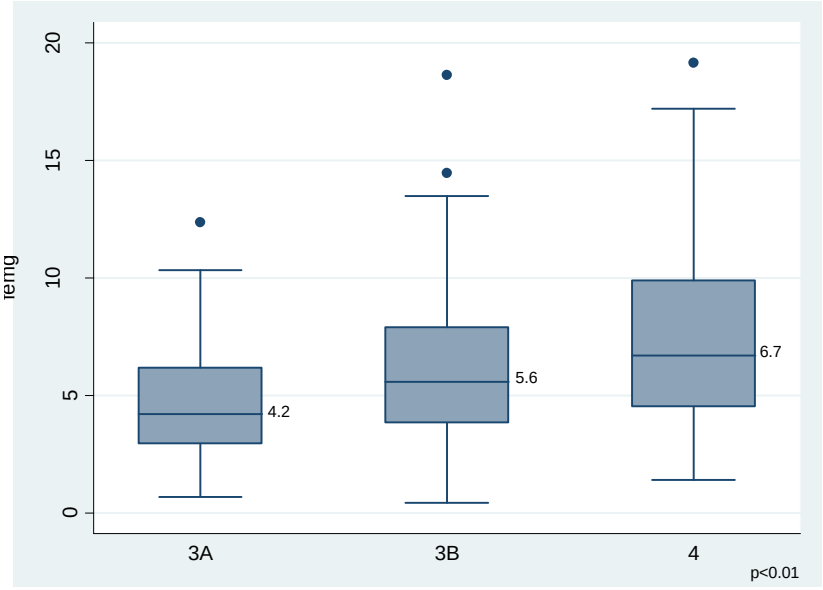

Figure No. 3: Median of FEMg values distributed by the CKD stages (3A, 3B and 4). Kidney Disease Prevention Center-HUUFMA, São Luís-MA, 2018.

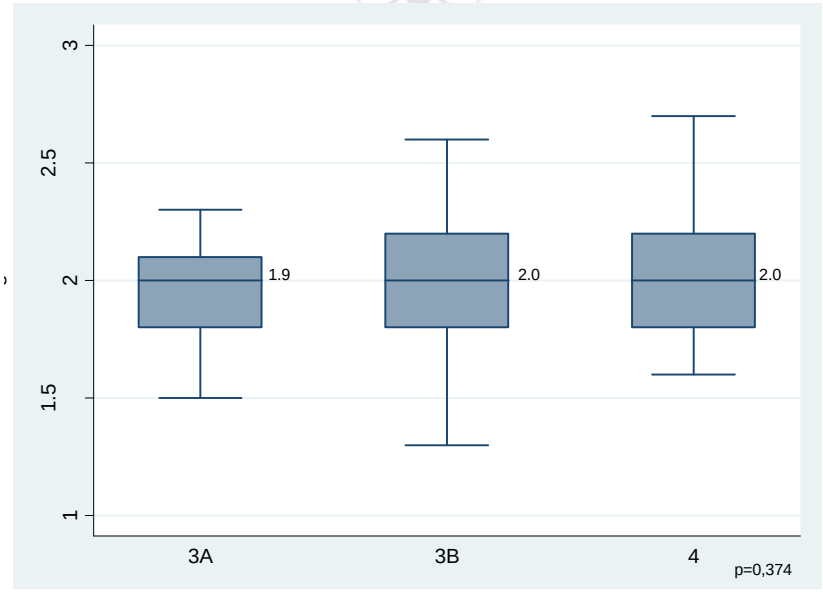

Figure No. 4: Average of serum magnesium values distributed by the CKD stages (3A, 3B and 4). Kidney Disease Prevention Center-HUUFMA, São Luís-MA, 2018.

Tables 4 and 5 show the association of renal and atherosclerotic disease markers with serum and urinary magnesium tertiles (FEMg). It was observed that the highest tertiles of FEMg were related to lower eGFR ($p < 0.01$) and to the highest levels of albuminuria ($p = 0.02$).

Table No. 4: Association of markers of kidney and atherosclerotic disease with serum magnesium tertiles among the 152 patients with CKD included in the study. Kidney Disease Prevention Center-HUUFMA, São Luís-MA, 2018.

Serum magnesium (mg/dL)				
Variables	1º tertile (1,3-1,9) p50 (p75-p25)	2° tertile (2,0-2,1) p50 (p75-p25)	3° tertile (2,2-2,7) p50 (p75-p25)	*p-valor*
CCS	35,50 (132,00-0,00)	16,00 (128,00-0,00)	16,00 (180,00-0,00)	0,78
cIMT	0,87 (1,00-0,75)	0,90 (1,05-0,85)	0,90 (1,00-0,80)	0,12
ABI	1,11 (1,17-1,03)	1,08 (1,16-1,00)	1,06 (1,13-0,97)	0,19
eGFR	37,65 (48,00-29,40)	38,40 (45,00-29,00)	33,20 (44,80-26,60)	0,28
Albuminuria	38,12 (291,63-4,40)	10,07 (351,28-0,85)	41,50 (162,34-3,79)	0,27

CCS: coronary calcium score; cIMT: carotid intimal medial thickness; ABI: ankle-brachial index; eGFR: estimated glomerular filtration rate

Table No. 5: Association of renal and atherosclerotic disease markers with FEMg tertiles among the 152 patients with CKD included in the study. Kidney Disease Prevention Center-HUUFMA, São Luís-MA, 2018.

Fractional excretion of magnesium (FEMg%)				
Variables	1º tertile (0,45-4,11) p50 (p75-p25)	2° tertile (4,19-6,81) p50 (p75-p25)	3° tertile (6,94-19,14) p50 (p75-p25)	p-valor
CCS	18,50 (141,00-0,00)	2,50 (111,00-0,00)	25,00 (191,00-0,00)	0,27
cIMT	0,90 (1,00-0,80)	0,85 (1,00-0,80)	0,90 (1,00-0,75)	0,77
ABI	1,08 (1,15-1,01)	1,10 (1,16-1,00)	1,07 (1,16-1,00)	0,89
eGFR	40,45^{A} (52,80-33,40)	41,35^{A,B} (46,40-29,40)	30,40^{a,b} (36,90-25,20)	<0,01
Albuminuria	11,53^{a} (47,10-1,66)	33,75^{A} (291,63-2,08)	58,73^{A} (431,73-4,96)	0,02

CCS: coronary calcium score; cIMT: carotid intimal medial thickness; ABI: ankle-brachial index; eGFR: estimated glomerular filtration rate

Kruskal Wallis according to Dunntest (A>a; B>b)

The aim of this study is to analyze the serum and urinary levels of magnesium and to investigate their association with the rate of progression of renal disease and atherosclerotic lesions in patients with non-dialysis CKD over 24 months. The initial sample majorly comprises elderly individuals, with an equal proportion of men and women, a high prevalence of hypertension, diabetes mellitus, overweight/obesity, and metabolic syndrome. More than half have increased levels of albuminuria, fasting glucose, and LDL (Low Density Lipoproteins), and approximately 50% have elevated PTH values. In addition, there is a high prevalence of the atherosclerotic disease, especially coronary disease, with almost 2/3 of the patients having coronary artery calcification. The findings demonstrate the complexity of factors associated with renal disease,

which increases the risk of morbidity and mortality, mainly because of cardiovascular disease. This indicates the need to determine the effect of new agents in this pathophysiological interaction, including the Mg ion.

Mg deficiency is an emerging public health concern [20]. Several lines of evidence suggest that low serum Mg level might be a risk factor for the development of kidney disease and cardiovascular complications [21]. Mg deficiency is related to oxidative stress, pro-inflammatory status, endothelial dysfunction, platelet aggregation, insulin resistance, hyperglycemia [22], and progression of renal disease [23].

In patients with CKD, lower serum magnesium levels have been associated with an increased risk of sudden death, usually due to cardiac arrhythmia [24]. In the Framingham Heart Study, a prospective cohort study that evaluated a population without previous cardiovascular disease, it was observed that individuals with serum Mg levels in the lowest quartile (≤0.73 mmol/L) had approximately 50% increased risk of atrial fibrillation than in those with levels in the highest quartile (≥ 0.82 mmol/L; HR 1.52 [1.00-2.31]) [25]. Another mechanism involved in the cardiovascular mortality in patients with CKD is cardiac remodeling and congestive heart failure. The effects of Mg on these mechanisms also suggest a causal relationship between serum Mg and cardiovascular mortality in patients with CKD. Previous publications have shown an association between low serum Mg and higher left ventricular mass index in patients with CKD [26,27].

Vascular calcification is an important pathological process in CKD and is a strong predictor of cardiovascular mortality [28]. An *in vitro* study showed that Mg strongly inhibits phosphate-induced smooth muscle cell apoptosis, a key process in vascular calcification [29]. This finding suggests that Mg also participates in other pathological conditions in which phosphate toxicity is involved, such as the progression of CKD [23]. Observational studies show that serum Mg concentrations are inversely related to vascular calcification, as assessed by the coronary calcification score [30,31]. Similarly, lower levels of Mg were associated with increased carotid intima-media thickness in patients with dialysis and non-dialysis-dependent CKD [27,32,33].

Moreover, Mg levels affect blood pressure and endothelial function. Evidence shows that higher serum Mg concentration is associated with an improvement in the endothelial dysfunction in

patients with CKD, demonstrated by increased dilation mediated by the brachial artery flow [34,35]. In patients without CKD, serum Mg is inversely related to the incidence of hypertension [36]. *In vitro* studies have indicated mechanisms that might explain the association between Mg and blood pressure, including the effect of Mg on the contractility of vascular smooth muscle cells in the arterial wall and the effect of Mg on the endothelial function through the processes of vasodilation and vasoconstriction. In vascular smooth muscle cells, Mg inhibits calcium inflow through the L-type calcium channels, which reduces the vascular tonus [37]. At the endothelial level, vasodilation induced by acetylcholine (via the release of endothelium-derived vasorelaxation factors, including nitric oxide) is Mg-dependent and increases in proportion to *in-vitro* Mg concentrations [38,39].

Despite the studies already cited to indicate a relationship between cardiovascular disease, endothelial injury and blood pressure levels with serum magnesium values, the present study has not demonstrated, until now, this relationship when assessing the coronary calcium score, carotid intima-media thickness and ankle-brachial index. Likewise, until the first stage of the study, these parameters had no association with FEMg.

The results found in the first phase showed that FEMg was significantly higher in patients with more advanced stages of renal dysfunction. The FEMg has been reported, as one of the most sensitive markers in the identification of initial stages of tubulointerstitial lesions [15]. Evidence of increased FEMg in patients with kidney disease is derived from clinical and experimental studies that have pointed out the fundamental role of the kidney in regulating magnesium excretion [40,41]. Chie Noiri et al. [42], FEMg showed an inverse correlation with the estimated GFR, suggesting that FEMg is strongly affected by the decrease in the number of functioning nephrons. Tubular damage leads to the development of atubular glomeruli and decreases the number of functioning nephrons, with a consequent reduction in GFR. The tubulointerstitial damage also reduces blood flow in the corresponding region and induces ischemic injury to the nephrons, with reduced renal plasma flow [43,44].

The relationship between serum magnesium and albuminuria has been analyzed in other studies, showing an inverse relationship between the two biomarkers. BaihuiXu et al. [45] evaluated Chinese diabetic individuals and showed that those with the lowest serum magnesium tertile

were 1.85 times more likely to have microalbuminuria when compared to the higher tertile. Our initial findings did not identify an association between serum magnesium and albuminuria, but they revealed a significant association between FEMg and albuminuria, as well as the study by Žeravica et al. [15] that identified a positive correlation between these two parameters ($r = 0.39$, $p < 0.01$). Both represent important biomarkers of early kidney injury, especially in patients with diabetes mellitus, which make up approximately half of our sample. The relationship between albuminuria and FEMg can be explained in part by the impairment of magnesium tubular reabsorption in situations of deficiency or insulin resistance, especially in diabetic individuals [46]. Possibly, a vicious circle is formed, between low magnesium and high insulin levels, which can increase the risk of albuminuria [47].

This study has some limitations: 1) It is performed at a single center with a small sample, which, however, it is statistically representative of the studied population; 2) Since some patients have advanced renal disease, it is impossible to discontinue diuretics for a period longer than 24 hours because of the risk of clinical decompensation; 3) The outcomes might not follow the natural course because preventive therapeutic interventions might be performed for ethical reasons during the follow-up period of patients. In contrast, the strong point of the MAG-PROGRESS study is that it proposes to evaluate not only Mg serum levels but also the relationship between fractional excretion of Mg, and the renal and cardiovascular outcomes in patients diagnosed with CKD in the non-dialysis stage.

CONCLUSION:

A high prevalence of atherosclerotic disease was observed in the patients followed by the study, mainly coronary heart disease. The initial findings showed an association of the FEMg only with markers of kidney disease. The future findings are expected to contribute new information on the role of Mg in the progression of CKD and in the development of cardiovascular complications in patients with renal dysfunction.

Conflict of Interest Statement

The authors have no conflicts of interest to declare.

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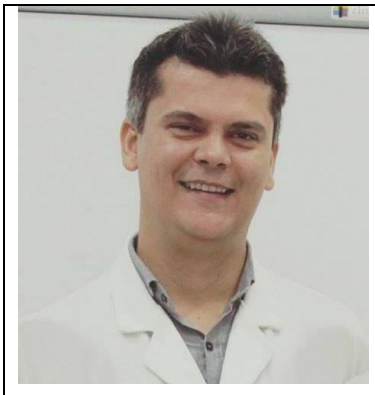

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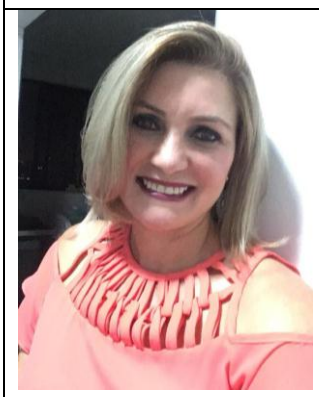	Elisangela Milhomem dos Santos Department of Nursey, Federal University of Maranhao, São Luis, (Maranhao) Brazil Institute address: Barão de Itapary Street, 227 - Downtown. São Luis, Maranhao, Brazil. ZIP Code: 65020-070.
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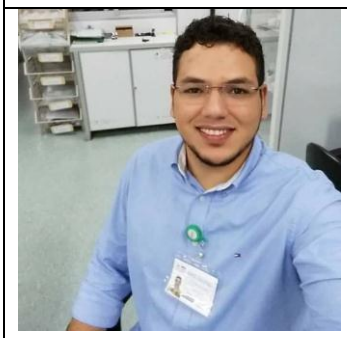
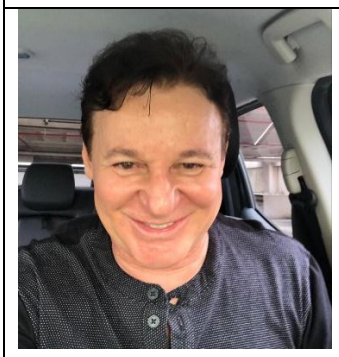
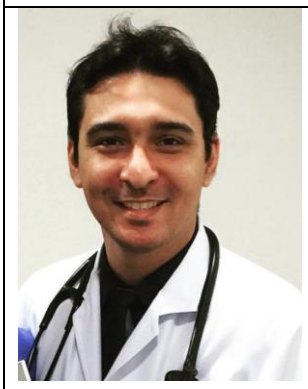
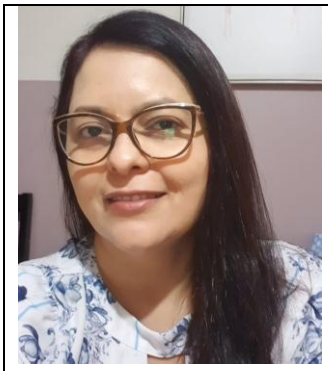
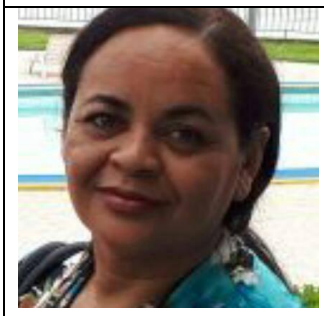
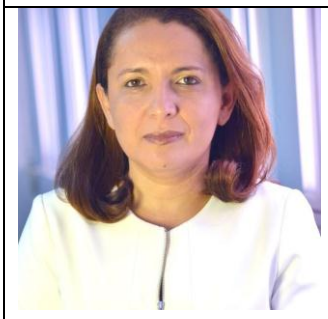
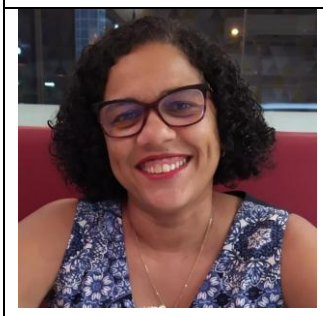
	Elton Jonh Freitas Santos Department of Nephrology, University Hospital of Federal University of Maranhao. Institute address: Barão de Itapary Street, 227 - Downtown. São Luis, Maranhao, Brazil. ZIP Code: 65020-070.
	Enedino Fontes da Silva-Neto Department of Nephrology, University Hospital of Federal University of Maranhao. Institute address: Barão de Itapary Street, 227 - Downtown. São Luis, Maranhao, Brazil. ZIP Code: 65020-070.
	Paulo Cezar Ferraz Dias Filho Department of Cardiology, University Hospital of Federal University of Maranhao. Institute address: Barão de Itapary Street, 227 - Downtown. São Luis, Maranhao, Brazil. ZIP Code: 65020-070.

	Alessandra Costa de Sales Muniz Department of Clinical Analysis, University Hospital of Federal University of Maranhao. Institute address: Barão de Itapary Street, 227 - Downtown. São Luis, Maranhao, Brazil. ZIP Code: 65020-070.
	Carla Dea Trindade Barbosa Department of Clinical Analysis, University Hospital of Federal University of Maranhao. Institute address: Barão de Itapary Street, 227 - Downtown. São Luis, Maranhao, Brazil. ZIP Code: 65020-070.
	Joyce Santos Lages Department of Public Health, Federal University of Maranhao. Institute address: Barão de Itapary Street, 227 - Downtown. São Luis, Maranhao, Brazil. ZIP Code: 65020-070.
	Alcione Miranda dos Santos Department of Public Health, Federal University of Maranhao. Institute address: Barão de Itapary Street, 227 - Downtown. São Luis, Maranhao, Brazil. ZIP Code: 65020-070.
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