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Original Article

Biochemistry Section

Serum Magnesium Levels in Chronic Kidney Disease with and without Diabetes Mellitus and its Correlation with Calcium and Phosphate Levels: A Case-control Study

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ABSTRACT

Introduction: Nearly 10% of the world population is affected by Chronic Kidney Disease (CKD). Hypermagnesaemia is seen in late stages of CKD whereas Diabetes Mellitus (DM) is commonly associated with hypomagnesaemia. Monitoring Mg levels and early correction may prevent the future risk for cardiovascular and CKD.

Aim: To compare the serum Mg levels in healthy controls, diabetic patients without CKD, late stages of CKD with and without diabetes and to evaluate its correlation with serum phosphate and calcium levels in CKD patients.

Materials and Methods: A case-control study was conducted in Vydehi Institute of Health Sciences and Research Centre, Bangalore, Karnataka, India from January 2018 to December 2019. A total of 100 participants were divided into four groups of 25 each. Group I were healthy controls, Group II were DM patients without CKD, Group III CKD stage 3 and more without DM and Group IV CKD stage 3 and more with DM. Serum creatinine, urea, estimated Glomerular Filtration Rate (eGFR), calcium, phosphorous and magnesium levels were estimated in all the groups. Analysis of Variance (ANOVA) and Pearson's correlation was used to statistically analyse the data.

Results: The Group III participants who had CKD but no DM were relatively younger in age (mean age 48.5±5.5 years) compared to other groups and there was a significant difference in the age group between four groups (p-value 0.0113). Though there was a male preponderance in all the four groups, the gender difference was not significant. Significant difference in the Mg was observed between the four groups (p-value <0.001). The DM group showed low serum magnesium. CKD patients with and without DM showed high but within reference range Mg levels. Sub-group analysis revealed DM patients on haemodialysis had lower Mg level as compared to non DM. Mg levels in CKD showed significant positive correlation with phosphorous levels (r-value 0.5002, p-value 0.0002).

Conclusion: High levels of Mg in late stages of CKD are expected but CKD with DM had relatively low Mg level. Low Mg level was also seen in DM patients on haemodialysis.

Keywords: Blood glucose, Haemodialysis, Hypermagnesaemia, Phosphorus, Renal failure

INTRODUCTION

The CKD is a state of progressive loss of kidney function diagnosed when the estimated Glomerular Filtration Rate (eGFR) is less than 60 mL/min/1.73 m^2, persisting for three months or more, irrespective of the cause [1]. Epidemiological update of 2022 states that the current total number of individuals affected by CKD stages 1-5 worldwide was estimated to be 843 million [2]. In India, the prevalence is approximately 800 per million populations [3]. The most common cause of CKD being diabetic nephropathy contributes to 41% of the cases [3].

Kidneys play a central role in the homeostasis of multivalent cations like calcium, phosphorous, and magnesium. Kidneys regulate the serum concentration of these ion through filtration, reabsorption and tubular secretion. Magnesium (Mg) is a cofactor for more than 300 enzyme systems that regulate protein synthesis, muscle and nerve function, blood glucose control, blood pressure regulation etc., [4].

In CKD stage 1-3, an increase in fractional Mg excretion which compensates for the reduced GFR so, Mg levels are regulated within the normal range. In advanced CKD stage, due to impaired tubular reabsorption, the compensatory mechanisms become inadequate and fractional excretion of Mg increases resulting in overt hypermagnesaemia [5,6]. Low magnesium levels stimulates the parathormone secretion which might result in increased vascular calcification. Vascular calcification is an independent predictor of cardiovascular disease, and thus has bad prognosis in CKD [7,8].

The DM is often associated with reduced Mg levels [9]. Nearly, 13-47% of the diabetics suffer from hypomagnesaemia and the incidence is common in patients with poor glycaemic control and longer duration of disease [10]. Few of the common causes are reduced dietary intake, hyperinsulinaemia, glomerular hyperfiltration, hypophosphataemia etc. Hypomagnesaemia in turn causes increased insulin resistance, atherosclerosis, endothelial dysfunction, inflammation, and dyslipidaemia [10].

Studies which have explored the Mg metabolism in CKD also states that hypermagnesaemia is more prevalent in late stages of CKD [11-13]. But studies comparing the Mg metabolism in CKD due to DM and causes other than DM are scarce. So, with such extremes of pictures in diabetes and CKD, the present study was aimed to evaluate the Mg level in advanced CKD patients with and without diabetes. Since Mg has many vital functions, knowledge of Mg homeostasis might reduce the risk of future CVD and CKD progression. The study has hypothesised that serum Mg level may be low in CKD due to diabetes compared to other causes of CKD.

Hence, present study was conducted to compare the serum Mg levels in healthy controls, diabetic patients without CKD, late stages of CKD with and without diabetes and to evaluate its correlation with serum phosphate and calcium levels in CKD patients.

MATERIALS AND METHODS

This case-control study was conducted from January 2018 to December 2019 by the Department of Biochemistry in Vydehi

Institute of Medical Sciences and Research Centre, Bangalore, Karnataka, India. The study was approved by the Institutional Ethics Committee (Ref No VIMS7RC/IEC/103). Informed consent was taken from all the participants after explaining the procedure of the study. Confidentiality of the data was maintained during the study.

Inclusion criteria: A total of 100 participants aged more than 18 years, recruited for the study, were divided into the following four groups.

- Group I consisted of 25 healthy controls.
- Group II consist of 25 known cases of DM with normal eGFR.
- Group III consist of 25 patients with stage 3-5 CKD without DM.
- Group IV with 25 CKD patients of stage 3-5 with DM.

Stage 3 of CKD is when the eGFR falls between 30-59 mL/min/1.73 m^2, Stage 4 is 15 to 29 mL/min/1.73 m^2, Stage 5 when eGFR less than 15 mL/min/1.73 m^2 [14].

Diagnosis of DM was done using American Diabetes Association (ADA) criteria using any of the following.

- Glycated Haemoglobin (HbA1c) level of 6.5% or higher.
- Fasting plasma glucose level of 126 mg/dL or higher.
- Two-hour plasma glucose level of 200 mg/dL or higher during a 75-g Oral Glucose Tolerance Test (OGTT).
- Random plasma glucose of 200 mg/dL or higher in a patient with symptoms of hyperglycaemia (polyuria, polydipsia, polyphagia, weight loss) or hyperglycaemic crisis [15].

For the sub-group analysis, patients with CKD (Group III and IV) were subdivided into CKD on haemodialysis and without haemodialysis.

Exclusion criteria: Participants with stage 1 and 2 CKD, known case of chronic liver diseases, inflammatory bowel disease, malabsorption syndrome, history of diuretic intake, Mg supplementation, antacid intake, recent intake of proton pump inhibitors were excluded from the study.

Sample size calculation: Estimation of the sample size was done by iterative procedure using the formula for Analysis of Variance (ANOVA) $N=\lambda/f^2$ where λ is non centrality parameter, f is effect size calculated from $f=\sigma k/\sigma$ where $\sigma k=\sqrt{\sum k}\ i=1\ (\mu i-\mu)2/k$. Assuming type I error 0.05, power(1-β) is 80, difference between the mean of the groups μ as 1.8 mg/dL and expected Standard Deviation (SD) σk as 0.45 the required sample size was 96 for four groups together [16].

Study Procedure

Basic demographic details like age and gender, history of diabetes, year diagnosed, medication history, and in CKD patient cause of CKD, whether under haemodialysis or not and frequency of dialysis were recorded in a structured case report form. A 5 mL of blood samples was collected from all the participants. The blood samples were estimated for serum creatinine, urea, calcium, phosphorous, magnesium and eGFR. Serum creatinine was estimated by modified Jaffe's method, reference range 0.60-1.24 mg/dL for males and 0.40-1.00 mg/dL for females. Serum urea was estimated using urease reference range 13-43 mg/dL. Serum calcium using ion selective electrode reference range 8.5-10.2 mg/dL. Serum Phosphorous using ammonium molybdate method reference range 2.5-4.5 mg/dL. Serum magnesium was estimated using calmagite chromogen method reference range 1.8-2.9 mg/dL. Magnesium forms a purple-coloured complex when reacts with Calmagite in alkaline solution. The intensity of the colour formed is proportional to the magnesium concentration in the sample. CKD was diagnosed using Modification of Diet in Renal Disease (MDRD) equation where eGFR in mL/min/1.73 m^2 is given by $175\times(\text{Serum creatinine})^{-1.154}\times(\text{Age})^{-0.203}\times(0.742\text{ if female})$ [17].

STATISTICAL ANALYSIS

Statistical Package for Social Sciences(SPSS) version 22.0 (IBM, Inc., Armonk, NY, USA) was used to analyse the data. Normally distributed data are expressed as mean±SD (SD). One-way analysis of variance (ANOVA) was used to compare normally distributed data between multiple groups. The Chi-square test was used to compare categorical variables. The relationships of Mg with calcium and phosphorous was analysed using Pearson's correlation. A p-value <0.05 was regarded as statistically significant.

RESULTS

The Group III participants who had CKD but no DM were relatively younger in age compared to other groups and there was a significant difference in the age group between four groups. Though, there was a male preponderance in all the four groups, the gender difference was not significant. There was a significant difference in the serum creatinine, urea, eGFR, serum phosphorous and serum magnesium, but there was no significant difference found in serum calcium levels among four groups. Highest serum creatinine and lowest eGFR was observed in the Group IV participants who had both DM and CKD. All groups except Group II had mean Mg values within the normal range [Table/Fig-1].

Characteristics	Group I DM (-), CKD (-)	Group II DM (+), CKD (-)	Group III DM (-), CKD (+)	Group IV DM (+), CKD (+)	p-value
n	25	25	25	25	
Mean age (years)	50.4±1.2	51.7±1.6	48.5±5.5	52.3±6.2	**0.0113**
Males	15	17	13	18	0.4696
Females	10	8	12	7	
Blood urea mg/dL	24.52±4.82	34.10±5.12	109.33±45.20	116.57±26.72	**<0.001**
Serum creatinine mg/dL	0.86±0.24	1.03±0.51	6.67±4.98	7.41±5.03	**<0.001**
eGFR mL/min/1.73 m^2	106.54±25.5	102.44±28.4	15.12±10.25	13.11±10.56	**<0.001**
Serum calcium mg/dL	9.31±0.68	9.10±0.49	8.91±0.85	8.87±0.55	0.0767
Serum phosphorous mg/dL	3.27±0.54	3.9±0.63	4.80±1.24	4.88±1.79	**<0.001**
Serum magnesium mg/dL	1.9±0.29	1.65±0.30	2.18±0.285	2.03±0.321	**<0.001**

[Table/Fig-1]: Demographic and biochemical parameters of the study groups. All data expressed as mean±Standard deviation. The p-value <0.05 considered significant. Analysis of Variance used to compare between groups. DM: Diabetes mellitus; CKD: Chronic kidney disease. eGFR: Estimated glomerular filtration rate

The overall prevalence of hypermagnesaemia was more compared to hypomagnesaemia. Hypermagnesaemia was the most common Mg disturbance is Group III and IV. Hypomagnesaemia was more prevalent in Group II as compared to ther groups. In group 1 (controls), out of 25 subjects, 23 (92%) had normal magnesium level [Table/Fig-2].

Variables	Group I	Group II	Group III	Group IV	p-value
Hypomagnesaemia	0	8 (32%)	1 (7%)	4 (15%)	**<0.001**
Normal magnesium levels	23 (92%)	14 (57%)	15 (59%)	14 (55%)	
Hypermagnesaemia	2 (8%)	3 (11%)	9 (34%)	7 (30%)	

[Table/Fig-2]: Spectrum of Mg disturbance in each group. The data expressed as absolute numbers (percentage). p-value <0.05 considered significant. Chi-square test used to calculate p-value for categorical variables

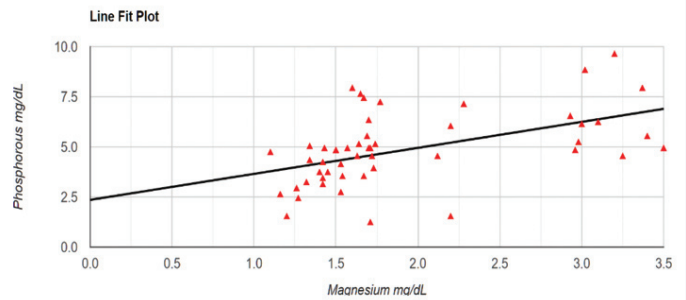
There were 22 patients in the CKD group who were on haemodialysis. Sub-group analysis of the patients with and without DM showed that there were significantly high Mg levels in patients without haemodialysis [Table/Fig-3].

A significant positive correlation was found between serum Mg and phosphorous levels in CKD patients (r=0.5002 and p-value 0.0002) [Table/Fig-4]. But there was non significant, negative

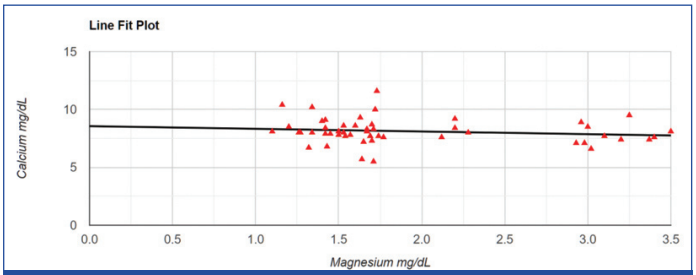
correlation between Mg and calcium among the CKD patients (r=-0.149, p-value 0.3) [Table/Fig-5].

	CKD without haemodialysis (n=28)	CKD on haemodialysis (n=22)		p-value
		CKD on haemodialysis with DM (n=10)	CKD on haemodialysis without DM (n=12)	
Magensium levels mg/dL	1.86±0.328	2.08±0.31	2.31±0.20	**0.0003**

[Table/Fig-3]: Mg levels in CKD patients (Group III+Group IV) on haemodialysis and without haemodialysis.
All values expressed as mean±Standard deviation. p-value <0.05 considered significant. Analysis of variance used to compare between the groups. CKD: Chronic kidney disease



[Table/Fig-4]: Showing the correlation of serum magnesium and phosphate levels in CKD patients.
Pearsons correlation coefficient used to calculate r and p-value



[Table/Fig-5]: Showing the correlation of serum magnesium and phosphate levels in CKD patients.
Pearsons correlation coefficient used to calculate r and p-value

DISCUSSION

The CKD is a highly prevalent condition with increase in incidence in the recent years [16]. Disturbances in phosphorous, calcium and magnesium start developing in the early stages and eventually affect most of the patient during the disease [14]. Many studies have mapped out the role of Mg in CKD [5,6,15]. But Mg metabolism in CKD with DM is not much explored. Despite this, it is well known that serum Mg levels increases when the GFR goes below 30 mL/min and hypomagnesaemia is one of the common disturbances observed in diabetics [5,10]. This study was determined to evaluate the Mg homeostasis in CKD patients with and without DM and to compare the same in healthy controls and in DM.

One of the major findings in present study was that the participants with CKD had a higher Mg level as compared to subjects with non CKD. This is in concordance with the fact that in later stages of CKD as the GFR falls, the filtered load of Mg falls. However, the resorption capacity remains same. This process ultimately leads to a picture of hypomagnesaemia especially in late stages of CKD. But present study did not show picture of overt hypermagnesaemia in the CKD group and the levels were within the normal range. The prevalence of hypermagnesaemia in Group III and IV was the highest. In a study done on 693 CKD patients by Afonso R et al., the mean Mg levels in CKD patients was 1.8 mg/dL [18]. Similarly a multicentric study done in China found that hypermagnesaemia was common in CKD stage 5 [19].

Another Important finding is that the CKD group with DM had relatively low Mg compared to CKD without DM. The common causes of CKD without DM in Group III were nephrotic syndrome, obstructive uropathy, hypertension etc. This finding is also concordant with the finding of reduced Mg levels in patients with diabetic nephropathy [20-22]. One of the study done in China by Feng J et al., showed that the Mg levels in diabetics with albuminuria was relatively low [21]. Another study conducted in India on diabetic nephropathy patients, the mean Mg levels were 1.98 mg/dL compared to non DM who had serum Mg level of 2.05 mg/dL [22].

Studies done by Eisenman K and Holley JL; and Gonella M et al., suggested that the Mg levels in End Stage Renal Disease patient depends on the dialysate Mg levels [23,24]. Even though the prescribed Mg in dialysate is 1.5 mg/dL most of the haemodialysis patients have hypermagnesaemia [24]. In the present study, patients on haemodialysis had a relatively higher Mg values compared to CKD not on haemodialysis. Sub-group analysis again revealed that DM on haemodialysis had low Mg levels. So, the overall finding is that the late CKD patient with DM has a relatively low Mg levels compared to non DM patients.

It is a well-known fact that Mg inhibits the calcium and phosphorous mediated vessel wall calcification and Mg inhibits the calcium absorption in intestines. So a correlation analysis to see the effect of Mg on calcium levels was also done. In present study, a significant positive correlation was found between serum Mg and phosphorous levels in CKD patients, but there was non significant, negative correlation between Mg and calcium among the CKD patients. In a study done by Sakaguchi Y et al., on 574 CKD patients no significant correlation between Mg and Ca was seen. However significant positive correlation between Mg and Phosphorus was seen [25].

Low Mg levels stimulate the parathormone secretion leading to hypercalcaemia. CKD patients having high phosphate and calcium levels are at high risk for future cardiovascular disease due to vessel wall calcification [22]. Many studies have proven that hypomagnesaemia causes increase in all-cause mortality in CKD patients and individuals with prolonged hypomagnesaemia progress to end stage renal disease quickly compared to others [11,26,27]. Also, high magnesium levels inhibit the Ca absorption in intestines. In the present study, the authors found a significant positive correlation between serum Mg and Phosphate levels and negative correlation between Mg and calcium. The high Mg levels in late stages of CKD disease indicates that the protective mechanism of Mg eventually falls as the disease progresses.

With all these findings, it imparts that Mg plays a predominant role in CKD also involved in the calcium and phosphorous homeostasis. So, it is mandatory to routinely look for Mg levels right from the onset of CKD and during the prepathological state which is DM. Routine monitoring and maintenance of Mg in early course of disease may slow down the progression.

Limitation(s)

One of the limitations in present study was the study design. A prospective study monitoring Mg levels at constant intervals would have shown if there is any dynamic change in Mg levels over a period of time. Also, vessel wall calcification was not measured. Studies on association of Mg levels with vessel wall calcification and risk for CVD in CKD should be done in future. In present study, controls were not age-matched with cases.

CONCLUSION(S)

The CKD patients with DM have a lower Mg level compared to CKD patients without DM. The sub-group analysis of CKD patients showed again the DM patients on haemodialysis had lesser Mg values compared to non DM patients. In later stages of CKD, the Mg levels have a positive correlation with phosphorous levels indicating the reduced antagonistic action of Mg on phosphorous. Since low Mg level is associated with early progression to CVD, in future studies,

on the association of Mg levels with inflammatory markers and risk scores can be done in CKD patients. Also, the association of Mg with proteinuria can also be done to predict the severity of CKD.

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