Original Article

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Biochemistry Section

Association of Serum Magnesium and Cardiovascular Events in End Stage Renal Disease Patients: A Prospective Cohort Study

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ABSTRACT

Introduction: End Stage Renal Disease (ESRD) patients display cardiovascular complications which is a significant feature for surge in death rate and morbidity. Vascular calcification manifests prematurely in renal failure patients and progresses often in an enhanced manner in subjects with hypomagnesaemia in comparison to the common herd.

Aim: The aim of the study was to evaluate if there is any association with serum Magnesium (Mg) levels and future cardiovascular events in Chronic Kidney Disease (CKD) stage-5 patients.

Materials and Methods: The present prospective cohort study was conducted on 60 CKD patients presenting to the Department of Nephrology at Government Medical College, Kottayam, Kerala, India, from 6th May 2016 to 5th May 2017. 5 mL of venous blood samples were collected and separated serum was used to determine Mg using the Xylidyl blue method in a fully automated clinical chemistry analyser, the Beckman Coulter AU 480. The study subjects were also grouped based on all causes of mortality due to cardiovascular events after follow-up for one year. Statistical analysis was performed by using Fischer's-exact test, Pearson's Chi-square, and Pearson's correlation test. The p-value <0.05 was considered statistically significant.

Results: Sixty subjects study population were in the age group ranging from 20-79 years, including 47 (78.3%) males and 13 (21.7%) females. The mean Mg level in those who developed an event was 2.32±0.41 mg/dL and in those who did not develop an event was 2.41±0.45. This difference in mean was not statistically significant with independent t test with t value=-0.69 and p-value=0.49. There was no significant association between low serum Mg levels and occurrence of cardiovascular event with p-value >0.05.

Conclusion: Although Mg has been shown to effectively slow down calcification in controlled settings, additional elements impacting calcium deposition in CKD individuals must not be overlooked.

Keywords: Arterial calcification, Atherosclerosis, Colourimetric method, Haemodialysis, Mortality

INTRODUCTION

The CKD is characterised by the presence of kidney damage or an estimated Glomerular Filtration Rate (eGFR) of less than 60 mL/min/1.73 m², persisting for three months or more, irrespective of the cause [1]. End-Stage Renal Disease (ESRD) is defined as a GFR of less than 15 mL/min [2]. Vascular arterial calcification in CKD and dialysis patients is a vital manifestation for increased morbidity and mortality [3]. Cardiovascular Disease (CVD) is present in 50% of patients in stages four to five of CKD, putting them at high risk for cardiovascular events. Cardiovascular reasons account for between 40 and 50 percent of all deaths in individuals with advanced disease CKD (stage 4) and ESRD (stage 5) [4].

Vascular calcification presents with thickening of arterial walls and loss of their elasticity [5]. The major arteries develop aortic arterial wall stiffness and raised pulse pressure because of the increased calcification of these arteries. It also leads to decreased myocardial perfusion during diastole, contributing to the incidence rate of cardiovascular casualties [6]. Typically, intimal or superficial calcification is associated with atherosclerotic plaques, whereas the medial calcification is represented by vascular stiffening and arteriosclerosis and is prevalent in patients with CKD [7].

Mg is an important mineral rendering various functions like stabilising abnormal nerve excitations or blood vessel spasms in conditions like eclampsia, regulating blood pressure, and supporting the immune system. In current times, Mg has gathered much attention as a cofactor in various biochemical reactions [8]. Mg has essential functions in the regulation of bone mineral metabolism, energy metabolism, releasing neurotransmitters, averting cardiac arrhythmias, and platelet-activated thrombosis [9]. Hypomagnesaemia causes changes in the lipoprotein framework by producing dyslipoproteinemia indicated by a rise in the compositions of Very Low-Density Lipoprotein (VLDL) and Low-Density Lipoprotein (LDL) and a fall in High-Density Lipoprotein (HDL) levels [10]. The lipoprotein component within a tissue itself may interact poorly with the lipase or receptor, producing hyperlipemia and initiating the pathogenesis of ischemic heart disease [11]. The prospective link between hypomagnesaemia and atherosclerosis, along with other risk factors like hypertension, diabetes, and left ventricular hypertrophy, including Cardiovascular Disease (CVD) and all-cause mortality has been studied in the general population [12,13]. Hypomagnesimia induced vascular calcification interconnects with hypokalemia and hypocalcaemia [14-16].

Research indicates higher mortality rates were also noticed with hypomagnesaemia in sustaining Hemodialysis (HD) patients [17,18]. There is two to five times the substantial risk of mortality in ESRD patients subjected to dialysis due to Coronary Artery Calcification (CAC) than in patients with known coronary artery disease [19]. Intimal and medial calcification are the direct or indirect sources of CVD-related mortality in CKD patients [20,21]. Several studies were conducted about the effect of hypomagnesaemia on mortality in ESRD patients [22,23], but literature about the Indian population is negligible. Hence, the present study was purposed to evaluate if there is any association with serum Mg levels and cardiovascular events in CKD stage-5 patients.

MATERIALS AND METHODS

The present prospective cohort study was conducted on CKD patients presenting to the Department of Nephrology at Government Medical College Kottayam, Kerala, India, from 6th May 2016 to 5th May 2017 after receiving approval from the Institutional Review Board (IRB No.60/2016).

Inclusion criteria: Patients between 20-80 years, on maintenance HD in CKD stage 5, clinically stable without any infection or infectious disease, were enrolled in the study after providing informed consent.

Exclusion criteria: Individuals with documented CVD, those taking Mg-containing antacids, and those unwilling to participate were excluded from the study.

Sample size selection: A cohort of 60 HD patients in CKD stage-5 attending the Department of Nephrology were chosen as participants using a convenient sampling method.

Study Procedure

The total sample of 60 selected subjects and all relevant details-demographic details, co-morbid conditions (diabetes, hypertension, and dyslipidaemia), history of smoking, duration of dialysis, and baseline clinical status of the patients-were noted in proforma. For the study patients, dialysis was performed using a polysulfone dialyser membrane where a dialyser made from a synthetic polymer called polysulfone is used to filter waste products and excess fluid from a patient's blood. The study subjects were followed up for one year and were divided into two groups based on their outcome as those who developed cardiovascular events and those who did not develop any events, also grouped based on all causes of mortality due to cardiovascular events after follow-up for one year as according to 2014 ACC/AHA key data elements and definitions for cardiovascular endpoint events in clinical trials [24].

A 5 mL of venous blood samples were collected from each subject in plain tubes. Blood was allowed to clot, and serum was separated by centrifugation at 3000 rpm for 10 minutes. Serum Mg was determined using the xylidyl blue method in the fully automated clinical chemistry analyser Beckman Coulter AU 480. The principle of the method is Mg reacts with xylidyl blue to form a colored compound in alkaline solution. The intensity of the color formed is proportional to the Mg in the sample. Reagent used was Mg reagent of Agappe Diagnostics Limited containing Xylidyl Blue with ethanolamine, which is linear up to 5 mg/dL. Normal reference range is taken as 1.6-2.2 mg/dL. Calibration of the assay was done using the Agappe Multicalibrator [25].

STATISTICAL ANALYSIS

Statistical data is inputted in Microsoft Excel, and statistical analysis is conducted in Statistical Package for Social Sciences (SPSS) 16.0. Microsoft Word and Excel are utilised for creating tables, graphs, and other visual representations. All variables were meticulously analysed and compared across these distinct groups. Continuous data is presented as mean±Standard Deviation (SD). Given the absence of skewness in the distribution of quantitative variables, an independent t-test was employed to evaluate the significance of hypomagnesaemia as a potential risk factor for vascular calcification in ESRD patients, which may lead to CVD in CKD patients. Gender, a qualitative variable, was scrutinised using Fischer's-exact test. Additionally, Pearson Chi-square and Pearson correlation analyses were employed. Statistical significance was determined at a 5% level, with a p-value of 0.05 or less deemed statistically noteworthy.

RESULTS

A total of 60 patients were included in the present study. The participants in the current study ranged in age from 20 to 79 years, with an average age and standard deviation of the study population being 46.13 years and 14.5, respectively. The study group consisted of 47 (78.3%) males and 13 (21.7%) females. Among them, 22 (36.7%) participants were under the age of 40, while 15 (25%) were between 41 and 60 and 23 (38.8%) were above 60-year-old [Table/Fig-1].

Parameters	Frequency	Percent
Completed age in years		
20-40	22	36.6
41-60	15	25.0
>60	23	38.8
Gender		
Male	47	78.3
Female	13	21.7
History of smoking		
Present	35	58.3
Absent	25	41.7
Diabetes		
Present	41	68.3
Absent	19	31.7
Hypertension		
Present	43	71.6
Absent	17	28.4
Dyslipidaemia		
Present	38	63.3
Absent	22	36.7
Total	60	100.0

[Table/Fig-1]: The demographic details and clinical past history of study population.

Out of the 60 study participants, 15 (25%) individuals experienced a cardiovascular event, while the remaining 45 (75%) participants did not encounter any such occurrences. Of the 60 participants, 12 (20%) individuals succumbed to cardiovascular events, while the remaining 48 (80%) participants are alive [Table/Fig-2].

Cardiovascular event	Frequency	Percentage
Developed	15	25
Not developed	45	75
Mortality due to cardiovascular events		
Yes	12	20
No	48	80

[Table/Fig-2]: Occurrence of cardiovascular event and resultant mortality due to it in study population.

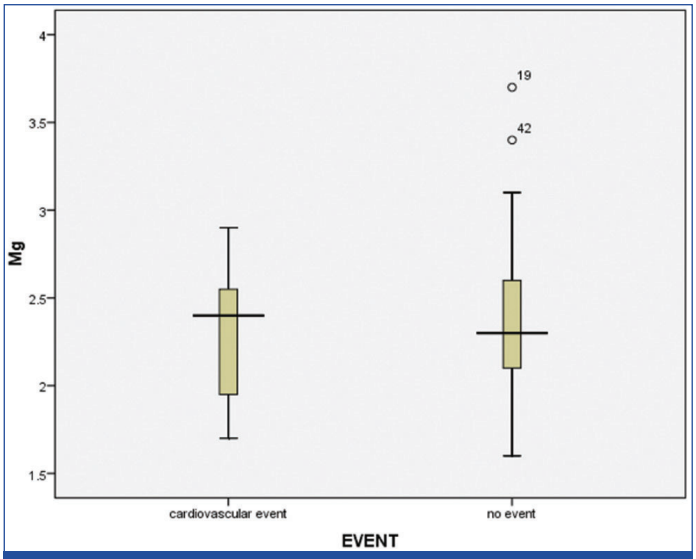
The median and interquartile range (Q3-Q1) for s.magnesium in cardivascular event group was 2.4 (2.6-1.9) and in no cardiovascular event group was 2.3 (2.65-2.10) [Table/Fig-3,4].

Serum Mg	Cardiovascular event	No cardiovascular event
Median	2.4	2.3
Interquartile range (Q3-Q1)	2.6-1.9	2.65-2.10

[Table/Fig-3]: Median and interquartile range of S.Mg (serum Mg).

Among those who developed a cardiovascular event, 15 (25%) had hypomagnesaemia reported in 7 (46.7%) subjects, whereas 8 (46.7%) had normal S.Mg levels. The mean Mg level in those who developed a cardiovascular event is 2.32±0.41; the mean Mg level in those who did not develop an event is 2.41±0.45. This difference in mean is not statistically significant with an independent t-test with a t-value of -0.69 and a p-value of 0.49 [Table/Fig-5].

There was no significant association between age and occurrence of cardiovascular event. There was no significant association between the duration of HD and the occurrence of cardiovascular events [Table/Fig-6].



[Table/Fig-4]: Box Whisker plot for S.Mg.

S.Mg	Cardiovascular event		Total	S.Mg		t value	p-value
	Event	No event		Mean	SD		
≤2.2*	7	17	24	2.32	0.41	-0.69	0.49.
	29.2%	70.8%	100%				
>2.2*	8	28	36	2.41	0.45		
	22.2%	77.8%	100%				
Total	15	45	60				

[Table/Fig-5]: Distribution of cardiovascular event according to Serum Mg values. Independent t-test

Completed age in years	Cardiovascular event		Total	p-value
	Event	No event		
20-40	3 (13.6%)	19 (86.4%)	22 (100%)	0.122
≥41	12 (31.6%)	26 (68.4%)	38 (100%)	
Total	15 (25%)	45 (75%)	60 (100%)	
Gender	**Cardiovascular event**		**Total**	**p-value**
	Event	**No event**		
Male	12 (25.5%)	35 (74.5%)	47 (100%)	0.35
Female	3 (23.1%)	10 (76.9%)	13 (100%)	
Total	15 (25%)	45 (75%)	60 (100.0)	
Duration of haemodialysis (Years)	**Cardiovascular event**		**Total**	**p-value**
	Yes	**No**		
≤1 y	5 (21.7%)	18 (78.3%)	23 (100%)	0.64
>1 y	10 (27%)	27 (73%)	37 (100%)	
Total	15 (25%)	45 (75%)	60 (100%)	

[Table/Fig-6]: Distribution of cardiovascular events according to age group, gender and duration of haemodialysis.
Pearson Chi-square test was done to compare age and occurrence of cardiovascular event, the duration of HD and the occurrence of cardiovascular events. Fisher's-exact test was done to compare gender and cardiovascular event

DISCUSSION

The present study compared Mg levels and the development of cardiovascular events in patients who were diagnosed with ESRD on HD. In the study, out of the 60 subjects, 60% had elevated S.Mg levels, 40% had normal levels of S.Mg, and none of them had low serum Mg levels. 25% of patients developed cardiovascular events during the follow-up period, and 75% did not develop any event. Also, it was found out that the occurrence of cardiovascular events among the patients who had elevated serum Mg levels and normal serum Mg levels was 22.2% and 29.2%, respectively. Since the p-value >0.05, no significant association existed between serum Mg level and occurrence of cardiovascular events.

Observational studies conducted on individuals with CKD have indicated that both hypomagnesaemia and hypermagnesemia are correlated with adverse outcomes [26,27]. Kanbay M et al., conducted an observational cohort study involving 283 CKD patients that evaluated the possible contribution of Mg on cardiovascular outcome in patients with moderate-to-severe CKD, which revealed that Mg could serve as an independent predictor for future cardiovascular events. This study was pioneering in highlighting such a significant role in patients diagnosed with CKD, spanning various stages [26]. Alhosaini M et al., reported that both CKD and ESRD patients on dialysis usually have normal serum levels of Mg and sometimes even low serum Mg concentrations (hypomagnesaemia) [27].

The primary discovery of this research indicates that the serum Mg level does not appear to serve as an independent prognosticator of forthcoming cardiovascular incidents in patients with CKD-stage 5 during short-term monitoring. Sakaguchi Y et al., have demonstrated in their studies that a diminished serum Mg level represents a significant and autonomous predictor of CVD mortality in individuals undergoing chronic HD [17]. The scope of clinical trials investigating Mg supplementation, excluding Mg-phosphorus binders, remains restricted [28,29]. The main finding of the clinical trial by Bressendorff I et al., was that oral Mg supplementation using slow-release Mg hydroxide30 mmol/d does not affect intracellular Mg as assessed by energy dispersive X-ray microanalysis after eight weeks of treatment, despite significant increases in serum Mg and urine Mg. Furthermore, this trial has shown for the first time that serum calcification propensity T50 can be improved by oral Mg supplementation in CKD stages 3 and 4 [28]. Sakaguchi Y et al., conducted a 2-year, open-label, randomised, controlled trial enrolling patients with stage 3-4 CKD with risk factors for CAC. Employing a two-by-two factorial design, they randomly allocated patients to either an MgO intervention group or a control group and to an AST-120 intervention group or a control group. The findings revealed that MgO demonstrated efficacy in decelerating the progression of CAC [29].

In the present study, patients who had undergone HD for <1 year had less occurrence of cardiovascular events (21.8%) when compared to those who had HD for >1 year, but no significant association between duration of HD and occurrence of cardiovascular event was found out. A similar finding to this study was found out by Negrea L et al., in which a cohort study involving 3,867 participants diagnosed with CKD revealed a non-linear correlation between serum Mg levels and overall mortality rates. Both low and high levels of Mg were linked to increased all-cause mortality rates, even after adjusting for various factors such as demographics, comorbidities, medications like diuretics, estimated Glomerular Filtration Rate (eGFR), and proteinuria (p<0.001). Interestingly, there were no significant correlations found between serum Mg levels and the occurrence of composite cardiovascular events. However, a notable association was established between low serum Mg levels and the onset of atrial fibrillation [30].

Serum Mg levels pose a significant challenge in observational studies. Various factors can impact serum Mg levels, such as intestinal absorption, hormonal regulation, bone health, GFR, and pharmaceutical treatments [31,32].

Limitation(s)

The present study is constrained by its observational nature, limited sample size, study period, and the measurement of serum Mg levels, which was conducted only once at the commencement of the study.

CONCLUSION(S)

A diminished serum Mg level is linked to escalated mortality rates, yet its role as a predictor of CVD remains ambiguous. The study showed no significant association between serum Mg level

and occurrence of cardiovascular events in patients with CKD. Randomised controlled trials investigating whether elevated serum Mg levels or increased Mg intake yield favorable outcomes in CKD patients are paramount. A thorough analysis of these effects transcends the confines of this study.

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