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Evaluation of serum Zinc and Magnesium in chronic kidney disease patients undergoing dialysis a case control study in Indian patients

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ABSTRACT

Chronic Kidney Disease patients suffer from numerous nutritional deficiencies which need to be supplemented. Two most commonly studied trace elements are Zinc and Magnesium whose excess or deficiency has been directly been linked to morbidity amongst the CKD patients. We conducted the matched case-control hospital study with objectives to estimate levels of serum Zn (Zinc) and Magnesium amongst the CKD population undergoing hemodialysis. A total of 150 cases and 100 controls were subjected to inclusion and exclusion criteria. Out of which only 50 cases and 50 controls fulfilled the criteria which were included in the study. Descriptive analysis was done and an Area Under Curve (AUC) was drawn, which showed significant difference between two groups. This evidence suggests these important points: (1) Routine determination of trace elements should be considered in the clinical evaluations by physicians (2) Determination of trace elements such as Zn and Mg, maybe of worth before or after dialysis and could be an indicator of trace element imbalance status.

Keywords: Chronic Kidney disease, Serum Zinc, Serum Magnesium, Dialysis

INTRODUCTION

Chronic Kidney Disease is a syndrome of persistent renal impairment involving loss of both glomerular and tubular function such that the kidneys homeostatic functions are compromised. (1) Reported age-adjusted incidence rate of End Stage Renal Diseases to be 229 per million populations. The prevalence of Chronic Kidney Disease was observed to be 17.2%. (2) In these patients hemodialysis is the most common technique used to treat advance and permanent kidney failure.

Chronic Kidney Disease patients suffer from numerous nutritional deficiencies which need to be supplemented. Until recently more stress is being given to trace elements and its impact on overall co-morbidity, complications and quality of life of CKD patients. Two most commonly studied trace elements are Zinc and Magnesium whose excess or deficiency has been directly been linked to morbidity amongst the CKD patients.

Zinc is the second most abundant trace element in human body. It acts as a structural and functional component of several metalloproteins. As well as, Zn is involved in many cellular metabolism reactions and acts as an anti-oxidant as well. (3, 4) Organ systems known to be affected clinically by zinc deficiency states include the epidermal, gastrointestinal, central nervous, immune, skeletal, and reproductive systems. Zn level. Low concentration of serum zinc is commonly seen in CKD patients. The cause of decrease is unclear but may be due to restricted or low protein diet is recommended to CKD patients.(5) The important factor which increase zinc deficiency is the ability of zinc to bind to proteins modified by uremia. (6)

Serum zinc concentration has some important characteristics that make it an excellent indicator of zinc status for population. It reflects dietary zinc intake, it responds consistently to zinc supplementation and reference data are available for most age and sex groups.

Mg is the fourth most abundant and second most prevalent intracellular cation in body. 60%of it is present in bones, in exchangeable and non-exchangeable pools. Mg functions intracellularly as a necessary cofactor for more than 300 enzyme systems. Hypermagnesaemia is frequently seen in CKD patients and particularly in patients with dialysis and also in acute renal disease. Magnesium deficiency increases the risk for several diseases like diabetes mellitus type 2, hypertension and atherosclerosis. Moderate hypermagnesaemia however, seems to have beneficial effects on vascular calcification and mortality rates in CKD patients.

In human beings, trace elements are essential nutrients with many important functions; they are the indispensable components of many enzymes, have some regulatory functions and may affect immune reactions.

To prevent complications in CKD patients, hemodialysis is very important to know and regulate the level of trace elements. (1) Keeping in view the altering observed in trace elements in CKD.

Not many studies have reported status of Zn & Mg status in Indian CKD population and to comment weather levels are in con-ordnance with the normal population. With the above background we conducted the study with objectives to estimate levels of serum Zn & Magnesium amongst the CKD population undergoing hemodialysis.

Materials & Methods

It was a hospital-based age and sex matched case-control study of one year duration.

Sample:

After obtaining clearance from the institutional ethics committee. All the patients attending Medicine OPD and IPD, of the Hospital in year 2015-16 were screened for inclusion or exclusion criteria for cases and controls. Inclusion criteria of cases: Age between 20-70 years and clinically diagnosed CKD patients undergoing dialysis for at least 01 month before being included in the study.

Inclusion criteria for controls: Each age & sex matched to that of case, with no history of any evidence of any kidney disease both clinically and biochemically or presence of any potential risk factors for any kidney disease.

Exclusion Criteria (for cases and controls): The patients suffering from other disease, such as inflammatory diseases, hepatic or respiratory diseases as well as smokers and alcoholics were excluded from the study. Patients on any Zinc and Magnesium supplemented diet, Acrodermatitis enteropathica, Anemic, Acute kidney disease, very sick patients and failure to obtain informed written consent.

Sample size:

The sample size was calculated used open epi software for calculating sample size. With the assumptions of Observational study, Power 80%, taking 1:1 ratio of cases to controls, hypothetical proportions of controls with exposure taking as 17% with assumption that 50% of cases have chances of impaired Zn & Mg levels from normal population. The sample size came out to be total 37 each of cases and controls, total sample size came out to be 74.

Sampling procedure:

After obtaining written consent from each enrolled subject (case or control). Using a standard questionnaire, a complete history and necessary information with the emphasis on kidney diseases risk evaluation obtained from both case and controls. It was followed by a detailed physical examination done by trained physician team members. All subjects were investigated routine examination and specifically for Blood Urea, Serum Creatinine and Serum Zinc and Serum Magnesium levels. In the morning (8 to 10 a.m.) after the participants had abstained from eating or drinking overnight, 5 ml of venous blood were taken in zinc-free containers form every participant in the investigation and immediately centrifuged to prevent the zinc efflux due to hemolysis from the cells into the plasma, or influx of zinc into the erythrocytes. All plastic and glassware used in the experiments were treated with deionized water and then dried.

The following methodology was used for analysis: Serum zinc was estimated by colorimetric method. Serum magnesium was estimated by Calmagite – method. Blood urea was estimated by enzymatic end-point colorimetric method. Serum creatinine was estimated by Jaffe`s method. (7)

Zinc determination: Zinc Monoliquid: Colorimetric method. **Principle**: Zinc present in the sample is chelated by 5-Br-PAPS 2-(5-bromo-2-pyridyiazo)-5-(N-propyl-N-sulfopropylamino)-phenol in the reagent. The formation of this complex is measured at a wavelength of 560nm. Mix properly and incubate for at 25°C or at 37°C for 5minutes. The cuvette should be of 1cm path length. Read final absorbance of test and standard against reagent blank at 546nm. **Calculation:** Zinc in (µg/dl) = Absorbance of Sample – Absorbance of blank / Absorbance of Standard – A Blank × Standard Conc. (µg/dl). **Normal values** being Male: 72.6-120 µg/dl

& Female: 70-114 µg/dl.

Magnesium determination Principle: Magnesium forms a colored complex when treated with calmagite in alkaline solution. EGTA eliminates the calcium interference. The blue calmagite-magnesium complex is measured at 520nm and the amount formed is directly proportional to the concentration of magnesium in the sample.

Working Reagent: Add 1volume of Reagent Calgamite (170mmol/L) to 1 volume of Reagent AMP (1mol/L & EGTA 310 µmol/L with standard being Magnesium 20mg/L. Gently mix &incubate for 5 minutes at 20°C or 25°C and measure the absorbance of standard and test against blank at 520 nm. Measure the Optical Density (OD) – against reagent blank within 1 hour. **Calculation** = OD of sample/ OD of Calibrator × Concentration of Calibrator. **Normal range:** 1.60-2.5mg/dl

Statistical analysis

When the data were distributed normally according to the Kolmogorov-Smirnov test, we used parametric tests, i.e. Student's t test for independent or related samples. For variables that required nonparametric testing we used the Wilcoxon test for related samples and the Mann-Whitney test for unrelated samples. Z test was used to find differences between the participants with low plasma levels of magnesium or zinc. Receiver operating characteristics (ROC) curves were plotted to evaluate analytical sensitivity and specificity of Zn and Mg determination in hemodialysis patients.

The area under the curve (AUC) was calculated with 95 % confidence interval (CI) to detect the best cut-off value. The optimal cut-off values which will be presented involve both highest sensitivity and specificity.

Table: I Descriptive statistics cases and controls.

Parameter	**Cases (Mean ± SD)**	**Controls (Mean ± SD)**	**P-Value**
Age	53.24 ± 10.07	50.36 ± 7.08	
Sex			
Male	39 (78%)	25 (50%)	
Female	11 (22%)	25 (50%)	
Serum Urea (mg/dl)	130.00 ± 53.42	25.02 ± 8.26	< 0.0001
Serum Creatinine (mg/dl)	4.95 ± 2.74	0.87 ± .34	< 0.0001
Serum Zinc (µg/dl)	83.44 ± 34.77	104.08 ± 25.41	0.001
Serum Magnesium (mg/dl)	3.47 ± 0.67	1.89 ± 0.40	0.0001

Results:

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A total of 150 cases and 100 controls were subjected to inclusion and exclusion criteria. Out of which only 50 cases and 50 controls fulfilled the criteria which were included in the study. All were subjected to Serum Zn & Serum Magnesium levels in there OPD visits to the institute and estimation of Serum Urea & Serum Creatinine.

Table-1 depicts age and sex distribution and assessment of investigations carried out.

Both cases and controls were age and sex matched as depicted with p-value > 0.05.

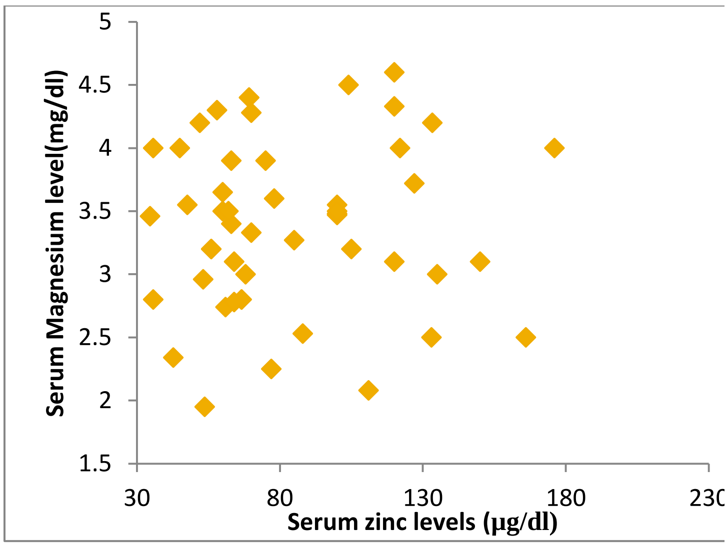

Figure 1 (a): Correlation between serum zinc and serum magnesium in cases. As shown in scatter diagram there was positive correlation between serum zinc and serum magnesium in cases. The data was not statistically significant. (r= 0.050, p= .732)

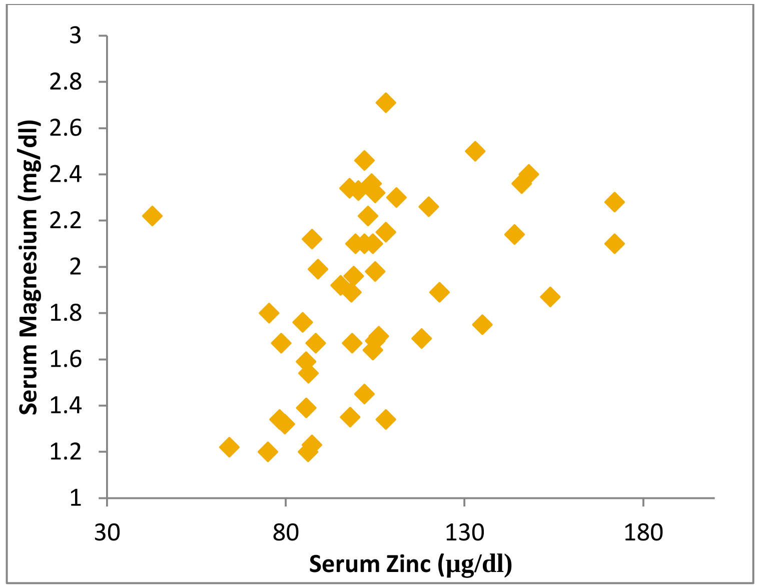

Figure: 1 (b): Correlation between serum zinc and serum magnesium in controls. As shown in scatter diagram there was positive correlation between serum zinc and serum magnesium in healthy controls. It was statically significant (r=.439, p= 0.001)

Correlation between Serum Zn & Mg is depicted in Fig. 1 (a) & Fig. 1 (b); which shows positive and statistically significant relationship in controls.

Figure: 02 depicts the AUC for Zn concentrations was equal to 0.514 (P = 0.815). AUC for Mg concentrations was equal to 0.065 (P = 0.000). Determination of cut-off values for the measurement of Zn and Mg before and after hemodialysis helps to evaluate the clinical significance of such measurement. According to ROC analysis when at least considering 90 % specificity, the cut-off and sensitivity values would be as shown in Table 2.

Table: II Area under the Curve.

Test Result Variable(s)	Area	Std. Error[a]	Sig.[b]	95% Confidence Interval
S.Zn	.514	.063	.815	0.391 - 0.637
S.Mg	.065	.023	.000	0.019 - 0.110

On calculating Pearson co-efficient of correlation between Serum Mg & S. Creatinine levels, there is a

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positive relationship and it is statistically significant i.e. Serum Mg levels rises as the S. creatinine levels rises. R value= 0. 5757 & P-value= 0.00012.

Pearson correlation co-efficient S. Zn & S. Creatinine- R= - 0.0967 & p-value= 0.56.

S.Zn & BU- R= -0.095 & p-value= 0.51

S.Zn & S. Mg; R = -0.1757 and p value 0.221.

S. Mg and Blood Urea; R = 0.6962 and p value < 0.00001.

DISCUSSION:

Overall, an estimated 5–10 million people die annually from kidney disease. (8, 9) CKD is worldwide public health problem, both for the number of patients and cost of treatment. (13)

Essential trace metals are involved the maintenance of undisturbed biological functions in a number of metabolic activities, including neuro-conduction, transport, excretory processes and serving as cofactors for enzymes. (10 -13)

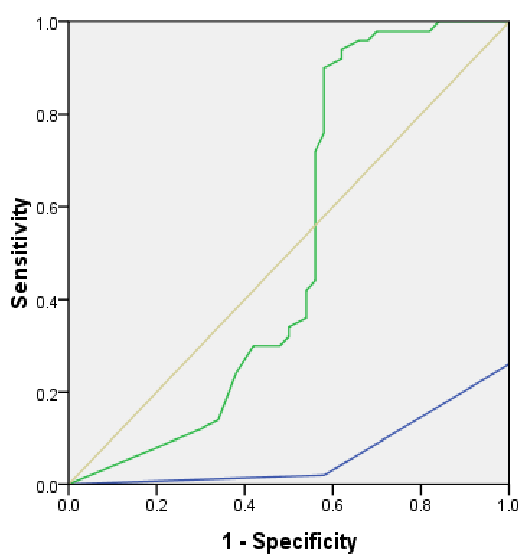

Figure: 2A: ROC curve of Zn, Mg serum levels amongst cases & controls, predicting lower limits. With AUC in Mg 0.065 & AUC in Zn is 0.514. Green line represents Serum Zinc and Blue line as Serum Magnesium levels.

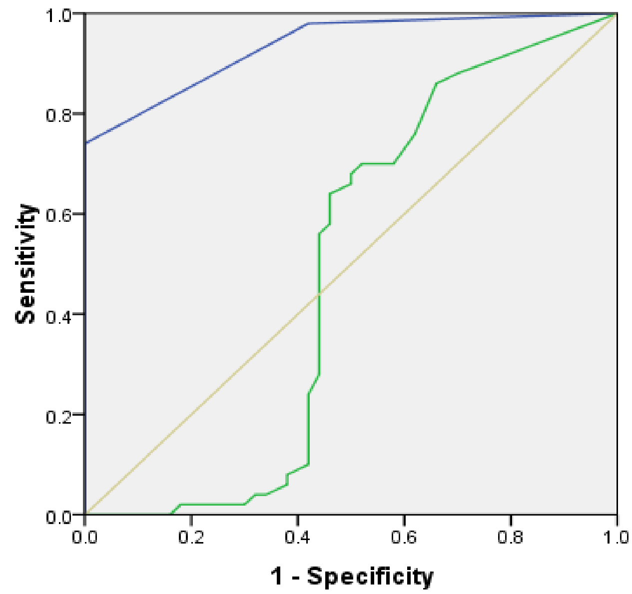

Figure: 02B: ROC curve of Zn, Mg serum levels amongst cases & controls, predicting higher limits. With AUC in Mg 0.935 & AUC in Zn is 0.486. Green line represents Serum Zinc and Blue line as Serum Magnesium levels.

Hemodialysis patients are at theoretical risk for both deficiency and accumulation of trace elements, depending on dietary intake, removal by dialysis, the composition of the source water used for hemodialysis, and residual kidney function. (9, 14)

The present study was aimed to evaluate the possible alterations in zinc (Zn) and magnesium (Mg) levels in CKD patients undergoing hemodialysis.

Some investigators have reported a reduction (15, 16) and others acute toxicity or increased levels of essential trace elements in hemodialysis patients. (21) Such discrepant results were also present in a review article by Tonelli et al. and various other worldwide reported articles. (6, 15)

Serum Zinc:

Studies conducted across world and India reporting decreased level of Serum Zinc in patients undergoing dialysis in concordance with our study. (18-20) Decreased S. Zinc levels in patients undergoing hemodialysis was purposed to be due to restriction of proteins, low dietary Zn intake, a specific Zn transport defect, and absence of intestinal Zn ligand. These studies also suggested that low serum zinc levels in renal failure patients are believed to be due to the shift of zinc into red blood cells under acidic conditions. (19, 20)

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Other theory is that since, the concentration of the trace elements in the dialysate is unknown. When their concentration in the dialysate is lower than in the blood, they may be removed during dialysis through semipermeable membranes. This can deplete biologically active minerals such as zinc. The mineral content of the water used in the dialysis sessions may also have interfered with the concentration of zinc in plasma. The levels of trace elements are not periodically controlled and this can lead to changes in the concentration gradient between blood and dialysate for some trace elements including zinc. (20)

Systemic review by Wang et al reported that zinc supplementation may benefit the nutritional status of hemodlyliasis patients and show a time-effect relationship. (21)

The correlation of serum zinc with urea and creatinine in the Youssef et al. shows that there was negative correlation with either urea or creatinine. (22) In our study it shows in healthy subjects serum zinc is positively correlated with blood urea and creatinine and in cases there were negative correlation with serum creatinine.

Serum Magnesium:

Hypomagnesaemia is usually observed in patients with creatinine clearance below 10 ml/min. In the case of patients on dialysis, the diffusion of Mg ions through dialysis or the peritoneal membrane, their concentration depending on the dialysate, is the main determinant of serum Mg levels.

Hypomagnesaemia in CKD may be due to the limited ability of kidneys to excrete excess of magnesium. (1, 23, 24)

This evidence suggests these important points: (1) Routine determination of trace elements should be considered in the clinical evaluations by physicians (2) Determination of trace elements such as Zn and Mg, maybe of worth before or after dialysis and could be an indicator of trace element imbalance status.

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