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Biochemical Characterization Of (Ca^{2+}-Mg^{2+})-Atpase And Ionic Imbalance In Patient With Chronic Renal Failure

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Abstract

Calcium and Magnesium homeostasis can be disturbed in many ways in the course of chronic renal failure (CRF) . Almost all calcium body content resides in the extracellular space , while the Magnesium almost intracellular space . The concentration of both ions is maintenance is possible via cooperation between many regulating systems in the cell membranes .

The aim of our study was to estimate the activity of Ca^{2+}–Mg^{2+}- ATPase (CMA) , creatinine, urea, sodium, potassium , magnesium , total calcium, inorganic phosphate , Parathyroid hormone, Vitamin D. , and total protein in blood of patients with CRF and evaluate the correlation of Ca^{2+}–Mg^{2+}- ATPase with Vitamin D , Parathyroid hormone , Ca+2 and Mg+2 .

Methods A total of 60 diagnosed CRF patients (30 males and 30 females) their ages ranged from 22-65 years its called G1 while G2 consisted of 60 healthy subjects , The chemicals and kits that were used in this study were of the highest purity.

Results in patients groups Ca^{2+}–Mg^{2+}- ATPase , magnesium , total calcium and Vit. D was Significant decrease in G1 compare with G2 while creatinine, urea, potassium , inorganic phosphate and Parathyroid hormone Significant increases

Keywords: Ca^{2+}–Mg^{2+}- ATPase ; chronic Renal Failure , Ionic imbalance.

Introduction

Calcium homeostasis can be disturbed in many ways in the course of chronic renal failure (CRF) . Almost all calcium body content resides in the extracellular space, where the ion concentration is around 10000 times higher than inside the cells , Calcium is also an important element in animal physiology and It is well established that intracellular calcium plays a vital role

in the control of many important aspects of cellular metabolism [Polak *et al*,2010; Muto *et al* ,1984].

Magnesium (Mg) is the fourth most abundant cation in the body and the second most important intracellular cation and Mg has gained much importance with the growing awareness that Mg is required as a cofactor in multiple enzymatic reactions and that it plays an important role in neuromuscular processes (van de *et al* , 2018) One of the major systems responsible for regulating the cytoplasmic calcium and magnesium level is Ca^{2+}-Mg^{+2} dependent ATPase (Muto *et al* ,1984) .

The (Ca^{2+} + Mg^{2+})-ATpase (E.C. 3.6.1.3) class of enzymes hydrolysis that catalyze ability to hydrolyze ATP to ADP and inorganic phosphate (Pi) . Its molecular weight 110-140 k Da protein whee to mediate calcium transport across the sarcoplasmic reticulum and plasma membrane , Ion transport ATPases constitute an essential part of the system controlling salt and water balance in living systems , It is a protein playing a role in the maintenance of the cellular Ca+2 and Mg+2 homeostasis (Dhalla *et al*, 1988 , tentes *et al* , 1992) Ca+2 Mg +2 ATPase is an important pump that extrudes calcium and magnesium out of cells and inside it (Carafoli , 1987 ,Schatzmann, 1999) and its normal function requires adequate ATP as a substrate (Carafoli , 1987, albyti *et al* 2016) .

Chronic Renal Failure (CRF) is defined as a progressive loss of renal function over time, the kidney is regulate the fluid, electrolyte, and pH balance of the extracellular fluids (Dhalla *et al*, 1988 ,Gonzales *et al* 2004 ,Hadtstien *et al* 2008) . CRF leads to calcium homeostasis disturbances and so the Calcium decreased in blood results in the decreased cell flexibility, elevated osmotic fragility and, finally hemolysis. (Dhalla *et al*, 1988 , tentes *et al* , 1992) .

MATERIALS AND METHODS

A total of 60 diagnosed adult chronic renal failure patients (30 males and 30 females) their ages ranged from 22-65 years for females and males. All pateints are suffering from chronic renal failure in end stage And undergo dialysis , were enrolled in the study , The clinical status of patients have been diagnosed by doctors specialized in artificial kidney department in general hospital of Tikrit . The blood samples were collected before dialysis . The control groups consisted of 60 healthy (30 males and 30 females), and their ages ranged from 22 to 65 years.

The chemicals and kits that were used in this study were of the highest purity. The determination of serum creatinine, urea, sodium, potassium , magnesium , total calcium, inorganic phosphate , Parathyroid hormone ,Vitamin D. , total protein and activity of (Ca 2+ - Mg2+)-ATPase were performed by approved methods.

Estimation of (Ca^{2+} - Mg^{2+})-ATPase Activity : The basis of (Ca 2+ - Mg2+)-ATPase activity measurement its ability to hydrolyze ATP to ADP and inorganic phosphate , Ca2+/Mg2+ ATPase breaks down ATP to generate ADP and inorganic phosphate, ATP activity is determined by measuring the amount of inorganic phosphorus .

Assay procedure : Measured according to the kit supplied from company mybiosource.

Added following reagents in the micro centrifuge tubes :

Reagent	Control	Sample
Reagent I	65 µl	45 µl
Reagent II	60 µl	60 µl
Reagent III	.....	20 µl
Sample	.....	100 µl
Mix, put it in the oven, 37 °C for 10 minutes.		
Reagent IV	25 µl	25 µl
Sample	100 µl	....
Mix, centrifuged at 8000g, room temperature for 10 minutes, take the supernatant into a new centrifuge tube.		

Added following reagents in the 96-Well microplate:

	Standard	Blank	Sample	Control
0.5µmol/ml Standard Solution	20 µl	....	....	....
Distilled water	....	20 µl	....	....
The supernatant	....	....	20 µl	20 µl
Working Solution	200 µl	200 µl	200 µl	200 µl
Mix, room temperature for 30 minutes, record absorbance measured at 660nm.				

Calculation : According to the volume of serum

Ca2+/Mg2+ ATPase activity (U/ml) = 7.5 × (OD Sample – OD Control) / (OD Standard – OD Blank)

.Estimation of electrolytes

was determined Inorganic phosphate using the colorimetric method of Fiske and Subbarow (King ,1992) , the total Calcium determined using the Moorehead and Briggs derived CPC (O-Cresol Phtalein Complexone) Method allows to determinate total calcium concentration in serum with chronic renal failure and compare with control (Meselson *et al,* 1968) Magnesium determined using the colorimetric method (Poenie *et al* ,1985).

Concentration of protein was measured using the method of Bradford et al. Lew et al , 2003) , and bovine albumin was used as a standard .

Determination of vitamin D and PTH

The LIAISON ®25 OH Vitamin D assay is a direct competitive chemilu -mine scence immunoassay (CLIA) for quantitative determination of total 25 OH vitamin D in Serum (Bradford , 1976) . as for the parathyroid hormone (PTH) was assay according to a method (Diasorien,1951).

Results

The mean values of total Ca^{2+}, Mg^{2+} , Na^{+1} , K^{+1} ,Vit D. , PTH , Ca^{2+} - Mg^{2+}-ATPase , Urea , Creatinine and inorganic phosphate in serum of patients from investigated groups are shown in Table (1-1) and Figures 1 and 2 .

In Table (1-1), the statistically significant differences ($P \leq 0.05$ and $P \geq 0.05$) between the examined groups are shown. The results of the our study indicated that was Ca^{2+} - Mg^{2+}-ATPase activity was Significant decrease In group of patients compare with the controls While Total calcium In examined Chronic Renal Failure groups , the concentrations of total calcium were significantly lower than those in the control group . The results show significant ($P< 0.05$) increase in urea and creatine concentration in chronic renal failure patients when compared with those of the control group. So do both Parathyroid Hormone (PTH) , Inorganic phosphate and Potassium In all examined Chronic renal failure groups, the concentrations of Vitamin D. and Magnesium were significantly lower than those in the control group , but The levels of Sodium concentrations were not significantly lower than those in the control group .

The results of our study showed as in table (1-2) An inverse correlation between Ca+2/Mg+2- ATPase With PTH , Ca+2 and Mg+2 in serum of Chronic Renal Failure but with Vit. D is direct . As shown this in figures (1-3) , (1-4) , (1-5) and (1-6) .

Table (1-1) Concentration of some biochemical Parameters in patients with Chronic renal failure compared with control group.

Parameters	Mean± SD		P-Value
	G1 patients (n= 60)	G2 controls (n=60)	-----
Ca^{2+} - Mg^{2+}-ATPase activity (U/ml)	1.086 ±0.217	2.436 ±0.397	0.000*
total Calcium (mg/dl)	6.290 ± 0.839	7.224 ± 0.676	0.000*
Magnesium (mg/dl)	1.187 ± 0.206	1.730 ± 0.277	0.000*
Potassium (mmol/l)	5.292 ± 1.019	3.883 ± 0.801	0.000*
Sodium (mg/dl)	128.911 ± 14.498	131.633 ± 9.408	0.372#
Inorganic phosphate(mg/dl)	6.245 ± 1.262	3.068 ± 0.443	0.000*
Creatinine (mg/dl)	3.217 ± 0.718	0.912 ±0.252	0.000*

Urea (mg/dl)	134.929 ± 24.217	23.238 ± 4.155	0.000*
Vitamin D. (ng/ml)	11.335 ± 1.984	16.131 ±2.707	0.000*
PTH (ng /ml)	101.591 ± 16.134	26.42 ±4.318	0.000*
Protein			

**** Highly Significant p ≤ 0.05 , # non Significant p ≥ 0.05***

Table (1-2) Correlation Between Ca+2/Mg+2- ATPase With Vitamin D. , PTH , Ca+2 and Mg+2 in serum of Chronic Renal Failure .

Ca+2/Mg+2-ATPase (U/mL) / Parameter	**Control**	**Patiens**
Ca (mg/dL)	-0.0821	-0.2066
Mg (mg/dl)	-0.1782	-0.1531
PTH(pg/ml)	-0.0471	-0.04090
Vit.D (ng/mL)	-0.02285	0.1768

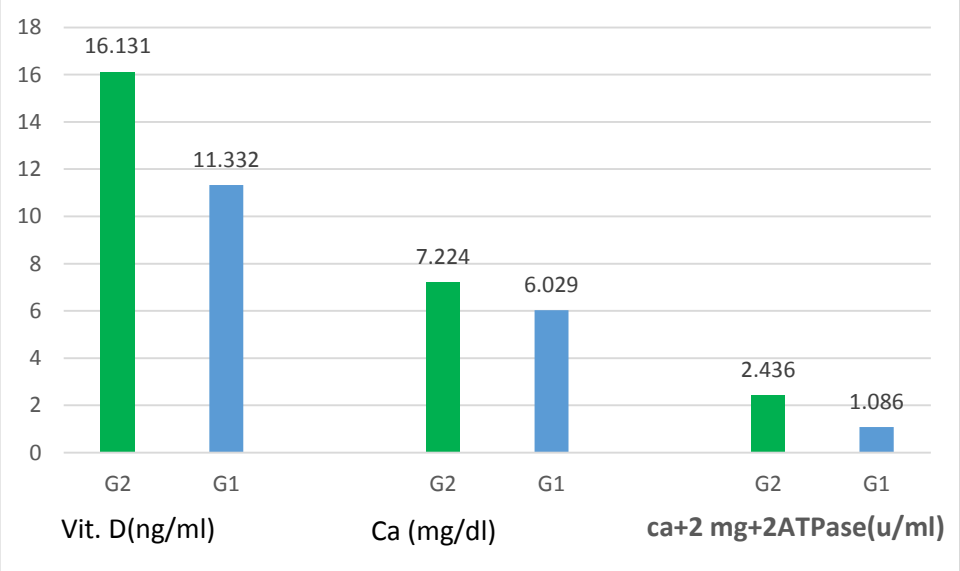


Fig.1 The mean values of ca+2 mg+2 ATPase, Vit.D and Ca in patient with CRF (G1 = pateints groups , G2 = control groups)

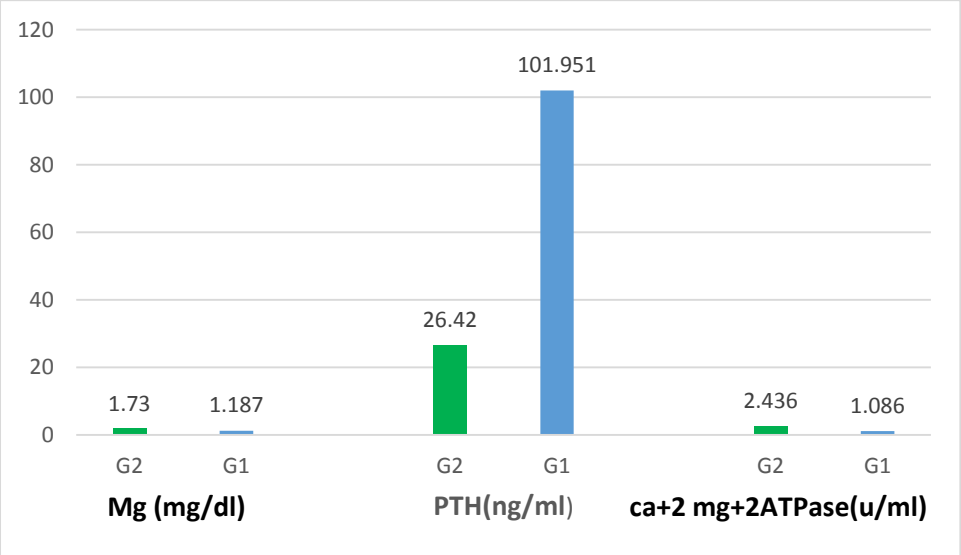
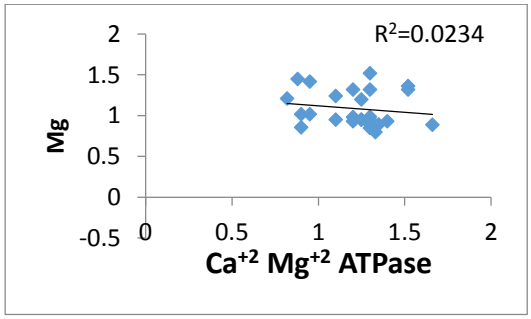
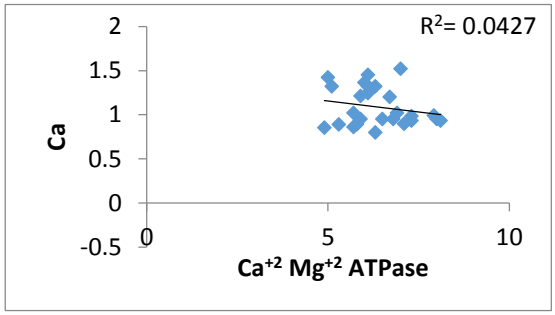


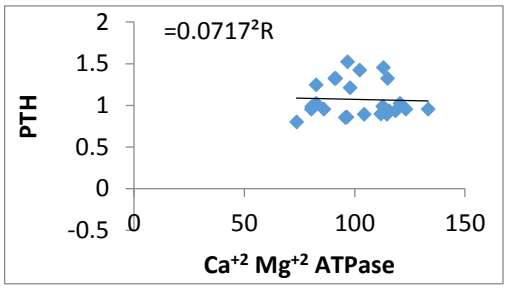
Fig. 2. The mean values of ca+2 mg+2 ATPase, Calcitonin Hormone and PTH in patient with CRF . (G1 = pateints groups , G2 = control groups)



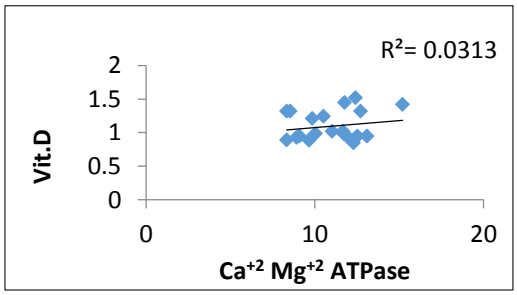
Fig(1-3) correlation between Ca^{2+}–Mg^{2+}- ATPase and Mg+2 patient with CRF



Fig(1-4) correlation between Ca^{2+}–Mg^{2+}- ATPase and Ca+2 in patient with CRF



Fig(1-5) correlation between Ca^{2+}–Mg^{2+}- ATPase and Vit. D in patient with CRF



Fig(1-6) correlation between Ca^{2+}–Mg^{2+}- ATPase and PTH in patient with CRF

Discussion

The chronic renal failure is causes to calcium and magnesium homeostasis disturbances , also on the cellular level , These disturbances increase with CRF

progression . An decrease in calcium concentration was found in serum of patient with CRF undergoing dialysis , this study in Our previous preliminary results have shown that Ca 2+ concentrations are decline in serum patient with CRF. (Cuwsse ,1996 , Mile *et al* , 1974 , Gafter *et al* 2018) .

Our previous result have shown that Ca^{2+} - Mg^{2+}-ATPase activity was lower than in the controls compare with all groups of patient with CRF . These results are in agreement with that of (Dhalla *et al* 1988 , Gafter *et al* , 1989) and This finding remains in accordance with other authors' observations concerning dialysed patients as well as with the results obtained by Grafter'setal (Gafter *et al* , 2018 , Jankowski *et al* , 2001). It is worth stressing that the decline in Ca^{2+} - Mg^{2+}-ATPase activity found in our study was similar in study groups (polka *et al* , 2001). Decreased Ca^{2+} - Mg^{2+}-ATPase activity in serum of patient with chronic renal failure compared with control may be caused by numerous factors. A direct cause might be a total calcium disturbed which due to glomerular filtration decline , This is a dangerous phenomenon , because its a messenger as well as a mediator between chemical and electrical signals that govern cell activity and it eventually may trigger apoptosis , and then a result of a disturbed balance of the ion's influx and efflux from the cell (Gafter *et al* , 2018 , Dhalla *et al* , 1988). On the other hand This phenomenon may be caused by a secondary hyperparathyroidism and elevation Calcitonin hormone concomitant with CRF since it has been proven that PTH intensifies Ca2+ influx into various types of cells , and thus may be one of the reasons for the decrease in the Ca+2 Mg+2 ATPase activity (Gafter *et al* , 2018) . There is no direct correlation between the Calcium ion , PTH and Calcitonin hormone in with chronic renal failure but were observed in patients group the Calcium ion , PTH and Calcitonin hormone ,where levels were much higher compared to the control groups , and This corresponds to a decrease in the Ca+2 Mg+2 ATPase activity ,

The progressive PTH increase observed in some the study patients correspondingly also Ca+2 / Mg+2 - ATPase activity and Vit. D decline and thus this due to the glomerular filtration tube (Jankowski *et al* , 2001).

1,2 dihydroxycholecalciferol and Subsequently the second factor changes in magnesium homeostasis may occur In patients with chronic renal failure and end-stage renal disease both hypomagnesaemia as well as hypermagnesaemia , an increase in fractional magnesium excretion compensates for the loss of renal function and as a consequence a situation occurs hypermagnesaemia especially with a glomerular filtration rate less than 10 mL/min , may be occurs decrease in Ca^{2+} - Mg^{2+}-ATPase activity in serum of patient with chronic renal failure compared with control (Polak-Jonkisz *et al* , 2007). The observed decreased Ca^{2+} - Mg^{2+}-ATPase activity in all Chronic renal failure stages could be caused by a 'shortening' of molecular memory of the Ca^{2+} - Mg^{2+}-ATPase isoform due to persistent calmodulin deficiency this accordance with authors Polak and et al (Jankowsk *et al* ,1998). .

The other factor a direct cause might be a calmodulin deficiency because It is known that physiological high calmodulin concentration permanently stimulates Ca^{2+} - Mg^{2+}-ATPase and keeps the enzyme in an active 'open' form (Soldati *et al* , 1999, Nieman *et al* , 1985). Further binding to calmodulin keeps the Ca^{2+} - Mg^{2+}-ATPase activity level independent from the pulsatile Ca2+ concentration changes (Albert *et al* , 1991, Coux *et al* , 2009).

The Ca^{2+} - Mg^{2+}-ATPase activity also depends on other endogenous protein regulators , for instance calmodulin and calpain CANP (a proteinase belonging to cysteine endopeptidases group) and its inhibitor— calpastatin CAST. Their activity is sensitive to intracellular Ca2+ concentration changes and is regulated by reversible phosphorylation that in turn build on specific phosphatases and kinases activity [Bonilla *et al* , 1991, Hajjar *et al* ,1991).

Conclusions

The The Ca^{2+} - Mg^{2+}-ATPase activity is decreased in patient with chronic kidney disease CKD. The reasons for progressive Ca 2+ concentration decrease are multifactorial and calmodulin deficiency as well as CANP–CAST system disturbances are all implicated moreover , A decrease in concentration is observed for , magnesium , total calcium and Vit. D was while creatinine, urea, potassium , inorganic phosphate and Parathyroid hormone Significant increases .

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