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LIST OF PROCEDURES DONE

PROJECT REPORT

TITLE OF THE PROJECT: SERUM AND RBC LEVELS OF MAGNESIUM IN ENDOMYOCARDIAL FIBROSIS

NAME: A. Nageswara Rao.

PROGRAMME: D.M. Cardiology.

MONTH & YEAR OF SUBMISSION: Dec. 1988.

Forwarded

25/11/88

Note:— (i) In the case compilation of procedures done, the contents and the subsequent pages should be made into different sections (a) Procedures done (b) Procedures assisted (c) Procedures participated (d) Procedures attended/participated etc in Other Centres. Each section should be preceded by a leaf carrying the name of the section that is succeeding.

(ii) The Contents page will carry into. as per model given under

PROCEDURES DONE

Closed Mitral valvotomy.....124 (say)
Patent ductus arteriosus-ligation.....10
Atrial septal defects.....20
.....
.....

PROCEDURES ASSISTED

Closed Mitral valvotomy.....100 (say)
.....

(iii) In the subsequent pages details of each procedure done/assisted should be given in the format given below:—

Heading: **Closed mitral valvotomy**

Date	Name of the patient	Age	Sex	Patient No.

(iv) In the case of Project Report in the page immediately following the Certificate page the under-mentioned details should be given:—

(a) Title
(b) Duration
(c) Aim and scope
(d) 50 word summary of work done

CERTIFICATE

I, Dr. A. Nageswara Rao. hereby declare that I have actually performed all the procedures listed/carried out the project under report.

Signature A.Rao.

Place: TRIVANDRUM

Name in A. NAGESWARA RAO. capital letters

Date: 9-11-88.

CONTENTS

ABSTRACT.

Endomyocardial Fibrosis is a restrictive cardiomyopathy of unknown etiology confined to certain tropical countries and in India to the Kerala State. Various factors have been incriminated as the etiological agent for EMF such as Viral, parasitic, Immunological and hypereosinophilia etc. It has been speculated that an excess of thorium in conjunction with magnesium deficiency may play a role in the causation of endomyocardial fibrosis.

We analysed serum and RBC magnesium levels in 21 patients suffering from EMF 23 healthy volunteers and 11 patients of rheumatic heart disease. Atomic absorption spectrophotometry was used for estimating serum and RBC magnesium. No significant difference was observed in the mean serum magnesium levels between the patients with EMF (1.5 ± 0.22 m.eq/L), RHD (1.48 ± 0.09 m.eq/L) and the controls (1.47 ± 0.136 m.eq/L).

When analyzed by the type of diuretic patients took, there was a difference in the mean Serum magnesium concentration between those receiving triamterene containing group (1.54 ± 0.12 m.eq/L) and those receiving frusemide (1.42 ± 0.23 m.eq/L). However these differences appear very small and obvious hypomagnesemia was uncommon.

We therefore conclude that the Serum and RBC magnesium levels are in normal range in EMF group of patients.

INTRODUCTION.

Magnesium has been recognized as an essential nutrient since early 1930. Adult human has a magnesium pool of 1mol. More than 99% of magnesium is either intra cellular or in the bone. Mg^{++}ion is essential to a large number of enzymatic processes particularly those involving adenosine triphosphate (ATP) and energy transfer. In addition the transfer of Na^{+}, K^{+} and Ca^{++}ions across the cell membrane as well as the regulation of intracellular binding of Ca^{++} are directly influenced by the cellular concentration of magnesium.

The daily requirement recommended by Seelig is 5mg/kg/day. Magnesium is mainly absorbed in the small intestine the proportion being dependent on dietary intake. Only 1/3 of dietary magnesium is absorbed in a balanced diet. The absorption increases to 70% in a poor diet and to 100% in a diet without magnesium. Non absorbed magnesium is excreted in stools representing 50-80% of total excretion. On average 1/3 of dietary magnesium is excreted in urine. Large quantities are

lost in the urine following diuretic therapy. In states of magnesium deficiency urinary Mg^{++} levels are low.

Magnesium distribution: Essentially an intracellular ion.

Plasma :- 55% of the plasma magnesium is in the free ionized form and the rest is bound to the proteins.

Saliva: Contains less than half of the plasma Mg^{++} level.

Sweat, Urine: Sweat contains 15mg/24hrs and urine 90mg/24hrs in normal individuals.

INTRA CELLULAR

Blood cells: RBC, WBC and Platelets contain stable levels of magnesium due to genetic factors

Bone: 50% of total magnesium is in bone. It is found in two forms. One representing 70-80% of bone Mg and is incorporated in apatite crystals. It is not affected by acute variation of Mg levels although it can be affected in chronic Mg deficiency. The second form exists on the surface of the apatite crystals and it is easily interchangeable.

Muscle: This represents 25% of total magnesium There is less magnesium in cardiac muscle when compared to the skeletal muscle.

Physiological functions : More than 300 enzymes depend on Mg^{++} especially those related with energetic metabolism. Mg^{++} plays an important role in reactions that involve ATP and in the hydrolysis and transfer of organic phosphate groups. It plays a pivotal role in the metabolism of carbohydrates, fats and proteins. Magnesium has an important interactive role influencing the intra and extra cellular balance of other ions in particular K^+ and Ca^{++}.

Magnesium is labelled as the nature's calcium blocker. Intracellular binding and membrane transport of Ca^{++} are influenced by the free Mg^{++} concentration. Hypomagnesemia causes a rise in free cytosolic calcium concentration. This is due to enhanced calcium permeability via the calcium channels and inhibition of calcium efflux due to reduced activity of the membrane bound Ca^{++}-ATPase pump mechanism responsible for the extrusion of calcium from the cell. Low intra cellular magnesium enhances the calcium binding to the troponin on the contractile proteins and initiates actin-myosin interaction which leads to muscle contraction. The higher concentration of intracellular calcium may lead to enhanced extrusion of calcium

from the cell via Na^+/Ca^{++} exchange pore which is not ATP dependent. Enhanced Na^+ influx due to increased Na/Ca exchange along with a reduction in Na/K ATPASE activity increases the electrical instability of the membrane and may lead to cardiac arrhythmias..

Myocardial Magnesium: Rogers and Mahn reported that in the exchange of plasma magnesium with tissue magnesium there are rapidly and slowly exchangeable forms of magnesium in the tissues of rats. In the heart, liver and kidneys the exchange was rapid reaching equilibrium in 3 hours. The rate of exchange is independent of rate of contraction or external work done by the ventricle. Most of the exchangeable magnesium is present as Mg complexes of adenine nucleotides ATP, ADP and AMP. Less than 15% is associated with mitochondria or myofibrils

In vivo myocardial cells accumulate a proportional increase in Mg^{++} in response to stimuli that cause cellular hypertrophy. Under such conditions there is also a disproportionate increase in sequestered

myofibrillar Mg^{++}. Polimeni and Page comment that a constant cellular Mg^{++} is essential to the myocardial cell. They observed that since a major proportion of Mg is complexed with adenine nucleotides, it may be the constancy of magnesium concentration that is related to the constancy of adenine nucleotide concentration which is necessary for normal myocardial cellular metabolism including ion exchange and energy production.

Uptake of Mg in the heart is 10 times that of the skeletal muscle. The greatest avidity was found in the IVS and in the Left Ventricle. The highest uptake in the septum might reflect the requirements of the conduction systems for impulse transmission.

The amount of myocardial magnesium might be the factor that determines the cardiac response to the many cardiopathic factors in environment. Dietary imbalances that increase the Mg requirement have been shown to lower the myocardial magnesium levels. In experimental animals both short term and long term magnesium deficiency has produced arrhythmias, Coronary

artery lesions and microscopic evidence of damage to the myocyte. Catecholamines, steroids and drugs that cause further loss of magnesium particularly when associated with retention of calcium exaggerate the cellular damage.

There are several clinical conditions associated with cardiac abnormalities that resemble those produced by experimental Mg deficiency. It has been demonstrated that loss of magnesium from the myocardial cells leads to ultrastructural changes such as enlargement and vacuolization of sarcoplasmic reticulum, mitochondrial degeneration which leads to a deranged cardiac metabolism resulting in myocardial degeneration, heart failure or fatal cardiac arrhythmias.

Apart from various factors which are known to be associated with dilated cardiomyopathy Hudson et al found that Magnesium deficiency can lead to a clinical syndrome of dilated cardiomyopathy. Cadell suggested that the

prolonged electrolyte deficiency particularly magnesium due to chronic malnutrition might be an important contributing factor in the pathogenesis of endo myocardial fibrosis.

In an attempt to correlate geochemical factors with cardiac tissue changes it has been hypothesized that hypomagnesemia with excessive thorium may play a role in the causation of endomyocardial fibrosis. The binding capacity of thorium towards the phosphate of the membrane phospholipid is very high. Thorium ion could replace or displace Mg ions which play a key role in the mechanism of myofibrillar contraction and relaxation. In effecting the ionic shift it is possible that strong electropositivity overcomes the possible mass retraction of thorium and enables the element to retain its chemical reactivity. The resulting conformational changes in the membrane phospholipid would be irreversible and could impair cardiac contractility and induce structural changes leading to EMF.

MATERIAL AND METHODS

The aim of the present study is to see whether the patients with EMF have an inherent deficiency of Magnesium in the serum when compared to the controls and rheumatic heart disease patients.

Serum and RBC magnesium levels are estimated by atomic absorption spectrophotometry in age and sex matched population.

The diagnosis of EMF is established by the clinical findings, x-Ray, ecg, echocardio gram and in some cases by cardiac catheterization A total of 21 patients with EMF (M11, F10) were included to estimate the serum magnesium levels.

The diagnosis of rheumatic heart disease was established by the clinical history associated cardiac findings and with the help of XRay, ECG and 2D echocardiogram. RHD group Comprised of 11 patients (5M, 6F)

Blood samples were collected from 23 (M14, F9) healthy volunteers which served as control.

Sample collection : Venous blood samples were collected in the morning after an overnight fasting in all the patients and controls. All the patients were asked to stop all the medicines 48 hours prior to the venous sampling. Magnesium levels are estimated on the same day

Serum was separated by allowing the blood to clot and settle for 3 to 4 hours, followed by centrifugation of clotted blood at 2000 RPM for 15 mts. The serum was pippetted out into a separate test tube. 1ml of serum was diluted with 100ml of triple deionised water for estimation of serum magnesium

For the collection of RBC 1ml of heparinized blood was centrifuged at 3500 RPM and the supernatant clear plasma was discarded. The cellular elements are washed three times with normal saline. The separated RBC pellet was dried overnight in autoclave and the weight of the pellet was measured. Weighed RBC pellet was dissolved in concentrated HCl (4ml). Once the whole pellet was completely dissolved 1ml of sample is diluted with 100ml of deionised water for the estimation of Mg.

Atomic Absorption Spectrophotometry.

Instrumentation laboratory Inc. 551 atomic absorption instrument was used under the standard operating conditions.

Light Source	:	Hollow cathode.
Lamp Current	:	3mA.
wave length	:	285.2 nm.
Slit width	:	320 µm.
Flame	:	Air Acetylene.

The samples were aspirated into the acetylene flame for determining the magnesium levels.

RESULTS

Estimated Serum magnesium values of the three groups of patients are shown in table one. Serum levels of magnesium in the control ranged from 1.20 to 1.70 m.eq/L (mean 1.48 ± 0.095). Magnesium levels in EMF group of patients ranged from 0.85 to 1.97 m.eq/L (mean 1.47 ± 0.136) and in rheumatic heart disease patients it ranged from 1.37 to 1.65 m.eq/L (mean 1.51 ± 0.22).

Table two shows the estimated Serum magnesium according to the age and sex in all the three groups. The mean value of Serum magnesium for the control is 1.47 m.eq/L in both Sexes. In the EMF group of patients the mean value of Serum magnesium in the male group is 1.54 m.eq/L and in female group it is 1.47 m.eq/L. In rheumatic heart disease group the mean Serum Mg in male group is 1.53 m.eq/L and in the female group it is 1.45 m.eq/L.

Of the 21 patients in the EMF group 8 patients were on Dytide (Triamterene + Benzthiazide) 11 patients were on Frusemide, one patient was on both the drugs and one patient was not on any drugs.

Table 3 Shows the estimated magnesium values in the Group of patient who are on diuretic and those who are on frusemide. Mean value of Serum magnesium in the Group on diuretic is (1.54 ± 0.12 m.eq/L) marginally higher than the group on furesemide (1.42 ± 0.23 m.eq/L) but it is of no Statistical Significance.

Table 4 Shows the estimated magnesium values in the RBC of Controls, EMF and Rheumatic heart disease patients. The mean RBC magnesium in the Control is 88.69 ± 55.7 µg/gm of RBC, in the EMF Group of patients it is 114.76 ± 103.54 µg/gm and in the RHD patients the mean value is 148.25 ± 122.58 µg/gm.

Table 1. Serum Mg. levels in M.eq/l in EMF, RHD and Controls.

Serial No.	Endomyocardial fibrosis	Rheumatic Heart Disease	CONTROLS.
1	1.4	1.38	1.61
2	1.69	1.52	1.44
3	1.97	1.50	1.48
4	1.58	1.58	1.29
5	1.37	1.40	1.39
6	1.25	1.65	1.20
7	1.60	1.48	1.50
8	1.56	1.46	1.45
9	1.67	1.60	1.30
10	1.42	1.37	1.33
11	1.46	1.40	1.65
12	0.85		1.56
13	1.40		1.65
14	1.70		1.49
15	1.63		1.56
16	1.48		1.51
17	1.52		1.70
18	1.70		1.35
19	1.34		1.43
20	1.44		1.60
21	1.60		1.43
22			1.45
23			1.64

S.No.	Disease									
	MALE		FEMALE		MALE		FEMALE		MALE		FEMALE.	
	Age	Mg	Age	Mg	Age	Mg	Age	Mg	Age	Mg	Age	Mg.
1	15	1.69	28	1.4	19	1.58	28	1.38	32	1.61	35	1.3
2	21	1.97	37	1.58	22	1.65	23	1.52	20	1.44	27	1.33
3	27	1.56	22	1.37	20	1.48	37	1.50	22	1.48	37	1.49
4	20	1.67	24	1.25	22	1.60	18	1.40	28	1.29	28	1.66
5	20	1.42	27	1.60	27	1.37	32	1.46	18	1.39	30	1.51
6	30	1.46	30	1.40			17	1.40	27	1.20	25	1.35
7	20	0.85	14	1.63					25	1.50	18	1.43
8	34	1.7	22	1.52					32	1.45	21	1.64
9	12	1.48	29	1.34					35	1.56	30	1.60
10	29	1.70	14	1.60					15	1.55		
11	14	1.44							28	1.70		
12									29	1.43		
13									20	1.45		
14									32	1.65		

Table 3. Serum mg (m.eq/L) in EMF as per the type of diuretic consumed.

	DYTIDE	FRUSEMIDE.
1	1.4	1.37
2	1.69	1.25
3	1.58	1.60
4	1.40	1.56
5	1.70	1.67
6	1.63	1.46
7	1.52	0.85
8	1.44	1.4
9		1.48
10		1.70
11		1.34
	1.54±0.12	1.42±0.23

Table 4. RBC Mg levels (ug/gm/lit) in Controls, EMF and RHD patients.

S. NO.	Controls	EMF.	RHD.
1	24	52	68
2	30	23	130
3	48	48	85
4	41.2	139	249
5	81.9	112.3	26.2
6	163	228	49
7	177	74	445
8	18	42.8	165
9	124	45	151.8
10	108.2	52	113.5
11	129	267	
12	120	48	
13		103.7	
14		106.7	
15		116.8	
16		76.4	
17		97.	
18		442	
Mean	88.69 ± 55.7	114.76 ± 103.54	148.25 ± 122.58

DISCUSSION.

The etiology of tropical endomyocardial fibrosis is not known but seems related to the tropical environment. The similarities in pathology of Endomyocardial fibrosis to the Loeffler's hypereosinophilic disease suggests that some of eosinophil may be common to both disorders. Profuse eosinophilia is seldom demonstrated in EMF unlike the Loeffler's disease. Immunological studies suggest that the inhabitants prone to develop EMF differ in their immunolo gic makeup from those who are less susceptible. A significantly elevated serum IgE levels in patients with EMF has been demonstrated earlier. The intestinal parasitism and filariasis could be the inducers of elevated serum IgE. High levels of antinuclear antibodies and antibodies to thyroid and gastric cell mucosa have been demonstrated. However these immunological patterns may be the result of infection and may not be etiologically important. It has also been suggested that EMF may represent an immunological response to streptococcal infection in individuals who are prone to malaria; although the nature of the relationship is unclear. Other hypotheses incriminated infection with viruses, filaria and ingestation with Loaloa.

EMF has been shown to occur in tropical countries and in India it is confined to the Kerala State. All the tropical countries from where EMF has been reported e located within 12° of latitude on either side of the equator in different continents. It is also an observation that te majority of the patients suffering from EMF are from ow socio economic group who will have associated features g malnutrition. Cadell hypothesized that EMF in Afriza is prevalent in areas where Protein Calorie malnutrition associated with magnesium deficiency is found and her suggestion that prolonged electrolyte imbalance might be contributory to the pathogenesis of EMF.

Earlier Valiathan et al have demonstrated an excess of thorium, Sodium and calcium and a deficiency of magnesium in the tissue samples obtained from EMF patients and speculated that an excess of thorium along with hypomagnesemia may play a role in the causation of EMF.

Hypomagnesemia has been noted to occur in various clinical disorders which include idiopathic cardiomyopathy, Peripartum cardiomyopathy

alcoholic cardiomyopathy, ischemic heart disease, Mitral valve prolapse and various cardiac arrhythmias. The prevalence of hypomagnesemia in congestive heart failure is as high as 50%. Long term diuretic therapy, Secondary hyperaldosteronism, poor nutrition and digoxin therapy which accompany the heart failure can independently contribute to the low serum magnesium levels. Concomitant conditions like Vomiting, diarrhea, alcoholism also lower the Mg^{++} levels. The type of diuretic also makes a difference. Thiazides and loop diuretics produce greater magnesuria than the potassium sparing diuretics like triamterine, Spironolactone which also spare magnesium.

Different thresholds have been used to define hypomagnesemia ranging from 1.2 to 1.4 m.eq/L the mean values differ from one laboratory to other. We determined the serum and RBC magnesium levels of 23 controls, 21 EMF patients and in 11 patient of rheumatic heart disease. we did not find any difference in the serum or RBC levels of magnesium in patients of EMF when compared with the controls.

CONCLUSIONS

In this study of small group of patients no significant difference was observed in the mean serum magnesium levels between the patients with EMF, Rheumatic heart disease and the controls. RBC and Serum magnesium levels are poor indicators of total body magnesium stores as they are influenced by various other factors. Skeletal muscle magnesium and WBC magnesium are better indicators of tissue levels of magnesium than serum or RBC levels of magnesium.

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