

ASSOCIATION OF MAGNESIUM LEVELS WITH LIPID PROFILE AND INFLAMMATORY MARKERS IN THIAZIDE INDUCED HYPOMAGNESEMIC RATS

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ABSTRACT

Objective: To study the effect of Magnesium supplementation on lipid profile (TC, HDL, LDL, TG, VLDL) and inflammatory markers (CRP s-VCAM 1) in thiazide administered rats.

Study Design: Randomized control trial.

Place and Duration of Study: Department of Biochemistry & Molecular Biology, Army Medical College Rawalpindi from Feb 2010 to Feb 2011.

Material and Methods: Ninety male Sprague Dawley rats, of age 90 to 120 days, divided into three groups: Control, Hydrochlorothiazide (HCTZ) administered control and Hydrochlorothiazide + Magnesium Oxide (HTCZ + Mg²⁺), administered experimental groups. Levels of inflammatory markers, Magnesium, lipid profile were measured in blood, after three weeks.

Results: Mean values in control group were: Serum TG 104.39 mg/dl, TC 151.86 mg/dl, HDL 76.91 mg/dl, LDL 52.52 mg/dl, VLDL 19.78 mg/dl, CRP 1784.27 mg/L, sVCAM-1 564.33 ng/ml and serum Magnesium 0.85 mmol/l. Mean lipid profile values in HCTZ administered control group were Serum TG 150.04 mg/dl, TC 182.92 mg/dl, HDL 71.77 mg/dl, LDL 76.40 mg/dl, VLDL 32.56 mg/dl, CRP 2923.33 mg/L, sVCAM-1 2003.00 ng/ml and serum Magnesium 0.49 mmol/l. Mean lipid profile values in HCTZ + Magnesium administered experimental group were Serum TG 131.69 mg/dl, TC 157.60 mg/dl, HDL 73.55 mg/dl, LDL 58.2 mg/dl, VLDL 25.05 mg/dl, CRP 2149.53 mg/L, sVCAM-1 1516.60 ng/ml and serum Magnesium 0.68 mmol/l.

Conclusion: Levels of sVCAM-1, CRP, TG, TC, LDL and VLDL are increased in HCTZ administered group, with negative correlation with serum magnesium levels. Levels of these parameters decreased in HTCZ + Mg²⁺ administered group, showing that magnesium supplementation helped in normalizing derangements ($p < 0.05$).

Keywords: CRP, Hyperlipidemia, Inflammation, Magnesium, sVCAM-1.

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INTRODUCTION

The most prevalent type of hypertension is Primary hypertension¹. Though no direct cause has been identified, many factors such as sedentary lifestyle, stress, potassium deficiency can be responsible². Obesity³, kidney diseases and metabolic disorders can cause secondary hypertension⁴. There has been 40% reduction in risk of stroke and a 15% reduction in risk of myocardial infarction by treatment of

hypertension⁵.

A thiazide-type diuretic is usually prescribed as first-line pharmacotherapy⁶. For long term treatment of hypertension, thiazides are often indicated⁷. This helps in reducing BP and cardiovascular events⁸. Use of hydrochlorothiazide, can lead to several unfavorable effects such as electrolyte disorders (hyponatremia, hypokalemia and hypomagnesemia), hyperlipidemia, hyperuricemia and impairment of glucose metabolism⁹.

The main intracellular divalent cation is Magnesium¹⁰. involved in a large number of biochemical processes¹¹. Normal serum

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magnesium has the range between 1.7 to 2.3 mg/dl (0.75-0.95 mmol/L)¹². Hypomagnesaemia is associated with considerable morbidity¹³. More recently, Mair et al discovered that low Mg²⁺

control group (n=30) and, provided with standard diet and water ad libitum, Group-2 (n=30) rats, administered with Hydrochlorothiazide (10mg/kg body weight) by

Table-1: Mean levels of lipid profile, inflammatory markers and serum magnesium in all the groups.

Parameters		Sample Size	Group 1	Group 2	Group 3
			Mean ± SD	Mean ± SD	Mean ± SD
Lipid Profile	Serum TG (mg/dl)	30	104.39 ± 8.55	150.04 ± 14.00	131.69 ± 7.77
	Total Cholesterol (mg/dl)		151.86 ± 14.53	182.92 ± 6.22	157.60 ± 6.05
	HDL (mg/dl)		76.91 ± 4.90	71.77 ± 3.59	73.55 ± 3.90
	LDL (mg/dl)		52.52 ± 4.47	76.40 ± 5.43	58.25 ± 4.07
	VLDL (mg/dl)		9.78 ± 2.39	32.56 ± 4.31	25.05 ± 4.09
Inflammatory Markers	sVCAM-1 (ng/ml)		564.33 ± 67.91	2003.00 ± 927.92	1516.60 ± 300.36
	CRP (ng/ml)		1784.27 ± 731.97	2923.33 ± 702.37	2149.53 ± 566.61
Serum Mg ²⁺	Serum Magnesium (mmol/l)		0.85 ± 0.11	0.49 ± 0.13	0.68 ± 0.10

Table-2: Pearson's product moment correlation between serum magnesium levels and various markers of lipid profile and inflammation of HCTZ + Mg²⁺ administered group-3.

Pearson's product moment correlation			
Correlation Between		Correlation Coefficient "r"	
		Positive	Negative
Serum TG	Serum Magnesium		-0.257
Total Cholesterol			-0.241
HDL		0.109	
LDL			-0.112
VLDL			-0.199
sVCAM-1			-0.155
CRP			-0.051

concentrations influence the inflammatory response and affect the endothelial proliferation, by up regulation of interleukin-1 (IL1) and sVCAM-1 (pro inflammatory cytokines)¹⁴. People who consume only slightly below the RDA of Mg²⁺ had a higher likelihood of elevation of CRP¹⁵. Studies demonstrate that increased intake of Mg²⁺ in diet may lower blood triglyceride and increase high density lipoprotein (HDL) levels¹⁶.

MATERIAL AND METHODS

It was a randomized control trial and duration of study was two years. Ninety Sprague Dawley rats were selected by purposive sampling. They had a weight 220 ± 30g and were kept at animal house of AMSON VACCINES & PHARMA, Pvt. (Ltd). They were divided, through random number table, into Group 1:

drenching through gavage needle, given standard diet and water ad libitum and Group 3 (n=30), administered with Hydrochlorothiazide (10mg/kg body weight) and Magnesium Oxide supplemented diet (1.075mg/kg body weight/day) with water ad libitum, for three weeks. Blood samples were drawn by intracardiac sampling. Levels of inflammatory markers were determined by ELISA and levels of serum Magnesium and lipid profile, by Automated analyzer (SELECTRA), using specific kits.

1.C - Reactive Protein was measured by using R&D Systems, Inc, USA. Quantikine® Human C-Reactive protein Immunoassay, Catalog No. DCRP00. Assay was performed on Awareness Technology Inc, USA, Complete system (Shaker incubator Stat Fax 2200,

automated washer Stat Fax 2600 & Microplate reader Stat Fax 2100).

100 μ l of Assay Diluent RD1F, was added to each well. We added 50 μ l of standard, control,

Incubated for 1.5 hours at room temperature, washed, with Wash Buffer (400 μ l) using an Automated washer. We added 100 μ l of Substrate Solution to each well, covered and incubated.

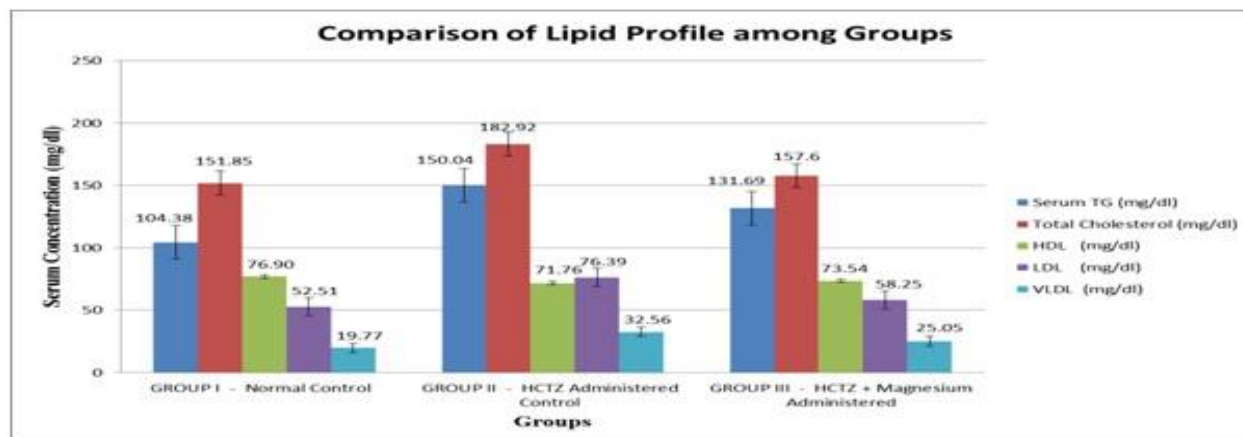


Figure-1: Comparison of lipid profile among the two controls and one experimental group.

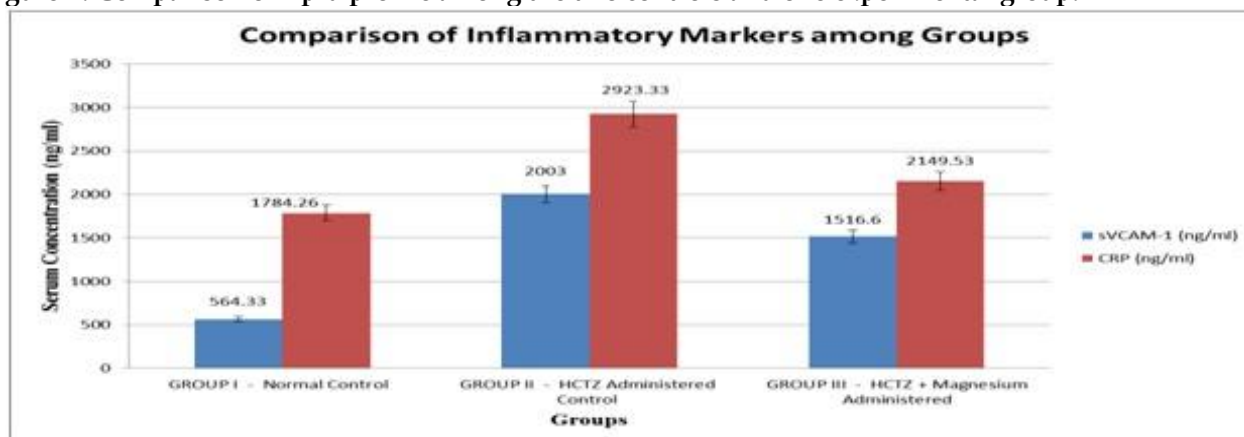


Figure-2: Comparison of inflammatory markers among the two controls and one experimental group.

sample per well. Wells were covered, incubated and washed. 200 μ l of CRP Conjugate was added to each well. It was covered and incubated. Added 200 μ l substrate solution and incubated for 30 minutes. Added 50 μ l stop solution. Color in wells changed to yellow. We determined the optical density, at 450 nm.

Soluble Vascular Cell Adhesion Molecule Type-1 was measured by (ELISA), using R&D Systems, Inc, USA. Quantikine® Human sVCAM-1 Immunoassay, Catalog No. SVC00. We added 100 μ l of sVCAM-1 Conjugate to each well and the added 100 μ l of standard, control and sample per well. Covered with the adhesive strip.

Added 50 μ l of Stop Solution to each well. Color in wells changed from blue to yellow. We determined optical density, at 450 nm.

For estimation of Total Cholesterol commercially available kit manufactured by Pioneer Diagnostics, New York, U.S.A. was used. Three test tubes, labeled blank, standard and sample, with one ml of monoreagent in each tube. 10 μ L of distilled water, 10 μ L of standard and 10 μ L of sample were added to each test tube respectively. Mixing and incubation for 5 minutes at 37°C was done. The results of standard and samples were measured at 500 nm wavelength against reagent blank by Selectra.

Estimation of TG was done, using Kit (Ready to use) by Pioneer diagnostics New York, U.S.A.

Three test tubes, labeled as blank, standard and sample, with 1 ml of monoreagent, in one tube. 10 μ L of distilled water, 10 μ L of standard and 10 μ L of sample were added by micropipette in each test tube respectively. Thorough mixing and incubation for 5 minutes at 37°C. Results of standard and samples measured at 500 nm wavelength against reagent blank by Selectra. Absorbance (A) of the sample and standard was read.

$A \text{ (sample)} / B \text{ (standard)} \times C \text{ (standard)} = \text{mg/dl Triglycerides.}$

Estimation of HDL-c was done by kits prepared by Linear Chemicals, Barcelona (Spain), direct enzymatic method. 200 μ L sample and 500 μ L precipitant were mixed, centrifuged for 10 minutes at 4000 r.p.m. Supernatant separated and cholesterol measured by CHOD-PAP method.

Estimation of (LDL) & VLDL was measured using Friedwald's formula.

$LDL = \text{Total Cholesterol} - \text{HDL} - \text{Triglycerides} / 5$

$VLDL-c = \text{Triglycerides} / 5$

Statistical Analysis

Data was entered in a database using SPSS version 16. Values were expressed as mean and standard deviation (SD). Independent Sample t-test was applied. A *p*-value of <0.05 and <0.01 was considered significant and highly significant respectively. Pearson's product moment correlation (*r*) was applied.

RESULTS

The mean values of lipid profile (serum TG, Total Cholesterol, HDL, LDL, VLDL), inflammatory markers (sVCAM1, CRP) and serum Magnesium in all three groups are shown in table-1.

Lipid Profile of control group 1 was compared with HCTZ administered control group 2 by using student's t-test. There was a

highly significant difference between the levels of Triglyceride (*p*<0.001), Total Cholesterol (*p*<0.001), HDL (*p*<0.001), LDL (*p*<0.001) and VLDL (*p*<0.001) among both the groups. Diseased control group 2 was also compared with Experimental group 3 by using student's t-test. There was a highly significant difference between the levels of Triglyceride (*p*<0.001), Total Cholesterol (*p*<0.001), LDL (*p*<0.001) and VLDL (*p*<0.001) among both the groups and an insignificant CRP (difference between HDL (*p*=0.228) - Fig-1. State of inflammation for control group 1 was compared with diseased control group 2 by using student's t-test. There was a highly significant difference between the levels of sVCAM - 1 (*p*<0.001) and insignificant difference between CRP (*p*=0.094). Diseased control group 2 was also compared with Experimental group 3 by using student's t-test. There was a significant difference between the levels of sVCAM-1 (*p*=0.004) and highly significant difference between *p*<0.001) fig-2. Pearson's product moment correlation (*r*) was applied to assess the correlation between parameters of serum magnesium levels, lipid profile and inflammatory markers in the experimental HCTZ + Mg²⁺ administered group. A positive and significant (*r*=0.109) correlation was found between Magnesium levels and HDL whereas negative and significant correlation was found between Magnesium levels and TG (*r*=-0.257), TC (*r*=-0.241), LDL (*r*=-0.112) and VLDL (*r*=-0.199). A negative and significant (*r*=-0.155) correlation was found between Magnesium levels and sVCAM -1 and CRP (*r*=-0.051) table-2.

DISCUSSION

In our study serum levels of Magnesium in the control group were in the range of 0.61-1.1mmol/l, with a mean value of 0.85 as compared to the range of 0.24-0.84mmol/l, with a mean value of 0.49 in the HCTZ administered control. There was a significant decrease in serum Magnesium levels between both groups. This fact is supported by John W in his study which shows that elderly hypertensive patients are at

particular risk because of their tendency to have significantly depressed serum magnesium levels, which decrease even further when treated with thiazide diuretics¹⁷. There was a significant decrease in serum Magnesium levels between both groups. Animal studies found that serum triglycerides were significantly higher and HDL cholesterol levels were significantly lower with magnesium deficiency¹⁸. Magnesium administration in our study resulted in the lowering of serum TG, TC, LDL, VLDL and increase in the levels of HDL. Acute magnesium deficiency leads to an inflammatory response¹⁹. Several cross-sectional studies have reported a link between both low dietary magnesium intake²⁰, and serum magnesium concentrations²¹ and elevated CRP. With regard to endothelial dysfunction, it was observed that dietary magnesium was inversely associated with plasma concentrations of sVCAM-1 independently of other dietary factors. Levels of inflammatory markers were also decreased in group 3 as compared to the HCTZ administered group, in our study.

CONCLUSION

This study demonstrates a significant decrease in inflammatory markers and lipid profile in association with serum magnesium levels.

CONFLICT OF INTEREST

This study has no conflict of interest to declare by any author.

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