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## Research Article

# Assessment of Serum Magnesium Level and Its relation with Atherosclerotic Carotid Intima Media Thickness in Post-Menopausal Women

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## ABSTRACT

**Objective:** A magnesium deficiency is known to be involved in many abnormalities in the post-menopausal women. Previous studies proposed that magnesium (Mg) might prevent atherosclerosis. In this study the efficacy of serum magnesium level and its relation with carotid intima media thickness (CIMT) in post-menopausal women was evaluated. **Methods:** Eighty Iranian Postmenopausal women were included in final analysis. The serum Mg level was evaluated. The CIMT was assessed bilaterally using an ultrasonography in the 4 points at 2 cm proximal to the bulb, the 4 points at 2 cm distal to the bulb and 2 points at the carotid bulb and averaged to obtain the mean IMT. A thorough history of age and duration of menopause was taken from each patients. Data was analysed by SPSS software. **Results:** In all the patients of case group, a significant increase in intima media thickness of common carotid artery was observed ( $p \leq 0.001$ ). Increased intima media thickness was associated with reduced serum level of Mg. **Conclusion:** This study shows that lower serum level of Mg are associated with higher CIMT in postmenopausal women. Nutritional status of the customary high intake of the marginal Mg might well be contributory to prevent the adverse effects of cardiovascular diseases. Therefore, patients with lower serum level of Mg may have a high risk of increased CIMT.

## Introduction

Atherosclerosis is a focal disease that constricts some areas of affected vessels and is a fundamental pathologic change of cardiovascular diseases (CVD). It implicates the systemic condition with increasingly abnormalities in many regional circulations such as the brain, kidneys, and limbs especially in women (Ross, 1986). Cardiovascular diseases are major causes of morbidity

and mortality in the elderly (Braithwaite et al, 2003; Wenger, 2003). These events are expected to rise in upcoming years owing to the increasing longevity of women in most of the industrialized countries of the world, especially in the United States (Soldo et al, 1988). Atherosclerosis would be the number one cause of death worldwide (Murray et al, 1997). Considerable programs has been developed for prevention and treatment of the clinical symptoms of atherosclerosis (Kavey et al, 2003).

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Prevention of atherosclerosis is paramount to the health of every woman and has caused severe financial burden on every nation (Manson et al, 2002). The cardiovascular events (CV) is a multi factorial diseases and may be due to risk factors including high blood pressure, cholesterol, overweight, lack of physical activity and diabetes (Knopman et al, 2001). It is reported that high blood pressure is a major risk factor among the elderly, particularly for the increased arterial stiffness (O'Rourke, 1990). The identification of high risk individuals by different independent predictors of cardiovascular risk factors along with timely referral is urged as more than two-thirds of women who suddenly die of cardiovascular events had no prior sign of the disease (Mathews et al, 1989).

Menopause is a risk factor for cardiovascular disease because estrogen withdrawal has a harmful effect on cardiovascular function and metabolism (Shlipak et al, 2000). Estrogen is believed to have a beneficial effect on the inner layer of artery wall to keep blood vessels flexible. Decrease in the natural hormone level of estrogen, is considered to be a substantial risk factor in CVD among post-menopausal women (Manson et al, 2003).

Magnesium is the fourth most abundant mineral found in the body which is essential for good health. Approximately 50% of its total in the body is found in bone. The other half is found predominantly inside cells of body tissues and organs. Mg is needed for more than 300 biochemical reactions in the body. It also helps regulate blood sugar levels, promotes normal blood pressure, and is known to be involved in energy metabolism and protein synthesis. There is an increased interest in its role in preventing and managing disorders such as hypertension, cardiovascular disease, and diabetes. Magnesium is one of the natural mineral that been reported to be related to risk factors associated with many aspects of the CVD (Arsenian, 1993). Magnesium is found in whole grains, green leafy vegetables and nuts. It is a critical cofactor for hundreds of enzymes and is reported to be a natural antagonist of intracellular calcium, affecting many aspects of the cardiovascular system. Moreover, magnesium may have a myocardial protective role (Chakraborti et al, 2002). It seems that Magnesium supplementation improves myocardial metabolism, inhibits calcium accumulation and myocardial cell death. Mg was reported to improve vascular tone, peripheral vascular resistance, after load and cardiac output, reduces cardiac arrhythmias and improves lipid metabolism (Hoorn et al, 2010). Several studies reported that lower serum level of Mg or

hypomagnesaemia found commonly in elderly hospitalized patients were suffering from coronary artery disease (Longo et al, 2001). Mg in the In vitro animal studies showed to prevents atherosclerosis by maintaining endothelial function and improving the lipid profile (Altura et al, 1990; Maier et al, 2004). Hypomagnesaemia has been found to be associated to the bowel disease, diabetes mellitus and metabolic syndrome. It appears that inappropriate medication use such as taking proton pump inhibitors is associated with Hypo-magnesia in elderly patients (Hoorn et al, 2010).

Carotid intima-media thickness (CIMT) is an indicator that has been proposed to be added to measure cardiovascular risk factors to improve risk prediction and individual risk assessments (Meinzer et al, 2006, Xie et al., 2011). Carotid artery disease has a major role in the etiology of stroke and CVD. Carotid ultrasound has been widely used to detect carotid atherosclerosis by measuring CIMT and carotid plaque (CP). CIMT and plaque together has been reported as imaginary biomarkers of subclinical atherosclerosis (Spence et al, 2002). In addition carotid atherosclerotic plaque, reported to be a powerful non-invasive imaging tool for vascular risk estimation, and stronger predictor for future ischemic stroke (IS) than CIMT (Mathiesen et al, 2011; Wannarong et al, 2013). Previous epidemiologic studies suggested that serum Mg concentration was inversely associated with general atherosclerosis, as indicated by CIMT (Kupetsky-Rincon et al, 2012).

In one of the study, relation between Mg-CIMT has not been fully assessed in healthy individuals and the findings in dialysis patients cannot be generalized to general population. To our knowledge, only a cross sectional study along with Atherosclerosis Risk community (ARIC) study has reported this association of higher serum Mg levels to be inversely associated with CIMT in the general population (Hashimoto et al, 2010; Ma et al, 1995). In ARIC research study, the relationship between Mg levels with CIMT (Ma et al, 1995) and ischemic stroke (Ohira et al, 2009) were reported to be increased among Whites than Blacks, while data in relation to Iranian women has not been assessed. Moreover, it was reported that dietary Mg was associated with higher risk of CHD mortality in Chinese women with a lower dietary calcium/Mg ratio (Dai et al, 2013).

Due to ethnic and environmental factors, Mg-atherosclerosis association may be modified; so many studies are to be conducted in a variety of populations to get the final assessment on this issue. Few studies and not much work about the role of magnesium-

atherosclerosis in postmenopausal women has been done in Iran.

So we conducted this study to assess the hypothesis that serum magnesium level of the post-menopausal women can be related with carotid intima media thickness; as the consequences has not been clearly recognized in Iranian women. It would be useful to provide some clinical recommendations for the prediction of CVD and implement prevention programs for high risk post-menopausal women.

## **Patients and method**

### **Study Design and Population**

This study was conducted on 80 Subjects between November 2013 and November 2015 in the Kashani care center, Isfahan, Iran The study protocol was approved by ethical committee of Isfahan University of Medical Sciences and written informed consent was obtained from the participants.

### **Demographic characteristics and traditional risk factors**

Iranian Postmenopausal women included in this study, where those who had at least 1 year duration passed since the last menstrual period were included .Exclusion criteria included ; alcoholics, problems interfering with the normal activities, hyperparathyroidism, diabetes mellitus, chronic kidney or liver dysfunction and history of taking drugs affecting the atherosclerosis, history of heart failure or obvious heart disease.

The diagnoses were confirmed by radiological and laboratorial findings in patients with CVD sign and symptoms. Thereafter, the participants were classified as depending on radiological findings into healthy patients or with atherosclerosis and enrolled in the following two groups.

In addition, participant's demographic information such as age, length time of the menopause, clinical, risk factor and other manifestations were collected. These forms contain detailed questions in relation to family history, personal and medical history, and hormone and medications intake.

### **Laboratory Assay**

#### **Measurement of serum Magnesium**

A venous blood sample were obtained in the morning after a 12 h fast from each participant. Serum was obtained by centrifuging at 3000 rev/min for 10 min at 4 degree C and was separated, aliquots were quickly frozen and stored at -80 degree C within 2h until analysis was performed. The measurement of serum levels of the Mg

levels was based on the reaction of Mg with xylydyl blue in an alkaline solution using a commercials kits (Roche Diagnostics, Mannheim, Germany) with a co-efficient variance of 1.2% for human serum .Mg concentration was estimated photo metrically via decrease in the xylydyl blue absorbance of all the patients (Hashimoto et al, 2010).

### **Carotid ultrasound**

The carotid arteries IMT was assessed bilaterally by well trained technician using an ultra sonograph (Siemens, Sonoline G40, and Germany) equipped with a 7.5-MHz linear array type-B-mode transducer (GE-Yokogawa Logiq S6). Each participant has rested for a while in supine position, then her neck was slightly hyper extended and the head was turned away from the sonographer. .CIMT is used as an outcome measure in intervention trials and has recently been promoted as a method for assessing cardiovascular risk in individual patients in clinical practice. In order to obtain CIMT on each right and left side of the far wall of the common carotid artery was measured in the 4 points at 2 cm proximal to the carotid bifurcation, internal carotid artery in the 4 points at 2 cm distal to the carotid bifurcation and 2 points at the carotid bulb and the average measurements of the bilateral values was calculated to obtain the patients mean intima media thickness (Fig. 1). A carotid plaque was defined as a focal thickening  $\geq 1.2$ mm at the level of carotid artery.

### **Statistical analysis**

Mean and SD values were measured for control and experimented groups. For determination of statistical significance of differences in means between two independent groups, Student's t test was performed.

To adjust the effect of the age and times since menopause, co-variance test was performed. For correlations between variances, Pearson correlation coefficient was used. Probability (p) values were two-tailed. Statistical analysis was performed using SPSS software.

## **Results**

The characteristics of the study were 80 patients of which 40 were atherosclerotic cases and 40 controls patients were included in our analysis.

The mean age of the participants was 69.9 and the mean length of time of menopause in two groups was 28.45 as presented in Table 1 with demographic characteristics of subject's age period of menopause.

The independent t test elucidated mean age ( $p=0.001$ ) and the mean length of time from menopause ( $p=0.004$ ) between two groups were found to be statistically significant.

The mean age and the average length of time for menopause was not the same. Co- variance test was used to adjust the effect of age and length of the time from menopause and for comparing of Right IMT, Left IMT and serum level of Mg between the two groups (Table 1). With controlling of the age and the average length of time for menopause, the RTIMT ( $p<0.001$ ) and LTIMT ( $p<0.001$ ) and the Mg ( $p<0.001$ ) was higher and showed

to be more statistically significant than control group (Table2).

In the case group, Pearson correlation coefficient showed the reverse relationship between the serum level of magnesium and the average length of time from the period of menopause ( $p=0.007$   $r=-0.419$ ).but there was no apparent relationship between the two varieties in the control group ( $p=0.47$   $r=-0.0117$ ) (Table.2).

The Pearson correlation showed that there is no significant relationship in two groups between RTIMT, LTIMT and serum level of magnesium (Ttable3, Fig.2).

**Table.1:**

The mean age and duration of menopause in the postmenopausal women.

Variance	Control group	Case group	p- value
	SD Average	SD Average	
age	11.9 66.7	9.8 73.2	0.001
Duration of menopause	11.2 25.6	10.1 31.3	0.004

**Table.2:**

The mean of serum magnesium level and CIMT in two groups of postmenopausal women.

Variance	case group	control group	p-value
RTIMT	0.84±0.15	0.6±0.1	< 0.001
LTIMT	0.88±0.13	0.62±0.1	< 0.001
Mg	2.04±0.29	2.26 ±0.33	0.001

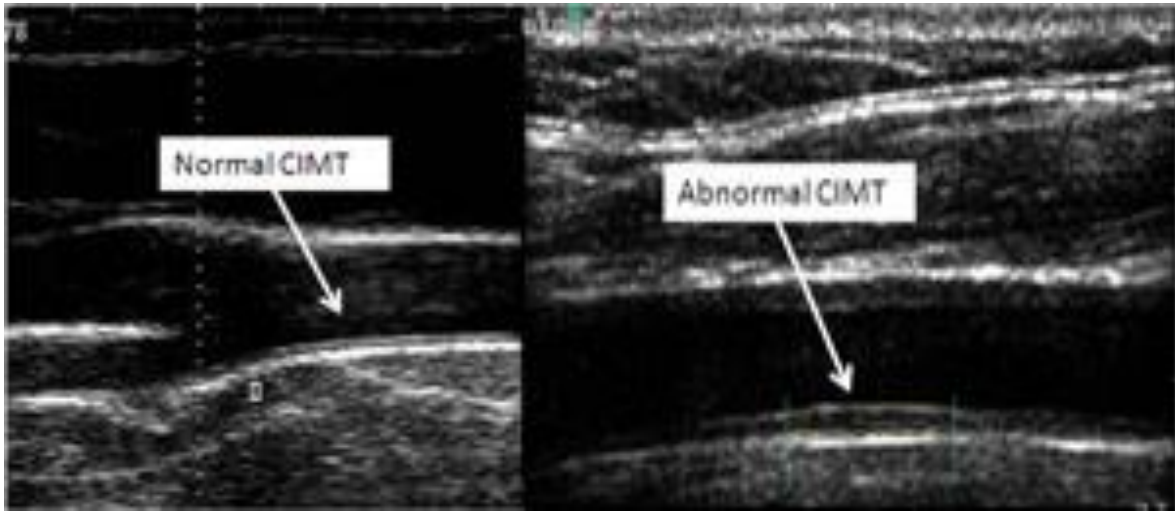
The Independent T-test showed that mean of RTIMT in case groups was statistically significant than control group ( $p<0.001$ ).

**Table .3:**

Pearson correlation between Mg, and CIMT in two groups.

	Case group	Control group
	Mg	Mg

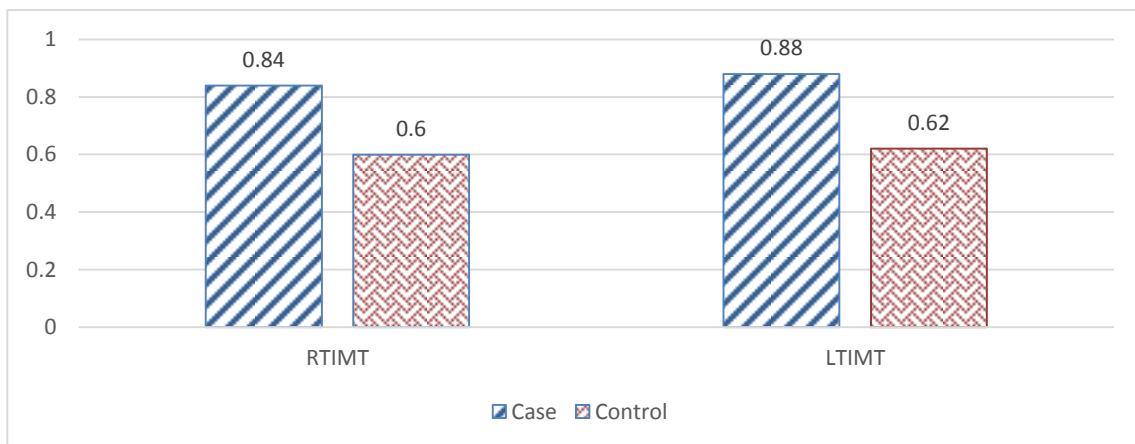
r	-0.123	0.19
RTIMT	0.45	0.24
P		
r	-0.06	-0.13
LTIMT	0.72	0.42
P		



A-control groups

B-case groups

**Figure 1:** CIMT of common carotid arteries in two groups of postmenopausal women.



**Figure 2:** Mean of RTIMT and LTIMT in two groups of postmenopausal women.

## Discussion

In the present study, it was found that age had a differential impact on the association between serum level of Mg and CIMT. The result of the present study was in agreement with a previous finding (Cao et al, 2015), table 1 and table 2 showed the apparent relationship between serum level of Mg and CIMT with age and statistical analyses revealed a significant interaction between serum level of Mg and CIMT with age. Subsequent mean age mean length of time from menopause between two groups of case and control were found to be statistically significant (Table 1). Overall the enzymatic processes using phosphorus as an energy source require magnesium for activation. At present, serum Mg is still the most commonly used parameter for assessing disorders of Mg metabolism in clinical practice (Reinhart 1998). The major sources of Mg in the food supply are dairy products (20%); grain products (18%); vegetables (16%); meat, poultry, and fish (15%); and legumes, nuts, and soya products (13%). Drinking water may also contribute up to 27% of total Mg intake in areas where the water is hard (Marier 1990).

Abnormalities of Mg levels, such as hypomagnesaemia, in nearly every organ system can cause some fatal complications such as ventricular arrhythmia, coronary artery vasospasm and with symptoms including growth failure, pallor, weakness, tremor, muscle and nerve irritability, electromyography changes, hypocalcaemia, and hypokalemia respectively. This abnormality is more important in Postmenopausal women. Mg deficiency in Postmenopausal women who have diabetes or in whom diabetes may develop are at higher risk for hip fracture than non-diabetic postmenopausal women (Lukaski et al, 2002). In post-menopausal women, Mg deficiency induces heart arrhythmias, impairs glucose homeostasis, and alters cholesterol and oxidative metabolism (Seelig 2012).

In the present study of the Iranian postmenopausal women, serum level of Mg was found to be inversely associated with CCA-IMT, which is an indicator of atherosclerosis. It was revealed that patients with increase serum level of Mg in control group had reduced risk of atherosclerosis showing normal CIMT. The CCA-IMT was significantly lower in the patients indicating higher serum level of magnesium. It was found that decrease serum level of Mg was associated with significantly increased CIMT values.

Our findings were in agreement with one community-based cross-sectional study in middle-aged and elderly Chinese people, Mg levels in serum and urine were found to be inversely associated with CCA-IMT, an indicator of

general atherosclerosis (Cao et al, 2015). In an animal experiments, Mg deficiency accelerated the atherosclerotic process (Altura et al, 1990) and orally administered Mg suppressed the development of atherosclerotic lesions (Altura et al, 1990; Orimo et al, 1990; Saito et al, 1990). In a cross-sectional study, the mean level of serum Mg was found to be inversely associated with age-adjusted average CIMT in women and White men but not in Black men (Folson et al, 1995). In a Japanese study, the mean CCA-IMT was significantly lower in patients with higher serum level of magnesium (Hashimoto et al, 2020).

The results of the present study, consistent with the previous studies, found that the mean level of serum Mg was inversely associated with age-adjusted average CIMT in Iranian postmenopausal women.

Although in one study of magnesium supplementation trial in middle aged and older American women, found no significant association between Mg intake and incidence of CVD, CHD or stroke. In contrast they suggested a lower risk for total stroke and ischemic stroke associated with larger intakes of Mg (Song et al, 2005). Several cohorts studies have prospectively examined the relation between dietary Mg intake and the risk for developing CHD in men and yielded mixed results (Abbot et al, 2003; Al-delaimy et al, 2004). Some animal studies, although not all, have suggested potential neuro-protective effects by Mg supplementation in rodent stroke models (Zhu et al, 2004). Overall, the evidence is not convincing because of sparse data, and further investigation in prospective studies with sufficient cases of incident stroke is warranted.

Serum Mg level is dependent on many varieties, for example the speed of blood flow, and serum level of calcium. It seems that, the role of Mg in CIMT might be controversial. Estrogen is associated with reduced subclinical atherosclerosis progression in healthy postmenopausal women. The pro-coagulant effect of estrogen is intensified by Ca; Mg—which counteracts many steps in the coagulation cascade and inhibits platelet aggregation and adhesion. Mg enhances estrogen's central nervous system protective effects which is important in improvement of cerebral blood flow (Chi et al, 1989). Low Mg levels was reported to be associated with a pro inflammatory state, oxidative stress, endothelial dysfunction and platelet aggregation, which may potentially accelerate the formation of plaque (Maier et al, 2004).

Considering the significantly inverse relationship of the serum Mg level with CIMT in postmenopausal women observed in our study, we suggest a protective role of Mg

in the process of atherosclerosis. Overall, the evidence is convincing about Mg favorable effect on atherosclerosis in terms of improving lipid metabolism. Further investigation in prospective studies are needed to elucidate the role of Mg in lipid metabolism.

### Conclusion

In the present study, measurement of average carotid intima-media thickness by B-mode ultra sound, an indicator of atherosclerosis, enabled us to examine the relationship of serum Mg level with early atherosclerosis in asymptomatic adults. Low serum Mg level seemed to be a risk factor for development of atherosclerosis. The associations of serum and dietary Mg with hypertension, diabetes, asymptomatic atherosclerosis, and CVD risk factors in these cross-sectional analyses cannot be inferred to be causal, as they may be biased due to non response or misclassification of variables. They also cannot clarify whether low serum Mg contributes to the etiology of these conditions or is a result of the diseases and their treatment.

While some race and gender inconsistencies remain to be explained, these data provide suggestive evidence of the role of Mg in atherosclerosis. While recommendations based upon data obtained from cross-sectional studies must be made cautiously. These results suggest that the clinical monitoring of Mg status among postmenopausal women with hypertension and CVD might be useful and foods rich in Mg (such as nuts, green vegetables, soya beans, and whole grains) may provide protection against these chronic diseases. In these consideration, this study suggests that the customary high intake of the Mg might well be contributory in the prevention and treatment of the adverse cardiovascular effects.

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