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Role of Magnesium Supplement on Hyperlipidemia and L-CAT Level in Patient on Atorvastatin Therapy

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Authors' contributions

This work was carried out in collaboration between all authors. Both authors are designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript and managed the analyses of the study and managed the literature searches. Both authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aim: This study aimed to evaluate the effect of magnesium supplement to atorvastatin on hyperlipidemic patients and to elucidate the possible ability of oral magnesium supplement to counteract or delay statins induced myalgia.
Study Design: Forty hyperlipidemic male and female patients were randomly divided into two groups: group one consisted of twenty patients, who received atorvastatin 10 mg once daily for 6 weeks then 20 mg once daily for another 6 weeks; group two consisted of twenty patients, who received the same dose of atorvastatin plus once daily oral low dose magnesium sulfate trihydrate 419.5 mg equivalent to 50 mg of magnesium.
Place and Duration of Study: The Laboratory of Pharmaceutical Research Center of Faculty of Pharmacy, Tanta University, Egypt, between July to December 2013.
Methodology: Two samples of venous blood (2 ml + 8 ml =10 ml total), were collected from all individuals, and were drawn from the antecubital vein before, 1.5 and 3 months after treatment. Sera and plasma were separated immediately for biochemical analyses of lecithin cholesterol acyltransferase (L-CAT) (ELISA), creatine kinase (CK), serum Ca^{+}, Mg^{++}, Na^{+}, K^{+}, lipid profile and aspartate transaminase (AST) (colorimetrically), and serum creatinine (S.Cr) spectrophotometrically.
Results: The statistical analysis revealed that, 3 months after treatment, both groups showed significant amelioration in lipid profiles and significant elevation in L-CAT level

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regarding to baseline data obtained before initiation of treatment. In addition, the patients received atorvastatin plus magnesium supplement showed significantly higher levels of serum magnesium, plasma L-CAT and HDL-cholesterol concentrations and significantly lower total cholesterol, LDL-cholesterol and triglycerides concentrations with non significant lower CK level as compared to the patients group received atorvastatin solely.
Conclusion: Mg^{++} supplement to atorvastatin improve all lipid profile and provide better control on dyslipidemia than atorvastatin alone. However, Mg^{++} supplement to atorvastatin doesn't prevent elevation in CK; it may delay and provide some protection against statin induced myopathy that in turn may increase patient compliance.

Keywords: Hyperlipidemia; atorvastatin; magnesium; lipid profiles; L-CAT.

1. INTRODUCTION

Hyperlipidemia is a disorder of lipid metabolism that results in abnormally high levels of cholesterol, triglycerides, and lipoproteins in the blood circulation. Statins lower cholesterol by inhibiting 3-hydroxy-3- methylglutaryl coenzyme A (HMG-COA) reductase, thus preventing the formation of the major building block of the cholesterol molecule. Statins are generally well tolerated; however some adverse such as myopathy and elevation of serum transaminase concentration are reported which in turn lead to omission medication error related to patients non-adherence [1,2]. Atorvastatin belongs to stations group which is 3-hydroxy-3-methylglutaryl coenzyme A (HMG COA) reductase inhibitors. Statins are the first-line and more effective treatment for patients with elevated LDL cholesterol [3]. Atorvastatin is an analogue of HMG, the precursor of cholesterol. Because of its strong affinity for the enzyme, it competes effectively to inhibit HMG COA reductase, the rate-limiting step in cholesterol synthesis. By inhibiting de novo cholesterol synthesis, it depletes the intracellular supply of cholesterol. Atorvastatin is the most potent LDL cholesterol lowering statin [4]. Depletion of intracellular cholesterol causes the cell to increase the number of specific cell-surface LDL receptors that can bind and internalize circulating LDL. So, the end result is a reduction in plasma cholesterol, both by lowered cholesterol synthesis and by increased catabolism of LDL. In addition, atorvastatin increases plasma high density lipoprotein (HDL) levels resulting in an additional lowering of coronary heart disease risk [5]. For both primary and familial hyper-cholesterolemia, the usual dose is 10 mg once daily, but if necessary, the dose may be increased to maximum 80 mg once daily. Statins should be used with caution in patients with liver disease. Liver-function tests should be carried out before and within 1-3 months of starting treatment and therefore at intervals of 6 months for 1 year. Treatment should be discontinued if serum transaminase concentration rises to 3 times the upper limit of the reference range. Statins should be used with caution in those with risk factors for myopathy or rhabdomyolysis [6].

Magnesium serves as a co-factor for enzymes involved in a variety of physiological processes including lipid metabolism [7]. Dietary magnesium has the ability to decrease activity of lipogenic liver enzymes, improve insulin action or increase lipoprotein lipase activity [8,9]. Also, magnesium is an antagonist of the N-methyl-D-aspartate (NMDA) receptor ion channel, and this may explain part of its analgesic activity [10] but no study investigates the role of Mg supplement on myopathy. Clinical studies on patients with metabolic syndrome have shown that individuals with low levels of magnesium have lower levels of HDL-cholesterol [11-13], but higher levels of triglycerides [11,12] and total

cholesterol [12,14,15]. Few short studies examining serum Mg levels have shown a positive correlation with triglycerides [16] and total cholesterol [17].

Lecithin cholesterol acyltransferase (L-CAT) is also believed to be a key enzyme in the reverse cholesterol transport (RCT) pathway because esterification of cholesterol on HDL increases the concentration gradient for the movement of free cholesterol from cells onto HDL by the various cell transports that efflux cholesterol [18]. HDL delivers its cholesteryl esters (CE) to the liver, and cholesterol is then excreted into the bile as cholesterol or as a bile salt, thus completing the RCT pathway [19]. Lecithin cholesterol acyltransferase was found to be positively correlated to ionized Mg in humans [20]. In the Mg-deficient rat [21] there was a reduced activity of plasma L-CAT. Severe Mg deficiency in weanling rats produces a marked hypertriacylglycerolaemia, a decrease in cholesterol transport by HDL-cholesterol and a reduction of L-CAT activity [22,23].

The purpose of this study was firstly to investigate the role of oral magnesium adjuvant therapy to atorvastatin on hyperlipidemia and secondly to elucidate the possible ability of oral magnesium supplement to counteract or delay statins induced myalgia.

2. MATERIALS AND METHODS

2.1 Materials

Atorvastatin (Ator®) was obtained from EPICO Pharmaceutical Company (10th of Ramaden City, Cairo, Egypt), magnesium (Spasmag®) was obtained from Global Napl Pharmaceutical Company (2nd industrial zone, 6th of October City, Egypt). Serum lipid profiles including triglycerides (TG), total cholesterol and high-density lipoprotein cholesterol (HDL-C) were measured using commercial kits (Biodiagnostic, Dokki, Giza, Egypt). L-CAT was measured using commercial kits (Sun Red, Shanghai). AST, Calcium, Sodium, CK, Magnesium, Potassium, Serum creatinine (SCr) were measured using commercial kits (SPECTRUM, Egyptian Company for Biotechnology (S.A.E), Obour city industrial area).

2.2 Subjects

Forty males and females recently hyperlipidemic patients were enrolled in the study. Their ages ranged from 33 to 50 years. Their body mass index was ranged from 25.11 to 32.92 kg/m^2. The subjects included in the study were not on any other medications and did not have any other definitive disease or family history for hyperlipidemia or cardiovascular accidents. Inclusion criteria were: both sexes, previously untreated hyperlipedemic patients, subjects with total cholesterol > 240 mg/dl, LDL > 160 mg/dl with or without triglycerides > 200 mg/dl. Exclusion criteria were: Cardiovascular disease, heart failure, diabetes mellitus, liver diseases, renal impairment, smokers, women on oral contraceptives, pregnant and nursing women and patients on antihyperlipidemic drugs. The protocol for this study was approved by the National Research Ethics Committee of Tanta University Egypt. Eligible patients gave their written, informed consent. After signing a consent form, patients were interviewed for complete history and clinical examinations, which were carried out by qualified physician from Internal Medicine Department, Tanta University Hospital.

2.3 Study Design

Hyperlipidemic patients were randomly divided into two groups: group one consisted of twenty patients, their mean age is 40.4±4.57 years and BMI is 29.75±3.18 kg/m^2 (16 males and 4 females, over weight to obese is 7:13) who received atorvastatin (Ator®)10 mg once daily for 6 weeks then 20 mg once daily for another 6 weeks; group two consisted of twenty patients, their mean age is 39.5±3.85 years and BMI is 29.68±2.71 kg/m^2 (15 males and 5 females, over weight to obese is 8:12) who received the same dose of atorvastatin (Ator®) for the same duration plus once daily oral low dose magnesium sulfate trihydrate 419.5 mg (Spasmag®) equivalent to 50 mg of magnesium. Patients were followed up every 2 weeks to ensure compliance and to report any dropout or adverse effects.

Two samples of venous blood (2 ml + 8 ml =10 ml total), were collected from all individuals, and were drawn from the antecubital vein before, 1.5 and 3 months after treatment. Sera and plasma were separated immediately for biochemical analyses. All blood samples were obtained between 9-11 hours to minimize possible diurnal variations.

2.4 Assay

Serum lipid profiles including triglycerides (TG), total cholesterol and high-density lipoprotein cholesterol (HDL-C) were measured colorimetrically (Enzymatic colorimetric method) using commercial kits (Biodiagnostic, Dokki, Giza, Egypt). Low-density lipoprotein cholesterol (LDL-C) was calculated. L-CAT was measured by enzyme-linked immunosorbent assay (ELISA) using commercial kits (Sun Red, Shanghai). AST was measured by colorimetric method. Calcium was measured by O-cresolphthaline complexone colorimetric method, Sodium and CK were measured colorimetric, Magnesium was measured by Xylidyl Blue, Colorimetric Endpoint and Potassium was measured by Turbidimetric Tetraphenylborate (TPB) method, Serum creatinine (SCr) was measured spectrophotometrically. (SPECTRUM, Egyptian Company for Biotechnology (S.A.E), Obour city industrial area).

2.5 Statistical Analysis

Data were statistically analyzed by paired Student's *t* test to compare between the results before (baseline) and after treatment within the same groups, and unpaired Student's *t* test to compare between means of the different groups using SPSS software for Windows version 10 (Chicago, IL, USA). Correlations between variables were evaluated by using Pearson correlations. All results were expressed as mean ± SD. The level of significance was set at < 0.05.

3. RESULTS AND DISCUSSION

Mean biochemical parameters in hyperlipedimic patients on atorvastatin therapy are shown in Table 1. Six weeks after initiation of atorvastatin therapy, group 1 patients showed significantly lower serum cholesterol and LDL-C concentrations compared to the baseline data obtained before initiation of therapy (221.3±15.9 mg/dl, 151.7±21.5 mg/dl versus 257.6±15.1 mg/dl, 184.3±19.8 mg/dl respectively). Three months after atorvastatin therapy, the statistical analysis revealed that, there was significant increase in L-CAT, CK, AST and HDL-C levels (9.2±2.4 µg/ml, 113.1±21.1 U/l, 23.8±5.5 U/ml, 43.1±4.4 mg/dl versus 6.55±2.3 µg/ml, 96.4±20.3 U/l, 18.1±6.1 U/ml, 37.3±3.85mg/dl respectively). Also, there was significant decrease in total cholesterol, TGs and LDL-C concentrations (171.5±23.5 mg/dl,

117.5±38 mg/dl and 104.9±24.4 mg/dl versus 257.6±15.1 mg/dl, 179.9±71.6 mg/dl and 184.3±19.8 mg/dl respectively). Comparing data obtained 1.5 and 3 months after atorvastatin therapy, the statistical analysis revealed that, three months after initiation of atorvastatin therapy, there was significant increase in AST and HDL-C levels (23.8±5.5 U/ml and 43.1±4.4 mg/dl versus 19.4±4.9 U/ml and 39.5±4.1 mg/dl respectively). In addition, there was significant decrease in cholesterol and LDL-C concentrations (171.5±23.5 mg/dl and 104.9±24.4 mg/dl versus 221.3±15.9 mg/dl and 151.7±21.5 mg/dl respectively).

Table 1. Biochemical parameters in hyperlipedimic patients on atorvastatin

Parameters	Group 1(n = 20) before treatment 0 time	Group 1(n = 20) 1.5 month after treatment	Group 1(n = 20) 3 months after treatment
L-CAT (µg/ml)	6.5±2.3	7.91±2.3	9.2*±2.4
CK (U/l)	96.4±20.3	102.2±20.8	113.1*±21.1
AST (U/ml)	18.1±6.1	19.4$^{\$}$±4.9	23.8*±5.5
S.Cr (mg/dl)	0.73±0.28	0.76±0.24	0.78±0.26
Cholesterol (mg/dl)	257.6±15.1	221.3*$^{\$}$±15.9	171.5*±23.5
HDL-C (mg/dl)	37.3±3.9	39.5$^{\$}$±4.1	43.1*±4.4
LDL-C (mg/dl)	184.3±19.8	151.7*$^{\$}$±21.5	104.9*±24.4
TGs (mg/dl)	179.9±71.6	150.3±56.8	117.5*±38.03
Ca^{++} (mg/dl)	9.6±0.61	9.59±0.69	9.53±0.64
K^{+} (mmol/l)	4.2±0.46	4.19±0.5	4.25±0.41
Na^{+} (mEq/l)	136.2±4.96	135.6±4.89	135.1±5.85
Mg^{++} (mg/dl)	1.63±0.08	1.65±0.09	1.68±0.15

Data are presented as mean ± SD, P < 0.05: significant
**Significantly differenent from baseline (before initiation of therapy) (P < 0.05)*
$^{\$}$Significantly different from data obtained 3 months after treatment (P < 0.05)
HDL-C, high density lipoprotein-cholesterol, LDL-C, low-density lipoprotein-cholesterol, TGs, triglycerides, L-CAT, lecithin cholesterol acyltransferase, CK, creatine kinase, S.Cr, serum creatinine and AST, aspartate transaminase

Mean biochemical parameters in hyperlipedimic patients on atorvastatin plus magnesium treatment are shown in Table 2. Six weeks after initiation of atorvastatin plus magnesium supplement therapy, group 2 patients showed significantly lower serum cholesterol and LDL-C concentrations as compared to baseline results obtained before initiation of treatment (218.3±16.9 mg/dl, 149.1±19.4 mg/dl versus 262±15.4 mg/dl, 189.5±24.9 mg/dl respectively). In addition, those patients showed significantly higher serum L-CAT, HDL-C and Mg^{++} levels compared to their base line obtained before treatment (9.3±2.3 µg/ml, 40.9±3.1mg/dl, 1.78±0.09 mg/dl versus 6.3±2.3 µg/ml, 37.9±2.6 mg/dl, and 1.66±0.11 mg/dl respectively).

Three months after atorvastatin plus magnesium supplement therapy, the statistical analysis revealed that, there was significant increase in L-CAT, AST, Mg^{++} and HDL-C levels as compared to baseline data obtained before initiation of therapy (12.2±4.1µg/ml, 22.4±3.7 U/ml, 1.95±0.11 mg/dl, 46.8±2.9 mg/dl versus 6.3±2.3 µg/ml, 17.7±5.5 U/ml, 1.66±0.11 mg/dl, 37.9±2.6 mg/dl respectively). Also, there was significant decrease in cholesterol, TGs and LDL-C levels (133.3±13.4 mg/dl, 95.9±20.8 mg/dl and 67.4±15.3 mg/dl versus 262±15.4 mg/dl, 173±68.6 mg/dl and 189.5±24.9 mg/dl respectively). Comparing data obtained 1.5 and 3 months after atorvastatin plus magnesium supplement therapy, the statistical analysis revealed that, three months after initiation of therapy, there was significant increase in L-

CAT, AST, Mg^{++} and HDL-C levels as compared to data obtained 1.5 months post treatment (12.2±4.1 µg/ml, 22.4±3.7 U/ml, 1.95±0.11 mg/dl, 46.8±2.9 mg/dl versus 9.3±2.3 µg/ml, 19.03±4.98 U/ml, 1.78±0.09 mg/dl, 40.9±3.1 mg/dl respectively). Also, there was significant decrease in cholesterol, TGs and LDL-C levels (133.3±13.4 mg/dl, 95.9±20.8 mg/dl and 67.4±15.3 mg/dl versus 218.3±16.9 mg/dl, 141.3±56.4 mg/dl and 149.1±19.4 mg/dl respectively).

Table 2. Biochemical parameters in hyperlipedimic patients on atorvastatin and magnesium supplement

Parameters	Group 2 (n = 20) Before treatment 0-time	Group 2 (n = 20) 1.5 month after treatment	Group 2(n = 20) 3 months after treatment
L-CAT (µg/ml)	6.3±2.3	9.3*$±2.3	12.2*±4.1
CK (U/l)	97.5±18.2	102.1±19.2	107.9±20.9
AST (U/ml)	17.7±5.5	19.03$±4.98	22.4*±3.7
S.Cr (mg/dl)	0.74±0.28	0.75±0.26	0.81±0.22
Cholesterol (mg/dl)	262±15.4	218.3*$±16.9	133.3*±13.4
HDL-C (mg/dl)	37.9±2.6	40.9*$±3.1	46.8*±2.9
LDL-C (mg/dl)	189.5±24.9	149.1*$±19.4	67.4*±15.3
TGs (mg/dl)	173±68.6	141.3$± 56.4	95.9*±20.8
Ca^{++} (mg/dl)	9.6±0.56	9.52±0.53	9.5±0.55
K^{+} (mmol/l)	4.2±0.35	4.15± 0.46	4.2±0.35
Na^{+} (meq/l)	137.1±4.9	135.5± 5.02	136.5±4.76
Mg^{++} (mg/dl)	1.66±0.11	1.78*$±0.09	1.95*±0.11

Data are presented as mean ± SD, P < 0.05: significant
**: Significantly different from baseline data (before initiation of therapy) (P < 0.05)*
$: Significantly different from data obtained 3 months after treatment (P < 0.05)
HDL-C, high density lipoprotein-cholesterol, LDL-C, low-density lipoprotein-cholesterol, TGs, triglycerides, L-CAT, lecithin cholesterol acyltransferase, CK, creatine kinase, S.Cr, serum creatinine and AST, aspartate transaminase

Table 3 shows the significant difference in the biochemical parameters between hyperlipedimic patients on atorvastatin (gp 1) and atorvastatin plus magnesium (gp 2).

There was no significance difference between the two groups before initiation of therapy ($P \geq 0.05$). The statistical analysis between the two groups carried out 1.5 months after treatment revealed that there was no significance difference in all parameters except for magnesium which was significantly ($P<0.05$) higher in group 2 as compared to group 1 (1.78±0.09 mg/dl versus 1.65±0.09 mg/dl).

The statistical analysis between the two groups after 3 months of treatment revealed that group 2 patients showed significantly ($P<0.05$) higher L-CAT, Mg^{++} and HDL-C levels as compared to group 1 patients (12.2±4.1 µg/ml, 1.95±0.11 mg/dl, 46.8±2.9 mg/dl versus 9.2±2.4 µg/ml, 1.68±0.15 mg/dl, 43.1± 4.4 mg/dl respectively). Also, group 2 patients showed significantly ($P<0.05$) lower cholesterol, TGs and LDL-C concentrations as compared to group 1 patients (133.3±13.4 mg/dl, 95.9±20.8 mg/dl and 67.4±15.3 mg/dl versus 171.5±23.5 mg/dl, 117.5±38.03 mg/dl and 104.9±24.4 mg/dl respectively).

Table 3. The significant difference in the biochemical parameters between hyperlipedimic patients on atorvastatin and atorvastatin plus magnesium

Parameters	At time 0		1.5 month after treatment		3 months after treatment	
	Atorvastatin	Atorvastatin+Mg	Atorvastatin	Atorvastatin+Mg	Atorvastatin	Atorvastatin+Mg
L-CAT (µg/ml)	6.5±2.3	6.3±2.3	7.91±2.3	9.3±2.3	9.2±2.4	12.2*±4.1
CK (U/l)	96.4±20.3	97.5±18.2	102.2±20.8	102.1±19.2	113.1±21.1	107.9±20.9
AST (U/ml)	18.1±6.1	17.7±5.5	19.4±4.9	19.03±4.98	23.8±5.5	22.4±3.7
S.Cr (mg/dl)	0.73±0.28	0.74±0.28	0.76±0.24	0.75±0.26	0.78±0.26	0.81±0.22
Cholesterol (mg/dl)	257.6±15.1	262±15.4	221.3 ±15.9	218.3±16.9	171.5 ±23.5	133.3*±13.4
HDL-C (mg/dl)	37.3±3.9	37.9±2.6	39.5 ± 4.1	40.9±3.1	43.1±4.4	46.8*±2.9
LDL-C (mg/dl)	184.3±19.8	189.5±24.9	151.7 ±21.5	149.1±19.4	104.9±24.4	67.4*±15.3
TGs (mg/dl)	179.9±71.6	173±68.6	150.3±56.8	141.3± 56.4	117.5±38.03	95.9*±20.8
Ca^{++} (mg/dl)	9.6±0.61	9.6±0.56	9.59±0.69	9.52±0.53	9.53±0.64	9.5±0.55
K^{+} (mmol/l)	4.2±0.46	4.2±0.35	4.19±0.5	4.15± 0.46	4.25±0.41	4.2±0.35
Na^{+} (meq/l)	136.2±4.96	137.1±4.9	135.6±4.89	135.5± 5.02	135.1±5.85	136.5±4.76
Mg^{++} (mg/dl)	1.63±0.08	1.66±0.11	1.65± 0.09	1.78$^{\$}$±0.09	1.68±0.15	1.95*±0.11

*No significant difference between the two groups before initiating therapy (at time zero) (P > 0.05) $^{\$}$: Significant difference between group who administer Atorvastatin alone and Atorvastatin + Mg^{++} after 1.5 months duration (P < 0.05) *: Significant difference between group who administer Atorvastatin alone and Atorvastatin + Mg^{++} after 3 months duration (P < 0.05)*

The Pearson correlation test was used to determine the correlations between magnesium plus atorvastatin, L-CAT level and lipid parameters as shown in Table 4. This Table shows a significant positive correlation between L-CAT and HDL-C (r = 0.540, P = 0.000) and magnesium (r = 0.347, P = 0.007). An inverse correlation was observed between L-CAT and total cholesterol (r = -0.623, P = 0.000), triglycerides (r = -0.331, P = 0.010) and LDL-C (r = -0.607, P = 0.000). Also Table 4 shows a significant positive correlation between Mg^{++} plus atorvastatin and HDL-C (r = 0.625, P = 0.000) and L-CAT (r = 0.347, P = 0.007). An inverse correlation was observed between Mg^{++} plus atorvastatin and total cholesterol (r = -0.719, P = 0.000), triglycerides (r = -0.554, P = 0.010) and LDL-C (r = -0.663, P = 0.000).

Table 4. Pearson correlation of magnesium plus atorvastatin, L-CAT and lipid parameters

Variables	Mg^{++}		Variables L-CAT		
	r	***P* value**	***r***		***P* value**
Cholesterol (mg/dl)	-0.719**	0.000	Cholesterol (mg/dl)	-0.623**	0.000
TG (mg/dl)	-0.554**	0.000	TG (mg/dl)	-0.331**	0.010
HDL-C (mg/dl)	0.625**	0.000	HDL-C (mg/dl)	0.540**	0.000
LDL-C (mg/dl)	-0.663**	0.000	LDL-C (mg/dl)	-0.607**	0.000
L-CAT (µg/ml)	0.347**	0.007	Mg^{++} (mg/dl)	0.347**	0.007

*** Correlation is significant at the 0.01 level (2-tailed). * Correlation is significant at the 0.05 level (2-tailed)*

High plasma cholesterol has been acknowledged, since the mid-20th century, as a major heart disease risk factor. Dyslipidemia refers to the abnormal levels of lipids in the blood, including low high-density lipoprotein cholesterol (HDL-C), which is known as good cholesterol, high low-density lipoprotein cholesterol (LDL-C), also known as bad cholesterol, and/or high TGs levels that contribute to the development and progression of atherosclerosis.

Regarding to the hyperlipedemic patients on atorvastatin, 6 weeks after treatment, our results showed that there were a significant decrease in both serum total cholesterol and LDL-cholesterol without significant decrease in serum triglyceride or significant elevation in both HDL-cholesterol and plasma L-CAT comparing with baseline data obtained before initiation of therapy. However, 12 weeks after initiation of atorvastatin therapy, there was significant amelioration in lipid profiles and significant increase in L-CAT plasma level as compared with baseline date for hyperlipedemic patients before initiation of therapy. Comparing the effect of atorvastatin 12 and 6 weeks after initiation of therapy, the data obtained 12 post treatment weeks showed significantly lower total cholesterol and LDL-cholesterol concentrations, significantly higher HDL-cholesterol with non-significantly higher L-CAT and non-significantly lower triglycerides levels as compared to the data obtained 6 weeks after atrovastatin therapy. These effects obtained by atorvastatin therapy is attributed to the following facts, statins competitively inhibit hydroxymethylglutaryl (HMG) CoA, decreasing cholesterol biosynthesis, up-regulating hepatic LDL receptors, and enhancing the clearance of apo B-containing lipoproteins [24]. In addition, statins decrease plasma triglyceride levels, and thereby may also alter the metabolic fate of HDL particles [25]. All of the statin drugs significantly lower mean LDL-C, some raise mean HDL-C and some lower mean triglycerides, especially in patient groups with high initial levels [26,27]. The effect of atorvastatin on blood lipid 12 weeks after initiation of therapy is significantly better than that

reported after 6 weeks of atorvastatin therapy may be due to dose response relationship as reported by Naci et al. [28].

The significant amelioration in lipid profile and the significant increase in plasma L-CAT concentration 12 weeks after atorvastatin therapy reported by our study is in accordance with previously reported finding by Heller et al. [29]. Heller et al. reported that ten patients with mixed hyperlipidemia (TC and TG greater than 250 and 200 mg/dl, respectively) were treated with atorvastatin, 10 mg/day for 1 month and then 20 mg/day for another month showed a strong reduction in plasma levels of total cholesterol (TC), LDL-cholesterol and triglycerides and a marked increase in the plasma L-CAT [29]. Our result are on line to great extent with that reported by Kassai et al. [30] who demonstrated that treatment of 33 patients with type II a and II b primary hyperlipoproteinemia with 20 mg atorvastatin for 3 months resulted in reduced levels of cholesterol, triglyceride, LDL-C and a significant elevation in L-CAT activity but not levels of HDL-C [30]. The effect of atorvastatin on L-CAT may be due to the up-regulation of HDL-apo A-I production by statin [31,32]. Apo A-I has been suggested to be the most potent cofactor for activation of L-CAT [33].

Regarding to the hyperlipedemic patients on atorvastatin plus magnesium supplement, 6 weeks after treatment, our results showed that there were a significant decrease in both serum total cholesterol and LDL-cholesterol and significant increase in HDL-cholesterol, plasma L-CAT and serum magnesium levels without significant decrease in serum triglyceride as compared with baseline data obtained before initiation of therapy. Twelve weeks after initiation of atorvastatin therapy plus adjuvant magnesium supplement, there was significant amelioration in lipid profiles and significant increase in serum magnesium and in L-CAT plasma level as compared with baseline date for hyperlipedemic patients before initiation of therapy. Comparing the effect of atorvastatin plus magnesium supplementation 12 and 6 weeks after initiation of therapy, the data obtained 12 weeks post-treatment showed significantly lower total cholesterol, LDL-cholesterol, triglycerides concentrations and significantly higher HDL-cholesterol, L-CAT and magnesium levels as compared to the data obtained 6 weeks post-treatment. These results may be attributed to the additive effect of both statin and magnesium on both lipids and L-CAT activity.

In comparing the effects of atorvastatin with that achieved by atorvastatin plus magnesium supplement 6 weeks after initiation of therapy, there were no significant difference between the two groups except for serum magnesium. Serum magnesium was significantly higher in patients received atorvastatin plus magnesium supplement than those administered atorvastatin alone; this was associated with non significant elevation of plasma L-CAT. This finding may be attributed to magnesium supplement. On other hand, 12 weeks after treatment, the patients received atorvastatin plus magnesium supplement showed significantly higher levels of serum magnesium, plasma L-CAT and HDL-cholesterol concentrations and significantly lower total cholesterol, LDL-Cholesterol and triglycerides concentrations as compared to the patients group received atorvastatin solely.

This promising result may be due to additive lipid lowering effect of both statin and magnesium supplement. It has been reported that statins regulate lipoprotein metabolism [34] and magnesium, as well as statins, targets the enzyme, HMG CoA Reductase [35]. Magnesium has effects parallel to those of statins whereas the enzyme that deactivates HMG-CoA Reductase requires magnesium, thereby making magnesium a reductase controller rather than inhibitor [35]. HMG CoA Reductase is an important enzyme in lipid and cholesterol metabolism, but it is not the only one. The statins act by inhibiting, the enzyme whereas the magnesium ion (Mg^{2+}) is an important part of a complex control and regulation

of this important pathway. Both lower LDL-C, some statins can raise HDL-C and lower triglycerides, but Mg supplements do both quite reliably [35]. Magnesium also activates desaturase and other important enzymes involved in lipid metabolism, which statins do not directly affect [36,37]. These promising results may be also due to the effect of magnesium on L-CAT activity. It has been postulated that, magnesium is necessary for the activity of lecithin cholesterol acyl transferase or L-CAT [38] a key enzyme for the production of cholesteryl esters in plasma that promotes the formation of high density lipoprotien (HDL) and promotes the Reverse Cholesterol Transport (RCT), the anti-atherogenic mechanism by which excess cholesterol is removed from cells by HDL and delivered to the liver for excretion [39,40,41]. L-CAT also, lowers LDL-C and triglyceride levels and raises HDL-cholesterol levels [38,42,43]. The results of the current study are in accordance with previously reported finding by Davis et al. [44]. Davis et al. found that, the treatment of sixteen hyperlipedemic patients for 118 day with an oral dose of 18 mmol Mg/day significantly reduced the total cholesterol, LDL-Cholesterol and VLDL-Cholesterol concentrations and increased HDL-cholesterol [44]. Also, our results are parallel with that reported by Itoh et al. [38] who reported a significant decrease in serum LDL-Cholesterol and a significant increase in serum L-CAT activity after 4 weeks supplementation with high oral doses of magnesium [38]. Guex et al. [45] reported that, dietary magnesium affects the activity of the enzyme involved in the esterification of free cholesterol to cholesterol ester. It has been reported that the L-CAT activity decreased significantly in atherosclerotic patients, in comparison with the healthy subjects and this decrease in L-CAT activity was accompanied by elevated levels of LDL-cholesterol and by moderate increase in triacylglycerol [46]. It has been demonstrated that, severe magnesium deficiency in weanling rats produces a marked hypertriacylglycerolemia, a decrease in cholesterol transport by HDL-cholesterol and a reduction of L-CAT activity [47,48]. All the above mentioned information support the promising results obtained by implication of magnesium supplement to atorvastatin which provided more better control on lipid profile than atorvastatin alone. These results may be attributed to the effect of magnesium supplement on L-CAT activity.

The kidney plays a major role in magnesium homeostasis and the maintenance of plasma magnesium concentration [49]. Hypermagnesaemia (magnesium > 2 mmol/L) is less frequent and results from failure of excretion or increased intake of magnesium. Magnesium reabsorption is proportional to sodium reabsorption and magnesium and potassium homeostases are closely related [50]. For all aforementioned information, we aimed to evaluate the electrolytes levels as well as the kidney function all over the current study. All over the study duration, no significant change in kidney function (Scr) and serum electrolytes was detected, except for serum magnesium which showed significant elevation in patient group received atorvastatin plus magnesium supplement (group 2) but its level till within the normal reference range.

Statin treatment has been associated with asymptomatic and usually transient elevation of serum aminotransferase levels that often occurs in the first 12 weeks of therapy. Most of the time, this biochemical finding does not meet criteria as a true indicator of liver injury [51]. Withdrawal or reduction of the statin dose resulted in normalization of the liver enzymes. Also, statins are associated myalgia with and without elevated creatine kinase (CK) levels [52]. For the previously mentioned information we evaluated the AST and CK levels. The AST levels were significantly higher 12 weeks after treatment for both groups as compared to baseline data for each group. No statistical significant difference was detected between the two groups. This elevation in AST is related to atorvastatin. In addition, the patients received atorvastatin only (gp 1) showed significant elevation of CK level 12 weeks after initiation of therapy compared to their baseline data. On other hand, those received

atorvastatin plus magnesium supplement (gp 2) showed non significant elevation of creatine kinase 12 weeks after initiation of therapy as compared to their baseline data. However 12 months after treatment patients received atorvastatin plus magnesium (gp 2) showed lower but non significant CK level than those on atorvastatin only (gp 1). This result gives indication about the ability of magnesium supplement to provide some protection against statin induced myopathy. These later results seem to be matched with previously mentioned finding demonstrated that, statins raise liver enzymes, can cause myopathy, whereas Mg supplements tend to protect against myopathy [35].

4. CONCOLUSION

This study showed that, magnesium supplement to atorvastatin resulted in better control on hyperlipidemia than that provided by atorvastatin therapy alone via raising HDL-C concentration, elevating L-CAT plasma level, decreasing total cholesterol, LDL-C, and TG levels. Regarding cost-effectiveness relationship, magnesium supplementation to atorvastatin provides a pharmacoeconomic importance since one should extend atorvastatin therapy to maintain its benefits. Atorvastatin raises liver enzyme AST which cannot counteracted by magnesium supplementation. The clinical spectrum of statin induced myopathy includes myalgia and increase in creatine kinase. Our results showed significant increase in levels of creatine kinase in patient received atorvastatin alone which may lead to poor adherence and omission medication error. On the other hand, magnesium supplement to atorvastatin tends to provide some protection against statins induced myopathy and more good control on lipid profiles, which in turn leads to decreased over all duration of therapy with subsequent decreased chance to develop myopathy and decreased risk of omission medication error. However, all of that need further looking into.

CONSENT

Eligible patients gave their written, informed consent.

ETHICAL APPROVAL

The protocol for this study was approved by the National Research Ethics Committee of Tanta University Egypt.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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