

Research Journal of Pharmaceutical, Biological and Chemical Sciences

A Study Of Serum Calcium, Magnesium, Phosphorus, And Lipid Profile In Pre-Menopausal Women In A Tertiary Care Hospital In Chennai, Tamil Nadu, India.

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ABSTRACT

Recent epidemiological evidence suggests that alteration in calcium, phosphorous, or magnesium metabolism may have direct cardiovascular consequences. However, it is unknown whether variations in serum values of these minerals are in relationship with lipid profile and adiposity as metabolic risk factors of cardiovascular events in premenopausal women independent of confounders. This study aimed to investigate the relationship between serum calcium, magnesium, and phosphorous with lipid profiles in healthy premenopausal women. This study was performed on 82 women aged 17-50 who were randomly selected from the general population Govt Stanley Medical College & Hospital, Chennai, Tamil Nadu, India in the year 2022. They were assigned into obese and non-obese groups. Weight and height for BMI calculation were measured using a calibrated Seca scale and cotton ruler which was attached to the wall. Body composition was analyzed by bioelectrical impedance analysis (BIA). Serum magnesium, calcium, and phosphorous were measured calorimetrically; fasting blood glucose (FBG) and serum lipids were assessed by enzymatic methods. : Obese woman had significantly lower serum magnesium ($P = 0.035$) and significantly higher fasting blood glucose ($P = 0.028$), total cholesterol ($P = 0.035$), triglyceride ($P = 0.019$), low density lipoprotein ($P=0.003$) and parathyroid hormone concentrations ($P = 0.031$) compared to non-obese women. In correlation coefficient analysis, serum calcium concentrations had a positive weak relationship with total cholesterol ($r = 0.267, P= 0.013$) and triglyceride ($r = 0.301, P = 0.005$) concentrations in all participants; whereas in a separate analysis of subjects as obese and nonobese groups, these relationships lost their significance. Serum phosphorous had a weak positive relationship with total cholesterol ($r = 0.31, P = 0.002$) and an inverse weak relationship with parathyroid hormone ($r = - 0.32, P = 0.002$). After adjusting for confounding variables by multiple regression analysis, the positive relationship between serum calcium, triglyceride, ME high-density lipoprotein, and low-density lipoprotein cholesterol was significant. Our results indicate that abnormality in serum calcium and phosphorous is significantly correlated with serum lipids. Further studies are warranted for the interpretation of these associations and understanding of the underlying mechanisms.

Keywords: calcium; phosphorous, magnesium, obesity, body composition, lipid profile.

<https://doi.org/10.33887/rjpbcs/2024.15.2.61>

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INTRODUCTION

Calcium as an essential regulator in many homeo- static systems plays an important role in controlling diverse biological processes such as hormone secretion, intermediary metabolism, and bone structure [1]. Recently it has been suggested that some metabolic disorders, hypertension, and cardiovascular disease are linked by common factors in the metabolism of some divalent cations such as calcium and magnesium [2]. The positive relationship between serum calcium and cardiovascular disease metabolic syndrome or myocardial infarction has been reported in previous studies. On a parallel note, several studies have reported an inverse relationship between serum magnesium concentrations and lipid profile. Low serum magnesium is associated with cardiovascular events and metabolic syndrome [3]. Although the exact mechanisms underlying these relationships are not fully understood, potential mechanisms are the basic role of these cations in metabolic pathways [4]. It can be concluded from the above introduction that serum calcium and magnesium are considered good predictors of lipid abnormality; however, one important unanswered question is whether this relationship can happen in healthy normal individuals without cardiovascular disease and other morbidities. Previous studies were carried out in diabetes, hypothyroidism, or metabolic syndrome and not in normal subjects [5].

METHODS AND MATERIALS

The Study Was Conducted At Department Of Biochemistry, Govt Stanley Medical College & Hospital, Chennai, Tamil Nadu, India In The Year 2022. A total of 82 healthy volunteer women participated in our study. Subjects were divided into obese (N = 40) and non-obese (N = 42) groups based on their body mass index. The inclusion criteria of the study were 17-50 years of age, BMI 30-34.9 kg/m² for obese and 18.5-24.9 kg/m² for non-obese group. The exclusion criteria included a history of hepatic or renal disease, cardiovascular disease, diabetes, hypertension, treatment with estrogen and contraceptives (OCP), loop diuretics, or corticosteroids. Subjects in the two groups were pair-matched for age. The matching limit for age was ± 3 years.

Biochemical measurements

Venous blood samples were collected from all subjects between 7 and 9 a.m. after overnight fasting in tubes without additives. These samples were centrifuged; the serum was obtained and stored at -70°C until the assay. Serum concentrations of calcium, magnesium, and inorganic phosphate were measured using standard colorimetric methods. The sensitivity of this test was 2 ng/L, and means inter and intra-assay coefficients of variation (CV) were 7.1 and 11% respectively [14]. Serum 25-hydroxy vitamin D was measured by Chemiluminescence Immuno Assay (DiaSorin Inc., Stillwater, MN, USA). The sensitivity of this test was 17.5 nmol/l and means inter and intra-assay coefficient of variation was 13.2% and 10.5% respectively. We also categorized subjects into three groups according to their serum calcium or phosphorous levels: groups with low, medium, and high serum calcium or phosphorous levels. Fasting blood glucose (FBG), total cholesterol (TC), triglyceride (TG), and high-density lipoprotein cholesterol (HDL-C) levels were analyzed using the enzymatic colorimetric method. The TG level in all subjects was lower than 4.52 mmol/L (400 mg/dL); therefore we used the Friedwald formula for the estimation of Low-Density Lipoprotein Cholesterol (LDL-C) concentrations based on serum TC, TG, and HDL levels. Mean inter and intra-assay CV was 1.14, 0.061 for TC; 1.60, 1.53 for TG, and < 4 for HDL.

Anthropometric And Dietary Assessments

Weight was measured to the nearest 0.1 kg using a calibrated Seca scale (Itin Scale Co., Inc. Germany) while subjects had light clothes and no shoes. Height was measured using a cotton ruler which was attached to the wall. Body mass index was calculated as weight (kg) / height (m)². Waist circumference (WC) was obtained by measuring the smallest area below the rib cage and above the umbilicus. Standing hip circumference (HC) was measured at the inter trochantric level. Waist to hip ratio (WHR) was obtained by dividing the mean WC by the mean HC. Each participant had a body composition analysis with the Bioelectrical Impedance Analysis (BIA) method (Human-IM Plus; DS Dietosystem, Milan, Italy). Demographic characteristics of subjects were determined using a screening questionnaire to provide information on general personal characteristics, health status, medication history, and a history of previous medical disease. To ensure that there is no difference between dietary calcium, magnesium, and vitamin D intake between obese and nonobese subjects, a three-day diet record was obtained from participants. Average daily nutrient intakes were calculated by Nutritionist III software (N-Squared analytical software computing, Ore., USA). Intake of magnesium, calcium, and vitamin D that are correlated with total energy intake was adjusted for total energy intake with residual method [6].

Statistical analysis

SPSS software (version 17, SPSS Inc., Chicago, IL, USA) was used for all analyses. The Kolmogorov-Smirnov test was used to verify the hypothesis of normal distribution, followed by the independent Student's t-test supposing normal distribution, and the Mann-Whitney U test was used when the supposition of normal distribution was not accepted. The association between serum calcium, magnesium, and phosphorous with fasting blood glucose and lipid profile was examined by using Pearson's correlation test or Spearman's rank correlation test.

RESULTS

The profile of obese and non-obese groups is shown in Table 1. All general characteristics are significantly different between groups except for age and dietary intake of calcium, magnesium, and vitamin D. Comparison of biochemical parameters between obese and nonobese women (table 2) shows that serum FBG, TC, TG, LDL-C, and PTH concentrations are higher and serum magnesium is lower in the obese group. There is no significant difference between serum calcium, phosphorous, HDL-C, and 25-hydroxy vitamin D concentrations between groups. In correlation coefficient analysis.

Table 1. Characteristics of study participants

Characteristic	Obese (N = 42)	Non obese (N = 40)	P
Age (years)	30 (17-51)	31 (19-45)	0.474
Weight (kg)	81.07 ± 10.13	58.19 ± 6.40	<0.001
Height (m)	1.57 ± 0.05	1.60 ± 0.05	0.036
BMI (kg/m ²)	32.95 ± 3.35	23.40 ± 4.12	<0.001
Fat mass (%)	41.64 ± 4.85	27.36 ± 5.34	<0.001
Fat-free mass (%)	58.06 ± 5.21	72.64 ± 5.34	<0.001
WHR	0.81 ± 0.05	0.74 ± 0.05	<0.001
WC (cm)	95.75 ± 8.0	74.68 ± 6.97	<0.001
Dietary calcium intake (mg/d)	511.03 ± 222.5	546.14 ± 319.78	0.673
Dietary magnesium intake (mg/d)	89.29 ± 56.92	103.14 ± 75.81	0.492
Dietary vitamin D (IU/d)	43.61 ± 8.03	47.03 ± 16.13	0.210

Table 2: Biochemical characteristics of study participants

Characteristics	Obese (N = 42)	Non obese (N = 40)	P
FBG (mmol/L)	4.26 ± 0.82	3.96 ± 0.61	0.028
Calcium (mmol/L)	2.22 ± 0.18	2.20 ± 0.17	0.459
Phosphorous (mmol/L)	1.11 ± 0.24	1.10 ± 0.24	0.561
Magnesium (mmol/L)	0.87 ± 0.11	0.97 ± 0.27	0.035
TC (mmol/L)	4.20 ± 0.95	3.85 ± 0.55	0.035
Triglycerides (mmol/L)	0.93 ± 0.26	0.81 ± 0.18	0.019
LDL-C (mmol/L)	2.84 ± 0.73	2.44 ± 0.48	0.003
HDL-C (mmol/L)	0.99 ± 0.13	0.98 ± 0.17	0.998
25 hydroxy vitamin D (nmol/L)	40.26 ± 19.12	41.42 ± 25.52	0.861
PTH (ng/L)	83.25 ± 39.84	65.53 ± 32.92	0.031

DISCUSSION

This study demonstrates that serum calcium is positively associated with lipid profiles in premenopausal women. This association remained significant after adjusting for potential confounders (BMI, WHR, PTH, and 25-

hydroxy vitamin D). Subjects with higher serum calcium levels had higher serum TC, TG, and LDL-C. Since menopausal status has a significant influence on lipid and calcium metabolism [6]. The average concentration of serum PTH in our study sample was 76.2 ± 39.01 ng/L, whereas the normal range for serum PTH concentrations is 10-65 ng/L this elevated PTH concentration leads to excessive calcium accumulation in the cytosol, PTH induced calcium entry into cells inhibits mitochondrial oxidation and ATP production and ultimately increasing intracellular calcium. Increased cytosolic calcium is responsible for disturbance in lipid metabolism and inducing hyperlipidemia [7]. Calcium ions are necessary for insulin production by islet cells of the pancreas and increased intracellular calcium concentrations may also induce insulin resistance and lipid abnormality. High serum PTH levels have also a suppressive effect on the lipoprotein lipase activity. Abnormality of this enzyme may result in increased triglyceride, low-density lipoprotein cholesterol, and decreased high-density lipoprotein cholesterol. These mechanisms can somewhat explain the relationship between serum calcium and lipid profile, however, due to the case-control design of the study, we are not able to clarify the causal nature of these relationships [8]. Subjects with higher serum phosphorous levels had significantly higher levels of triglyceride concentrations than their controls. Other anthropometric or biochemical variables were not different between serum phosphorous groups except in the case of PTH (In low, medium, and high phosphoro-groups: 89.91 ± 43.03 , 76.17 ± 39.16 and 63.88 ± 31.45 ng/L respectively; $P = 0.040$ [9]. The biological mechanisms of the phosphate and triglycerides relationship are not fully understood. Another observation of our study is the significant difference between serum magnesium concentrations between obese and non-obese women [10]. The relationship between low serum magnesium and high tumor necrosis factor α (TNF- α) in obese subjects. The cause-and-effect relationship between serum magnesium and inflammatory markers in obesity is poorly known. One possible mechanism is that inflammatory factors especially tumor necrosis factors increase renal 1- α -hydroxylase activity and 1,25-(OH) $_2$ vitamin D. This active metabolite enhances renal calcium reabsorption and so higher magnesium urinary excretion [11]. In addition, low serum magnesium can promote weight gain by increasing intracellular calcium. Increased calcium in adipocytes enhances lipogenesis. It also activates phosphodiesterase 3B and decreases lipolysis [12]. Similarly, elevated serum PTH concentrations in the obese group of the present study which has also been reported previously by other investigations can be attributed to elevation in calcium influx into adipocytes due to elevation of 1,25-(OH) $_2$ vitamin D production by PTH; this can promote lipid storage in adipose tissue [13]. Some potential limitations of our study are as follows: first, only women and not men were enrolled in this study, therefore we cannot generalize the results to the total population, second, insulin resistance and inflammatory cytokines were not evaluated, whereas these markers have physiological relations with both mineral metabolism and lipid abnormalities, finally, an interventional study rather than case-control one could better explain the causal relationship between variables [14]. Despite these limitations, one important clinical consideration of our study is that calcium and phosphorous levels should be controlled as risk factors for lipid abnormality and consequently metabolic syndrome- or cardiovascular disease [15].

CONCLUSION

As the atherosclerosis is the main culprit for development of CAD. And the lipid profile shows significant increase in total cholesterol, LDL-C, TG as well as slight increase in VLDL-C, along with decreased levels of HDL-C in pre-menopausal women. This study showed a significant association between serum calcium and lipid profile even after adjusting for potential confounders (BMI, WHR, serum PTH, serum 25-hydroxy vitamin D,) in a group of premenopausal women. In summary, the current study analyzed the relationship between the abnormalities of serum lipids and serum calcium. A positive association was found between serum Ca $^{++}$, phosphorus and magnesium level. Total cholesterol, TG, HDL-c, and LDL-c in women from the general Newfoundland population. Demonstrating a relationship between calcium and serum lipids despite comprehensive control of major confounding factors makes this study unique and further strengthens the possibility of a direct relationship.

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