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RESEARCH ARTICLE

A cross-sectional study to find out the role of lipid peroxidation in the alteration of serum calcium and magnesium levels with aging

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ABSTRACT

Background: In the forthcoming decades, there will be a tremendous increase in the elderly population in India. According to free radical theory, aging is a result of cumulative damage incurred by free radical reactions (reactive oxygen species [ROS]). Antioxidant system (superoxide dismutase, catalase, glutathione, etc.) performs the function of neutralizing of these ROS activities. Free radicals cause peroxidation of membrane lipids and serum malondialdehyde (MDA) can be used as a biomarker to assess the overall lipid peroxidation level. Lipid peroxidation will alter the membrane permeability to electrolytes/other ions and may lead to alteration in the electrolyte or mineral levels. **Aims and Objectives:** This study aims to compare the serum levels of calcium and magnesium in different age groups and to find out its correlation with serum MDA level. **Materials and Methods:** A total of 150 healthy subjects (20–90 years) were recruited and divided into three groups of 50 each; as young (20–30 years), middle aged (40–59 years), and the elderly (60–90 years). Serum MDA, calcium, and magnesium levels were determined and compared. The correlation between serum MDA and with calcium and magnesium levels was analyzed with appropriate statistical tests. **Results:** The present study revealed a significant fall in serum calcium and magnesium levels in the elderly. A negative correlation between serum MDA with calcium and magnesium was observed. **Conclusion:** It is evident that lipid peroxidation plays a role in alteration in the serum calcium and magnesium levels with aging.

KEY WORDS: Lipid Peroxidation; Aging; Calcium; Magnesium; Malondialdehyde; Antioxidants

INTRODUCTION

The recent UN projections reveal that the aging population will increase by about 4 times by the middle of the 21st century, to about 324 million in 2050. The Indian aged population is the second largest in the world. These demographic facts and trends make the elderly in India an increasingly important segment of the population pyramid in the coming years. A demographic transition is now seen with a shift from high mortality/high fertility to low mortality/low fertility pattern resulting in an increased proportion of older people in the total population with a consistently increased old-age dependency ratio. India is also showing such a demographic transition.[1] These changes in population have significant social, economic, and political effects on the society.

The analysis of the process of aging in an integrated fashion is known as gerontology. There are various theories put forward to explain the phenomenon of aging. August Weismann, in 1881, was the first to analyze the causes of senescence in terms of evolution by natural selection[2] and proposed that longevity was programmed by "the needs of the species." The free radical theory of aging proposed by Harman;[3] hypothesized aging as single common process,

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modifiable by genetic and environmental factors, in which the accumulation of endogenous oxygen radicals generated in cells could be responsible for the aging and death of all living beings. Several evidences are there in support to the free radical theory of aging.[4]

Reactive oxygen species (ROS) are highly reactive intermediary metabolites that are normally produced in the course of oxygen metabolism. Almost all biological macromolecules are damaged by free radicals. Uncontrolled production of ROS often leads to damage of DNA, lipids, and proteins. Humans have evolved a highly complex antioxidant systems – enzymatic and non-enzymatic, which work synergistically, and in combination with each other to protect the cells and organ systems of the body against free radical damage.[14] It can be endogenous or exogenous obtained from diet or as dietary supplements.[5]

Free radical attack of lipids leads to peroxidation of polyunsaturated fatty acids (PUFAs) which are important constituents of plasma membrane. Cell membrane damage causes increased permeability to sodium ions, rapid influx of calcium ions, and entry of water into the cell by osmosis, leading to cell damage. Malondialdehyde (MDA), a by-product of lipid peroxidation, is used as an index of lipid peroxidation.[6] Lipid peroxidation has been implicated in aging process and the pathogenesis of many age-related disorders such as hypertension, diabetes mellitus, Alzheimer's disease, parkinsonism, and cancers.[7]

Calcium helps in the activation of various enzymes, secretion of hormones such as insulin, parathyroid hormone (PTH), and calcitonin from the cells acts as a second messenger in signal transduction and is important in hemostasis. Calcium mediates excitation and contraction in skeletal muscles. It also decreases neuromuscular excitability. Moreover, it is essential for the development of bone and teeth.[8]

The elderly are more likely to have insufficient dietary calcium intakes and is also at risk for Vitamin D deficiency, due to their reduced mobility and decreased exposure to sunshine. The capacity of the skin to synthesize Vitamin D also decreases with age. In the presence of inadequate Vitamin D status, calcium absorption is lower than optimal and there is a compensatory increase in PTH levels (secondary hyperparathyroidism), with a consequent stimulation of bone resorption and accelerated bone loss.[9] Low calcium level is associated with increased incidence of osteoporosis. Regulation of intracellular calcium plays a key role in hypertension, insulin resistance, and obesity. Dysregulation of intracellular calcium may be a fundamental factor linking these three conditions. McCarron's first reported an inverse relationship between dietary calcium and blood pressure regulation, with diets lower in calcium serving to increase the risk and diets higher in calcium exerting a protective effect. Review of data about the age-related changes in antioxidant levels has shown that one or more antioxidant enzymes or molecules decrease with aging.[10]

Lipid peroxidation is a deleterious process resulting in structural modification of complex lipid-protein assemblies associated with cellular malfunction. It has been reported that lipid peroxidation contributes local membrane destabilization that alters receptor-mediated ligand uptake and produces damages to cell membrane ion transporters. Inactivation of these ion channels may produce physical and functional disability with age.[5] ROS cause inactivation of the sarcolemmal Ca^{2+} pump, Na^{+}-K^{+} ATPase, and sarcoplasmic reticulum Ca^{2+}-ATPase (Kim and Akera, 1987) and all these mechanisms are involved in the elevation of intracellular calcium levels.[11]

Magnesium serves as a cofactor for all enzymatic reactions that require adenosine triphosphate and as a key component in various reactions that require kinases. It is also an essential enzyme activator for neuromuscular excitability and cell permeability, a regulator of ion channels and mitochondrial function, a critical element in cellular proliferation and apoptosis, and an important factor in both cellular and humoral immune reactions. Moreover, some studies reported that Mg lowers free radical activity. Magnesium deficit has been observed in elderly people. Primary magnesium deficit originates from two etiological mechanisms: Deficiency and depletion. Deficiency is due to insufficient intake and depletion is due to deregulation of factors controlling magnesium status such as intestinal magnesium hypoabsorption and urinary leakage. Magnesium absorption seems to decrease with age and magnesium exchange pools are reduced in elderly people. Magnesium deficit may accelerate aging through its various effects on different organ systems such as neuromuscular, cardiovascular, endocrine systems, bone, kidney, immune, and antioxidant systems.[12,13]

Aging itself constitutes a risk factor for Mg deficit. Recent studies have shown that culture in low magnesium (Mg) accelerates the senescence of human endothelial cells and fibroblasts. Mg stabilizes DNA, promotes DNA replication and transcription, influences RNA translation, and induces ribosome assembly. Mg deficiency also induces oxidative stress and promotes cellular senescence by promoting the shortening of telomeres. Increased oxidative stress may result, in part, from alteration of mitochondrial function. Mg in adequate amount is essential for normal mitochondrial function. Its deficiency results in mitochondrial dysfunction. Hence, Mg deficiency promotes mitochondrial dysfunction and oxidative stress. This is a common accompaniment in aging.[12,14] Mg deficiency thus can lead to a chronic state of increased free radical production, which in the long run results in depletion of antioxidants. Furthermore, it is hypothesized that Mg deficiency leads to a decrease in the synthesis of antioxidants, leading to increased oxidative stress.[15]

Magnesium deficiency has been associated with a wide variety of clinical conditions such as atherosclerosis, cardiovascular disorders, development of cancers, and diabetes mellitus. Oxidative stress is an important pathogenic factor associated with these diseases in aging.[15] Evidence suggests that the "Westernized diet" is relatively deficient in Mg, whereas the "Oriental diet" which is characterized by a greater intake of fruits and vegetables is rich in Mg. Studies in rats prove that a long-term moderate Mg-deficient diet aggravates the cardiovascular risk associated with aging by causing a significant increase in the blood pressure.[14]

The beneficial effect of antioxidants in slowing the aging process and its role in preventing further deterioration of body functions in the elderly is a topic of considerable interest now. The objectives of the present study are to find out the alterations in serum calcium and magnesium levels with aging and to find out the correlation between serum MDA with calcium and magnesium levels.

MATERIALS AND METHODS

The present study was designed as a cross-sectional observational study to find out the role of lipid peroxidation in the alterations of serum levels of calcium and magnesium with aging. Serum MDA level was determined as a marker of lipid peroxidation.

After getting approval from the institutional ethics committee, the study was conducted for a period of 9 months from March 2010 to November 2010 in Government Medical College, Kozhikode.

In the present study, 150 healthy subjects were selected from among the hospital staff, students, and bystanders of patients admitted in the wards at Government Medical College, Calicut. The subjects selected were not on any medication or supplemental therapy.

After getting informed consent, a detailed history was taken and screening was done using a pro forma. Both males and females were included in equal proportion. Subjects were selected randomly and categorized into three groups (50 in each group).

- Group 1 – Included young controls of age group 20–30 years
- Group 2 – Included middle-aged subjects of age group 40–59 years
- Group 3 – Included elderly subjects of age group 60–90 years.

Subjects with a history of hypertension, diabetes mellitus, renal disease, heart disease, stroke, neurodegenerative diseases, diarrhea, or fever (during the previous 1 week) were excluded from the study. Body mass index (BMI) was calculated and those with BMI >25 were excluded from the study.

Serum MDA was assayed using ultraviolet-visible spectrophotometer 118 (Systronics). Serum magnesium and calcium were estimated using photoelectric colorimeter (Systronics 114).

RESULTS

The present study was conducted to find out the changes in the levels of lipid peroxidation product – MDA, serum calcium, and magnesium with aging. Data were analyzed using the Statistical Package for the Social Sciences (SPSS) version 16 and the results are summarized in Tables 1-3 and Figures 1 and 2. Results were expressed as mean ± standard deviation (SD). Mean differences between the groups were analyzed using ANOVA (analysis of variance). It is used to test whether there is a significant difference among two or more independent groups. $P < 0.05$ was taken as the level of statistical significance. To find out the association between two variables, coefficient of correlation was calculated.

Table 1: Comparison of serum calcium levels of three age groups (n=150)

Calcium [mg/dL], Mean±SD			
Group 1 (20–30 years)	**Group 2 (40–59 years)**	**Group 3 (60–90 years)**	***P* value**
9.74±0.88	9.19±0.67	_	0.002 (significant)
9.74±0.88	_	8.52±0.80	0.001 (significant)
_	9.19±0.67	8.52±0.80	0.001 (significant)

SD: Standard deviation

Table 2: Comparison of serum magnesium levels of three age groups (n=150)

Magnesium (mg/dL), Mean±SD			
Group 1 (20–30 years)	**Group 2 (40–59 years)**	**Group 3 (60–90 years)**	***P* value**
2.45±0.19	2.11±0.23	_	0.001 (significant)
2.45±0.19	_	1.90±0.20	0.001 (significant)
_	2.11±0.23	1.90±0.20	0.001 (significant)

SD: Standard deviation

Table 3: Comparison of serum MDA levels of three age groups (n=150)

Mean±SD	Group 1 (20–30 years)	Group 2 (40–59 years)	Group 3 (60–90 years)
MDA	80.28±2.95	84.58±8.62	138.57±20.25

SD: Standard deviation, MDA: Malondialdehyde

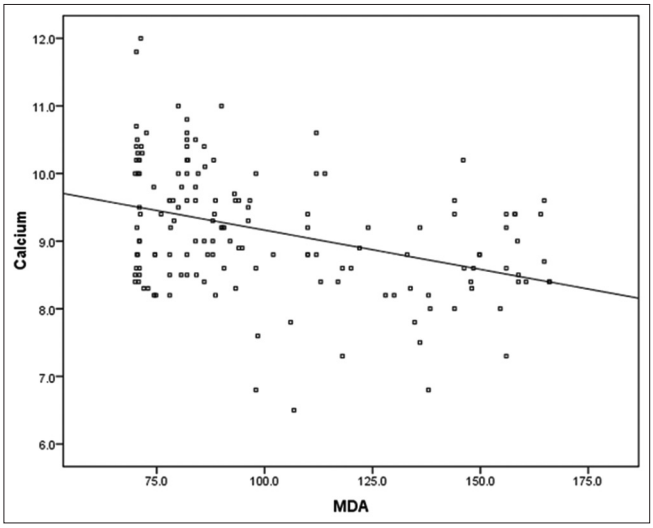

Figure 1: Scatter plot showing the correlation between serum malondialdehyde (nmol/dL) and calcium (mg/dL)

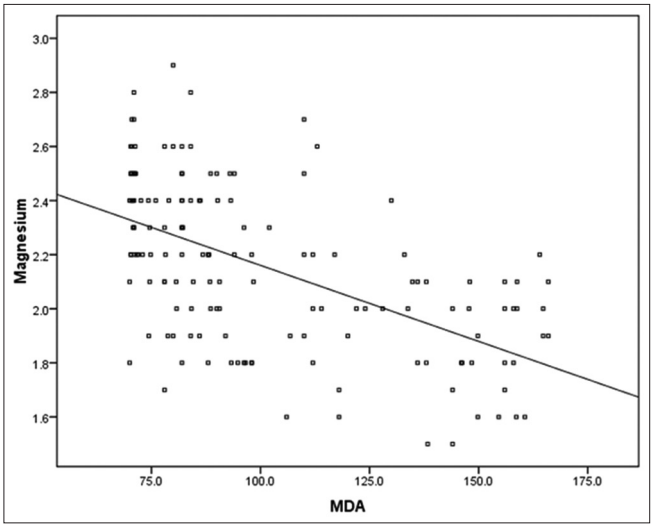

Figure 2: Scatter plot showing the correlation between serum malondialdehyde (nmol/L) and magnesium (mg/dL)

The elderly had significantly lower calcium [Table 1] and magnesium [Table 2] levels compared to the other two age groups. The mean values of serum calcium in Group 2 (9.19 ± 0.67) and Group 3 (8.52 ± 0.80) were lower than that of Group 1 (9.74 ± 0.88). The intergroup differences were statistically significant. The mean values of serum magnesium in Group 2 (2.11 ± 0.23) and Group 3 (1.90 ± 0.20) were lower than that of Group 1 (2.45 ± 0.19). The intergroup differences were statistically significant.

The mean (± SD) value of MDA in Group 1, Group 2, and Group 3 was 80.28 (± 12.95), 84.58 (± 8.62), and 138.57 (± 20.25), respectively [Table 3].

On analysis, it was found that there exists a significant negative correlation of MDA with calcium (Figure 1; correlation coefficient $r = -0.379$; $P = 0.000$) and magnesium (Figure 2; correlation coefficient $r = -0.560$; $P = 0.000$).

DISCUSSION

The 21st century is often called the "era of aging". Aging is a complex process characterized by a gradual decline in body functions and decreased ability to maintain homeostasis. It affects all physiological processes. Aging has been shown to be associated with increased free radical (ROS) activity.[16,17]

The present study was conducted to determine the role of lipid peroxidation in the age-related alteration in serum calcium and magnesium levels. In this study, the mean ± SD values of serum calcium in middle aged (9.19 ± 0.67 mg%) and the elderly (8.52 ± 0.80 mg%) were lower than that of young controls (9.74 ± 0.88 mg%) [Table 1].

A progressive decrease in serum magnesium levels was observed with mean ± SD levels as 2.45 ± 0.19 mg% in young controls, 2.11 ± 0.23 mg% in middle aged, and 1.90 ± 0.20 mg% in the elderly [Table 2]. Serum MDA is an excellent marker of lipid peroxidation. The mean (± SD) MDA levels in young controls (Group 1), middle aged (Group 2), and elderly (Group 3) subjects were 80.28 (± 12.95), 84.58 (± 8.62), and 138.57 (± 20.25) nmol/dL, respectively [Table 3], which shows an elevated level of MDA in the elderly. A negative correlation was obtained between serum MDA levels with calcium and magnesium levels [Figures 1 and 2].

Calcium is essential for bone health throughout life and is available to the body mainly through dietary source. Osteoporotic fractures are a common health-care problem in the aged population. This study revealed a decline in serum calcium levels with aging. Masoompour *et al*.[17] also reported a decrease in serum calcium in the elderly. Correlation studies by Pawade *et al*. showed that the serum ionized calcium levels varied negatively with age.[18] Vitamin D status is a major determinant of the intestinal absorption of calcium. The elderly are at a risk of Vitamin D deficiency due to dietary deficiency and decreased exposure to sunlight. The capacity of the skin to synthesize Vitamin D also decreases with age.[9] All these factors may lead to decreased intestinal absorption of calcium in the elderly. Calcium and Vitamin D supplementation is an essential component of management strategies for the prevention and treatment of osteoporosis and osteoporotic fractures. It improves bone mineralization, corrects secondary hyperparathyroidism, and reduces risk of fractures.[9]

The present study showed a progressive decrease in serum magnesium levels with aging. Decreased magnesium levels in the elderly have been reported by Henrotte *et al*.[19] and Durlach *et al*.[13] Several studies have found that elderly people have relatively low dietary intakes of magnesium. Decreased intestinal absorption and increased urinary loss of magnesium increase the risk of magnesium depletion in the elderly.[20] Low magnesium might accelerate cellular senescence by compromising DNA stability, protein synthesis, and cell energy metabolism.[14] Hypomagnesemia is associated with age-associated memory decline, neurodegenerative diseases, decreased muscle performance, insulin resistance, cardiac diseases, osteoporosis, and development of some cancers. Various studies have shown the effectiveness of magnesium supplementation in reducing atherogenic dyslipidemia and insulin refractoriness.[14,15]

ROS/free radicals produced in the body as a by-product of aerobic metabolism damage cellular macromolecules that include lipids, proteins, DNA, and mitochondrial components.[2] Lipid peroxidation is an important biological consequence of oxidative cellular damage. It is a chain reaction and leads to generation of more and more free radicals which, in turn, cause further peroxidation of other PUFAs and results in greater cell damage and dysfunction. Serum MDA is an excellent marker of lipid peroxidation. Increased oxidative damage with aging as reflected by elevated MDA levels could result from repeated exposure to ionizing radiations, mitochondrial dysfunction, and reduction in antioxidant defense mechanisms. A progressive increase in serum MDA levels was seen with increase in age, indicating the presence of increased oxidative damage with aging. Similar findings were also reported by Singh *et al*.[21] and Adak and Nazri.[20]

Cell membranes which are made up of large amounts of PUFA are highly susceptible to oxidative attack. Studies have reported that lipid peroxidation contributes to local membrane destabilization that alters the proper trafficking of intracellular vesicles, phagocytosis, degranulation, antigen presentation, receptor-mediated ligand uptake, etc., leading to age-related deterioration in many cellular functions. Correlation analysis revealed a significant negative correlation between serum levels of MDA with calcium [Figure 1] and magnesium [Figure 2). Our findings were in agreement with the findings of Saxena and Lal.[5] It has been well documented that the ROS generated during oxidative stress exert their cytotoxic effects on the cell membrane through lipid peroxidation and also by damaging ion transporters. Recent evidences showed that hypomagnesemia induces oxidative stress in aging by promoting mitochondrial dysfunction and telomere shortening.[14]

CONCLUSION

Aforementioned observations are suggesting that there might be an association between lipid peroxidation and the age-related disturbances in the serum levels of calcium and magnesium which are essential for maintaining fluid-electrolyte balance, pH, and proper functioning of various enzymes in our body.

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