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Research Article

A study on the relation between sex steroids and divalent cations in women of menopausal and reproductive age group

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ABSTRACT

Background: Menopause is the time when permanent cessation of menstruation occurs following the loss of ovarian activity. Though there is a relation between the divalent cations and sex hormones, and the lack of sufficient literature on their relation with sex hormones, the present study was conducted to compare and correlate the relation between levels of sex hormones and divalent cations in normal healthy menopausal women and the different phases of menstrual cycle in healthy women of reproductive age group.
Methods: The study was performed after institutional ethical clearance and informed consent from all the subjects. The study included three groups of 30 subjects each of reproductive age and postmenopausal women with less than 10 years of menopause and more than 10 years of menopause. Blood samples were obtained from the control group, one each in early follicular phase, ovulatory phase and during luteal phase for estimation of calcium, magnesium and the level of sex hormones and compared with that of postmenopausal group using commercially available kit. The values are expressed as mean ± S.D. The comparison between the different phases of menstrual cycle in reproductive age group with menopausal women was performed using student t test. Correlation between different parameters were done using Pearson correlation test. P value less than 0.05 was considered significant.
Results: The correlation of progesterone with magnesium and calcium/magnesium ratio in women of reproductive age group was significant. The correlation of estrogen with calcium and magnesium in women more than 10 years after menopause was found to be significant.
Conclusions: A low level of estrogen supplementation may be more acceptable, because of its negative correlation with serum magnesium, and thus helping to maintain an adequate protective level of Mg^{2+} circulating in the blood. Therefore, it is necessary for maintaining optimum Ca^{2+}/Mg^{2+} ratio, to increase the efficacy of HRT and decrease the complications of high calcium intake in women after menopause.

Keywords: Serum Ca^{2+}, Serum Mg^{2+}, Calcium-magnesium ratio, Estrogen, Progesterone, Menopausal women

INTRODUCTION

Menopause is the time when permanent cessation of menstruation occurs following the loss of ovarian activity. The years prior to menopause that encompasses the change from normal ovulatory cycles to cessation of menses are known as peri-menopausal transitional years. It takes twelve months of amenorrhea to confirm that menopause has set in and therefore it is a retrospective diagnosis.

During menopause, the levels of estrogen and progesterone are very low. In menopausal women the levels of Mg^{2+} were sufficiently higher than the levels seen in normal cycling women. It was concluded that Mg^{2+} level was inversely and significantly related to serum estrogen levels in menopausal women and in reproductive age group.

It has been well documented that during the menopausal stage of a women's life, bone metabolism is increased to a point where breakdown is more prevalent than build up.

Serum Ca^{2+} levels typically rise with the cessation of menstrual function. This is due to an increased propensity to osteoporosis in the menopausal women. It was found that in menopausal women, mean levels of Mg^{2+} and total magnesium were similar to that seen during the early follicular phase of the healthy cycling women. During this phase the estrogen and progesterone levels were comparatively low in the healthy women. The level of Mg^{2+} was sufficiently higher than the levels seen in healthy cycling women during high estrogen phase (ovulatory phase). Muneyyirci et al had reported that that Mg^{2+} level was inversely and significantly related to serum estrogen levels in healthy cycling women and menopausal women.

During menopause, women develop symptoms of estrogen deprivation, for the relief of which hormone replacement therapy (HRT) is usually advised. In addition to HRT, usually calcium is also supplemented for the prevention of menopausal osteoporosis. The postmenopausal bone loss is commonly attributed to the hyperparathyroidism developing as a result of loss of inhibition of estrogen on PTH secretion. High calcium intake interferes with magnesium absorption. Since estrogen increases intracellular levels of Mg^{2+}, the major cardiovascular benefits of estrogen treatment derive[1] from activities that are shared with Mg^{2+}. There is an imbalance between hormonal levels and divalent cations Ca^{2+} and Mg^{2+} after hormone supplementation. This intensifies the risk for cardiovascular and skeletal complications in postmenopausal women undergoing hormone replacement therapy.

Postmenopausal women who are treated with low dose replacement estrogen to prevent or slow down osteoporosis are considered to be at less risk of thromboembolic events compared to those receiving high dose estrogens. Magnesium counteracts the calcium dependant conversion of prothrombin to thrombin. Magnesium stabilizes fibrinogen and decreases fibrin formation and increases fibrinolytic activity[2]. Furthermore, it has been shown to participate in the correction of lipid and prostanoid abnormalities that increase the risk of thrombosis. Combined calcium and estrogen treatment of such patients may lower serum magnesium further, thereby increasing the risk of excess plasma calcium and intravascular coagulation. Thus magnesium supplementation has been suggested to reduce the risk of intravascular coagulation due to increased calcium and possibly to improve the anti-osteoporotic effect of calcium.

Though there is a relation between the divalent cations and sex hormones, there is no sufficient literature on their relation with sex hormones at different phases of menstrual cycle of young women with their level in menopausal women. Hence, the present study was conducted to compare and correlate the relation between levels of sex hormones and divalent cations in normal healthy menopausal women and the different phases of menstrual cycle in healthy women of reproductive age group.

METHODS

The present study was undertaken after the institutional ethical clearance. The study was performed in two groups of subjects with a control group of 30 healthy women of reproductive age group and a postmenopausal group of 30 women with varying durations of menopause (less than and more than 10 years after menopause).

Selection of subjects

The control group consists of 30 healthy women of reproductive age group (25-30 and 30-35 years). The study group consists of 30 menopausal women each of durations of menopause less than 10 years and more than 10 years.. The women on hormonal treatment and those with history of diabetes, hypertension, or systemic illnesses were excluded from the study. A prior written informed consent was obtained from all the subjects.

Collection of blood samples

Disposable syringes and needles were used to collect blood samples from the subjects. Under all aseptic precautions, 5 ml of blood was drawn from antecubital vein and was collected in two clean dry bottles. The 28 - 29 day menstrual cycle was divided into 5 day menstruation, 8 day follicular phase,6 day ovulatory period, and a 10 day luteal phase.

Three blood samples were obtained from the control group –one each in early follicular phase, ovulatory phase and during luteal phase for estimation of calcium, magnesium, estrogen and progesterone. One sample was taken from the menopausal age group for the estimation of calcium, magnesium, estrogen and progesterone.

Levels of estrogen and progesterone were assayed by chemiluminescence method using commercially available kit according to the manufacturer's guidelines.

Serum ionized calcium was calculated from the formula:

Serum ionised Ca (mg %) =

$$\frac{6\text{x total Ca (mg \%)} - \frac{K}{3}}{K + 3}$$

Where K is total protein in g%

Total magnesium concentration was assayed calorimetrically. Ionised magnesium was obtained by calculation.

Statistical analysis

The values are expressed as mean ± S.D. The comparison between the different phases of menstrual cycle in reproductive age group with menopausal women was performed using student t test. P value less than 0.05 was considered significant.

RESULTS

The correlation of estrogen with calcium, magnesium and calcium/magnesium ratio in women of reproductive age group is not significant (r = -0.46, -0.1 and +0.2 respectively). This correlation is negative with magnesium and calcium but positive with calcium/magnesium ratio (Table 1and 2). The correlation of progesterone with calcium in women of reproductive age group is not significant (r= -0.1, p>0.05) But with magnesium and calcium/magnesium ratio is significant (r = -0.3 and + 0.2 p=0.04, Table 1, 2). The correlation of estrogen with calcium, magnesium and calcium/magnesium ratio in women less than 10 years of menopause is not significant (r=+0.06, +0.1, - 0.2 and p>0.05 respectively). This correlation is positive with magnesium and calcium but negative with calcium/magnesium ratio (Table 3). The correlation of progesterone with calcium, magnesium and calcium/magnesium ratio in women less than 10 years after menopause is not significant (r=+0.2, +0.5, -0.4 and p>0.05 respectively). This correlation is positive with calcium and magnesium but negative with calcium/magnesium ratio (Table 3).

Table 1: Comparison of serum estrogen, progesterone, calcium, magnesium and calcium-magnesium ratio of menopausal women with that in the different phases of menstrual cycle in women of reproductive age.

	Menopausal women	Women of reproductive age group		
		Follicular Phase	Ovulatory Phase	Luteal phase
Estrogen (pg//ml)	20.05±7.81	121.87±21.79 p=0.000	327.35±61.17 p=0.000	208.1±36.25 p=0.000
Progesterone (ng/ml)	0.27±0.21	0.43±0.21 p=1.000	10.04±2.8 p=0.000	18.29± 3.42 p=0.000
Calcium (mmol/l)	1.14±0.7	1.17±0.1 p=0.8	1.16±0.06 p=1.000	1.16±0.06 p=1.000
Magnesium (mmol/l)	0.6±0.08	0.59±0.1 p=1.000	0.55±0.11 p=0.3	0.52±0.11 p=0.01
Calcium-Magnesium ratio	1.86±0.42	2.02±0.42 p=0.96	2.18±0.48 p=0.04	2.31±0.5 p=0.01

Table 2: Correlation of serum estrogen and progesterone level with calcium, magnesium and calcium-magnesium ratio of women of reproductive age.

	Calcium (mmol/l)	Magnesium (mmol/l)	Calcium-Magnesium ratio
Estrogen (pg//ml)	r = -0.46 p=NS	r = -0.1 p=NS	r= +0.2 p=NS
Progesterone (ng/ml)	r = -0.1 p=NS	r= -0.3 p=0.04	r=+0.2 p=0.04

The correlation of estrogen with calcium and magnesium in women more than 10 years after menopause is significant (r=+0.6, -0.3 and p=0.01, 0.03 respectively). But, its relation with calcium/ magnesium ratio is not significant (r= -0.02, p>0.05). This correlation is positive with calcium but negative with magnesium and calcium/magnesium ratio (Table 4).

Table 3: Correlation of serum estrogen and progesterone level with calcium, magnesium and calcium-magnesium ratio of women of less than 10 years of menopause.

	Calcium (mmol/l)	Magnesium (mmol/l)	Calcium-Magnesium ratio
Estrogen (pg//ml)	r=+0.06 p=>0.05 (NS)	r=+0.1 p=>0.05 (NS)	r= -0.2 p=>0.05 (NS)
Progesterone (ng/ml)	r=+0.2 p=>0.05 (NS)	r=+0.5 p=>0.05 (NS)	r= -0.4 p=>0.05 (NS)

The correlation of progesterone with calcium, magnesium and calcium/magnesium ratio in women more than 10 years after menopause is not significant (r=+0.4, +0.2, +0.06 and p>0.05 respectively). The correlation is positive with calcium, magnesium and calcium/magnesium ratio (Table 4).

Table 4: Correlation of serum estrogen and progesterone level with calcium, magnesium and calcium-magnesium ratio of women of more than 10 years of menopause.

	Calcium (mmol/l)	Magnesium (mmol/l)	Calcium-Magnesium ratio
Estrogen (pg//ml)	r=+0.6 p=0.01	r= -0.3 p=0.03	r= -0.02 p=>0.05 (NS)
Progesterone (ng/ml)	r=+0.4 p=>0.05 (NS)	r=+0.2 p=>0.05 (NS)	r=+0.06 p=>0.05 (NS)

DISCUSSION

The present study was conducted to compare and correlate the relation between levels of sex hormones and divalent cations in normal healthy menopausal women and in healthy women of reproductive age group. The estrogen levels showed cyclical fluctuations during different phases of menstrual cycle in normal cycling women, with the lowest levels during early follicular phase. Serum estrogen levels were significantly lower in menopausal women, when compared with the early follicular phase value in women of reproductive age group (Table 1). The progesterone levels also showed cyclical fluctuations during different phases of menstrual cycle in normal cycling women, with the lowest levels during early follicular phase. Serum progesterone levels were significantly lower in menopausal women, than those of women in reproductive age when compared with the early follicular phase value in women of reproductive age group (Table 1), which was consistent with the findings of Muneyyirci et al.[3]

Serum Mg^{2+} levels in women of reproductive age showed cyclical fluctuations during the menstrual cycle, with the highest levels being in the follicular phase. Serum Mg^{2+} levels were higher in the menopausal women than normal cycling women during their ovulatory and luteal phase. No significant difference was seen between the values in menopausal women and in follicular phase (table-1). During the early follicular phase, levels of Mg^{2+} were comparatively elevated. As the estrogen level rises and reaches ovulatory values, the Mg^{2+} levels fell. During menopause, the levels of estrogen and progesterone were very low. In menopausal women the levels of Mg^{2+} were sufficiently higher than the levels seen in normal cycling women. It was concluded that Mg^{2+} level was inversely and significantly related to serum estrogen levels in menopausal women and in reproductive age group. Pandya et al, Das k et al, Muneyyirci et al observed that magnesium levels were lowest at the time of ovulation, when the estrogen levels peaked.[3-5] The interrelationship of magnesium with estrogen was studied extensively by Seelig MS et al and hypothesized that estrogen induced shifts of magnesium can be deleterious when estrogen levels are high and magnesium intake is low.[6] In menopausal and postmenopausal women mean levels of Mg^{2+} and total magnesium were similar to those in cycling women during the early follicular phase, when estrogen and progesterone levels are comparatively low (Table 2, 3).

Estrogen showed a negative correlation with Mg^{2+} in menopausal women more than 10 years of menopause (Table 4). Serum Mg^{2+} levels were similar in menopausal women and normal cycling women during their low estrogen and progesterone phase that is in the early follicular phase. Serum Mg^{2+} levels were higher in the menopausal women than normal cycling women during their ovulatory and luteal phase. However Mg^{2+} level were significantly higher in menopausal women than in healthy cycling women during the high estrogen stage (around the ovulation). In addition, in the postmenopausal women, the Mg^{2+} level was inversely and significantly related to the estrogen level. Lindsay et al[7] reported an increase in the total magnesium level in serum and in urine but found no change in erythrocyte magnesium related to menopause or estrogen therapy. Therefore it was concluded that the increases in divalent cations concentrations were due to increased bone breakdown. Mcnair et al interpreted their findings of increased magnesium in blood and urine as indicative of increased absorption of cations from the gut.[8] No significant correlation was obtained between progesterone and Mg^{2+} in menopausal age (Table 1), whereas a significant positive correlation existed with progesterone and Mg^{2+} in reproductive age (Table 1).

In the present study, no statistically significant cyclical changes of serum Ca^{2+} were observed, during different phases of menstrual cycle in normal cycling women, but the highest levels were seen during the follicular phase. Numerous studies were performed worldwide to show the cyclical fluctuations of serum calcium and calcium regulating hormones. Studies of Pitkin et al and Gray et al confirmed that it was the calcium regulating hormones which had cyclical fluctuations throughout the cycle.[9,10] Similarly, Tjellesen et al and Buchanan et al noted increased 1, 25 $(OH)_2$ D concentrations when serum estrogen levels peaked.[11,12] Chiu et al demonstrated that PTH was negatively correlated with estradiol.[13] Animal investigations in rodents, chicks, and quails have also provided evidence supporting cyclical changes in calcium metabolism during the oestrous cycle.[14-16] No significant difference was observed between the values of ionized calcium concentrations of menopausal women and during different phases of menstrual cycle. Lori et al established that menopause did not affect the serum ionized calcium levels; instead it affected the total serum calcium levels only.[17] This was attributed to an age-related decrease in serum albumin concentration in women. Estrogens showed a significant positive correlation with Ca^{2+} in menopausal women more than 10 years of menopause (Table 4). There was no correlation between progesterone and Ca^{2+} in women after menopause and in reproductive age.

In the present study, the Serum Ca^{2+}/Mg^{2+} ratio was elevated significantly in luteal phase of menstrual cycle when compared to follicular and ovulatory phase. Mauskop and Altura found significant rise of the ratio during the luteal phase when progesterone levels peaked and predicted its possible role in menstrual migraines.[17,18] The Serum Ca^{2+}/Mg^{2+} ratio in menopause was significantly lower than that in ovulatory and luteal phases of normal cycling women in the present study. No significant positive correlation was obtained between estrogen and progesterone with Ca^{2+}/Mg^{2+} ratio in menopausal women (Table 1–4). It may be due to the fact that, the Ca^{2+}/Mg^{2+} ratio was increased simultaneously with the peak of estrogen and progesterone confirms that its effect is present throughout premenstrual period and suggests that this ratio may be related to premenstrual syndrome complaints, which many women have. It is possible that women who have low serum levels of magnesium could be at the risk of developing cardiovascular disorders.[19,20] In patients with low levels of magnesium, the ratio will be invariably high. Studies of Mauskop et al, Altura et al have proved that there is a high Ca^{2+}/Mg^{2+} ratio in women with diabetes, hypertension and stroke.[18] High doses of estrogen when given as contraceptive or as HRT during menopause can induce hypercoagulability and thromboembolic disease.[21,22] This may be attributed to the virtual loss of serum Mg^{2+} with high serum estrogen levels, so that the Ca^{2+}/Mg^{2+} ratio is raised.[23] It has been shown repeatedly that this ratio is important for blood vessel tone, excitation secretion coupling, synaptic transmission and bone metabolism.[24] On the other hand, it is known that low doses of estrogen are protective and beneficial to the cardiovascular system, protective against osteoporosis, and protective to the central nervous system, including retarding the onset of Alzheimer's disease. So in any case, a low level of estrogen supplementation (not more than the value that circulates in early follicular phase) may be more acceptable, the most important reason being the negative correlation it exerts with serum magnesium, and thus helping to maintain an adequate protective level of Mg^{2+} circulating in the blood. Therefore it would be efficacious to study more postmenopausal women and to study some of them in series, to strengthen this hypothesis.

CONCLUSION

This study highlights the need for magnesium supplementation in postmenopausal women on HRT. Usually high calcium is recommended to avoid bone demineralisation in postmenopausal women. Considering the calcium blocking effect of magnesium, supplementation of magnesium along with calcium is useful in optimising the intake of calcium, and to maintain adequate plasma calcium levels in women on HRT. Hence it is necessary for maintaining optimum $Ca^{2}+/Mg^{2}+$ ratio, to increase the efficacy of HRT and decrease the complications of high calcium intake in women after menopause.

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