Altered Levels of Serum and Urinary Calcium, Phosphate and Magnesium in Natural Menopausal Versus Surgical Menopausal South Indian Women: A Case Control Study

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ABSTRACT

Background: *Women who undergo hysterectomy alone are known to attain menopause 3.7 years earlier than those who attain natural menopause due to altered blood supply to ovaries[1]. Very few studies are conducted to know the effect of earlier onset of endocrinological changes associated with hysterectomy on the serum and urinary levels of calcium, magnesium and phosphate. Hence the present study was conducted to compare the levels of calcium, magnesium and phosphate in serum and urine of hysterectomised vs. natural postmenopausal women.*
Methods: *The case control study included 26 healthy premenopausal, 22 early surgical menopausal (who were hysterectomised before the age of 50 years) and 21 natural post menopausal women aged above 50 years. Women suffering from endocrine disorders (hypo/hyper parathyroidism, hypo/ hyperpituitarism, ovarian disorders, diabetes), renal failure and malignancy were excluded. Calcium, magnesium and phosphate levels were estimated in serum using calcium-cresoptholone complex, magnesium-calmagite complex and Ammonium phosphomolybdate method respectively. Creatinine was estimated in random urine by kinetic jaffe's method. Calcium/creatinine, magnesium/creatinine and phosphate/creatinine ratio were estimated in random urine using same methods. Estimations were done spectrophotometrically in a fully automated Cobas c311 analyser after appropriate quality control check. Statistical analysis for significance of difference between means was done using ANOVA and kruskal Wallis test by SPSS 13 software.*
Results: *Natural post-menopausal(serum calcium:9.12±0.46 mg/dl; magnesium:2.1±0.23 mg/dl) (urine calcium/creatinine: 0.18±0.13; magnesium/creatinine: 0.09±0.05) and hysterectomised women (serum calcium: 8.83±0.63 mg/dl; magnesium: 2.0±0.16 mg/dl) (urine calcium/creatinine: 0.16±0.1; magnesium/creatinine: 0.07±0.04) have decreased levels of serum calcium and increased serum magnesium, urine calcium/creatinine, magnesium/creatinine compared to normal premenopausal women (serum calcium:9.38±0.46 mg/dl; magnesium:1.96±0.14 mg/dl)(urine calcium/creatinine:0.07±0.05; magnesium/creatinine: 0.06 ± 0.03).*
Conclusions: *Natural post-menopausal and hysterectomies women have decreased levels of serum calcium and increased serum magnesium, urine calcium/creatinine, magnesium/creatinine compared to normal premenopausal women. Large population based study with dietary details can establish the above findings and find the relationship with oestrogen for diet and hormone supplementation.*
Abbreviations: *ER-E Strogen receptor; CBD-Calbindin; TRPV-Transient receptor potential cation channel; NCX-Sodium cation exchange channel.*

Keywords: *Oestrogen; Calcium; Phosphate; Magnesium; Surgical menopause*

INTRODUCTION

Onset of natural menopause is associated with various endocrinological changes and alteration in bone and mineral metabolism[1]. Estrogen levels decrease significantly after menopause. Decrease destrogen affects the serum and urinary levels of calcium, magnesium and phosphate indirectly at various levels. Decreased estrogen alters the intestinal absorption, bone resorption and renal re-absorption of calcium, magnesium and phosphate[2]. All these changes are gradual after natural onset of menopause. However after hysterectomy blood supply to the ovaries are affected. Hence women undergoing hysterectomy at an early age also undergo various endocrinological changes and attain menopause 3.7 years earlier than those who attain natural menopause[1]. Decreased estrogen is also observed in them. However the onset of the endocrionological changes after surgical menopause is very sudden unlike natural menopause. Very few studies have been conducted to know the effect of suddenness and earlier onset of endocrinological changes (decreased estrogen) associated with hysterectomy, on the serum and urinary levels of these minerals, especially in south Indian women. Hence the present study is aimed at comparing and correlating the levels of calcium, magnesium and phosphate in serum and urine of hysterectomised and natural menopausal south Indian women.

MATERIALS AND METHODS

This is an observational case control study. The study was carried out in the Biochemistry Central Laboratory, Department of Biochemistry,

Father Muller Medical College, Mangalore. Ethical clearance was obtained from institutional ethical clearance committee. The samples were collected in Father Muller Medical College Hospital, Mangalore after obtaining a clearly written informed consent from women attending the obstetrics and gynaecology outpatient department, women coming for normal health check-up and from women who were admitted to inpatient wards of obstetrics and gynaecology.

This study was carried out in women of south Indian origin. The study involved 3 groups. First group comprised of 26 normal healthy premenopausal women as control. Second group comprised of 22 women who have undergone complete hysterectomy before the age of 50 years. Women who have family history of early menopause were excluded from the study. Third group comprised of 21women who have attained natural menopause and were aged above 50 years.

Any women suffering from hyper/hypo parathyroidism, hyper/hypo thyroidism, hyper/hypo pituitarism, hypothalamic failure, osteomalacia, Cushing's syndrome, Addison's disease, malabsorption syndrome, polycystic ovarian disease, any form of renal disease, diabetes mellitus, conditions causing hyper/hypocalcaemia, conditions causing hyper/hypomagnesaemia, conditions causing hyper/hypo phosphatemia and women who attained menopause secondary to medical causes were excluded from the study to rule out the confounding factors.

Demographic variables like area of residence, age in years at the time of collection, age at the time of hysterectomy, time in months or years after hysterectomy and time of collection of sample, reason for hysterectomy and age at menopause were noted. In a woman who had intact uterus and not pregnantor lactating, complete cessation of menstruation at least for 12 months was considered as menopause. In women who were not having uterus (who had undergone hysterectomy with or without ovariectomy) the samples was collected 1 week after the hysterectomy.

All the blood samples were collected in a plain vacutainer under strict aseptic conditions. Serum was separated after twenty minutes of collection by centrifuging the vacationer at3000 g for 10 minutes. Random sample of urine was collected in a sterile container which is later capped tightly. In case of storage, serum was separated from blood and was stored in a vial at 4 degree Celsius till estimations are done. The urine collected was

centrifuged to remove precipitants and was stored immediately in refrigerator in 4 degree Celsius till the estimations are done. All the estimations were done within two days of storage. Urine is diluted appropriately and dilution factor is considered for calculation wherever required.

Total calcium, magnesium, phosphate were estimated in serum and in a random sample of urine which was acidified prior to estimation. All the tests were done in Roche cobas c 311 fully automated chemistry auto analyser. The entire test was estimated photometrically after the relevant quality control checks.

Serum and urinary creatinine were estimated using jaffe's principle by spectrophotometric method. Serum and urinary calcium were estimated spectrophotometrically by calcium-cresopthalene complex method. Serum and urinary phosphate were estimated spectrophotometrically by ammonium phosphomolybdate method. Serum and urinary magnesium were estimated spectrophotometrically using magnesium-calmagite complex method. Urinary calcium/ creatinine ratio, magnesium/ creatinine ratio and phosphate/ creatinine ratio were calculated.

STATISTICAL ANALYSIS

The significance of the difference between the means of age, serum calcium, magnesium and phosphate between normal control, hysterectomy and post menopausal group were analysed by ANOVA test.

The significance of the difference between the means of urinary calcium/creatinine ratio, magnesium/creatinine ratio, phosphate/creatinine ratio between normal control, hysterectomy and post menopausal group were analysed by Kruskal Wallis test.

Multiple group comparisons for the significance of difference between means of urinary calcium/creatinine ratio, magnesium/creatinine ratio and phosphate/creatinine ratio were analysed by Mann Whitney test.

Multiple group comparisons for the significance of difference between means of age, serum calcium, magnesium and phosphate were analysed after bonferroni corrections.

Pearson's correlation test was used to assess the correlation of different parameters. p value less than 0.05 is considered as significant. Statistical analysis of the results was done using SPSS13 statistical package software.

RESULTS

The all groups mean difference is presented in Table 1.

Table 1: Difference between Natural Post-Menopausal, Hysterectomies and Normal Premenopausal Women

	Natural post menopausal women (Mean ± Standard deviation)	Hysterectomies women (Mean ± Standard deviation)	Healthy premenopausal women (Mean ± Standard deviation)
Age in years ***	62.64 ± 7.78	43.81 ± 4.44	20.69 ± 0.97
Serum calcium (mg/dl) **	9.12 ± 0.46	8.83 ± 0.63	9.38 ± 0.46
Serum magnesium (mg/dl) **	2.1 ± 0.23	2.0 ± 0.16	1.96 ± 0.14
Serum phosphate (mg/dl) *	5.1 ± 3.1	3.8 ± 0.67	3.9 ± 0.59,
Urinarycalcium/creatinine ratio ***	0.18 ± 0.13	0.16 ± 0.11	0.07 ± 0.05
Urinary magnesium/creatinine ratio *	0.09 ± 0.05	0.07 ± 0.04	0.06 ± 0.03
Urinary phosphate/creatinine ratio ***	0.54 ± 0.16	0.56 ± 0.26	0.38 ± 0.11

Legend to Table 1: *$p<0.05$, **$p<0.01$, ***$p<0.001$. There is a significant difference in all the variables between the groups. The significance of the difference between the means of age, serum calcium, magnesium and phosphate between normal control, hysterectomy and post-menopausal group were analysed by ANOVA test. The significance of the difference between the means of urinary calcium/creatinine ratio, magnesium/creatinine ratio and phosphate/creatinine ratio between normal control, hysterectomy and post-menopausal group were analysed by Kruskal Wallis test.

Table 2: Mean Group Difference of Various Variables

	Natural post menopausal women vs. Healthy premenopausal women (Mean difference ± Standard error)	Natural post menopausal women vs. Hysterectomised women (Mean difference ± Standard error)	Hysterectomised women vs. Healthy premenopausal women (Mean difference ± Standard error)
Age in years	41.9 ± 1.5***	18.8 ± 1.5***	23.12 ± 1.5***
Serum calcium (mg/dl)	-0.26 ± 0.15	0.29 ± 0.15	-0.55 ± 0.15**
Serum magnesium (mg/dl)	0.17 ± 0.15**	0.13± 0.05	0.04 ± 0.05
Serum phosphate (mg/dl)	1.2 ± 0.52	1.2 ± 0.55	-0.03 ± 0.53
Urinary calcium/creatinine ratio	0.11***	0.02	0.09*
Urinary magnesium/ creatinine ratio	0.03*	0.02	0.01
Urinary phosphate/ creatinine ratio	0.16**	-0.125	0.19**

Legend to Table 2: *$p<0.05$, **$p<0.01$, ***$p<0.001$. There was a significant difference in age between each group. Serum calcium was significantly lower and Urinary calcium creatinine and phosphate creatinine ratio was significantly higher in Hysterectomies women when compared with Premenopausal women. Variables in the Hysterectomies women were similar to Postmenopausal women. Serum magnesium, Urinary magnesium, calcium and phosphate creatinine ratio was significantly higher in postmenopausal women when compared with Premenopausal women. Multiple group comparisons for the significance of difference between means of urinary calcium/creatinine ratio, magnesium/creatinine ratio and phosphate/creatinine ratio were analysed by Mann Whitney test. Multiple group comparisons for the significance of difference between means of age, serum calcium, magnesium and phosphate were analysed after bonferroni corrections.

DISCUSSION

Oestrogen is known to act on intestinal cells through oestrogen receptors ERα and ERβ. Studies have shown that decreased oestrogen lowers the expression of intestinal apical calcium channels (TRPV5, TRPV6), calcium binding proteins (CBD$_{28K}$) and basolateral channels (PMCA, NCX). This causes decreased calcium absorption from intestine[3]. This is corroborated by our study. In our study also we found significantly low levels of serum calcium in natural postmenopausal women (Table 1). This can be attributed to decreased intestinal absorption of calcium secondary to decreased oestrogen associated with aging[4]. In our study serum calcium was significantly low even in hysterectomies women (Table 1, Table 2) and there was no significant difference in levels of serum calcium between natural postmenopausal and hysterectomies women (Table 2). This suggests that even in early surgical menopause, serum calcium level gets altered similar to natural menopause. This effect is seen as early as within a week after hysterectomy.

Oestrogen is known to act on renal tubular cells through oestrogen receptors ERα and ERβ. Studies have shown that oestrogen up regulates the expression of apical calcium channel (TRPV5) independently of calcitriol[8]. Studies have also shown that deficient levels of oestrogen seen in natural postmenopausal women decrease the renal tubular reabsorption of calcium[5]. This is corroborated by our study. In our study also we found significantly high levels calcium/creatinine ratio in natural postmenopausal women (Table 1, Table 2). This can be attributed to decreased renal tubular reabsoprtion of calcium secondary to oestrogen deficiency in menopause. In our study urinary calcium/creatinine levels were low even in hysterectomies women (Table 1, Table 2) and there was no significant difference in levels of urinary calcium/creatinine between natural postmenopausal and hysterectomies women (Table 2). This suggests that even in early surgical menopause, urinary calcium/creatinine level gets altered similar to natural menopause. This effect is seen as early as within a week after hysterectomy.

Not much is known regarding the effect of oestrogen on intestinal expression of magnesium uptake apical membrane receptors (TRPM6, magnesium^{2+} / cation exchange, magnesium^{2+} / anion co transport)[5], intracellular magnesium binding and transport protein and basolateral magnesium receptors (magnesium^{2+} / sodium exchange)[6]. In our study we found significantly high magnesium levels in natural postmenopausal women (Table 1, Table 2). This could be due to a high dietary intake in the south Indian women. There was no significant alteration in serum magnesium levels in hysterectomised women (Table 2). However this needs to be established by correlating dietary intake of magnesium with serum levels of magnesium in large population studies. This is a limitation of our study.

Oestrogen increases the renal reabsorption of magnesium. This effect is evidenced by a study in which, oestrogen replacement therapy in postmenopausal women decreases urinary magnesium indicating the positive effect of oestrogen on magnesium reabsorption in kidneys[7]. Dietary magnesium restriction in humans increases the renal reabsorption of magnesium and decreases the urinary magnesium, whereas high dietary intake and increased plasma levels of magnesium decreases the renal reabsorption and increases the urinary magnesium level[8]. Studies done on mice shows that dietary restriction of magnesium increase the expression of magnesium uptake receptors (TRPM6) in distal convoluted renal tubular cells[8]. The high serum magnesium level observed in natural postmenopausal women could be due to a high dietary intake in the south Indian women. Also the high dietary intake could have possibly increased the urinary magnesium excretion. Detailed dietary intake studies are needed to establish the above.

In our study we found significantly high magnesium/ creatinine in urine of natural postmenopausal women (Table1, Table 2). High magnesium/creatinine in urine could be attributed to decreased renal reabsorption of magnesium secondary to low oestrogen observed in natural postmenopausal women. However there was no significant alteration in urinary magnesium/creatinine levels in hysterectomies women (Table 2).

Oestrogen is known to act on osteoblasts and osteoclasts through oestrogen receptors ERα and ERβ. Oestrogen promotes mineralisation by stimulating the action of osteoblasts and inhibiting the action of osteoclasts. It also decreases the reactive oxygen species concentration and decreases the degree of demineralisation. Decrease in oestrogen causes decrease in the activity of osteoblasts and increase in the activity of osteoclasts and hence causing demineralisation and eventually in postmenopausal osteoporosis[9]. This is corroborated by our study. In our study we found high level of serum phosphate in natural postmenopausal women. The observed alteration was not statistically significant (Table 1, Table 2). Observed higher serum phosphate levels could be due to increased demineralisation observed in postmenopausal women secondary to decreased oestrogen. However there was no significant alteration in serum phosphate levels in hysterectomies women (Table 2).

In our study we found significantly high levels of urinary phosphate/creatinine in natural post menopausal women. High phosphate/creatinine in urine could be due to increased filtered load secondary to increased phosphate in serum[10] (Table 1, 2). In our study we also observed significantly high

levels of urinary phosphate/ creatinine in hysterectomies women. However there was no significant difference in levels of urinary phosphate/ creatinine between natural postmenopausal and hysterectomies women. This suggests that even in early surgical menopause, urinary phosphate/ creatinine level gets altered similar to natural menopause. This effect is seen as early as within a week after hysterectomy.

Our study did not demonstrate any significant correlation of age with serum calcium, serum magnesium, serum phosphate, urinary calcium / creatinine ratio, urinary magnesium/ creatinine ratio and urinary phosphate creatinine ratio amongst all the three groups. This could be due to less number of subjects studied. Large population based study is needed to establish the relationship of the above. Also effect of oestrogen, vitamin D, Parathormone, calcitonin and dietary intake on the serum and urinary levels of calcium, magnesium and phosphate needs to needs to be considered.

CONCLUSION

Both natural post-menopausal and hysterectomies women have lower levels of serum calcium associated with increased urinary calcium/ creatinine ratio, phosphate / creatinine and magnesium/ creatinine ratio. Also serum magnesium levels were higher in natural post menopausal women. There were no significant differences between natural postmenopausal and hysterectomies women. There were no significant correlations between age of the women and the serum and urinary levels of calcium, magnesium and phosphate.

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