Effect of Oestrogen on Altering the Serum and Urinary Levels of Calcium, Phosphate and Magnesium in Hysterectomised Women Compared to Natural Menopausal South Indian Women: A Case Control Study

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Abstract Given the paucity of studies conducted to know the effect of suddenness and earlier onset of endocrinological changes associated with hysterectomy, on the serum and urinary levels of calcium, magnesium and phosphate the present study was conducted to compare the levels of calcium, magnesium and phosphate in serum and urine of hysterectomised and natural menopausal south Indian women. This is a cross-sectional observational study. The study included three groups of 30 healthy premenopausal, 30 early surgical menopausal and 30 natural post menopausal women. Women suffering from any endocrine disease were excluded. Analysis was performed in serum and urine sample. The levels of calcium, magnesium and phosphate in serum and calcium/creatinine, magnesium/creatinine and phosphate/creatinine ratio were estimated in urine by spectrophotometric method. Hysterectomised women (serum calcium: 8.7 ± 0.09 mg/dl; urine calcium/creatinine: 0.16 ± 0.02) have significantly low serum calcium ($p < 0.001$) and high urinary calcium/creatinine ($p = 0.002$) ratio and post menopausal women (serum magnesium: 2.1 ± 0.03 ; serum phosphate: 4.4 ± 0.16 ; urinary calcium/creatinine: 0.17 ± 0.02 ; urinary magnesium/creatinine: 0.09 ± 0.01) have significantly high serum magnesium ($p = 0.016$), serum phosphate ($p = 0.043$) and high urinary calcium/creatinine ($p = 0.002$), magnesium/creatinine ratio ($p = 0.025$) compared to healthy pre

menopausal women. Post menopausal women (serum calcium: 9.1 ± 0.08) have significantly high serum calcium and phosphate compared to hysterectomised women (serum phosphate: 3.93 ± 0.11). Hysterectomised women have significantly low serum calcium, oestrogen and high urinary calcium/creatinine ratio compared to healthy premenopausal women and low serum calcium and low serum phosphate compared to natural postmenopausal women. Natural postmenopausal women had low serum oestrogen and high serum magnesium, serum phosphate, urinary calcium creatinine ratio and urinary magnesium creatinine ratio compared to healthy premenopausal women.

Keywords Oestrogen · Calcium · Phosphate · Magnesium · Surgical menopause

Introduction

Menopause is associated with various endocrine changes and variations in bone and mineral metabolism. Homeostasis of calcium, magnesium and phosphate is achieved by regulation at the level of intestinal absorption, bone formation, bone resorption and renal reabsorption. Estrogen deficiency in menopause causes alteration in the homeostasis of calcium, magnesium and phosphate by directly and indirectly affecting the regulation of these minerals at the levels of intestine, bone and kidney. Studies have shown that calcium absorption decreases with increasing age after menopause and certain studies also suggest that increase in urinary calcium in postmenopausal women is not due to increased filtered load but due to decreased renal reabsorption of calcium caused by estrogen deficiency [1, 2]. It has also been reported that the hypermagnesuria observed in post menopausal women is decreased by estrogen supplementation [3]. Hysterectomy

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with bilateral oophorectomy is mainly responsible for early surgical menopause. Women who undergo hysterectomy alone are also known to attain menopause 3.7 years earlier than those who attain natural menopause due to altered blood supply to ovaries. Both natural menopausal and hysterectomised women undergo endocrine changes but differ in suddenness and age of onset. Very few studies have been conducted to know the effect of suddenness and earlier onset of endocrinological changes associated with hysterectomy, on the serum and urinary levels of these minerals. 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 a cross sectional observational study. The study was carried out in the Department of Biochemistry, Father Muller Medical College, Mangalore. The samples were collected after obtaining a clearly written informed consent from women attending the obstetrics and gynecology outpatient department, women coming for normal health check-up and from women who were admitted to inpatient wards of obstetrics and gynecology.

This study was carried out in women of south Indian origin. The study comprised of 30 normal healthy premenopausal women, 30 women who have undergone complete hysterectomy before the age of 50 years, and 30 women 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 (hypercortisolism), Addison's disease (hypocortisolism), malabsorption syndrome, polycystic ovarian syndrome, any form of renal disease, diabetes mellitus, conditions causing hyper/hypocalcaemia, conditions causing hyper/hypomagnesaemia, conditions causing hyper/hypo phosphatemia, women on medications (contraceptives, hormones diuretics) and women who attained menopause secondary to medical causes were excluded from the study to rule out the confounding factors. Women who have family history of early menopause were also excluded from the study.

Demographic variables like area of residence, age in years at the time of collection, age at the time of hysterectomy, time in days after hysterectomy and time of collection of sample, reason for hysterectomy and age at menopause were noted. In a women who had intact uterus and not pregnant or lactating, complete cessation of menstruation at least for 12 months was considered as natural 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.

Early morning fasting blood samples were collected under strict aseptic conditions. Serum was separated after 20 min of collection by centrifuging the vacutainer at 3000g for 10 min. In case of storage, serum was separated from blood and was stored in a vial at 4 °C till estimations are done. Early morning sample of urine was collected in a sterile container which is later capped tightly. The urine collected was centrifuged to remove precipitants and was stored immediately in refrigerator in 4 °C till the estimations are done. Urine is diluted appropriately and dilution factor is considered for calculation wherever required. Total calcium, magnesium, phosphate were estimated in serum and urine which was acidified prior to estimation. Samples of normal premenopausal women were collected on the tenth day of their menstrual cycle. All the tests were done using a Roche cobas c 311 fully automated chemistry auto analyser photometrically. Relevant quality control checks were performed.

Serum and urinary creatinine were estimated using Jaffe's principle by spectrophotometric method. Serum and urinary calcium were estimated spectrophotometrically by calcium-cresolphthalene 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.

Levene's statistic was used to check homogeneity of variance. ANOVA was used for analysis of mean difference in serum calcium, magnesium and phosphate. ANOVA (Welch) was used for analysis of mean difference in age, serum oestrogen, urinary calcium/creatinine, and magnesium/creatinine and phosphate/creatinine ratio.

Multiple group comparison was done for serum calcium, magnesium and phosphate by ANOVA post hoc after Bonferroni corrections. Multiple group comparison was done for age, serum oestrogen, urinary calcium/creatinine, and magnesium/creatinine and phosphate/creatinine ratio by ANOVA post hoc (Games Howell).

Pearson correlation test were 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 SPSS 21 statistical package software.

Results

The all groups mean difference is presented in Figs. 1, 2, 3 and 4. Demographic group characteristics are mentioned in Table 1. The multiple groups mean difference is presented in Table 2.

Fig. 1 Comparison of mean serum calcium level in healthy premenopausal, hysterectomised and natural postmenopausal women

Fig. 3 Comparison of mean serum phosphate level in healthy premenopausal, hysterectomised and natural postmenopausal women

Fig. 2 Comparison of mean serum magnesium level in healthy premenopausal, hysterectomised and natural postmenopausal women

Fig. 4 Comparison of mean serum oestrogen level in healthy premenopausal, hysterectomised and natural postmenopausal women

Table 1 Demographic characteristic of natural post menopausal, hysterectomised and normal premenopausal women

	Natural post menopausal women (mean $\pm$ standard error)	Hysterectomised women (mean $\pm$ standard error)	Healthy premenopausal women (mean $\pm$ standard error)	<i>p</i> value
Age (years)	62.5 $\pm$ 1.6	43 $\pm$ 0.7	21.3 $\pm$ 0.29	<0.001
Age at menarche	12 $\pm$ 1.8	12.4 $\pm$ 1.5	12.8 $\pm$ 1.6	>0.05
Age at menopause	48 $\pm$ 3.8	NA	NA	
Time since menopause	10 $\pm$ 3.2	NA	NA	
Time since hysterectomy	NA	7 days	NA	
Height (cm)	157 $\pm$ 6	159 $\pm$ 8	158 $\pm$ 7	>0.05
Weight (kg)	59 $\pm$ 1.6	62 $\pm$ 1.8	55 $\pm$ 1.9	>0.05
Body mass index	23.9 $\pm$ 1.1	24.5 $\pm$ 1.3	22 $\pm$ 1.2	>0.05
Medications	None	None	None	
Reasons for hysterectomy	NA	Ovarian tumor, fibroid uterus, dysfunctional uterine bleeding	NA	
Parity	2 $\pm$ 1.5	2 $\pm$ 1.6	Nulliparous	
Diabetes mellitus	None	None	None	
Thyroid disease	None	None	None	

Group comparison of mean age, age at menarche, height, weight and body mass index were done using ANOVA. All groups were significantly different in their mean age, and there were no significant differences in mean age at menarche, height, and weight and body mass index

Table 2 Mean multiple group difference of various variables

	Natural post menopausal women versus healthy premenopausal women (mean difference $\pm$ standard error) (<i>p</i>)	Natural post menopausal women versus hysterectomised women (mean difference $\pm$ standard error) (<i>p</i>)	Hysterectomised women versus healthy premenopausal women (mean difference $\pm$ standard error) (<i>p</i>)
Age (years)	41.2 $\pm$ 1.5 (<i>p</i> < 0.001)	19.5 $\pm$ 1.5 (<i>p</i> < 0.001)	21.6 $\pm$ 1.4 (<i>p</i> < 0.001)
Serum calcium (mg/dl)	−0.26 $\pm$ 0.12	0.31 $\pm$ 0.12 (<i>p</i> = 0.043)	−0.58 $\pm$ 0.12 (<i>p</i> < 0.001)
Serum magnesium (mg/dl)	0.13 $\pm$ 0.04 (<i>p</i> = 0.016)	0.09 $\pm$ 0.04	0.04 $\pm$ 0.04
Serum phosphate (mg/dl)	0.46 $\pm$ 0.18 (<i>p</i> = 0.043)	0.46 $\pm$ 0.18 (<i>p</i> = 0.045)	0.0 $\pm$ 0.18
Serum oestrogen (pmol/l)	−155.5 $\pm$ 23.9 (<i>p</i> < 0.001)	−9.37 $\pm$ 4.37	−146.18 $\pm$ 24.29 (<i>p</i> < 0.001)
Urinary calcium/creatinine ratio	0.09 $\pm$ 0.02 (<i>p</i> = 0.002)	0.01 $\pm$ 0.03	0.08 $\pm$ 0.02 (<i>p</i> = 0.002)
Urinary magnesium/creatinine ratio	0.03 $\pm$ 0.01 (<i>p</i> = 0.025)	0.02 $\pm$ 0.01	0.01 $\pm$ 0.01
Urinary phosphate/creatinine ratio	0.1 $\pm$ 0.04	0.01 $\pm$ 0.06	0.1 $\pm$ 0.05

Multiple group comparison was done for serum calcium, magnesium and phosphate by ANOVA post hoc after Bonferroni corrections. Multiple group comparison was done for age, serum oestrogen, urinary calcium/creatinine, and magnesium/creatinine and phosphate/creatinine ratio by ANOVA post hoc (Games Howell). Hysterectomised women have significantly low serum oestrogen and calcium and high urinary calcium/creatinine ratio and post menopausal have significantly low serum oestrogen, high serum magnesium and phosphate and high urinary calcium/creatinine, magnesium/creatinine ratio compared to healthy pre menopausal women. Post menopausal women have significantly high serum calcium and phosphate and no significant difference in serum oestrogen compared to hysterectomised women

Discussion

Menopause is a physiological state characterised by cessation of menstruation for at least more than 1 year in non lactating and non pregnant women with an intact uterus. Menopause is associated with various endocrinological, physiological, anatomical and psychological changes. There is a gradual but definite decrease in oestrogen secreted from the ovaries, also an irregular and erratic but definitive increase in the follicle stimulating hormone and

luteinizing hormone. It usually occurs during the age of 45–55 years. If women undergoes menopause earlier than the expected it is called as premature ovarian failure. Various medical causes like polycystic ovarian syndrome, chemotherapy, extensive pelvic radiation, auto immune diseases, thyroidal dysfunction, diabetes mellitus etc. are responsible for this. Ovariectomy with or without complete or partial hysterectomy is the major surgical cause of early menopause. This results in sudden cessation of production of ovarian oestrogen and a rise in follicle stimulating

hormone and Luteinizing hormone. This is responsible for all the changes including cessation of menstruation. Even women who undergo hysterectomy alone are known to attain menopause 3.5 years earlier than the natural menopausal age.

Apart from its known action on the reproductive system and secondary sexual organs, oestrogen also acts on bone intestine and kidney. Various oestrogen receptors like ER α and ER β are found in the intestinal cells, osteoblasts, osteoclasts and renal tubular cells. Oestrogen binds to its intracellular receptors and exerts direct effects as well as indirect transcriptional effects on various proteins involved in the metabolism of calcium, magnesium and phosphate in these cells.

Studies have shown that the absorption of calcium in small intestine takes place by a estrogen regulated metabolically driven active transcellular pathway and a saturable gradient dependent passive paracellular pathway across the intestinal cells [4], and generally the global calcium absorption in postmenopausal women is decreased more so in case of postmenopausal women with osteoporosis. Calcium absorption decreases in older age due to decrease in oestrogen, calcitriol and increase in end organ resistance to calcitriol. In postmenopausal women this can be attributed to decreased oestrogen associated with aging [5]. In our study we found a similar effect, hysterectomised women had significantly lower serum calcium compared to healthy premenopausal and natural postmenopausal women. These changes seen in calcium level could be due to lower level of estrogen that is usually seen in menopause and hence decreased intestinal absorption of calcium. Natural postmenopausal women had lower serum calcium compared to healthy premenopausal women but it was not significant (Fig. 1; Table 2).

Estrogen deficiency in postmenopausal women results in elevated plasma calcium secondary to increased bone resorption, which increases the filtered load of calcium in the kidney and urinary calcium [6]. But certain studies also suggest that increase in urinary calcium in postmenopausal women is not due to increased filtered load but due to decreased renal reabsorption of calcium caused by estrogen deficiency [2]. Elevated filtered load of calcium observed in postmenopausal women is due to decreased renal tubular reabsorption of calcium secondary to decreased estrogen levels. Our study also demonstrated that the urinary calcium/creatinine ratio were significantly higher in natural post menopausal women and in hysterectomised women when compared to normal healthy premenopausal women. But there was no significant differences in urinary calcium/creatinine ratio of natural post menopausal women and hysterectomised women indicating a very similar rate of renal free absorption of calcium in them (Table 2). In our study serum calcium was lower in hysterectomised women

and the urinary calcium/creatinine ratio was higher, suggesting that this could be due to lowered tubular absorption of calcium secondary to low estrogen and not due to higher filtered load as observed in other studies [2]. No significant difference was observed in the serum calcium levels of natural postmenopausal and healthy premenopausal women (Table 2).

The magnesium absorption across the intestine is similar to calcium absorption. Studies have shown that the absorption of magnesium in small intestine takes place by a metabolically driven active transcellular pathway and a saturable gradient dependent passive paracellular pathway across the intestinal cells [7]. Not much is known in detail regarding the specificities of absorption of magnesium by the intestinal epithelium and the effect of various hormonal changes associated with menopause on its absorption. Kidney plays a major role in the regulation of magnesium homeostasis [8]. The transport of magnesium across the tubules is similar to the transport across intestinal cell. In our study the serum magnesium levels and urine magnesium creatinine ratio however were found to be significantly higher in natural post menopausal women when compared to normal healthy premenopausal women. No significant difference was observed between other groups (Fig. 2; Table 2). The detailed mechanisms responsible for this are yet to be elucidated. Hypermagnesuria observed here can be due to decreased renal reabsorption secondary to reduced oestrogen as is observed in postmenopausal women. This can be corroborated by a study which shows that oestrogen replacement therapy in postmenopausal women decreases urinary magnesium indicating the positive effect of oestrogen on magnesium reabsorption in kidneys [3].

Studies have shown that 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]. 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. In our study we have not included the dietary intake, which is a limitation. Detailed dietary intake studies are needed to establish the above.

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]. In our study serum phosphate

levels were higher in natural post menopausal women compared to hysterectomised and healthy premenopausal women (Fig. 3; Table 2).

No significant difference was observed in the urine phosphate creatinine ratio between any groups (Table 2).

In our study serum oestrogen was significantly lower in natural postmenopausal as well as hysterectomised women compared to healthy premenopausal women. However the serum oestrogen levels of hysterectomised women was very similar to that of natural postmenopausal women and there was no significant difference between them (Fig. 4). This indicates that within a week after hysterectomy the serum oestrogen level decreases and reaches a very similar level seen in natural postmenopausal women. This could be due to the alteration in the blood flow to the ovaries that is severely altered after hysterectomy. The only difference being the suddenness in the onset of lower serum oestrogen levels post hysterectomy unlike the natural postmenopausal women in whom the lowering of serum oestrogen observed is gradual.

Conclusions

Hysterectomised women have significantly low serum calcium and high urinary calcium/creatinine ratio compared to healthy premenopausal women and low serum calcium and low serum phosphate compared to natural postmenopausal women. Natural postmenopausal women had high serum magnesium, serum phosphate, urinary calcium creatinine ratio and urinary magnesium creatinine ratio compared to healthy premenopausal women.

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Compliance with Ethical Standards

Conflict of interest Sonu Yeldose has received research Grants from ICMR. Sonu Yeldose, Avinash S S, Sreekantha, Arun Kumar K,

Malathi M, Shivashankara A R declares that we have no conflict of interest.

Human and Animal Rights Statement All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards.

Ethical Approval Ethical clearance was obtained from institutional ethical committee of Father Muller Medical College, Mangalore (FMMC/IEC/716/2012).

Informed Consent All authors declare that written informed consent was obtained from the patient (or other approved parties) for publication of this case report and accompanying images. A copy of the written consent is available for review by the editorial office/chief editor/editorial board members of this journal.

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