

Profile of estrogen receptor alpha gene (XbaI genotype) and vitamin D in pre and postmenopausal women in northern India

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Abstract: *Background:* In the present study XbaI gene polymorphism was exposed with the use of PCR and its co-relation was established with the level of vitamin D in the pre-menopausal and post-menopausal women of north Indian population. There are several pre and post-menopausal women in this region are suffering from vitamin D deficiency. *Objective:* The main objective of this research was to find out XbaI gene polymorphism and % of its genotypes in respect to vitamin D in the pre-menopausal and post-menopausal women of north Indian population. This is a novel study of XbaI gene polymorphisms in the north Indian women. *Methods:* Patients suffered with vitamin D deficiency was calculated as protocol proposed by WHO and calculated total cholesterol level, BMI etc. For XbaI gene variation DNA was isolated with Qaigen kit and isolated DNA was amplified with PCR. Amplified DNA was separated with 1% agarose gel electrophoresis. The Hardy-Weinberg equilibrium, OR, CI and P-value were estimated using standard protocols. *Results:* XbaI gene polymorphism was found co-related significantly with vitamin D deficiency in the pre-menopausal and post-menopausal women. The GG genotype frequency was found three fold more prone for vitamin deficiency with (P<0.0001) in patients. Logistic regression analysis was estimated for AG and GG genotypes with respect to AA.

Keywords: Allelic frequency, Gene expression, PCR, Polymorphism, Vitamin D, XbaI.

Introduction

In women there are two major phases are found. In the first stage known as premenopausal, in which menstruation occurs and characteristic of woman's sexual maturation as a biological signal of continuation of reproduction. While in second stage known as postmenopausal in which women feel sterile conditions *i.e.* end of menstruation normally this stage occur after the age of 45-50 years [1].

At the reproductive stage a women experiences an important anthropological change, which generates the exposure of estrogen and thus has major impacts for a woman's health later in life. Sometimes, early menarche is related to an increased risk of breast and endometrial cancer [2] while postponed menarche increases the chances of Alzheimer's disease [3] and osteoporosis [4]. After the menopause

osteoporosis and vitamin D deficiency may be recorded. Bone marrow density is useful indicator for the measurement of osteoporosis. In 50-80% cases of osteoporosis are found linked with the changes of genetic factors. Estrogen has found to play a major role in the menstruating and post-menstruating women [5]. Estrogen serum level get decline drastically at the stage of post menstruation. Decline serum level disturbs the bone metabolism and increased the production of inflammatory cytokinins such as IL-1, IL-6 and TNF- α . These cytokinins promote the oestiolast of bones and results the bone reabsorbing [6].

The function of estrogen is mainly governs through its receptor (estrogen stimulating receptor-I, ESR-I). In the literature it has mentioned that up-expression of ESR-I gene create severe bone loss and reduces BMD in

mice [7]. ESRs are group of nuclear receptors proteins and works as ligand-activated transcription factors. Estrogen actions on target tissues are mediated by the ESR [8]. Estrogen is a main endocrine hormone, playing a central role all through the pregnancy *i.e.* fetal development, implantation, ruling of reproduction, and synthesis of progesterone [9].

It has been reported that an enormous quantity of estrogen is generated by human placenta throughout pregnancy. To stop the estrogen receptor function which leads the pregnancy inhibition or termination of pregnancy in monkey [10]?. There are two types of receptors named ER1 and ER2, which are encoded by ESR1 and ESR2 genes located on chromosome 6 and chromosome 14 respectively [11]. The ER1 gene receptors are common in all human reproductive tissues [12]. Expression of ER2 gene has been observed in cultured human trophoblast cells before and after differentiation [13].

The objective of this work was to study *Xbal* gene polymorphism in premenopausal and postmenopausal woman and make conclusion with respect to level of vitamin D. This study is novel and not available in literature so far in detail. The relation among the polymorphism of *Xbal* gene and other related biochemical data such as lipid profile, total lipid content, low density lipoprotein cholesterol (LDL), high density lipoprotein cholesterol (HDL), and triglycerides (TG) serum levels and BMI in premenopausal and postmenopausal women are still unknown. Similarly, in the few studies, it has reported that alpha gene (*Xbal*) also plays an important role in the metabolism of vitamin D in premenopausal and postmenopausal woman. But the information provided in literature is not sufficient and yet to be confirmed.

In this study, we have tried to establish a relationship between polymorphism of *Xbal* gene (alpha gene) and vitamin D along with the related biochemical parameters in premenopausal and postmenopausal woman of northern India. We have also studied the factors which influence the variation of estrogen level such as genetic and environmental factors, including nutrition, exercise, socioeconomic conditions in premenopausal and postmenopausal woman in this area.

Material and Methods

This study was performed at Rama Medical College and Research Centre, Kanpur (India). This is a tertiary health care centre of India. All the reagents and chemicals were procured from standard companies such as Sigma Aldrich, Fermentas, Bio-Rad and Qaigen. For this study total 400 samples (200 cases and 200 controls) were taken with following standard protocols. Prior to taking the samples a written consent was obtained from all the cases and controls. During the collection of samples a standard questionnaire form was filled from each patients and controls.

This study was performed between June 2018 to September 2019 and entire protocol was got approval by the Ethics Committee of the Rama University. Cases and control age were selected either >12 years or > than 45 years after the confirmation of premenopausal and postmenopausal condition of women. Family history of cases and controls were also considered along with diabetes and obesity record. The amount of vitamin D was estimated as the protocol provided by Ott *et al.*, 1990 [14].

Before collection of samples an informed written consent forms were collected from willing participants.

Sample size: Sample size was estimated with minimum power of significance level *i.e.* up to 80% to and 95% using following formula [15]:

$$N \geq \frac{(Z_{1-\alpha/2} + Z_{1-\beta})(\sigma_1^2 + \sigma_2^2)}{(\mu_1 - \mu_2)^2}$$

$\mu_1 = 64.27$ $\mu_2 = 70.615$
 $\sigma_1 = 13.505$ $\sigma_2 = 16.36$
 $Z_{1-\alpha/2} = 1.96$ for 95% confidence,
 $Z_{1-\beta} = 1.84$ for 80% power

The calculated minimum sample size for our study is 162. After calculation we have agreed to analysis 200 for this study.

Inclusion criteria: Menstruating female above the age of 18 were recruited as controls. Women who have naturally attained postmenopause stage with no confounding factors

will be recruited as subjects for the study. Female, who attained the stage of puberty also included as subjects for this study.

Exclusion criteria: The following exclusion criteria were set up for the subjects as mentioned below.

1. Women who are having previous history of diabetes mellitus.
2. Women with known risk of thyroid disease e.g. hypothyroidism or hyperthyroidism and taking thyroid medication.
3. Women with forced menopause *i.e.* hysterectomy
4. Women with hormonal replacement therapy, taking vitamin D supplements.
5. Patients with viral infections, tuberculosis, cancer, mental illness, epilepsy and acute inflammation.

Biochemical measurement: Non-diabetic control patients were chosen at the time of health examination at the hospital according standard protocol of WHO *i.e.* fasting plasma glucose <7.0 mmol/l, and without having a history of management with hypoglycemic agents. For the anthropometric estimation height, weight, waist diameter, hip diameter, systolic and diastolic blood pressure were also calculated as per standardized protocols. BMI and waist-to-hip ratio were also measured.

Biochemical measurements pertaining levels of glycosylated hemoglobin, fasting plasma glucose, two hours post-load plasma glucose, fasting plasma insulin, C-peptide, total cholesterol (TC), high-density lipoprotein-cholesterol, low-density lipoprotein-cholesterol, triglyceride, blood urea, creatinine, C-reactive protein, and uric acid were calculated using standard laboratory assays test [18]. To find out the serum concentration of 25(OH) D, an exclusively automated analyzer by (ECLIA) Electro Chemi Luminescence Immuno

Assay process was used. We find different amount of vitamin D in the population, by followed standard protocol as; deficiency (<20 ng/ml), insufficiency (20-29 ng/ml) and normal range of vitamin D (30-100 ng/ml) was considered [16].

In the molecular biology study, for the identification of estrogen receptor alpha gene (*Xbal* genotype) first of all DNA was isolated from the fresh blood of patients and controls. Fresh blood (1ml) was composed in sterile EDTA vial to isolate DNA by using Qaigen kit with following user manual standard protocol. Purity of DNA was estimated by running 1% agarose gel and by calculating the 260/280 nm absorbance. Primers were got synthesized from Chromous Biotech Pvt. Ltd, Bangaluru. DNA was amplified with the use of PCR (Bio-RAD) and the amplified DNA was resolved with 1.0% agarose gel containing ethidium bromide and bromophenol blue. Gel photographs were recorded in software connected gel documentation system (Bio-RAD).

The PCR protocol was as below: Initial denaturation-94 °C for 5 min.

- Denaturation- 94°C for 30 Sec.
- Annealing-51°C for 30 Sec
- Synthesis- 72°C for 45 Sec
- Final synthesis-72°C for 7 min
- 35 no. of cycles were set

For the rapid fragment length polymorphism (RFLP) *Xbal* restriction endonuclease enzyme was used with following standard digestion protocol [17]. The PCR product were digested with 10 units of MspA1I (New England BioLabs) restriction endonuclease for more than 1 hour. The digested PCR products were separated on 2% agarose gel.

Sl. No.	Primer sequences	Tm (°C)
1.	<i>Xbal</i> F (5'- CTGCCACCCTATCTGTATCTTTTCCTATTCTCC-3')	53
2.	<i>Xbal</i> R (5'- TCTTTCTCTGCCACCCTGGCGTCGATTATCTGA-3')	55

The statistical analyse was conducted using SPSS software version 20 (USA). Statistical power of the study was calculated under log-additive

model, assuming 10% population possibility by Quanto. Hardy-Weinberg equilibrium for *Xbal* genotypes was analysed by χ^2 analysis.

P-value of <0.01 was measured significant after Bonferroni correction. Means and standard deviations values were estimated by inverse normal units of the parameters. Association with obesity and quantitative traits was performed only with controls. Allele frequencies of cases and controls of the study population were analysed by equal opportunity of proportions Z-test. All the data analyses were accustomed for age, sex and BMI as required. The odds ratio (OR) and 95% confidence interval (CI) were estimated with respect to minor alleles in the study [18].

Results

Clinical and biochemical measurement: The study contained data of 200 cases and 200 controls unrelated pre-menopausal and post-

menopausal women were considered. Total numbers of the patients (200) were considered along with controls (200) as mentioned in the Table-2. Many biochemical parameters (Table-3) were selected for the analysis and authentication of study. The clinical parameters such as BMI, total cholesterol, systolic and diastolic blood pressures, urea, uric acid, creatinine, triglycerides were analysed with significant P value <0.0001. The most of the analysed data (serum creatinine, diabetic record, uric acid, BMI, urea) were found significantly greater in cases with respect to controls. The BMI was calculated according to the WHO Asia Pacific guidelines as non-obese (BMI <25 kg/m²) and obese (BMI >25 kg/m²).

Table-2: Sample Distribution

S.N.	No of pre-menopausal women	No of post-menopausal women	Control	P-value
1	100	100	200	0.005
2	Total no of cases=200		Total no of control=200	

Table-3: Selected Biochemical Analysis

S.N.	Characteristics	Pre-menopausal women	Post-menopausal women	Control	P-value
1.	Average age (years)	≥32	≥51	≥40	<0.0001
2.	Diabetic record (No.)	18.3±0.6	29.9±1.6	14.7±0.6	<0.005
3.	BMI (Kg/m ²)	20.13±1.4 / 23.57±0.5	21.16±1.6 / 25.00±0.6	20.42±1.4 / 23.61±1.1	0.710
4.	Total cholesterol (nmol/l)	4.25±1.0	4.37±1.2	4.34±2.1	<0.0001
5.	Urea (mmol/l)	8.70±1.3	9.00±0.4	8.60±2.4	<0.0001
6.	Uric acid (mmol/l)	287.01±1.2	289.00±1.3	288.82±0.4	<0.0001
7.	Creatinine (mmol/l)	61.71±3.5	64.03±2.4	63.00±6.0	<0.0001
8.	Systolic BP (mmHg)	120±3.0	124±3.5	121±1.9	<0.005
9.	Diastolic BP (mmHg)	80±3.1	82±4.5	81±2.0	<0.005
10.	Triglycerides (mmol/l)	1.27±3.0	1.52±3.6	1.29±4.0	<0.0001

*Xba*I gene was detected and correlated with vitamin D with Hardy-Weinberg equilibrium. The genotype frequencies of the *Xba*I gene in cases were found significant statistically with vitamin D. Allele frequencies at the three polymorphic sites of cases were summarized in Table-4. The *Xba*I gene genotypes frequency of AG and GG were noted significantly higher in vitamin D

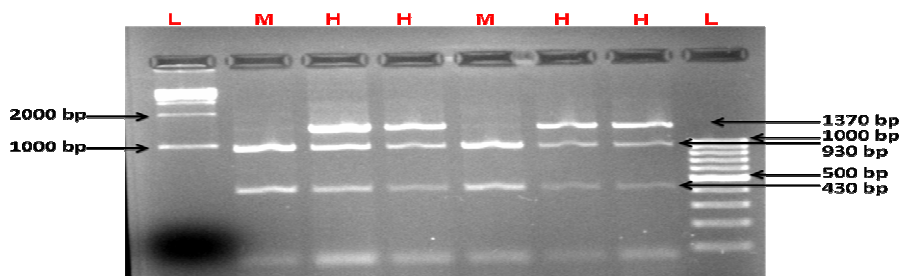
deficient cases with P value <0.0001. Logistic regression analysis was estimated for AG and GG genotypes with respect to AA, which was considered as a reference genotype. The comparison between the AG and GG genotypes recorded an unadjusted OR of 1.74, which was statistically significant (0.001), furthermore significance was retained after

adjusting for age and vitamin D level. When we compared the AA genotype with the GG genotype, the OR remained significant, conferring 1.50 times higher risk with the amount of vitamin D. The variation of frequencies of

Xbal gene alleles was also found significant for vitamin D (36.5%) of cases with P values <0.001. 95% CI value was calculated for both cases and control and mentioned in the Table-4.

S. N.	<i>Xbal</i> genotype	Pre-menopausal women (%)	Vitamin D (ng/ml)	Post-menopausal women (%)	Vitamin D (ng/ml)	Control (%)	Adjusted OR value	95% CI	P-value
1.	GG	47	30	51	10	86	Reference	Reference	0.005
2.	AG	40	35	33	15	12	1.74	1.16-1.89	0.001
3.	AA	13	32	16	12	2	1.02	1.25-2.40	0.002
	Total	100		100		200	-	-	-

Fig-1: PCR-RFLP of *Xbal* gene variants



(L- Ladder, M-Mutant, H-Hetrozygous)

In Fig.-1, the alleles of the *Xbal* polymorphism were defined as A and G: heterozygote AG (fragments: 1370 bp, 930 bp, and 430bp), wild type GG (fragment: 1370bp), and mutant AA (fragments: 930 bp and 430bp).

Discussion

The gene *Xbal* which is present on intron 1 of ESR1 gene have been associated to several estrogen-sensitive traits in menopausal woman. *Xbal* plays many pathological effects such as abortion, breast cancer, endometriosis, myocardial infarction, and thromboembolism in women [19]. The vitamin D level is regulated by VDR gene may be another prospective source of vitamin D synthesis at the premenopausal and postmenopausal woman. The expression of VDR has been studied in reproductive organs of women [20]. Due to deficiency of vitamin D in mice, it has been observed that uterine hypoplasia with deteriorated folliculogenesis [21].

In the literature there are many genetic studies have been reported in the relation with *Xbal* genotype. But *Xbal* genotypes variation in the respect to estimation of vitamin D level in pre-menopause and post-menopausal women are not studied well so far. Estrogen plays a crucial role during menstruation of women. In the many studies it has been reported that a considerable amount of estrogen is raised in the women throughout menstruation period [22].

Several studies have been mentioned strong linkage disequilibrium in the *Xbal* polymorphisms. *Xbal* gene polymorphisms located in the first intron of ESR1, and found the linkage disequilibrium with vitamin D [19]. The main differences between this study and other reported studies may be due to differences in ethnic background, sample size, region of study and statistical methods. In the present study in addition to the genotypes of *Xbal* gene along with the some environment

factors such as BMI, total cholesterol, systolic and dia-systolic BP have found significant role in the studied cases. The polymorphism of *Xbal* gene is found strongly co-related with the level of vitamin D in the cases. Present study has clear message that the magnitude and the direction of the effects of genotypes at one locus of gene may be affected by the definite genotype at the other loci of the gene [23].

The data obtained from this study is also indicating the association of genotypes at numerous loci may be more effective than a single one. From the physiological point the estrogen related *Xbal* gene may influence the level of vitamin D and its receptors [24]. The level of vitamin D may play a balance between androgens and estrogens level. The vitamin D may play a crucial role at several points of estrogen response pathway which may affect the levels of estrogen receptor in the menstruating women [25].

The data obtained in this study has strong co-relation with the finding of Hong *et al.*, 2005 [26] in the Chinese population. In this study original statistical results were presented for genetic investigations. In the present study the level of vitamin D was found substantially low which indicated that the expression of *Xbal* gene has strong co-relation with it in the menopausal women. In the present study, unrelated subjects were selected from similar living environment [27]. In this study the obtained GG genotype has indicated three fold higher risk of vitamin D deficiency in post-menopausal than pre-menopausal women and controls. With the

adjustment of OR value the GG genotype frequency was found high risk of vitamin D deficiency in the studied population. This finding is strongly supported with the study of Soares *et al.*, 2018 [28].

However, in this study methods have definite limitations, including instruments and technology. The present used PCR-RFLP method is well acceptable, convenient, inexpensive and a high-throughput method for genotyping of a small population size.

Conclusion

To our knowledge, this is the first study to report on *Xbal* gene variants in North Indian population. In our sense, such type of studies are needed to know the strength and exact nature of the genetic variation to determine which of the variant within a haplotype cluster could be functionally related to vitamin D in post-menopausal women. This study has showed a statistically significant co-relation in vitamin D deficiency in post-menopausal women in the studied population. In this study applied PCR-RFLP method is accurate, inexpensive, rapid, and easy to perform than other method such as PCR sequencing method.

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References

1. Peter I, Shearman AM, Zucker DR, Schmid CH, Demissie S, Cupples LA, et al. Variation in estrogen related genes and cross-sectional and longitudinal blood pressure in the Framingham Heart Study. *J. Hypertens.* 2005; 23:2193-2200.
2. Rees M. The age of menarche. *Orgyn.* 1995; 4:2-4.
3. Sutherland MK, Somerville MJ, Yoong LKK, Bergeron C, Haussler MR, McLachlan DR. Reduction of vitamin D hormone receptor mRNA levels in Alzheimer as compared to Huntington hippocampus: correlation with calbindin-D28K mRNA levels. *Mol. Brain Res.* 1992; 13:239-250.
4. Ito M, Yamada M, Hayashi K, Ohki M, Uetani M, Nakamura T. Relation of early menarche to high bone mineral density. *Calcif. Tissue Int.* 1995; 57:11-14.
5. Riggs BL, Khosla S, Melton LJ. A unitary model for involuntional oosteoporosis; estrogen deficiency causes both type I and type II oosteoporosis in post-menopousal women and contributes to bone loss in ageing men. *J. Bone Minor Res.* 1998; 13: 763-773.

6. Eghbali-Fatourehchi G, Khosla S, Sanyal A, Borel WJ, Lacey DL, Riggs BL. Role of RANK ligand in the mediating increased bone re-absorption in the early post-menopausal women. *J. Clin. Invest.* 2003; 111:1221-1230.
7. Hewitt SC and Korach KS, Oestrogen receptor knockout mice: roles for oestrogen receptors and in reproductive tissues. *Reproduction.* 2003; 125(2):143-149.
8. Kuiper GG, Carlsson B, Grandien K, Enmark E, Nilsson S et al. Comparison of the ligand binding specificity and transcript tissue distribution of estrogen receptors alpha and beta. *Endocrinology.* 1997; 138:863-870.
9. Albrecht ED and Pepe GJ. Estrogen regulation of placental angiogenesis and fetal ovarian development during primate pregnancy. *The Int. J. of Develop. Biol.* 2010; 54(2-3):397-407.
10. Ravindranath and Moudgal NR. Use of tamoxifen, anantio estrogen, in establishing a need for oestrogen in early pregnancy in the bonnet monkey (*Macaca radiata*). *J. of Reprod. and Fertility.* 1987; 81(2):327-336.
11. Menasce LP, White GRM, Harrison CJ and Boyle JM. Localization of the estrogen receptor locus (ESR) to chromosome 6q25.1 by FISH and a simple post-FISH banding technique. *Genomics.* 1993; 17(1):263-265.
12. Maruyama H, Toji H, Harringtonetal CR. Lack of an association of estrogen receptor α gene polymorphisms and transcriptional activity with Alzheimer disease. *Archives of Neurology.* 2000; 57(2):236-240.
13. Du JW, Tao XR, Xu KY, Fang LY, and Qi XL. Polymorphisms in estrogen receptor- α are associated with idiopathic female in fertility. *Mol. Med. Reports.* 20011; 4(6):1239-1242.
14. Ott SM, Chestnut CH. Tolerance to the doses of serum 1, 25-dihydroxy vitamin D is associated with improved bone density in women with postmenopausal osteoporosis. *J. Bone Miner. Res.* 1990; 5:745.
15. Gomes-Rochette NF, Souza LS, Tommasi BO, Pedrosa DF, Eis SR, Fin IDCF, et al. Association of PvuII and XbaI polymorphisms on estrogen receptor alpha (ESR1) gene to changes into serum lipid profile of postmenopausal women: Effects of aging, body mass index and breast cancer incidence. *Plos One.*2017; 12(2):1-13.
16. Yang J, Loos RJ, Powell JE, Medland SE, Speliotes EK, Chasman DI, et al. FTO genotype is associated with phenotypic variability of body mass index. *Nature.* 2012; 490:267-272.
17. Jungert A, Roth HJ & Neuhauser-Berthold M. Serum 25-hydroxyvitamin D₃ and body composition in an elderly cohort from Germany: a cross-sectional study. *Nutr. Metab. (Lond.).* 2012; 9:42.
18. Pouryasin M, Sharafi H, Behnava B, Alavian SM, Keshvari M, Pouryasin A. A simple PCR-RFLP method for genotyping of *IFNL4* rs368234815 polymorphism in patients with chronic hepatitis C. *Lab. Med.* 2017; 48(1):51-56.
19. Gennari L, Merlotti D, De Paola V, Calabro A, Becherini L, Martini G et al. Estrogen receptor gene polymorphisms and the genetics of osteoporosis: A HuGE review. *Am. J. Epidemiol.* 2005; 161:307-320.
20. Kitagawa I, Kitagawa Y, Kawase Y, Nagaya T, Tokudome S. Advanced onset of menarche and higher bone mineral density depending on vitamin D receptor gene polymorphism. *Eur. J. Endocrinol.* 1998; 139:522-527.
21. Yoshizawa T, Handa Y, Uematsu Y, Takeda S, Sekine K, Yoshihara Y, et al. Mice lacking the vitamin D receptor exhibit impaired bone formation, uterine hypoplasia and growth retardation after weaning. *Nat. Genet.* 1997; 16:391-396.
22. Corp IBM. Released 2011. IBM SPSS Statistics for Windows, Version 20.0. Armonk, NY: IBM Corp 2011.
23. Swami S, Krishnan AV, Feldman D. Alpha, 25-Dihydroxyvitamin D₃ down-regulates estrogen receptor abundance and suppresses estrogen actions in MCF-7 human breast cancer cells. *Clin. Cancer Res.* 2000; 6:3371-3379.
24. Zhang YY, Long JR, Liu PY, Liu YJ, Shen H, Zhao LJ, and Deng HW. Estrogen receptor α and vitamin D receptor gene polymorphisms and bone mineral density: association study of healthy pre- and postmenopausal Chinese women. *Biochemical and Biophysical Research Communications.* 2003; 308(4):777-783.
25. Motawi TMK, EL-Rehani M, Rizk SM, Ramzy MM, el-roby DM. Genetic polymorphism of estrogen receptor alpha gene in Egyptian women with type II diabetes mellitus. *Meta Gene.* 2015; 6:36-41.
26. Hong XU, Ji-rong NG, Miao-xin LI, Hong-wen DENG. Interaction effects between estrogen receptor α and vitamin D receptor genes on age at menarche in Chinese women. *Acta Pharmacologica Sinica.* 2005; 26(7):860-864.
27. Wietzke JA, Welsh J. Phyto-estrogen regulation of a vitamin D₃ receptor promoter and 1, 25-dihydroxyvitamin D₃ actions in human breast cancer cells. *J. Steroid Biochem. Mol. Biol.* 2003; 84:149-157.
28. Soares JM, Holanda FS, Matsuzaki CN, Sorpreso ICE, Veiga ECE, Abreu LC, Carvalho KC and Baracat EC. Analysis of the PvuII and XbaI polymorphisms in the estrogen receptor alpha gene in girls with central precocious puberty: a pilot study. *BMC Med. Genet.* 2018; 9:84.

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