



Original Research Article

Serum Vitamin D Status and hs-CRP in Pre- and Postmenopausal Women

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Abstract

Aim: Vitamin D deficiency is one of the major health problems in all age groups especially in elderly women. Menopause marks an important change in woman's life. With an increasing age, women are at high risk of several metabolic diseases. Therefore, the aim of this study is to find the status of serum vitamin D and hs-CRP in pre- and postmenopausal women.

Methods: Total 500 female subjects were taken, out of which 400 pre- & postmenopausal women were considered as cases while 100 subjects were considered as control healthy women. Serum Vitamin D was estimated by chemiluminescent micro particle immunoassay method while hs-CRP was assayed in serum by using latex-enhanced turbidimetric immunoassay method. Serum Estradiol (E₂) was estimated by competitive immunoassay method in all studied female subjects. Data were analyzed by using SPSS software, version 22.0.

Result: Serum Vitamin D levels were significantly decreased while hs-CRP was significantly increased ($p < 0.001$) in pre- and postmenopausal women as compared with control healthy women. Serum estradiol was also significantly decreased in postmenopausal women. We also found a significant negative correlation between vitamin D deficiency and hs-CRP levels in both pre- and postmenopausal women.

Conclusion: Postmenopausal women at increased risk of vitamin D deficiency as compared to premenopausal women. Low-grade systemic inflammation precedes the risk of CVD, diabetes and metabolic syndrome in postmenopausal women.

Keywords: Vitamin D, hs-CRP, Estradiol, premenopausal, postmenopausal women.

Introduction

Menopause is not considered a disease, it's the transition period in woman's life when her ovaries become inactive and she is not able to bear children. It usually occurs between the ages of 45-55 years^[1]. Due to aging and decline in estrogen hormone levels, postmenopausal women are at

high risk of several health issues. Menopause is also considered as the phenomenon of aging, as the aging of skin leads to reduce the efficiency of skin to synthesize vitamin D even upon sun exposure^[2].

Vitamin D is a fat-soluble vitamin synthesized photochemically in the skin from 7-

dehydrocholesterol. It plays an important role in calcium, phosphorous and bone metabolism^[3]. Nowadays vitamin D seems to have an anti-inflammatory and immune modulating properties. This immune modulating property of vitamin D and its inverse link with inflammation drawn more attention toward osteoporosis, metabolic syndrome, cardiovascular disease (CVD) and type 2 diabetes mellitus (T2DM) in postmenopausal women^[4,5]. Everything from depression to CVD to cancer and autoimmune disorders is theorized as having inflammation as a core etiological factor^[6-10]. In measuring that inflammation, C-reactive protein (CRP), an acute phase protein synthesized by hepatocytes are used as a classical marker of inflammation^[6]. In recent years hs-CRP has evolved as an important marker of low-grade systemic inflammation and predictor for CVD, obesity, and T2DM^[11]. Therefore, the importance of serum vitamin D and hs-CRP in postmenopausal women has been focused in this study

Material and Method

Study Design and sample selection

The present cross-sectional study was designed and conducted in the Department of Biochemistry at G.R. Medical College, Gwalior (M.P.) and Medanta- The Medicity, Gurgaon. Total 500 women who were visited for executive health checkup were included. Premenopausal women with age group of 30-45 years and with normal menstrual cycle while postmenopausal women with age group of >45 years and amenorrhea or whose last menstruation occurred at least 12 months prior were included. Women who were either suffering from or had a prior history of CVD, metabolic bone disorder, chronic liver and renal disease and malignancy were excluded from the study. Women using hormone replacement therapy was also excluded. The study was approved by the institutional ethical committee and was carried out after obtained informed consent voluntarily from all the participants.

Sample collection and assessment

After initial enrolment, 5ml of fasting venous blood samples were drawn from all the studied women. Blood was allowed to clot at room temperature 37°C for 30 minutes, and serum was obtained immediately by centrifugation at 3000 rpm for 10 to 20 minutes. The serum sample was analyzed for hs-CRP based on latex-enhanced turbidimetric immunoassay method and estradiol-based on competitive immunoassay method by using an automated VITROS 5600 analyzer (Ortho-Clinical Diagnostics®, Rochester, NY, USA). Serum vitamin D levels were measured by chemiluminescent micro particle immunoassay (CMIA) with an automated Architect® i2000 SR analyzer (Abbott Diagnostic Division, Barcelona, Spain). Internal and external quality control was run routinely before performing the assay analysis. Serum concentration of vitamin D less than 30 ng/mL were classified as deficient and more than 30 ng/mL as sufficient based on the latest recommendation of international endocrine society clinical guideline^[12]. Body mass index (BMI) was calculated by formulae Weight (Kg)/Height (m²).

Statistical Analysis

Data were analyzed by the statistical package for social science (SPSS) software, version 22. Results of the study were expressed in mean±SD and percentage. Student's t-test was used to compare between two groups. Pearson's correlation was used to find out the correlation. A p-value less than <0.001 was considered statistically significant.

Results

In our study, we observed a significant decrease in serum vitamin D levels and estradiol levels while significant increase (p<0.001) was found in BMI and hs-CRP levels in both pre- & postmenopausal women when compared to control healthy women (Table No. 1). Serum vitamin D levels and estradiol levels were also significantly decreased

while hs-CRP levels were significantly increased ($p < 0.001$) in postmenopausal women as compared with premenopausal women. BMI was also significantly increased ($p < 0.001$) in postmenopausal as compared with premenopausal women. hs-CRP was significantly increased in deficient vitamin D group as compared to sufficient vitamin

D group of both pre- & postmenopausal women (Table No. 2). We also observed a significant negative correlation between hs-CRP and vitamin D deficiency in premenopausal ($r = -0.365$, $p < 0.001$) and postmenopausal women ($r = -0.952$, $p < 0.001$) (Table No. 3).

Table No. 1: Showing the significant changes in premenopausal & postmenopausal when compared with control healthy women.

Parameters	Control healthy women (n=100)	Premenopausal Women(n=200)	Postmenopausal Women (n=200)	p-value
Age (years)	28±5.56	35.32±4.85**	57.03±8.41**	a. <0.001 b. <0.001 c. <0.001
BMI (Kg/m ²)	22.94±1.74	25.98±4.09**	27.67±4.51**	a. <0.001 b. <0.001 c. <0.001
Serum Vitamin D levels (ng/mL)	46.87 ±13.64	27.95±15.16**	22.02±17.53**	a. <0.001 b. <0.001 c. <0.001
hs-CRP (pg/mL)	2.19 ±0.62	4.02±2.16**	4.85±2.60**	a. <0.001 b. <0.001 c. <0.001
Estradiol (E ₂) (pg/mL)	76.89±15.21	76.44±22.88 ^{NS}	23.61±7.79**	a. >0.05 b. <0.001 c. <0.001

** Statistically Significant ($p < 0.001$), ^{NS}Non Significant ($p > 0.05$) Student's t-test was used to compare between a. Control vs. premenopausal women, b. Control vs. postmenopausal women, c. premenopausal vs. postmenopausal women

Table No. 2: Showing the significant changes between deficient and sufficient vitamin D levels in pre- & postmenopausal women.

Parameter	Premenopausal (n=200)		Postmenopausal (n=200)	
	Sufficient Vitamin D levels (n=73) 36.5%	Deficient Vitamin D levels (n=127) 63.5%	Sufficient Vitamin D levels (n=59) 29.5%	Deficient Vitamin D levels (n=141) 70.5%
hs-CRP (pg/mL)	2.37±1.04	4.94±2.09**	2.48±0.81	5.84±2.44**

** Statistically Significant ($p < 0.001$)

Table No. 3: Showing the Pearson's correlation between serum vitamin D deficiency and the hs-CRP in pre- & postmenopausal women.

Vitamin D Deficiency				
Inflammatory marker	Premenopausal Women		Postmenopausal Women	
	r value	p-value	r value	p-value
hs-CRP	-0.365	0.000**	-0.952	0.000**

** Statistically Significant ($p < 0.001$)

Discussion

Menopause marks an important health transition in woman's life. The shift from pre- to postmenopausal was associated with the emergence of various health problems due to hormonal changes with declining estrogen and

alteration of its ratio with testosterone-hormone^[13]. In postmenopausal women, the two major causes of bone loss are estrogen deficiency after menopause and age-related processes^[14]. Aging is one of the important risk factors for vitamin D deficiency because aging affects

multiple steps of metabolism of vitamin D. Vitamin D is important for the maintenance of calcium, phosphorous and bone metabolism^[2,3]. Bone turnover increases to high levels and estrogen deficiency may induce calcium loss by indirect effects on extraskeletal calcium homeostasis^[14]. One important role of estrogen is to stimulate the enzyme 1α hydroxylase which is responsible for the production of the active form of vitamin D i.e. 1,25 dihydroxyvitamin D in the kidney^[15]. Therefore, postmenopausal women are at increased risk of vitamin D deficiency due to fall in estrogen hormone and aging. In our study, we also found significantly decreased in serum vitamin D levels and estradiol levels in postmenopausal women as compared to premenopausal. The prevalence of vitamin D deficiency was more in postmenopausal women (70.5%) as compared to premenopausal women (63.5%) was also observed in the present study. Menopausal transition is associated with unfavorable changes in body composition and abdominal fat deposition^[16]. It is suggested that the loss of ovarian function induces a reduction in resting metabolic rate, fat-free mass and an increase in fat mass and abdominal adipose tissue accumulation^[17,18]. In our study, we found a significant increase in BMI in postmenopausal women compared with premenopausal women. BMI related metabolic factors, especially adipokines, can induce the expression and release of inflammatory factors. In adipose tissue, vitamin D also inhibits the inflammatory response by decreasing the production of proinflammatory cytokines via phosphorylating and inhibiting the translocation of the nuclear factor- κ B into the nucleus^[19]. We also observed that the inflammatory marker hs-CRP was significantly increased in deficient vitamin D group as compared to sufficient vitamin D group in both pre- and postmenopausal women. Inflammation is considered as an important causative factor in the development of CVD and numerous metabolic diseases^[20]. Studies have revealed that a low-grade systemic inflammation

precedes and predicts the onset of diabetes in adults and inflammatory markers like hs-CRP is significantly elevated in individuals with obesity & T2DM and are associated with measures of adiposity. The role of adiposity in the regulation of the inflammatory response is well known. Adipose tissue itself is a source of CRP formation and also a major producer of interleukin-6, which is a key stimulator of CRP secretion. CRP is produced in response to inflammation, infection, and injury and has been correlated with complications related to conditions like hypertension, CVD, and diabetes^[11, 21]. In obesity, adipose tissue contains an increased number of resident macrophages and T cells, which interact closely with adipocytes to modulate the inflammatory response^[21] and released a high amount of tumor necrosis factor- α (TNF- α) and interleukin-6 (IL-6) into the circulation, which stimulate the production of hs-CRP by the liver and induces insulin resistance^[22]. We also found a significant increase in BMI and hs-CRP levels in postmenopausal women when compared with premenopausal. We conducted a correlative study between vitamin D deficiency and hs-CRP and observed a significant negative correlation of hs-CRP with low levels of vitamin D in both pre- and postmenopausal women. This significant correlation showed that vitamin D deficiency and inflammation played an important role in the progression of various health issues with advancing age.

Conclusion

With the rapid transition in nutrition and lifestyle, postmenopausal women are at increased risk of vitamin D deficiency and obesity due to aging, the decline in estrogen hormone and also low-grade systemic inflammation. Thus the significant increase and positive correlation of hs-CRP with vitamin D deficiency in both pre- & postmenopausal suggest that inflammation, vitamin D deficiency and obesity are the key players for developing CVD, diabetes and metabolic syndrome.

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