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RESEARCH ARTICLE

"STATUS OF SERUM VITAMIN D IN PRE MENOPAUSAL INDIAN WOMEN"

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Abstract

Vitamin D promotes absorption of calcium in the intestine as well as renal tubules and maintains adequate levels of calcium and phosphate in serum to maintain normal bone mineralization and prevents osteoporosis. This vitamin can be produced endogenously when ultraviolet (UV) rays in sunlight hit the skin and promotes its synthesis [1]. Deficiency of this vitamin is common in India, the rate of prevalence range from 40 – 90 percent [2]! Geographically India is partly tropical and subtropical and receives sunlight round the year. More than 30ng/mL was the biological reference interval for vitamin D but it was derived from western population and it is using by the diagnostic laboratories across the country. Because of this scale majority of healthy individuals shows insufficient Vitamin D levels [3].

Objectives: To assess the status of vitamin D in the premenopausal women. To evolve a reference range in Indian sub-population and to correlate the findings with the reference interval established by National institutes of health (NIH), USA.

Methodology: A cross sectional study consisting of 127 adult pre-menopausal women (29 – 45 years of age) was conducted. Their total Vitamin D levels were measured by Chemiluminescence immunoassay technique.

Result: 26 ± 8.5ng/mL was the biological reference interval (BRI) for Vitamin D, established by NIH but the in-house obtained BRI was 18.2 ± 11.5ng/mL. The obtained BRI was significantly decreased (p <0.001) than NIH established values.

Conclusion: 18.2 ± 11.5ng/mL of Serum Vitamin D was observed in the adult premenopausal Indian sub population.

Introduction:-

India is well-known for its tradition, culture and lingual diversity and it is a wide tropical country located from 8.4º N to 37.6° N latitude and 68.7°E to 97.25°E longitude[4]. Deficiency of Vitamin D (Vit.D) in Indians is expected to be uncommon because the population gets the adequate sun light throughout the year[5]. However from the data available in the published literature, Vit.D deficiency is common in India in all the age groups and both the genders across the country [6,7]. Currently in the country, there is no health program that fulfills the specific health need of premenopausal women. Around 103 million Indian women are expected to attain post menopausal state by the year 2026[8]. Therefore there is an urgent need to focus on the pre menopausal women health care. The present study is therefore expected to unmask the magnitude of suffering due to health issues in pre menopausal women associated with vit.D status. Because of the health risks associated with low levels of vit.D in postmenopausal women, the

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primary objective of this study was to establish the biological reference interval in premenopausal women of Indian sub population and to validate the hypotheses among apparently normal healthy asymptomatic adult population that vit. D deficiency of < 20ng/mL in compare to western reference range. [9, 10]

Materials And Methods:-

About One hundred and twenty seven Adult premenopausal women who were attending Padmashree diagnostics, Department of Gynecology, Vijayanagar Main Road, Bangalore, for elective diagnosis and treatment for osteoporoses were constituted for the study.The study was completely explained to the subjects before obtaining a written informed consent. The study proposal has been approved by Institutional Review Board, Padmashree Institute of Clinical Research. Bangalore.

The study population (n= 127) were screened for entry into study by measuring indicators of liver and kidney function, blood calcium, phosphorus and alkaline phosphatase. 10 failed the initial screening; left one hundred and seventeen subjects the mean age of 39 ± 3.4 were recruited for the study.

Inclusion and Exclusion criteria :

	Selection Criteria :
Inclusion Criteria :	**Premenopausal Women**
Age in years	29 - 45
Sex	Female
Healthy Individuals	Yes
Women who had menstruation cycle at regular duration	Yes
Not on Hormone Replacement Therapy (HRT)	Yes
	Exclusion Criteria:
Metabolic disorders like hypo/ hyperparathyroidism and Diabetes mellitus Type I /Type II.	No
Auto immune disorders like Systemic Lupus Erythematosus(SLE) and Rheumatoid arthritis	No
Surgery like gastrointestinal resection or malabsorption	No
Chronic liver or Renal diseases	No
Drugs like, Glucocorticoids, aluminium containing antacids, Frusemide, bisphosphonate, calcium, Vitamin A, Vitamin D, Calcitonin, lithium, antiepileptics and anticoagulant, hypercalcaemia of malignancy, which effects the metabolism of bones	No
Chronic granulomatous disorders like Sarcoidosis and Tuberculosis	No
Paget's disease of bone tissue	No
Cigarette smoking, Alcohol abuse	No
History of recent fractures (in the past six months)	No

Specimen handling and analysis

The blood specimen received in the laboratory was centrifuged (1800 x g /15mins) to separate the cellular components and the cell free serum processed for the analysis of routine biochemical parameters sought by the treating clinicians. Remaining specimens were aliquoted, labeled and stored at – 20ºC till further analysis. The Aliquots of samples, once thawed were analyzed on the same day. Repeated freezing and thawing was not done to avoid any pre-analytical variables.

Type of study:

Randomized control study

Research design:

Non Interventional

Analytical Method:

Total serum Vit.D level was estimated by Chemiluminescence immunoassay technique.

Sources of Data
Available literature information from recent publications was updated during the course of study. The study design to be employed was standardized /modified depending upon the situation before applying the same for the sample analysis. Information with respect to study outcome was procured from the patient medical records and clinical expertise opinion was sought before relating the study outcome.

Statistical analysis:
SPSS Version 19 Data analysis package was used and applied to analyze the obtained data after discussion with the Biostatistician. The values were expressed in terms of mean ± SD. For the statistical comparison Student "t" test was used. The student's 't' test *p< 0.05; **p< 0.01; ***p< 0.001 was considered as significant.

Analytical Method:
Device Description:
ADVIA centaur - XP – Chemiluminescence immunoassay system was used for the estimation of Vit.D levels.

Principle:
Vit.D test was an eighteen – minute antibody competitive immunoassay technique that uses an anti-fluorescein monoclonal antibody of mouse covalently bound to paramagnetic particles (PMP), an Anti-25 hydroxy Vit.D monoclonal antibody of mouse labeled with acridium ester (AE), and a Vit.D analog labeled with fluorescein. An inverse relationship present between the amount of Vit.D present in the sample and the amount of relative light units (RLU) detected by the sensor in the system.
The **ADVIA Centaur XP** Vit.D Ready kit consists of the following reagents:
1. **Lite Reagent:** (5.0mL Reagent pack) Anti-Vit.D (monoclonal mouse) antibody labeled with acridinium ester (~0.8 µg/mL) in buffer with bovine serum albumin and sodium azide (< 0.1%).
2. **Primary reagent:**Acridinium ester(~0.8µg/mL) in buffer with bovine and serum albumin.Mouse IgG and Sodium azide(<0.1%)
3. **Solid phase:** (10.0mL reagent pack) Anti-fluorescein (monoclonal mouse)- coated paramagnetic particles (PMP) (~0.60 mg/mL) in buffer with bovine serum albumin, surfactant, and sodium azide (< 0.1%).
4. **Vit.D ancillary well reagent:** (5.0mL/reagent pack) Vit.D-analog conjugated to fluorescein (~0.2 µg/mL) in buffer with bovine serum albumin and sodium azide (< 0.1%).
5. **Vit.D ancillary Pack reagent:** (25.0 mL/reagent pack) Releasing agent in buffered saline with sodium azide (< 0.1%).
6. **Vit.D Diluent:**Phosphate buffer with BSA, Cholesterol and Sodium azide(<0.1%)
7. **Wash 1 reagent:** Phosphate-buffered saline with sodium azide(<1%) and surfactant.

Assay Protocols
Performance characteristics : The assay was highly specific and sensitive for the estimation.

Sensitivity and Detection limit	:	4.2 – 150 ng/mL
Specificity	:	0.3 % cross reactivity
Precision	:	It was evaluated according to the CLSI guidelines.

Quality control: Randox company controls were used. Two levels of controls were run (normal and abnormal) before analyzing the samples. Values of control samples were with in acceptable limits (± 2SD).

Procedural steps:
1. Load primary tube container into rack, ensuring that the barcode labels were clearly visible through the slot in the rack.
2. Place the rack in the sample compartment.
3. Press/clik the Start option.
4. The instrument was fully automated and analyzes the sample automaticly.
5. Values released from the instrument were interphased through LIS.
6. The values were noted.

Results:-
The obtained serum Vit.D Reference interval was **18.2 ± 11.5 ng/mL**, which was significantly lower (***p < 0.001) compared to the reference interval established by NIH ie 26 ± 8.5ng/mL.

Serum vitamin D level in Premenopausal women

Sl No.	Parameter	Vitamin D		n
		Range	Mean ± SD	
1	S_VIT.D	6.7 – 29.7	18.2 ± 11.5	117

Reference ranges of Vitamin D

SL No.	Reference	Mean ± SD
1	NIH, USA	26 ± 8.5
2	Established	18.2 ± 11.5

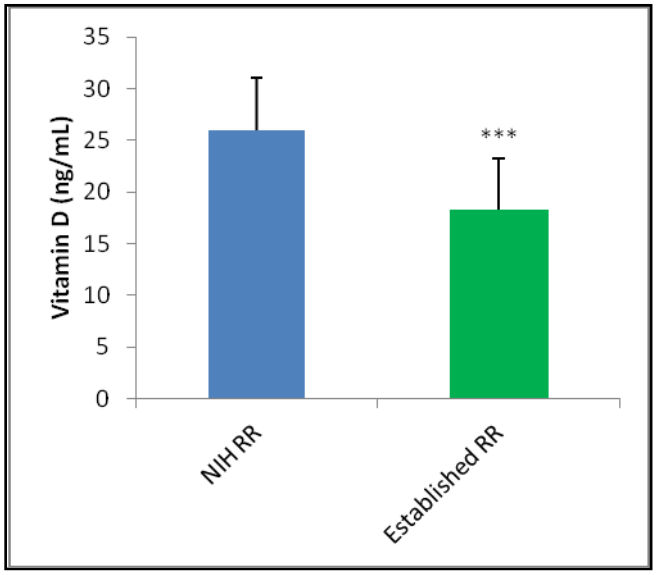

Fig 1:- Histogram represents the Vit.D reference interval of NIH Compared with premenopausal women. Expressed as Mean ± SD. Student's 't' test: *: $p < 0.05$; **: $p < 0.01$: ***: $p < 0.001$.

Thus, in present study measured values of vit.D in premenopausal women was compared with NIH reference interval (**Fig 1**). The observed finding suggests that, the measured Vit.D in premenopausal women significantly lower (***$p < 0.001$) than the NIH reference interval.

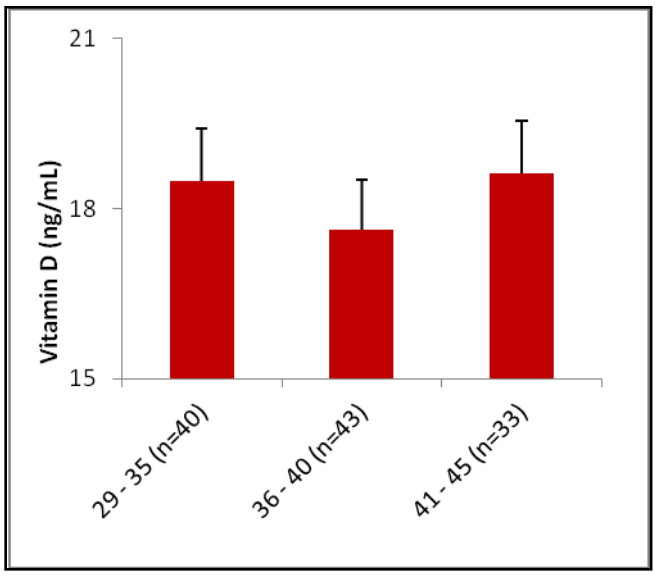

Fig 2:- Histogram represents the Vitamin D reference interval of premenopausal women from 3 sets age group Expressed as Mean ± SD. Student's 't' test: *: $p < 0.05$; **: $p < 0.01$: ***: $p < 0.001$.

In the present study, the premenopausal healthy subjects (n = 117) have provided the reference interval of vit.D for Indian sub-population with distinct aged premenopausal groups range from 29 – 45 years. Many factors involves in vit.D normal level including age, gender and body mass index(BMI). Hence, to minimize the bias the healthy subjects aged 29 – 45years were further sub-divided into 3 groups (29 – 35, 36 – 40 and 41 – 45 years). According to **Fig 2**, the histograms represents as 18.5 ± 12.1ng/mL, 17.6 ± 9.6ng/mL and 18.6 ± 13.1ng/mL between the 3 group respectively. The age related analysis did not showed any significant difference among the three groups.

Discussion:-

Serum Vit.D plays an important role in number of biological processes. Its deficiency also causes serious consequences. Inadequacy of the vitamin is the primary dilemma and inclined to osteoporosis in post menopausal women, in recent dogma. Vit.D became the most often sought after investigation to confirm or to rule out the suspected osteoporosis cases.

Vit.D is a unique nutrient because its requirement is met from diet and skin. In the year 1922, Dr. EV. Mc Collum et al were able to discover to prove that existence of second fat soluble vitamin [11], he did not called the common name Vit.D but he called as the Ca++ depositing vitamin. Nearing to a century of its existence in the field of medicine, still it is under stern scrutiny of defining the sufficiency and insufficiency for its optimum functional role in human beings.

In the past, Vit.D deficiency was defined as the concentration if less than 10ng/mL in serum because both the Vit.D and Ca++ absorption decline at this threshold [12]. In the 2003, the World health organization defined, Vit.D insufficiency as- lower than 20 ng/mL in serum and normal as 20 to 30 ng/mL[11]. The Institute of Medicine (IOM) reiterates that recommendation in 2011. The Endocrine Society proposed an alternative, defining insufficiency as less than 30 ng/mL and normal as higher than 30 ng/mL [13]. Why does it matter if the recommended cutoff for insufficiency is 20 ng/ mL or 30 ng/ mL? The reason is that meeting this target serum Vit.D changes the Recommended Dietary Allowance (RDA). The present study made an attempt to establish the in-house normative data (reference interval) of premenopausal women Vit.D value and it was compared with the available reference interval.

Summary and Conclusion:-

There had been an ample increase in measuring serum vit.D over the past decade. Hence, there is an urgency in developing our own reference interval of Vit.D to arrive a good indicator of osteoporosis to rule out its sufficiency and insufficiency in Indian sub-population. Our study involves obtaining normative Vit.D data from premenopausal women sub-population from India. Our study once again underscores the essentiality of our own in-house laboratory reference interval data for Vit.D may help us in identifying the individuals who are at increased risk for frequent bone fractures. In the present premenopausal women Vit.D level did not showed any astounding level which is normally called as sufficiency (>30ng/mL) by all available literature. It clearly indicates in our study that the pre menopausal women had 18.2 ± 11.5ng/mL concentration of Vit.D.

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Conflicts of Intrest

Authors of the study had no financial interest nor received any financial support from the companies manufacture reagents and instruments for the estimation of Vit.D levels.

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