Prof. Dr. C.V. HARI NARAYAN – DETAILS OF RESEARCH WORK DONE

VITAMIN D STATUS IN PRIMARY HYPERPARATHYROIDISM IN INDIA (1990) - CHANGING CLINICAL PROFILE WITH CHANGING VITAMIN D STATUS OF THE POPULATION (2022) – THREE DECADES LATER.

1. ***Harinarayan CV,*** **Gupta N, Kochupillai N. Vitamin D status in primary Hyperparathyroidism in Northern India.** ***Clinical Endocrinology*** **1995;43:351-358.**

2. ***Harinarayan CV,*** **Ashok H, Munigoti SP, Tandon S, Sarma KVS, Tandon A, *et al*. Vitamin D status in primary hyperparathyroidism in 1990 and thence – Emergence of normocalcaemic presentation and diagnostic challenges – Utility of parathyroid function index.** ***J Clin Sci Res*** **2022;11:167-74**

The first documentation of low vitamin levels In the Indian population was observed while evaluating patients with primary hyperparathyroidism (PHPT) as a part of my Doctor of Medicine (DM) thesis (1990). Primary hyperparathyroidism is a disease of bone and stones. In some patients, it is complicated by nephrolithiasis, nephrocalcinosis, and renal insufficiency. In India, it primarily presents as a symptomatic bone disease (sup periosteal resorption and fractures) in contrast to the West where they are picked up asymptomatic during routine evaluation with elevated serum calcium on bone loss not seen in radiology but only detectable by bone densitometry.

We found the prolonged low vitamin D levels along with low dietary calcium as the cause of bone disease (osteitis fibrosa cystica). Bone disease was the standard mode of presentation. Ninety percent of them had overt bone disease, 60 % had brown tumors, and 40% had pathological fractures. Renal stones and nephrocalcinosis were found in 50% of the patients. Only 50 % had hypercalcemia. However, 90% of the whole group (both normo and hypercalcemic PHPT) had hypercalciuria. The 25 hydroxy vitamin D levels were (mean $\pm$ SD) 8.4$\pm$ 5.1 ug/l (7.94 $\pm$ 1.94 ng/ml). The 25 hydroxy vitamin D levels of healthy age and sex-matched controls were comparable (8.3 + 2.5 ug/l). The parathyroid adenomas were large and weighed in grams. The median weight of parathyroid adenomas was 4.6 g (mean $\pm$ SD 3.75 $\pm$ 1.6) g. The age and sex-matched controls were none other than the doctors and nursing staff of the department. This striking observation contradicted the then-existing impression of normal Vitamin D status in Indians – a tropical Country with plenty of sunshine. *(Vitamin D status in primary Hyperparathyroidism in Northern India. Clinical Endocrinology 1995;43:351-358).*

This led to further evaluation of the vitamin D status of the Indian population with a planned survey of various rural villages in and around Tirupati (semi-urban population). This survey revealed about two-thirds of the population had low levels of vitamin D. Inadequate dietary calcium intake associated with a high phytate/calcium ratio reduces the bioavailable calcium in the gut. *(High prevalence of low dietary calcium and low vitamin D status in healthy south Indians. Asia Pac J Clin Nutr 2004;13 (4):359-365).* Around the same time, the population survey was extended to more rural villages, and data was gathered on dietary calcium intake along with 25 hydroxy vitamin D levels of the population. *(Table 1 & 2 below: High prevalence of low dietary calcium, high phytate consumption, and vitamin D deficiency in healthy South Indians, Am J Clin Nutr 2007;85:1062–7.)* This was the time when the normal vitamin D range changed from a "population-based reference range" to a "health-based reference range" (based on that level of 25 hydroxy vitamin D which elicits a rise in the parathyroid hormone levels – 25 hydroxy vitamin D of 30 ng/ml was considered normal). The subjects were classified as vitamin D – D-deficient, – insufficient, or – sufficient based on 25(OH)D concentrations of 20 ng/mL, 20-30 ng/mL, and 30 ng/mL, respectively, according to recent consensus. Data on dietary calcium intake and vitamin D status is shown below.

TABLE 1
Comparison of dietary intake of urban and rural groups[1]

	Men		Women	
	Urban (n = 32)	Rural (n = 109)	Urban (n = 476)	Rural (n = 96)
Dietary calcium (mg/d)	323 ± 8 (307, 340)	271 ± 3 (263, 280)	306 ± 2 (302, 310)	262 ± 3 (253, 271)
Dietary phosphorus (mg/d)	674 ± 17 (640, 707)	493 ± 9 (475, 511)	651 ± 9 (643, 660)	481 ± 10 (462, 501)
Phytate-to-calcium ratio	0.5 ± 0.02 (0.47, 0.54)	0.76 ± 0.01 (0.74, 0.78)	0.51 ± 0.01 (0.50, 0.52)	0.76 ± 0.01 (0.74, 0.78)

[1] All values are $\bar{x}$ ± SEM; 95% CIs in parentheses. Recommended dietary allowance of calcium in diet recommended by the Indian Council of Medical Research is 400 mg/d in adults. There was no significant interaction between sex and location (urban and rural). The main effects of sex and dietary calcium were significant. $P < 0.012$. Significant location (urban and rural) × dietary calcium., location (urban and rural) × dietary phosphorus, and location (urban and rural) × phytate-to-calcium ratio interactions were observed. $P < 0.0001$.

TABLE 2
Comparison of biochemical and hormonal profiles of urban and rural groups[1]

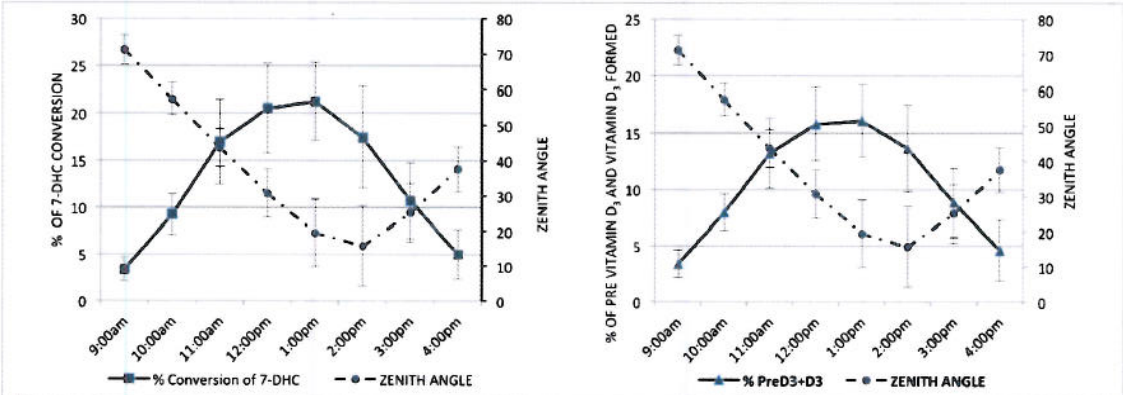
	Men		Women		
	Urban	Rural	Urban	Rural	P
Serum calcium (mg/dL)	9.74 ± 0.06 (9.63, 9.85) [100]	10.06 ± 0.06 (9.95, 10.2) [109]	9.68 ± 0.02 (9.64, 9.73) [678]	9.98 ± 0.06 (9.87, 10.15) [96]	< 0.001[2]
Serum phosphorus (mg/dL)	3.50 ± 0.07 (3.37, 3.64) [99]	2.84 ± 0.07 (2.27, 2.97) [109]	3.64 ± 0.03 (3.59, 3.69) [679]	2.74 ± 0.07 (2.79, 3.09) [96]	< 0.001[2]
SAP (IU/L)	84.87 ± 3.87 (78.85, 90.9) [98]	55.67 ± 2.07 (49, 61) [109]	80.4 ± 3.07 (78, 90.17) [683]	62.7 ± 3.41 (56, 69.4) [96]	< 0.001[2] 0.032[3]
25(OH)D (ng/mL)	18.54 ± 0.8 (17, 20) [134]	23.73 ± 0.8 (22, 25) [109]	15.5 ± 0.3 (14.9, 16) [807]	19 ± 0.89‡ (17.54, 21) [96]	< 0.001[2] < 0.01[4]
N-tact PTH (pg/mL)	27 ± 1.6 (23.9, 30) [135]	29.24 ± 1.6 (26, 32.35) [109]	28.35 ± 0.6 (27, 29.5) [803]	29.21 ± 1.7 (25.75, 32.7) [96]	

[1] All values are $\bar{x}$ ± SEM; 95% CIs in parentheses; n in brackets. SAP, serum alkaline phosphatase; 25(OH)D, 25-hydroxyvitamin D; N-tacts-PTH, immunoreactive parathyroid hormone. To convert 25(OH)D from ng/mL to nmol/L, multiply by 2.5.
[2] Main effect of location (urban and rural).
[3] Interaction between sex × location (urban and rural).
[4] Main effect of sex.

This study brought out a very interesting observation that the rural population (agricultural workers), who exposed more than 80% of their torso to sunlight working in the agricultural fields for 6-8 hours per day had 25 hydroxy vitamin D deficiency. They also had low dietary calcium intake.

This made us undertake a study, to establish the ability to synthesize vitamin D in sunlight. *(Extract from: C.V. Harinarayan, et al Vitamin D status and sun exposure in India. Dermato Endocrinology 2013; 5(1):130-141. http://dx.doi.org/10.4161/derm.23873).* The study was conducted in Tirupati latitude 13.40° N and longitude 77.2° E from May 2007 to August 2008. Sealed borosilicate glass ampoules containing 50 µg of 7-DHC in 1 ml of methanol were exposed to sunlight hourly beginning from 8 a.m. until 4 p.m. An ampoule was placed outside each hour so that the photolysis of 7-DHC could be studied in a time-dependent fashion for the whole day. From 12 p.m. to 1 p.m., a control ampoule was placed together with an ampoule for study. The satellite picture of the country on the day of study at 11:30 a.m. was downloaded from the website *www.hinduonnet.com* under section miscellaneous—weather report from the archives of the newspaper.

Casual exposure to solar radiation wavelengths 290–315 nm results in the cutaneous production of previtamin D_3. During sun exposure the UVB photons (290–315 nm) that enter the epidermis cause a photochemical transformation of 7-dehydrocholesterol (7-DHC) (provitamin D_3) to previtamin D_3. The relationship between the solar zenith angle, the percent conversion of 7-DHC to previtamin D_3 and its photoproducts, lumisterol, and tachysterol, and the percentage of previtamin D_3 and vitamin D_3 formed, against each day studied are shown in the figures below.



	ZENITH ANGLE °				% CONVERSION OF 7-DHC				% PREVITAMIN D_3 AND VITAMIN D_3 FORMED			
TIME	Mean	SD	Min	Max	Mean	SD	Min	Max	Mean	SD	Min	Max
9:00am	57	5	53	67	3.40	1.25	1.27	5.68	3.40	1.25	1.27	5.68
10:00am	44	5	39	55	9.25	2.13	4.57	11.44	7.97	1.61	4.57	10.80
11:00am	31	7	25	44	16.98	4.50	7.69	22.91	13.24	3.08	7.69	17.59
12:00pm	20	10	11	38	20.50	4.70	10.30	26.97	15.78	3.21	9.68	20.50
1:00pm	17	11	5	37	21.26	4.11	14.96	28.22	16.08	3.16	11.86	21.40
2:00pm	26	8	18	42	17.46	5.37	6.82	25.62	13.65	3.83	5.97	19.71
3:00pm	38	6	32	50	10.71	4.06	2.76	18.34	8.83	3.08	2.76	14.29
4:00pm	51	5	46	61	5.02	2.65	2.02	10.44	4.91	2.47	2.02	9.86

Figure 2. Showing the mean ± SD of the zenith angles, percent conversion of 7-Dehydrocholesterol (7-DHC) to previtamin D_3 and photoproducts, and the percentage of previtamin D_3 and vitamin D_3 against time (for the study duration). The table below gives the individual values, minimum and maximum of the variables.

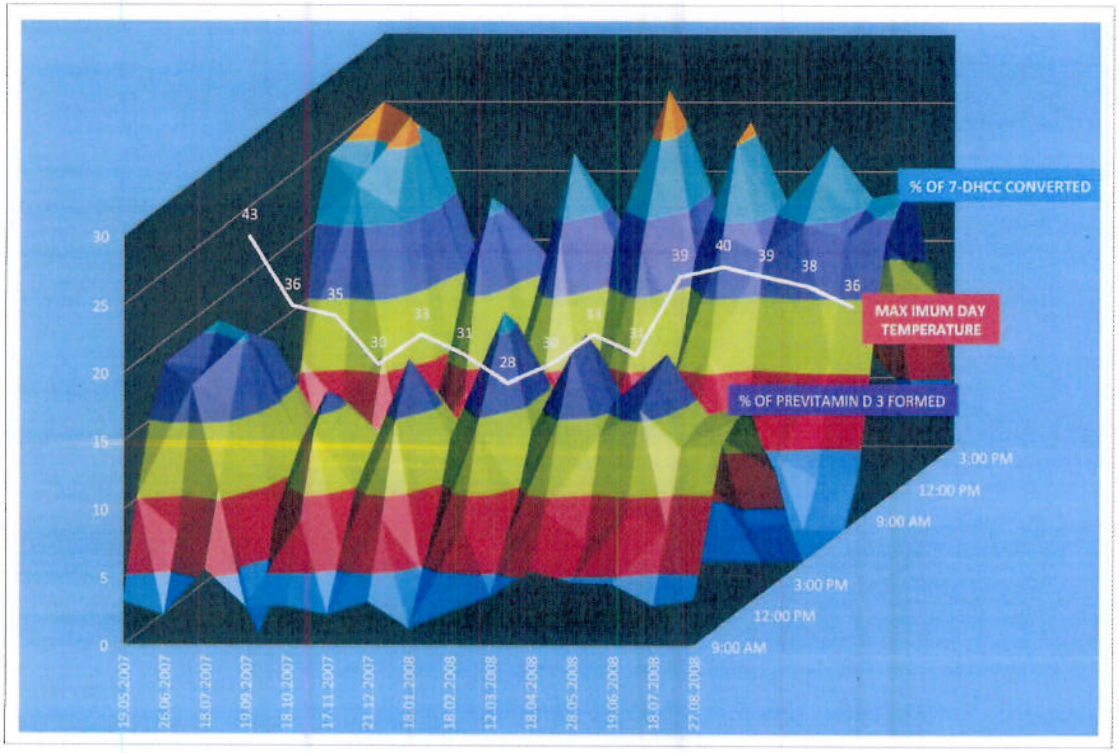


Figure 4. Three-dimensional depictions of the percent conversion of 7-DHC to previtamin D_3 and photoproducts and percentage of previtamin D_3 and vitamin D_3 along with zenith angles of various time of the day during the whole study period. Maximum temperature on the day of the study is also depicted in the same graph.

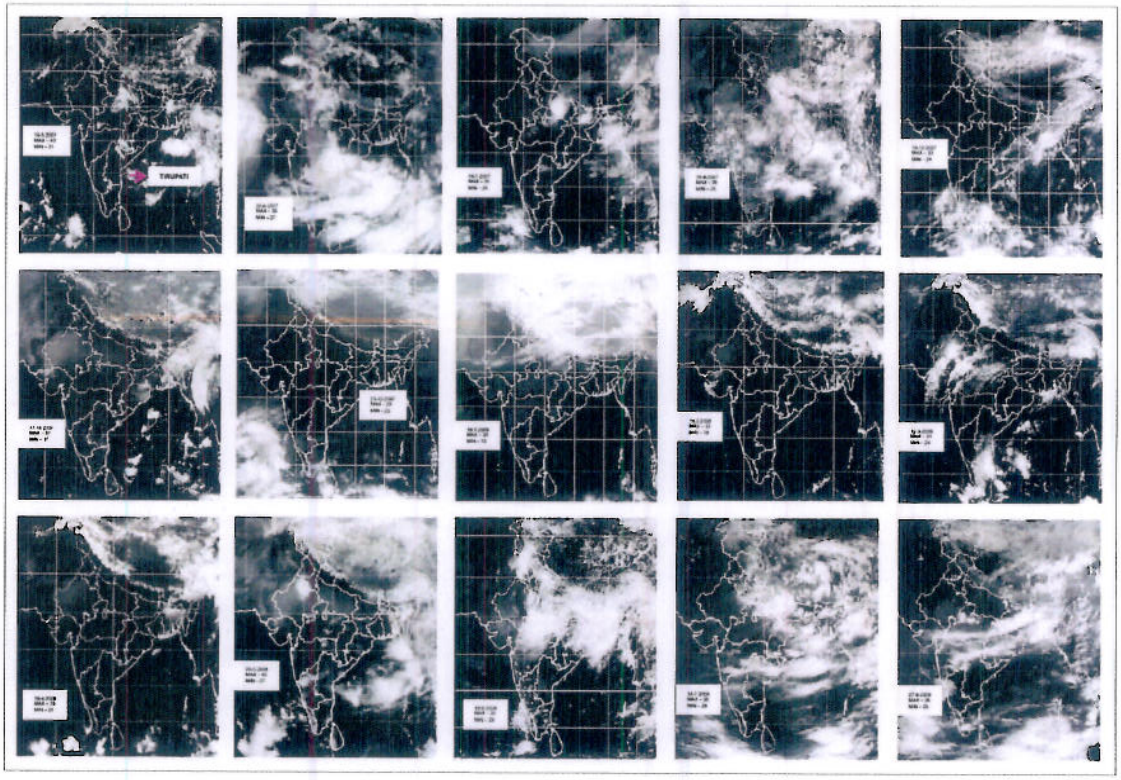


Figure 5. Satellite picture of the country on the day of study at 11.30 h. First picture on the left upper panel (row 1) shows the location of study site (TIRUPATI—latitude 13.40° N and longitude 77.2° E). The date and maximum and minimum temperature on the day of study is shown in each picture. The satellite picture is downloaded from www.hinduonnnet.com under section miscellaneous—weather chart.

Hence, we as Indians on exposure to sunlight between the hours of 11 a.m. and 2 p.m. (mid-day sun) will synthesize enough vitamin D in the skin year-round. The low vitamin D status in agricultural workers is due to low dietary calcium intake.

This led to us review the vitamin D and dietary calcium intake of India in the past half a century – to look in retrospect, introspect, and in prospect – projecting the remedial measures India should take to combat this "Twin Nutrient deficiency". The references are given below:

- *Harinarayan CV and Akhila H. Modern India and the tale of twin nutrient deficiency–calcium and vitamin D–nutrition trend data 50 years-retrospect, introspect and prospect. Front. Endocrinol., 09 August 2019.* https://www.frontiersin.org/articles/10.3389/fendo.2019.00493/full

- *Harinarayan CV, Akhila H, Shanthisree E. Modern India, and dietary calcium deficiency – half a century nutrition data – retrospect-introspect and the road ahead. Front. Endocrinol.* https://www.frontiersin.org/articles/10.3389/fendo.2021.583654/abstract. *Front. Endocrinol. 12:583654.doi: 10.3389/fendo.2021.583654.*

The **impact of his research work led to** the revision of national guidelines of Recommended Dietary Allowances for calcium and vitamin D by the ICMR. There is widespread awareness of vitamin D and

calcium deficiency. This is evident by the fact that every private laboratory is testing for 25 hydroxy vitamin D levels, which was once a research tool and now is available everywhere. Because of the increased awareness, there is self-supplementation of vitamin D and calcium. This is evident from the study *"Changing Trend in Vitamin D Status from 2008 to 2016: An Experience from a Tertiary Care Institute in North India. Indian Journal of Endocrinology and Metabolism 24(2):p 150-154, Mar–Apr 2020. | DOI: 10.4103/ijem.IJEM_634_19"* which showed the prevalence of vitamin D deficiency is on the decline. This is also supported by our review article mentioned above about various fortification measures taken by the government and measures yet to be implemented.

With this background, we decided to study the same disease Primary hyperparathyroidism after three decades which we first documented in 1990. Patients with Primary hyperparathyroidism attending to Department of Endocrinology and Metabolism from 2014 to 2022 were studied. *(Vitamin D status in primary hyperparathyroidism in 1990 and thence – Emergence of normocalcaemic presentation and diagnostic challenges – Utility of parathyroid function index. J Clin Sci Res 2022;11:167-74)* The clinical presentation of PHPT has undergone a paradigm shift. In the present cohort, the subjects were older, with none of them having an overt bone disease. The subjects have improved 25-hydroxy vitamin D levels, lower serum alkaline phosphate, and Calcium/Creatinine ratio (Ca/Cr ratio) with a smaller size and lower weight of the adenoma compared to the 1990 cohort documented earlier. Improved vitamin D status and dietary calcium intake, which we believe is thought to be the reason for less severe manifest bone disease, lower SAP levels and Ca/Cr ratio, and lowered size and weight of parathyroid adenoma.

Supplementary Table 2: Comparison of biochemical parameters between 1990 cohort and 2021 cohort of patients with PHPT

	NORMICALCEMIC PHPT		HYPERCALCEMIC PHPT	
	1995 (n=8)	**2021 (n=21)**	**1995 (n=12)**	**2021 (n=31)**
AGE	37.25 ± 5.3	52.86±3.48 [d]	36.83± 4.25	55.06± 2.68 [b]
S.ALB *gm/dl*	4.15 ± 0.1	3.86± 0.17	3.74± 0.12	3.77± 0.13
S Cr *mg/dl*	1.11 ± 0.12	0.90 ± 0.13 [c]	1.12± 0.14	0.88± 0.12[d]
S.Cal *mg/dl*	10.02 ± 0.24	9.25± 0.26	11.47± 0.17	12.18± 0.35
ACSC mg/dl	9.9 ± 0.26	9.52± 0.16	11.68± 0.18	12.51± 0.45
S Phos *mg/dl*	3.00 ± 0.24	2.69± 0.18	3.20± 0.29	2.52 ± 0.16[$]
SAP *IU/L*	352.63 ± 90.85	101.01± 12.3 [b]	473.42± 70.11	150.79± 37.72*
25 OHD3 *ng/ml*	7.94 ± 1.94	34.19± 3.46*	8.7± 1.16	22.58± 1.97*
PTH-MM *pmol/l*	281.29 ± 68.22		547.3± 128	
Intact PTH *pmol/l*		194.30± 40.93		343.11± 89.17
Cr/cl	49.8 ± 4.43	86.47± 6.12 [a]	35.83± 6.79	91.37± 4.75*
Ca/Cr	1.37 ± 0.22	0.03± 0.02*	1.71± 0.18	0.07± 0.03*
Wt of gland *gms*	3.75 ± 1.6	0.22± 0.08 [e]	9.96± 3.55	1.73± 0.41*

PHPT=Primary Hyperparathyroidism; Serum(S)ALB=S. Albumin; S.CRE=S. Creatinine; S.CAL= S. Calcium; ACSC=Albumin Corrected Serum Calcium; S.PHOS=Phosphorous; SAP=Serum alkaline phosphatase; PTH MM=Parathyroid hormone Mid molecule; PTH-intact=parathyroid hormone – intact molecule; Cr/Cl=Creatinine clearance; Ca/Cl=Calcium/creatinine ratio; CP ratio=Calcium phosphate ratio; PF-index=Parathyroid function index, All values are mean ± SEM. Comparison between two groups by Mann- Whitney U test.
*$P < 0.0001$, [$]$P < 0·001$, [b]$P < 0·002$, [c]$P < 0·02$, [d]$P < 0·03$, [e]$P < 0.004$, [$]$P < 0.05$ denotes significant difference in the row between the groups

Earlier attempts to define PHPT had not considered the 25OHD levels as part of the definition. In the recent past, it has been a part of the investigation panel.

His research work has provided

1. New perspectives in the correct diagnosis and treatment of metabolic bone diseases in India.

2. Documentation on the low vitamin D status as the cause of bone disease in primary hyperparathyroidism. His work on primary hyperparathyroidism is quoted in many textbooks on metabolic bone disease.

3. Documentation of the low vitamin D levels in urban and rural subjects through population survey. His work on dietary calcium and vitamin D status of the Indian population changed the Recommended Dietary Allowances (RDA) of India.

4. Documented by in vitro studies on the ampoule model of previtamin D synthesis have shown that we as Indians can synthesize enough vitamin D on exposure to sunlight from 11 am to 2 pm, by exposing 10 to 15% of body surface area for 15 to 30 minutes.

5. His research work has been translated into National Health programs to address the problem of vitamin D and dietary calcium deficiency in India and has paved the way for the fortification of Food with vitamin D and calcium.

6. Developed the first guideline for the treatment of vitamin D deficiency in the sun-drenched country-of India.

7. Documentation of the renal tubular damage as the cause of bone disease in fluorosis. First to characterize that bone disease in fluorosis is primarily due to renal tubular damage and is amplified due to low vitamin D levels in the Indian population. His **original research work** has shown the pivotal link played by the kidney in the causation of flurotoxic bone disease. It was astuteness in the bedside investigation of the patients under my care that led to this original observation of the study. *(Harinarayan CV, et al. Endemic Skeletal Fluorosis in India. Fluorotoxic metabolic bone disease: an osteo-renal syndrome caused by excess fluoride ingestion in the tropics. Bone 2006; 39:907-14). (Ranked top 10 publications - year 2006 by Internet).*

8. Indigenously develop intact IRMA radioimmunoassay for parathyroid hormone in India along with BARC Mumbai. – MAKE IN INDIA CONCEPT. The kit can be taken up for commercialization after clearance of logistics like transport, shelf life, etc. *(Prasad UV, Krishna Mohan R, Grace Samuel, Harinarayan CV, Sivaprasad N and Venkatesh M. Standardization of a two-site PTH immunoradiometric assay using various solid phase formats. Indian J Med Res. 2012;136(6):963-70).*

9. Development of "Clinical Practice Guidelines on the management of Postmenopausal osteoporosis (2012-13) – Executive summary and Recommendations" for the Indian Menopause Society.

10. Documented that correction of vitamin D deficiency can improve the pancreatic beta cell secretory function – First to document the improvement in pancreatic β-cell function with vitamin D and calcium supplementation in vitamin D-deficient non-diabetic subjects. *(Harinarayan CV, et al. Improvement in pancreatic β-cell function with vitamin D and calcium supplementation in vitamin D-deficient non-diabetic subjects. Endocr Pract.2014;20(2):129-38.)*

11. First to document that the serum 25OHD levels fall in patients with epilepsy therapy irrespective of the antiepileptic drug used even at therapeutic concentrations of the drugs *(Menon B,*

Harinarayan CV. The effect of antiepileptic drug therapy on serum 25-hydroxyvitamin D and parameters of calcium and bone metabolism – a longitudinal study. Seizure. 2010 Apr;19(3):153-8. Epub 2010 Feb 7).

12. In a Collaborative study with AIIMS, New Delhi it was shown that Vitamin D Receptor gene polymorphisms and hypovitaminosis D might predispose to multidrug-resistant tuberculosis (MDR-TB). Lower serum 25OHD may increase the time to MDR-TB sputum smear negativity *(Rathored J, Sharma SK, Singh B, Banavaliker JN, Sreenivas V, Srivastava AK, Mohan A, Sachan A, Harinarayan CV, Goswami R. Risk and outcome of multidrug-resistant tuberculosis: vitamin D receptor polymorphisms and serum 25(OH)D. Int J Tuberc Lung Dis. 2012;16(11): 1522-8).*