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Original Research Paper

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SERUM ALKALINE PHOSPHATASE- A SCREENING TOOL FOR VITAMIN D DEFICIENCY?

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ABSTRACT

Background: Vitamin D is a secosteroid synthesized in the skin when exposed to sun light. Sun light is the major source of this vitamin in the body. Vitamin D is collective term given to Vitamin D_3 (Cholecalciferol) and Vitamin D_2 (ergocalciferol). Cholecalciferol is synthesized from the skin when exposed to UV radiation. Although alkaline phosphatase is considered to be a factor required for the synthesis and mineralization of new bone, its exact function is still unknown. Being a product of osteoblasts raised serum levels of alkaline phosphatase indicate state of increased bone turnover. That is why it is used as a bone formation marker.

Methods: This Cross Sectional, Observational Study was done in the Department of Biochemistry, RNT Medical College,Udaipur. 300 medical students were selected via simple random sampling.

Results: Out of 300 samples, 275 had Vitamin D deficiency (85.0%), 22 (12.6%) had Insufficient Vitamin D and only 3 (2.4%) had vitamin D sufficiency. All the subjects in the three groups had alkaline phosphatase within normal limits and the total mean value of the enzyme was 103.05 ± 25.13 I U/L. The inter group comparison showed highest values of alkaline phosphatase in the vitamin D deficiency group. The correlation coefficient of alkaline phosphatase and serum vitamin D levels was r = 0.143 (p-0.013).

Conclusion: Serum vitamin D levels may not be correlated with increased serum alkaline phosphatase levels. Therefore, alkaline phosphatase may not be used as a screening test to rule out vitamin D deficiency.

KEYWORDS : Vitamin D, Alkaline Phosphatase, Calcium, Phosphorus

INTRODUCTION

Vitamin D is a secosteroid synthesized in the skin when exposed to sun light. Sun light is the major source of this vitamin in the body. Vitamin D is collective term given to Vitamin D3 (Cholecalciferol) and Vitamin D2 (ergocalciferol). Cholecalciferol is synthesized from the skin when exposed to UV radiation. Ergocalciferol is obtained from fungi. Two consecutive hydroxylation reactions, one in liver and another in kidney converts it to active form 1,25dihydroxy Vitamin D (Calcitriol). 25-hydroxy vitamin D is the major circulating form of Vitamin D in blood, its evaluation in the body gives clear indication of vitamin D status in the body (Weaver and Fleet 2004).

Vitamin D is mandatory for the maintenance of health, due to the presence of its highly specific receptors, VDRs (vitamin D receptors) in all body tissues and a regulatory role in the encoding of more than 200 genes. The deficiency of vitamin D therefore, could affect any tissue or body system (Holick MF 2007).

Alkaline phosphatase is a group of identical enzymes that are native to four homologous alkaline phosphatase genes (Henthorn P *et al.* 1999). Three out of these four genes encode for tissue specific enzymes, while the remaining one is present in many body tissues like bone, kidneys and liver. In an adult with normal hepatic function, the total serum pool of alkaline phosphatases is furnished equally by liver and bone. However, in children and pubertal age groups, bone specific isoenzyme is present in abundant form due to rapidly growing bones (Magnusson P *et al.* 1999). Although alkaline phosphatase is considered to be a factor required for the synthesis and mineralization of new bone, its exact function is still unknown. Being a product of osteoblasts raised serum levels of alkaline phosphatase indicatestate of increased bone turnover. That is why it is used as a bone formation marker (Van Straalen JP *et al.* 1991). One of the causes of high levels of serum alkaline phosphatase is osteomalacia and these levels are positively correlated with the severity of the disease (Cardinal RN *et al.* 2009). Osteomalacia and its counterpart rickets in children are caused by vitamin D deficiency Boonen S *et al.* 2007).

AIM & OBJECTIVE:

Vitamin D anaylsis has been used clinically to diagnose hypovitaminosis D for the last 16 years. In the pevious studies raised alkaline phosphatase levels were correlated with low vitamin D levels. (Peacey SR 2004; Sara F *et al.* 2007 and Allen SC *et al.* 2004). Keeping in view the core role of vitamin D in bone metabolism and its use along with serum alkaline phosphatase, this study was carried out to determine the correlation between these two screening tools.

METERIAL AND METHOD:

The present cross sectional institution based study was conducted on 300 healthy students of RNT Medical College, Udaipur.

Inclusion criteria for the study are as follows:-

- Eligible participants will be medical students aged 18-26 years (I, II and III year MBBS students).
- Both sexes.

Exclusion criteria included those with:-

- Non willing for participation.
- Liver disorders.

- Receiving any form of supplements containing calcium, Vitamin D since past 3 months.
- Any acute or chronic illness.
- Malabsorption syndrome.
- Endocrine disorders.

Institutional ethical committee approval was taken and written consent was taken from Each and every student.

Collection of Blood Samples:

5 ml of blood sample was drawn from antecubital vein. The collected sample was incubated at 37°C for 15 minutes in the incubator and then centrifuged for 10 minutes at approximately 3000rpm. Vitamin D, Calcium, Phosphorus and Alkaline Phosphatase were estimated.

Analysis of Blood for various analytical parameters:

1. Vitamin D: Electrochemiluminescence (Holick MF, 2007)
2. Calcium: Modified O- Cresolphthalein complexone (OCPC) method (Burtis C, 2001)
3. Phosphorus: Modified Phosphomolybdate method (Fiske C H, 1925)
4. Alkaline Phosphatase: PNPP method (Rej R, 1977)

Biological Reference Range:

- Vitamin D: Deficiency Less than 20 ng/dl; Insufficiency 21-29 ng/dl; Sufficiency
- Equal to or more than 30 ng/dl.
- Calcium: 8.5-10.1 mg/dl
- Phosphorus: 2.5-4.5 mg/dl
- Alkaline Phosphatase: 50-136 mg/dl

Statistical Analysis:

The comparisons between the groups were analyzed by ANOVA. All parameters were given as mean± standard deviation. The criterion for significance was $p<0.05$. Data analysis was performed with the statistical package for the social sciences version 17.00. Pearson coefficient was applied to see correlation between levels of Vitamin D and that of Calcium, Phosphorus and Alkaline Phosphatase.

RESULTS

Three hundred medical students of I, II and III year were selected via random sampling according to the inclusion criteria. The frequency of different groups of vitamin D has been shown in Table 1. Mean serum levels of vitamin D, calcium, phosphorus and Alkaline Phosphatase of the total samples (n = 300) has been mentioned in Table 1. All subjects in the three vitamin D groups had alkaline phosphatase within normal limits. The highest and the lowest levels of alkaline phosphatase were seen in deficiency and vitamin D sufficiency groups respectively (Table 1). Intergroup comparison showed a significant difference between serum alkaline phosphatase levels of Vitamin D deficiency, Insufficient and sufficient groups ($p = 0.037$).

By applying Pearson Correlation coefficient (r) we found that Vitamin D and Alkaline Phosphatase were negative correlated, $r = 0.143$ (p-0.013) (Figure2).

Table1: Comparison of serum levels Calcium, Phosphorus and Alkaline Phosphatase with Vitamin D groups (n=300)

Biochemical Parameters	VITAMIN D DEFICIENCY		VITAMIN D INSUFFICIENT		VITAMIN D SUFFICIENCY		Total		p value
	Mean	± SD	Mean	± SD	Mean	± SD	Mean	± SD	
calcium	9.6	0.6	9.4	0.5	9.2	1.3	9.5	0.6	0.227
phosphorus	3.7	0.5	3.7	0.3	3.4	0.2	3.7	0.5	0.514
Alkaline Phosphatase	104.1	25.7	93.7	12.5	77.7	2.9	103.1	25.1	0.037

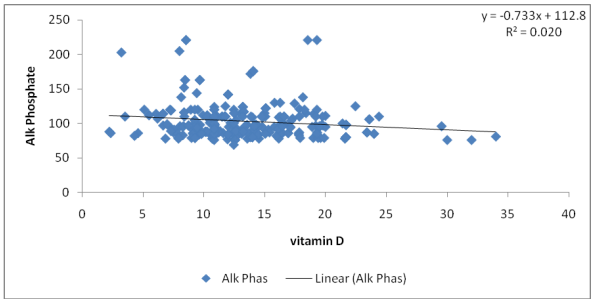

Figure1: Correlation between serum Vitamin D and Alkaline Phosphatase levels (n=300)

DISCUSSION

Kover *et al.* 1982 were the first to establish the role of alkaline phosphatase as a marker for vitamin D deficiency in premature infants. This is in contrast to all of the previous studies in which serum alkaline phosphatase levels showed a significant but inverse correlation with serum vitamin D levels as evidenced by raised levels of later one in vitamin D deficiency states (Peacey SR 2004; Sara F *et al.* 2007 and Allen SC *et al.* 2004).

In a retrospective study conducted by Peach H *et al.*1982 on histologically diagnosed cases of osteomalacia; alkaline phosphatase alone was regarded as a good indicator of vitamin D deficiency, though some of the subjects had false positive results. Contrary to all above mentioned researches, all the vitamin D deficient patients in the present study had serum alkaline phosphatase levels within normal range. It was also in contrast to a recent study conducted by Baig *et al.*2007 in which 19% of vitamin D deficient patients had raised serum alkaline phosphatase levels.

Though serum alkaline phosphatase levels were seen to be normally distributed among the sample; nevertheless the mean values of this enzyme had very high standard deviations (Table I). It was because our sample had a very wide range of alkaline phosphatase levels (18-301 IU/L).

The highest levels of alkaline phosphatase were seen in the group with severe vitamin D deficiency. The lowest levels of enzyme were seen in the group with moderate vitamin D deficiency rather than in the group having mild deficiency form. In the current clinical practice alkaline phosphatase is still used as a marker of vitamin D deficiency due to the low cost of the test which could be misleading.

Therefore, there is a need to estimate cut off values in our population in order to diagnose and treat true vitamin D deficient individuals. Furthermore, the specific role of alkaline phosphatase in bone metabolism besides a housekeeping enzyme should also be determined

CONCLUSION

Serum vitamin D levels may not be correlated with serum alkaline phosphatase levels. On this basis serum alkaline phosphatase may not be considered as a screening tool to rule out vitamin D deficiency states in population. The only reliable marker to estimate this deficiency could be serum vitamin D levels. Further large population based multicentre studies are required to determine the cut off values for vitamin D to diagnose and treat exact fraction of population that has true vitamin D deficiency.

REFERENCES

1. Allen SC, Raut S. Biochemical recovery time scales in elderly patients with osteomalacia. J R Soc Med 2004; 97:527-30.
2. Baig MA, Anjum MP, Khani MK, Islam NU, Rahman AU. Pattern of serum vitamin D in OPD patients. Pak J Surg 2007; 23:145-9.
3. Boonen S, Lips P, Bouillon R, Bischoff-Ferrari HA, Vanderschueren D, Haentjens P. Need for additional calcium toreduce the risk of hip fracture with

vitamin D supplementation:evidence from a comparative meta-analysis of randomized controlled trials. J Clin Endocrinol Metab 2007; 92:1415-23. Epub 2007 Jan 30.
4. Burtis C, Ashwood E. Tietz Fundamentals of Clinical Chemistry, 5th Ed. Philadelphia: WB Saunders, 2001: 797-799.
5. Cardinal RN, Gregory CA. Osteomalacia and vitamin D deficiency in a psychiatric rehabilitation unit: case report and survey: BMC Research Notes 2009; 2:82.
6. Fiske CH, Subbarow U. The colorimetric determination of phosphorus, J Biol Chem. 1925; 66-375.
7. Henthorn P, Millan JL, Leboy P, Seibel MJ, Robins SP, Bilezikian JP, editors. Principles of bone biology. San Diego: Academic Press; 1999.
8. Holick MF. "Vitamin D deficiency". The New England J. of Medicine. 2007; 357:266-81.
9. Kovar I, Mayne P, Barltrop D. Plasma alkaline phosphatase activity: a screening test for rickets in preterm neonates. Lancet 1982; 1:308-10.
10. Magnusson P, Larsson L, Magnusson M, Davie MW, Sharp CA. Isoforms of bone alkaline phosphatase characterisation and origin in human trabecular and cortical bone. J Bone Miner Res 1999 14:1926-33.
11. Peacey SR. Routine biochemistry in suspected vitamin D deficiency. J R Soc Med 2004; 97:322-5.
12. Peach H, Compston JE, Vedi S, Horton LWL. Value of plasma calcium, phosphate, and alkaline phosphatase measurements in the diagnosis of histological osteomalacia. J Clin Pathol 1982; 35:625-30. Principles of bone biology. San Diego: Academic Press; 1999.
13. Rej R. Effect of incubation with Mg ++ on the measurement of alkaline phosphatase activity. Clin Chem. 1977; 23: 1903-1911.
14. Sara F, Saygili F. Causes of high bone alkaline phosphatase. Biotechnol Biotechnol Eq 2007; 2:194-7.
15. Van Straalen JP, Sanders E, Prummel MF, Sanders GT. Bone alkaline phosphatase as indicator of bone formation. Clin Chim Acta 1991; 201:27-33.
16. Weaver CM, Fleet JC. Vitamin D requirements: current and the future. Am J Clin Nutr, 2004;80(suppl):1735S-9S.