ORIGINAL ARTICLE

Serum Alkaline Phosphatase Screening for Vitamin D Deficiency States

Shehla Shaheen[1], Syed Shahid Noor[2] and Qamaruddin Barakzai[1]

ABSTRACT

Objective: To determine whether serum vitamin D levels are correlated with serum levels of alkaline phosphatase or not.

Study Design: Cross-sectional, observational study.

Place and Duration of Study: Multi-centre study, conducted at Liaquat National Hospital and Medical College, National Medical Centre and Medicare Hospital, Karachi, from January to October 2009.

Methodology: Patients attending the Orthopaedic OPDs with complaints of pain in different body regions and serum vitamin D_3 levels of ≤ 30 ng/ml were included in the study. Patients with vitamin D deficiency were further categorized into mild deficiency or insufficiency (vit. D_3 = 20-29 ng/ml), moderate deficiency (vit. D_3 = 5 - 19 ng/ml) and severe deficiency forms (vit. D_3 < 5 ng/ml). Pearson correlation was applied to test the correlation of serum alkaline phosphatase levels with serum vitamin D_3 levels. P-value < 0.05 was considered to be significant.

Results: Out of 110 samples, 26 had mild (23%), 61 had moderate (55%) and 21 had severe (19.1%) vitamin D deficiencies. All of the patients in the three groups had alkaline phosphatase with in normal limits and the total mean value of the enzyme was 135.97 ± 68.14l U/L. The inter group comparison showed highest values of alkaline phosphatase in the moderate vitamin D deficiency group. The correlation coefficient of alkaline phosphatase and serum vitamin D_3 levels was r =0.05 (p =0.593).

Conclusion: Serum vitamin D_3 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.

Key words: *Alkaline phosphatase. Vitamin D_3 deficiency. Serum calcium.*

INTRODUCTION

Vitamin D, a steroid hormone, well known as sun vitamin due to the requirement of sunlight for its synthesis. Skin is a natural source of 7-dehydrocholesterol which transforms 7-dehydrocholesterol into cholecalciferol (vitamin D_3) after exposure to ultraviolet rays of sunlight. Vitamin D deficiency is prevalent all around the world and according to a recent report about one billion people are diagnosed with vitamin D deficiency (serum vitamin D_3 levels < 30 ng/ml).[1] Despite the fact that South Asia receives sunlight throughout the year, it is surprising that vitamin D deficiency is endemic in this region. In Karachi 55% of infants and 45% of nursing mothers were found vitamin D deficient and their serum 25 (OH) D levels were < 10 ng/ml.[2] Another multicentre study carried out at Karachi that encompassed all age groups came with different ailments, revealed that 90% of their subjects had vitamin D deficiency. Even those with normal vitamin D levels had values just touching the cut offs.[3] Siddiqui *et al.* stated in 2005 that rickets was highly prevalent among infants and children of Pakistan's northern areas.[4]

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.[5]

Alkaline phosphatase is a group of identical enzymes that are native to four homologous alkaline phosphatase genes.[6] 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.[7] 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.[8] 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.[9] Osteomalacia and its counterpart rickets in children are caused by vitamin D deficiency.[10]

[1] *Department of Pharmacology, Ziauddin Medical College, Ziauddin University, Karachi.*

[2] *Department of Orthopaedic, Liaquat National Hospital and Medical College, Karachi.*

Correspondence: *Dr. Shehla Shaheen, D-17, 6th Floor, Jumani Arcade, Block -14, Gulshan-e-Iqbal, Off-Edhi Centre, Karachi. E-mail: write2shehla@yahoo.com*

Received August 06, 2010; accepted April 19, 2012.

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.[11-13] 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.

METHODOLOGY

It was an observation based cross-sectional and multi-centre study carried out at Orthopaedics Clinics of Liaquat National Hospital and Medical College, National Medical Centre and Medicare Hospital, Karachi, from January to October 2009. Blood samples of all adult females (ages > 12 years) were taken that came with the complaints of pain in different body regions and had other clinical features of vitamin D deficiency. Before drawing blood their informed consents were also taken. It was a purposive convenient sampling. Blood tests were used to measure CBC, serum calcium, serum phosphorus, serum creatinine, serum vitamin D_3, serum alkaline phosphatase and other routine tests. Blood samples were first allowed to clot and then centrifugation was done to separate the serum. Levels of serum alkaline phosphatase were measured by kinetic photometric analyzer (Architect Abbott USA, Germany), whereas vitamin D_3 was analyzed by ELISA (Bio Vendor Laboratories Ltd. Germany, Catalogue no.: REA300/96) at Ziauddin University Hospital Laboratory, North Nazimabad, Karachi.

Patients having serum vitamin D_3 levels < 30 ng/ml were included in this study. Those who had chronic renal / liver diseases or taking any medicines that could affect liver or bone metabolism were excluded.

Patients with Vitamin D deficiency were further categorized into groups of mild form or insufficiency (vit.D_3 = 20-29 ng/ml), moderate form (vit.D_3 = 5 - 19 ng/ml) and severe deficiency form (vit.D_3 < 5 ng/ml).The reference levels of serum alkalaine phosphatase were taken as 65-306 IU/L, according to the laboratory criteria. Information about their life style patterns, marital status, number of children, exercise, sun exposure, vitamin D and calcium intake during pregnancy and lactation were collected on a proforma and then processed on Statistical Package for Social Sciences (SPSS) version 12.0. One-sample Kolmogorov-Smirnov test was used to check the normality of alkaline phosphatase distribution. ANOVA was applied to compare the significance of serum alkaline phosphatase levels in mild, moderate and severe vitamin D deficiency groups. Pearson correlation was performed to see the correlation of serum alkaline phosphatase levels with serum vitamin D_3 levels. P-value ≤ 0.05 was considered to be significant.

RESULTS

One hundred and ten subjects were included in the study according to the inclusion criteria in a 10 month's period. Their ages range from 12 to 80 years and their mean age was 45.33 ± 14.30 years. The highest frequency, 32 of vitamin D_3 deficiency was seen in 50 - 59 years of age group (29.1%), while lowest one was 1 in 80 - 89 years of age group (0.9%). When age groups were compared in mild, moderate and severe vitamin D deficient groups the difference was not significant (p = 0.51).

The frequency of different forms of vitamin D deficiencies has been shown in Figure 1. Mean serum levels of vitamin D_3, calcium, phosphorus and creatinine of the total sample (n = 110) has been mentioned in Table I. There was no significant difference in the serum levels of calcium, phosphorus and creatinine between the three vitamin D deficient groups (Table I). Alkaline phosphatase was normally distributed according to one-sample Kolmogorov-Smirnov test (p = 0.066). All patients in the three vitamin D deficient groups had alkaline phosphatase within normal limits. The highest and the lowest levels of alkaline phosphatase were seen in severe and moderate vitamin D deficient groups respectively (Table I). Intergroup comparison showed a significant difference between serum alkaline phosphatase levels of moderate and severe vitamin D_3 deficient groups (p = 0.001*). Serum vitamin D_3 levels were also significantly different in all of the groups (p = 0.001*). The correlation coefficient (r) of serum alkaline phosphatase and vitamin D was -0.05 (p = 0.593) which was statistically non-significant (Figure 2).

Table I: Comparison of blood test results in vitamin D_3 deficient groups (n=110).

Serum levels	Vitamin D_3 deficiency states				p-value
	Mild n =26 (Mean ± SD)	Moderate n = 63 (Mean ± SD)	Severe n = 21 (Mean ± SD)	Total n = 110 (Mean ± SD)	
Serum calcium mg/dL	9.158 ± 0.5672	9.089 ± 0. 5329	9.120 ± 0.4979	9.111 ± 0. 5307	0.854
Serum phosphorus mg/dL	3. 3396 ± 0.5730	3. 3513 ± 0. 5708	3.266 ± 0. 5769	3.438 ± 0. 5754	0.216
Serum creatinine mg/dL	0.766 ± 0.2212	0. 863 ± 1.876	0.792 ± 0.1788	0. 827 ± 0.1975	0.69
Haemoglobin g/dL	11.473 ± 0.7780	11.716 ± 1.2515	11.5 62 ± 1.0220	11.629 ± 1.1101	0.618
Alkaline phosphatase IU/L	157.85 ± 68.250	112.30 ± 56.915	179.90 ± 70.902	135.97 ± 68.140	0.001*

** Statistically significant p < 0.05*

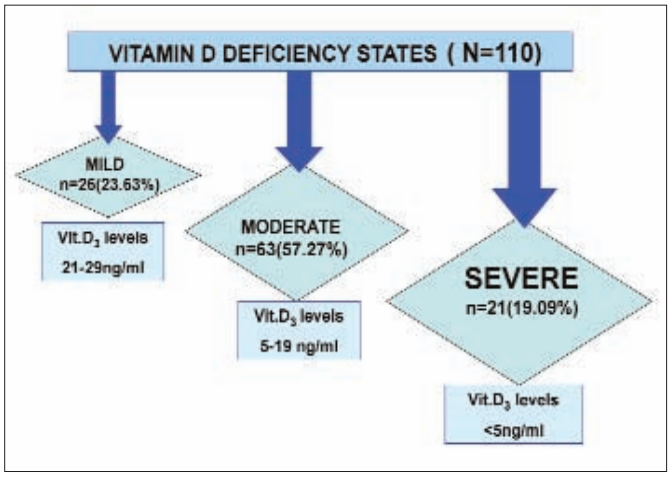

Figure 1: Frequency and percentage of different vitamin D deficiencies in 110 patients.

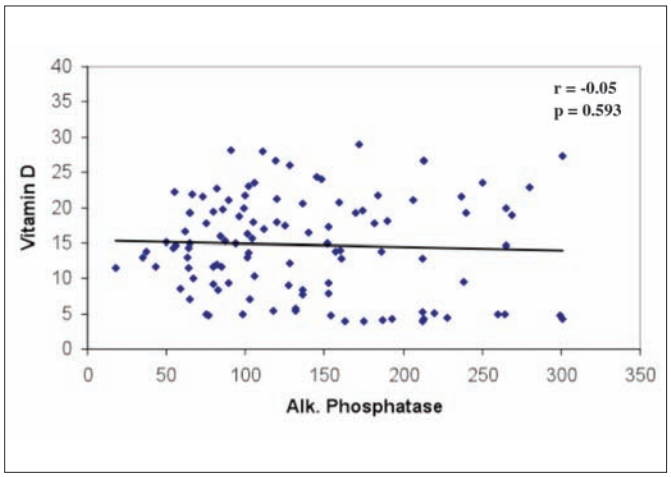

Figure 2: Correlation of serum vitamin D_3 and alkaline phosphatase levels (n = 110).

DISCUSSION

Kover *et al.* were the first to establish the role of alkaline phosphatase as a marker for vitamin D_3 deiciency in premature infants.[14] In the present study there was no correlation between serum alkaline phosphatase and serum vitamin D_3 levels (r = 0.05, p = 0.593).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_3 levels as evidenced by raised levels of later one in vitamin D deficiency states.[11-13] In a retrospective study conducted by Hedley *et al.* 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.[15] 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.* in which 19% of vitamin D deficient patients had raised serum alkaline phosphatase levels.[3] Although the study conducted by Faerk *et al.* also established that alkaline phosphatase is not a predictor of degree of bone mineralization but it was shown only in preterm infants.[16] This study is the first one to display normal serum alkaline phosphatase levels in patients belonging to diverse age groups (10-80 years) that had low serum vitamin D levels. The justification of this variant result is beyond the scope of this study; as the exact role of alkaline phosphatase at molecular level is still not well defined and needs extensive studies. However, the prevalence of vitamin D deficiency in all age groups shown by this study is consistent with other studies.[17-19] This study also showed moderate vitamin D deficiency to be more frequent (57%) than other forms of vitamin D deficiency among the studied subjects, which is consistent with the results of Baig *et al.*[3]

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.

Currently researchers have found that vitamin D has a preventive role in type I diabetes, heart diseases,[20] hypertension[21] and some of the autoimmune diseases.[22] In one study vitamin D deficient subjects were shown to have higher levels of triglycerides and lower levels of HDL-cholesterol in contrast to those that had normal vitamin D levels.[23] Vitamin D was also shown to have effects against aging and inflammation. Some studies have established that vitamin D plays an imperative role in different cancers by inhibiting cell cycle progression; which also modulates the actions of antigen presenting cells in addition to T-cells.[22] In some studies vitamin D was regarded to be essential for mental well being and its deficiency was related to Alzheimer's disease, schizophrenia[24] and depression.[25]

Due to the vital role of vitamin D for the regulation of normal body functions, it is mandatory to rule out its deficiency. In the clinical setup Western population-based reference levels of vitamin D are used to diagnose hypovitaminosis D. On the other hand clinician often recommend vitamin D medication to all patients presented with body pains without excluding other causes of body pains (for e.g. fibromyalgias, chronic fatigue syndrome, depression etc.). In this scenario, the question arises whether we are intoxicating our population with vitamin D or not? 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.

The limitation of this study was that this sample consisted of only females because majority of patients coming to orthopaedic OPDs with sign and symptoms of vitamin D deficiency were females.

CONCLUSION

Serum vitamin D_3 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_3 levels. However, the cut off values of vitamin D_3 for our population might be different from the Western population. Further large population based multicentre studies are required to determine the cut off values for vitamin D_3 to diagnose and treat exact fraction of population that has true vitamin D deficiency.

REFERENCES

1. Holick MF. Vitamin D. A millennium perspective. *J Cell Biochem* 2003; **88**:296-307.
2. Atiq M, Suria A, Nizami SQ, Ahmed I. Vitamin D status of breast-fed Pakistani infants. *Acta Paediatr* 1998; **87**:737-40.
3. 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.
4. Siddiqui TS, Rai MI. Presentation and predisposing factors of nutritional rickets in children of Hazara division. *J Ayub Med Coll Abottabad* 2005; **17**:29-32.
5. Holick MF. Vitamin D. Deficiency. *N Engl J Med* 2007; **357**:266-81.
6. Henthorn P, Millan JL, Leboy P, Seibel MJ, Robins SP, Bilezikian JP, editors. Principles of bone biology. *San Diego: Academic Press*; 1999.
7. 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.
8. Van Straalen JP, Sanders E, Prummel MF, Sanders GT. Bone alkaline phosphatase as indicator of bone formation. *Clin Chim Acta* 1991; **201**:27-33.
9. Cardinal RN, Gregory CA. Osteomalacia and vitamin D deficiency in a psychiatric rehabilitation unit: case report and survey: *BMC Research Notes* 2009; **2**:82.
10. Boonen S, Lips P, Bouillon R, Bischoff-Ferrari HA, Vanderschueren D, Haentjens P. Need for additional calcium to reduce 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.
11. Peacey SR. Routine biochemistry in suspected vitamin D deficiency. *J R Soc Med* 2004; **97**:322-5.
12. Sara F, Saygili F. Causes of high bone alkaline phosphatase. *Biotechnol Biotechnol Eq* 2007; **2**:194-7.
13. Allen SC, Raut S. Biochemical recovery time scales in elderly patients with osteomalacia. *J R Soc Med* 2004; **97**:527-30.
14. Kovar I, Mayne P, Barltrop D. Plasma alkaline phosphatase activity: a screening test for rickets in preterm neonates. *Lancet* 1982; **1**:308-10.
15. 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.
16. Faerk J, Peitersen B, Petersen S, Michaelsen KF. Bone mineralisation in premature infants cannot be predicted from serum alkaline phosphatase or serum phosphate. *Arch Dis Child Fetal Neonatal Ed* 2002; **87**:F133-6.
17. Bondar LM, Simhan HN, Powers RW, Frank MP, Cooperstein E, Roberts JM. High prevalence of vitamin D insufficiency in black and white pregnant women residing in the northern United States and their neonates. *J Nutr* 2007; **137**:447-52.
18. Harinarayan CV. Prevalence of vitamin D insufficiency in postmenopausal south Indian women. *Osteoporsis Int* 2005; **16**:397-402.
19. Andersen R, Molgaard C, Skovgaard LT, Brot C, Cashman KD, Jakobsen J, *et al.* Pakistani immigrant children and adults in Denmark has severely low vitamin D status. *Eur J Clin Nutr* 2008; **62**:625-34. Epub 2007 Apr 18.
20. Judd S, Tangpricha V. Vitamin D deficiency and risk of cardiovascular disease. *Circulation* 2008; **29**:503-11.
21. Forman JP, Giovannucci E, Holmes MD, Bischoff-Ferrari HA, Tworoger SS, Willet WC. Plasma 25-hydroxyvitamin D levels and risk of incident hypertension. *Hypertension* 2007; **49**:1063-9. Epub 2007 Mar 19.
22. Kuryowicz A, Bednarczuk T, Nauman J. The influence of vitamin D deficiency on cancers and autoimmune diseases development. *Endokrynol Pol* 2007; **58**:140-52.
23. Botella-Carretero JI, Alvarez-Blasco F, Villafruela JJ, Balsa JA, Vázquez C, Escobar-Morreale HF. Vitamin D deficiency is associated with the metabolic syndrome in morbid obesity. *Clin Nutr* 2007; **26**:573-80. Epub 2007 Jul 10.
24. Wilkins CH, Scheline YI, Roe CM, Birge SJ, Morris JC. Vitamin D deficiency is associated with low mood and worse cognitive performance in older adults. *Am J Geriat Psychiat* 2006; **14**:1032-40.
25. Berk M, Sanders KM, Pasco JA, Jacka FN, Williams LJ, Hayles AL, *et al.* Vitamin D deficiency may play a role in depression. *Med Hypotheses* 2007; **69**:1316-9. Epub 2007 May 11.