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Lack of correlation between vitamin D and alkaline phosphatase levels among Saudi population

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ABSTRACT

Objective: The present study evaluated the correlation between Vitamin D and Alkaline phosphatase levels among the Saudi population. *Methods:* Blood samples of patients with a complaint of pain in various body parts and other clinical symptoms of Vitamin D deficiency were tested for the levels of Vitamin D and ALP. The analyzes were carried out using SPSS version 20.0 software. *Results:* Vitamin D and ALP levels of 460 patients were analyzed. The mean levels of Vitamin-D and ALP were found to be 22.03±13.5 and 89.02±44.9. About 356 patients (77.4%) out of 460 had Vit-D deficiency, whereas 421 patients (91.5%) out of 460 had ALP at normal ranges. From the analysis based on the nationality, Saudi 223 patients out of 280 showed vitamin D deficiency and 257 patients out of 280 had normal ALP levels. No correlation between serum ALP and vitamin D levels was observed ($r = 0.05$, $p = 0.2$). *Conclusion:* serum alkaline phosphatase cannot be considered as a useful marker to represent the Vitamin D deficiency among the population.

Keywords: Vitamin D, Alkaline phosphatase, Saudi population

1. INTRODUCTION

Vitamin D is a prohormone steroid that belongs to the family of fat-soluble vitamins. They are also involved in neuromuscular function, hormonal release, and they are necessary for calcium absorption and bone mineralization. They play a vital role in the endocrine and paracrine process. Stimulation of growth, promoting the metabolism of calcium and phosphate and enhances remodelling of bone and teeth; these physiological functions are regulated by Vitamin D (Holick, 2017). Vitamin D deficiency is commonly accompanied by bone ailments (like rickets, osteoporosis and osteomalacia). Vitamin D intake has a desirable effect in reducing the mortality rate under different parameters regulating the cardiovascular system, immune system and tumour proliferation, which has been demonstrated in various studies. Vitamin D deficient people are predominant worldwide and on observation with recent reports about more than one billion people have been spotted with vitamin D deficiency. The vitamin deficiency (≤10ng/mL) has been considered as one of the epidemics global health problem, and insufficiency of vitamin D (10.1 ng/mL to 30 ng/mL) also plays the role (Martineau et al., 2017; Randev et al., 2018).

The homologous alkaline phosphatase genes include an identical group of a native enzyme called alkaline phosphatase. The one out of four genes exists in body tissues like liver, bone and kidney, while the three gene codes specifically for enzymes. The level of an alkaline phosphatase present in an adult is furnished equally by liver and bone under normal hepatic function. The isoenzyme, i.e. the bone-specific enzyme, is present in plentiful cases of children because of the rapid growing bones. However, alkaline phosphatase is regarded as a factor essential for the development and mineralization of new bone. Alkaline phosphatase shows the increased level of bone turnover when osteoblasts raise the serum level of alkaline phosphatase (Zaher et al., 2020; Rader, 2017). That is why it is being marked as a marker of bone formation. One of the major reasons for high levels of serum alkaline phosphatase (ALP) is osteomalacia, and these elevated levels are constructively associated with the severity of the disease. Osteomalacia and its counterpart rickets observed in children are triggered by vitamin D deficiency. Vitamin D analysis has been carried out clinically to analyze hypovitaminosis D in recent decades (Bover et al., 2018).

Alkaline phosphatase (ALP) levels were observed to be interrelated with lower vitamin D level, which was observed in the previous studies (Peacey, 2004; Sara and Saygili, 2007; Allen and Raut, 2004). The present study explains the correlation between ALP and Vit-D among various groups of Saudi and non-Saudi patients.

2. METHODOLOGY

Research design

An observation-based cross-sectional study was carried out at Prince Sattam bin Abdulaziz University Hospital, Saudi Arabia from January 2019 to October 2020. All the patients were categorized based on their gender (male & female), age (children, young adults, middle-aged adults & old-aged adults) and nationality (Saudi & Non-Saudi).

Procedure

Blood samples were collected from the patient's arrived with the complaint of pain in various body parts and other clinical symptoms of Vitamin D deficiency. The collected blood samples were utilized to evaluate the PTH, Vitamin D, ALP, calcium and phosphate levels. Blood samples were permitted for clotting, and serum was separated using centrifugation process. Levels of serum ALP were estimated by the kinetic photometric analyzer, whereas vitamin D was analyzed by ELISA at Prince Sattam bin Abdulaziz

University Hospital, Saudi Arabia. The patients were classified as a deficient, adequate, optimal, therapeutic and excess group based on Vitamin D levels. Similarly, ALP levels were classified as low, normal and high.

Statistics

All the data were processed on the Statistical Package for Social Sciences (SPSS) version 12.0. Mean and Standard deviation values of the Vit-D and ALP levels were calculated. Chi-square test, Independent T-test and Tukey HSD were performed and determined. ANOVA was used to compare the significance of serum ALP and Vit-D levels at different age groups. Pearson correlation was applied to observe the correlation of serum ALP levels with Vit-D levels. P-value ≤ 0.05 was considered to be statistically significant.

3. RESULTS

The present study was investigated between 460 patients, 234(50.9%) were males, and 226(49.1%) were females. Among them, 26(5.7%) were children, 122(26.5%) were young adults, 154(33.5%) were Middle-aged adults, and 158(34.3%) were old-aged adults. Table 1 represents the description of the study population based on gender, age and nationality.

Table 1 Description of the study population

	No. and %
Gender	
Female	226(49.1%)
Male	234(50.9%)
Age	
Children	26(5.7%)
Young Adults	122(26.5%)
Middle-aged Adults	154(33.5%)
Old-aged Adults	158(34.3%)
Nationality	
Saudi	280(60.9%)
Non-Saudi	180(39.1%)

The mean levels of Vitamin-D and ALP for 460 patients were found to be 22.03±13.5 and 89.02±44.9 with minimum and maximum levels of 3.7 to 83.3 for Vit-D and 34.0 to 368.0 for ALP (Table 2).

Table 2 Mean levels of Vitamin D and ALP among the study population

	N	Mean (Std)	Minimum	Maximum
Age	460	39.51(16.319)	1	100
VITAMIN D	460	22.03(13.528)	3.750	83.360
ALP	460	89.02(44.94)	34.0	368.0

Table 3 shows the levels of Vit-D and ALP of the study population classified based on deficient, adequate, optimal, therapeutic and excess levels of Vit-D; Low normal and high for ALP. About 356 patients (77.4%) out of 460 had Vit-D deficiency, whereas 421 patients (91.5%) out of 460 had ALP at normal ranges.

Figure 1 shows the levels of Vit-D and ALP based on gender. Male groups tended to have higher levels of Vit-D than females. Contrastingly Females had higher ALP levels than males. Middle-aged groups showed higher Vit-D level and children had higher ALP levels than other groups (Figure 2 & 3).

Table 4 shows the Chi-square test based on gender and age groups. Independent T-test values have been demonstrated in the Table 5. The correlation of coefficient (r) of the Vit-D and ALP levels was 0.05 (p=0.23) (Figure 4). ANOVA with age groups was presented on Table 6. Vit-D and Alp levels were found to be significantly different. Significance for Vit-D was 0.001, and ALP was 0.000. Tukey HSD was carried out to evaluate the Mean difference, std. Error and significance of Vit-D and ALP levels of different age

groups. ALP Tukey HSD used Harmonic Mean Sample Size (67.249) although the group's sizes are unequal. Type I error levels are not assured.

Table 3 Classification of Vitamin-D and ALP levels

	No. and %
Vit D reference level	
Deficient	356(77.4%)
Adequate	59(12.8%)
Optimal	34(7.4%)
Therapeutic	9(2%)
Excess	2(0.4%)
ALP range	
Low	0(0%)
Normal	421(91.5%)
High	39(8.5%)

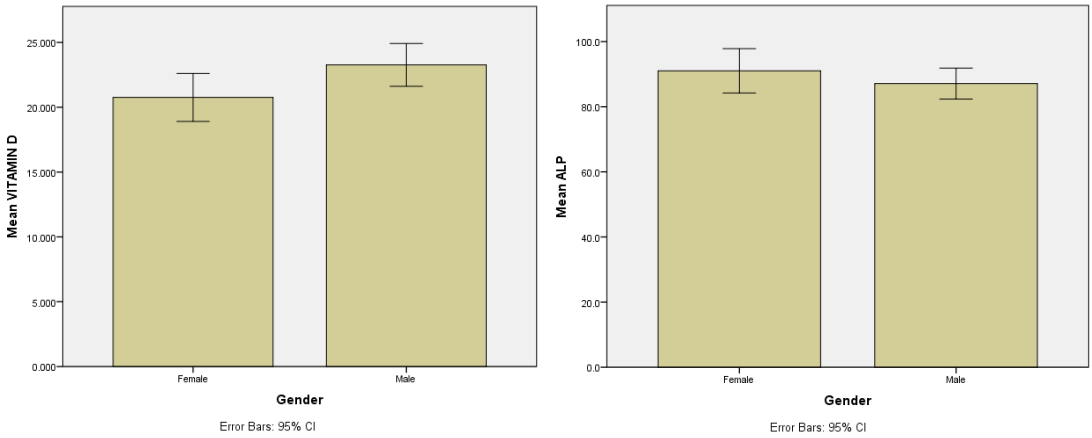

Figure 1 Vit-D and ALP levels based on gender

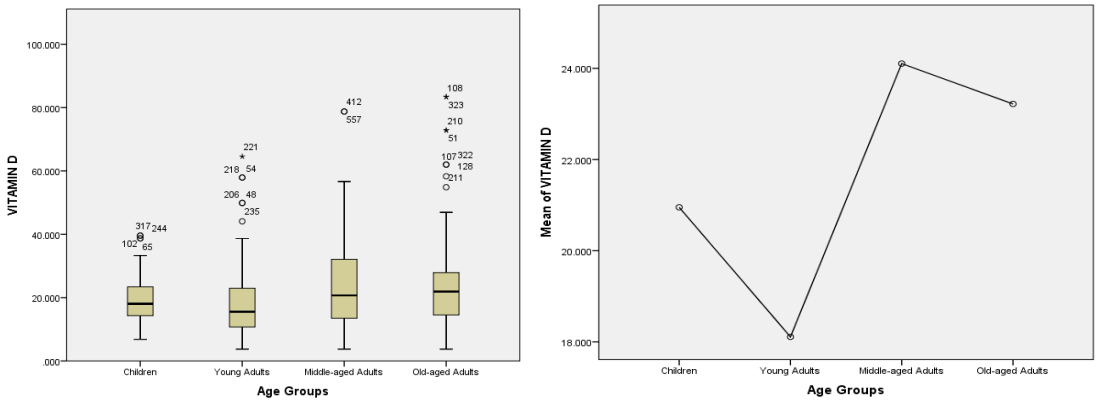

Figure 2 Vit-D levels based on age groups

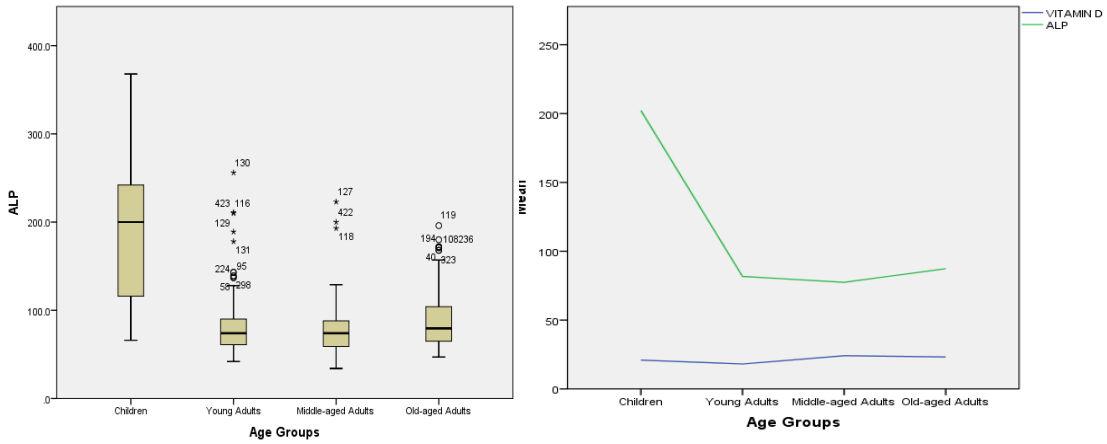

Figure 3 ALP levels based on age groups

Table 4 Chi-square test

	Chi-square	df	Sig.
Gender			
Vit D reference level	2.525	4	.640[a,b]
ALP Ranges	1.652	1	.199[a]
Age Groups			
Vit D reference level	23.934	12	.021[a,b,*]
ALP Ranges	150.411	3	.000[a,*]

Table 5 Independent T-test

Gender		N	Mean (Std)	Std. Error Mean	t	Sig. (2-tailed)
VITAMIN D	Female	226	20.76(±14.128)	.939750	-1.993	.047*
	Male	234	23.27(±12.832)	.838839		
ALP	Female	226	91.03(±52.032)	3.4611	.936	.350
	Male	234	87.09(±36.831)	2.4077		

Table 6 ANOVA with age groups

		Sum of Squares	df	Mean Square	F	Sig.
VITAMIN D	Between Groups	2791.98	3	930.66	5.226	.001*
	Within Groups	81204.32	456	178.08		
	Total	83996.31	459			
ALP	Between Groups	360656.92	3	120218.97	96.794	.000*
	Within Groups	566357.82	456	1242.01		
	Total	927014.737	459			

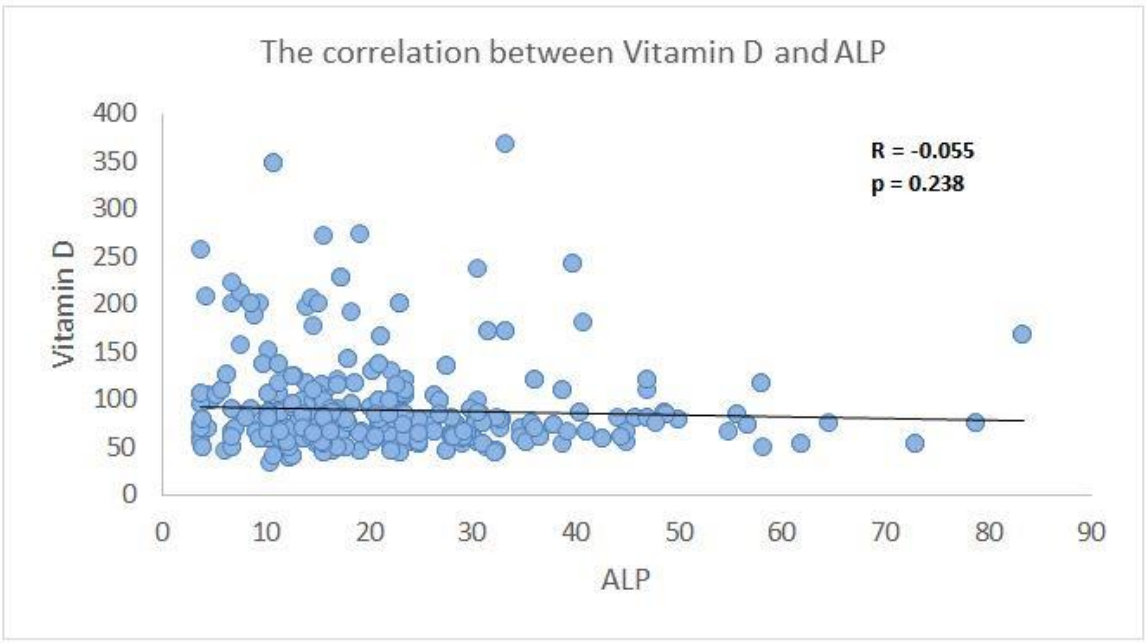

Figure 4 Correlation between Vitamin D and ALP levels

Table 7 Tukey HSD

Dependent Variable			Mean Difference (I-J)	Std. Error	Sig.
VITAMIN D	Children	Young Adults	2.842415	2.882514	.757
		Middle-aged Adults	-3.152817	2.829413	.681
		Old-aged Adults	-2.267950	2.824235	.853
	Young Adults	Children	-2.842415	2.882514	.757
		Middle-aged Adults	-5.995232*	1.617415	.001*
		Old-aged Adults	-5.110365*	1.608339	.009
	Middle-aged Adults	Children	3.152817	2.829413	.681
		Young Adults	5.995232*	1.617415	.001*
		Old-aged Adults	.884867	1.511108	.936
	Old-aged Adults	Children	2.267950	2.824235	.853
		Young Adults	5.110365*	1.608339	.009
		Middle-aged Adults	-.884867	1.511108	.936
ALP	Children	Young Adults	120.4956*	7.6125	.000*
		Middle-aged Adults	124.7572*	7.4723	.000*
		Old-aged Adults	114.8379*	7.4586	.000*
	Young Adults	Children	-120.4956*	7.6125	.000*
		Middle-aged Adults	4.2617	4.2715	.751
		Old-aged Adults	-5.6577	4.2475	.543
	Middle-aged Adults	Children	-124.7572*	7.4723	.000*
		Young Adults	-4.2617	4.2715	.751
		Old-aged Adults	-9.9194	3.9907	.064
	Old-aged Adults	Children	-114.8379*	7.4586	.000*
		Young Adults	5.6577	4.2475	.543
		Middle-aged Adults	9.9194	3.9907	.064

VITAMIN D Tukey HSD

Age Groups	N	Subset for alpha = 0.05	
		1	2
Young Adults	122	18.10951	
Children	26	20.95192	20.95192
Old-aged Adults	158	23.21987	23.21987
Middle-aged Adults	154		24.10474
Sig.		.119	.519

ALP Tukey HSD[a,b]

Age Groups	N	Subset for alpha = 0.05	
		1	2
Middle-aged Adults	154	77.435	
Young Adults	122	81.697	
Old-aged Adults	158	87.354	
Children	26		202.192
Sig.		.362	1.000

Means for groups in relatively homogeneous subsets are shown.

a. Uses Harmonic Mean Sample Size = 67.249.

b. The group sizes are unequal. The group-sized harmonic average is used. Type I error levels are not guaranteed

The final analysis of Vit-D and ALP levels between Saudi and Non-Saudi patients were carried out. Mean Vit-D levels were more or less similar between two groups, and ALP levels were slightly higher for Non-Saudi patients (Figure 5). Levene's Test for Equality of Variances and t-test for equality of Means among Saudi and Non-Saudi population was presented in Table 9. Among the Saudi patients' majority of the people showed Vit-D deficiency and normal ranges of ALP (Figure 6).

Table 8 Independent T-test among Saudi and Non-Saudi population

	NAT	N	Mean	Std. Deviation	Std. Error Mean
Vitamin D	Saudi	280	21.90689	14.521834	.867846
	Non-Saudi	180	22.22806	11.853025	.883472
ALP	Saudi	280	87.104	42.0396	2.5123
	Non-Saudi	180	92.011	49.0862	3.6587

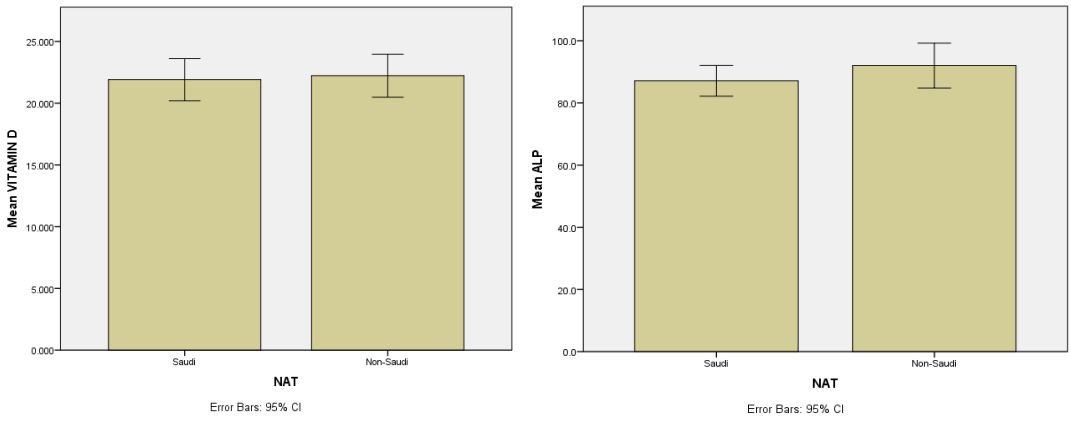

Figure 5 Vit-D and ALP levels for Saudi and Non-Saudi patients

Table 9 Levene's Test for Equality of Variances and t-test for equality of Means among Saudi and Non-Saudi population

02	Levene's Test for Equality of Variances		t-test for Equality of Means						
	F	Sig.	t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	95% Confidence Interval of the Difference	
								Lower	Upper
Vitamin D	4.682	.031	-.259	432.657	.796	-.321163	1.238418	-2.755226	2.112901
ALP	.238	.626	-1.143	458	.253	-4.9075	4.2920	-13.3419	3.5268

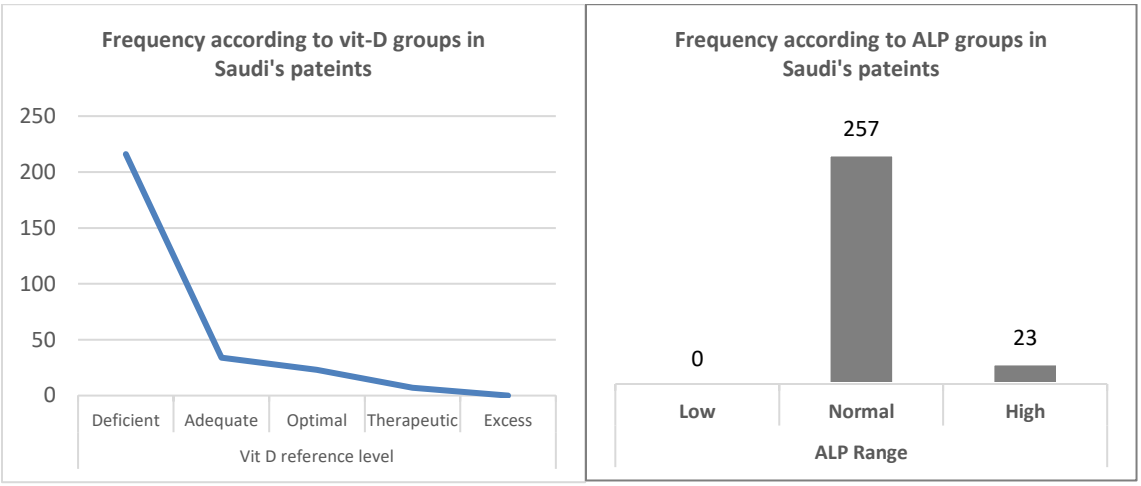

Figure 6 Frequency of Vit-D and ALP levels among Saudi patients

4. DISCUSSION

This is the first study to show the lack of correlation between ALP and Vit-D levels among the Saudi population. Kovar *et al.* (1982) established the functions of alkaline phosphatase (ALP) as a potential marker for vitamin D deficiency in premature babies. In the present study, no correlation between serum ALP and serum Vit-D levels (r = 0.05, p = 0.2) was observed. These results are in contrast to all other previous studies in which ALP levels showed an evident but inverse correlation with Vit-D levels as observed by higher levels of ALP in Vit-D deficiency conditions.

In a back dated cohort study carried out by Peach *et* al. (1999) biological tissues were diagnosed for osteomalacia; there was a false-positive result in some cases, but the majority of results showed that for vitamin D deficiency ALP was recognized as a superior indicator. In reverse, the present study shows that people with deficiency of vitamin D have alkaline phosphatase serum levels in their normal range, which is contradictory to the research mentioned above. The raised ALP was seen in 19% of Vit-D deficient patients which was examined by Baig *et al.*, (2007) Even though the study investigated by Faerk *et al.,* (2002) also proved that ALP is not an interpreter of the degree of bone mineralization. However, it was observed only in preterm children.

A total of 132 articles assessing vitamin D deficiency in Saudi Arabia have been found using the PubMed search criterion. Several reports have proven that healthy individuals are living with vitamin D deficiency in Saudi Arabia for the last decade. These reports showed a markedly high prevalence of Vit-D deficiency in Saudi women (41.2–100 %) compared to Saudi men (32.5–92.6 %). This condition was not only seen in adults, but it was also found in newborn and teenagers about (40-98 %). Though there are several internal and external factors responsible for the lower levels of Vit-D, the population remains to be healthy.

Similarly, an analysis conducted by Shaheen *et al.,* 2009 at Karachi, Pakistan showed no correlation between Vit-D and ALP was observed. The total patients analyzed were 110. The deficiency was divided into three groups, and all the three groups had ALP with normal range limits where 21 patients had excess vitamin D deficiency (91%), followed by moderate with 61 patients (55%) and 26 had mild (23%) the total mean value of the enzymes were 135.97 ± 68.14I U/L. The level of an alkaline phosphatase was seen high in the moderate Vit-D deficiency group compared with the inter groups. The coefficient correlation of the serum ALP and Vit-D was observed to be r =0.05 (p =0.593). These results well correlate with the present study.

5. CONCLUSION

Vitamin D and ALP levels of 460 patients were analyzed. The mean levels of Vitamin-D and ALP were found to be 22.03±13.5 and 89.02±44.9. About 356 patients (77.4%) out of 460 had Vit-D deficiency, whereas 421 patients (91.5%) out of 460 had ALP at normal ranges. From the analysis based on the nationality, Saudi 223 patients out of 280 showed vitamin D deficiency and 257 patients out of 280 had normal ALP levels. From this analysis, it is concluded that serum ALP cannot be considered as a useful marker to represent the Vitamin D deficiency among the population.

Ethical approval

The study proposal was approved by the Ethics and Research Committee of the Medical College at Prince Sattam Bin Abdulaziz University (ethical approval number: REC-HSD-006-2018).

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Authors' contributions

All authors contributed equally to this work, and they have read and agreed to the final manuscript.

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Conflict of interest

The authors declare that they have no conflict of interest.

Data and materials availability

All data associated with this study are present in the paper.

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