

Biochemical Analysis and Frequency of Vitamin D Deficient People in Different Age and Gender

Jamila¹, Rizwan Ahmed Kiani², Tehmina Qamar³, Sajjad Ahmed Khan², Ishtiaq Ahmed², Muhammad Noor ul Amin², Wasif Mehmood², Muhammad Mohsin², Irshad Hussain⁴

¹Associate Hematologist Islamabad diagnostic Centre. Associate Professor Pathology Wah Medical College.

² Department of Biochemistry and Biotechnology, PMAS Arid Agriculture University, Rawalpindi.

³Rawalpindi Medical University, Rawalpindi, ⁴Medical Legal Officer, PIMS

Correspondence: Muhammad Noor ul Amin
noorulaminamin@yahoo.com

Abstract

Objective: To analyze different biochemical Parameters in Vitamin D deficient population and to estimate prevalence of vitamin D deficiency in different age and gender.

Methodology: This cross sectional study was conducted at Islamabad Diagnostic Centre (IDC), Islamabad from January 2017 to March 2018. In this study a total of 721 participants were recruited. Vitamin D3, total calcium, phosphorus, urea, creatinine and uric acid were analyzed on Architect 1000. Statistical analysis was analyzed by SPSS version 19.0.

Results: Out of 721 recruited patients, there were 374 (51.8%) male and 347 (48%) were female. The mean values of vitamin D, total calcium, phosphorus, urea, creatinine and uric acid were 23.4 ng/ml (± 18.40 SD), 9.1 mg/dl (± 0.66 SD), 3.4 mg/dl (± 0.60 SD), 30.2 mg/dl (± 1.4 SD), 0.94 mg/dl (± 0.68 SD) and 5.6 mg/dl (± 1.5 SD) respectively. The overall vitamin D deficiency (Deficient + Insufficient) was seen in 558 (77.4%) study participants while 160 (22.1%) participants having sufficient level. Vitamin D toxicity was seen in only 03 (0.41) patients. It was observed that there is statistical significant difference ($p < 0.05$) in the mean value of all parameters.

Conclusion: Vitamin D deficiency in Pakistan is becoming endemic and it is much predominant in elder and female population. Female are more deficient due to social and religious limitations. They need more care of vitamin D blood levels due to less exposure to sun light in cities. Based on our finding renal function tests, serum calcium and phosphorus levels are not the conjecturer of vitamin D deficiency.

Keywords: Vitamin D, Sun light, Calcium, Phosphorus

Cite this article as: Jamila, Kiani RA, Qamar T, Khan SA, Ahmed I et al., Biochemical Analysis and Frequency of Vitamin D Deficient People in Different Age and Gender. J. Soc. Obstet. Gynaecol. Pak. 2018; Vol 8(4):249-254.

Introduction

Vitamin D or the “sunshine” vitamin is soluble in fat and ability of this vitamin is to enhance absorption of calcium, magnesium, and phosphate in intestine. In humans, Vitamin D3 (cholecalciferol) and D2 (ergocalciferol) are the vital compounds.¹ The history of vitamin D and some other vitamin deficiencies are known for centuries. Deficiency of this vitamin causes Rickets, also known as “English Disease” due to

sudden eruption in 17th century and was described in detail by F. Glisson in 1650.² The biologically inactive vitamin D, which is from skin and diet required enzymatic conversion in kidney and liver for activation. Quantification of 25-hydroxyvitamin D or 25(OH) D in serum confirms individual vitamin D status.³⁻⁴ In the absence of vitamin D, only about 60% of phosphorus and 10–15% of dietary calcium absorb.

Authorship Contribution: ¹Manuscript revision, ²Manuscript Drafting, Data analysis & interpretation of results ³Active participation in active methodology,

Funding Source: none

Conflict of Interest: none

Received: Sept 24, 2018

Accepted: Jan 11, 2019

While the adequate serum levels of Vitamin D raise calcium by 30-40% and phosphorus absorption by 80%.⁵⁻⁶

Vitamin D2 and D3 can be taken from the diet and supplements. The sources of Vitamin D3 & D2 are different. The richest sources of vitamin D3 are egg yolk, butter, liver, fish oil and dietary supplements whereas plant sources like UV light grown mushrooms, fortified foods & dietary supplements contain vitamin D2.⁴⁻⁷ Vitamin D3 increase blood levels of vitamin D more effectively than vitamin D2. Many studies revealed that inadequate solar exposure lead to vitamin D deficiency. Insufficient sunlight exposure is primary cause of vitamin D deficiency in adults and in children.⁷⁻⁹

Vitamin D plays important role by facilitating the gut to absorb calcium, maintain phosphate and calcium levels at right concentration and support & maintain healthy bone growth. Due to inadequate body levels of vitamin D, bones become brittle & weak and also associated with wide range of other conditions like cardiovascular diseases and malignancy.¹⁰⁻¹¹ There is protective relationship between low risk of malignancies and adequate serum vitamin D levels. Vitamin D receptors are present in many cell types that can be activated by 1, 25(OH) D. It regulates cell growth and promotes differentiation in normal functioning cells and impedes tumor growth, its invasion and spread to other sites.⁹⁻¹¹

Vitamin D deficiencies are commonly expressed in all age-groups, geographic regions, and seasons.¹²⁻¹³ Vitamin D deficiency is common in obese peoples, since their fat cells readily absorb and trap vitamin D due to its fat solubility nature.¹⁴ Children with deficiency of vitamin D suffer from retarded growth and bone disease and in adults, vitamin D deficiency will trigger and worsen osteopenia and osteoporosis and lead to greater the risk of fracture. Patients suffering from frequent bone discomfort along with pains in joints and muscles¹⁵⁻¹⁸. These patients may be incorrectly diagnosed with musculoskeletal pain, fatigue, and tenderness in localized areas, depression, degenerative joint disease, joint stiffness, and other diseases.¹⁹⁻²⁰ It is well known that skeletal muscles perform their activity at their peak in the presence of sufficient levels of vitamin D in the body due to presence of vitamin D receptors on skeletal muscle tissues.²¹⁻²²

The short wave ultraviolet rays of sunlight, when reaches the earth surface has striking consequence. During winters and in the early morning and late afternoon, scanty vitamin D₃ synthesis occurs therefore appropriate dietary supplements of vitamin recommended.^{23, 24} There is also increase risk of developing vitamin D deficiency / insufficiency in some cultures, where females use the clothing that covers the whole body, including the face, they do not get sufficient sunlight, and is common in adults and children both.^{25, 26} In aged people when compare to young adults, there is very low ability to make vitamin D3 due to low D3 precursor 7-dehydrocholesterol, in the aged skin.²⁷

The significant role of vitamin D in our body should not be ignored. Since vitamin D deficiency is increasing on wide scale, it is difficult to get sufficient levels only from natural sources so it has to be supplemented through diet.²⁸

Methodology

This cross-sectional study was conducted at Islamabad Diagnostic Centre (IDC), Islamabad from January 2017 to March 2018. Study was approved from the ethical committee of IDC and a total of 721 patients were selected in this study. All these patients were walked in cases and they had no major illness. Blood samples were collected from recruited patients after taking written inform consent in serum separating tubes. Vitamin D3, total calcium, phosphorus, urea, creatinine and uric acid were analyzed on Architect 1000. Vitamin D level was reported as deficient if it was <20 ng/ml, insufficient if it was between 20-30 ng/ml and requiring treatment. Desirable level is >30 ng/ml and levels >110 ng/ml reported as toxic levels. Statistical analysis was analyzed by SPSS version 19.0. Descriptive statistic, ANOVA and bivariate correlation were performed and for this a p-value less than 0.05 was considered as statistically significant.

Results

Out of 721 recruited patients, there were 374 (51.8%) male and 347 (48%) were female. The mean values of vitamin D, total calcium, phosphorus, urea, creatinine and uric acid were 23.4 ng/ml (\pm 18.40 SD), 9.1 mg/dl (\pm 0.66 SD), 3.4 mg/dl (\pm 0.60 SD), 30.2 mg/dl (\pm 1.4 SD), 0.94 mg/dl (\pm 0.68 SD) and 5.6 mg/dl (\pm 1.5 SD) respectively (Table I).

Table I: Descriptive Statistical Analysis

Parameter	Mean	Minimum	Maximum	Standard Deviation(±)
Vitamin D	23.4	1.3	151.2	18.4
Total Calcium	9.1	7.1	11.7	0.6
Phosphorus	3.4	1.8	5.9	0.60
Urea	30.2	12.0	183.0	1.4
Creatinine	0.94	0.4	10.6	0.68
Uric Acid	5.6	1.7	16.1	1.5

The overall vitamin D deficiency (Deficient + Insufficient) was seen in 558 (77.4%) study participants while 160 (22.1%) participants having sufficient level. Vitamin D toxicity was seen in only 03 (0.41%) patients (Table 2). The collected data was further categorized on the basis of age groups and vitamin D status, the most common age group was 46-65 year followed by 26-45 and then 66-85 with the frequency of 315 (43.7%), 240 (33.3%) and 125 (17.4%) respectively (Table 2). The vitamin D deficiency was most common in the age group >85 years followed by age group 66-85 year with the prevalence of 8 (100%) and 109 (87.2%) individually (Table II).

The study participants were further categorized into two main groups on the basis of gender, there were 347 (48.1%) male and 374(51.9%) were female patients. It was observed that vitamin D deficiency was more common in female population as compared to male, as shown in table 3 that Vitamin D was deficient in 210 (56.1%) females while 150 (43.2%) male were having deficient vitamin D levels.

Hypervitaminoses noted in only 3 (0.4%) patients and were all males (Table III).

In this study we have Analysis of variance (ANOVA) was done to check whether there is statistical difference between the mean values of vitamin D and age, serum phosphorus, serum total calcium, serum urea, serum creatinine and serum uric acid. It was observed that there is statistical significant difference ($p<0.05$) in the mean value of all parameters (Table IV).

We also assessed other laboratory parameters (Serum Urea, Creatinine, Uric acid, Total calcium (Ca) and phosphorus (PO_4) in blood samples of recruited patients along with vitamin D status, out of 721 patients, 351 (97.5%) cases with normal calcium level had vitamin D deficiency, 194 (98.0%) patients had insufficient levels of vitamin D, and 154 (96.2%) patients had desirable levels, whereas 3 (100%) cases showed toxic levels of vitamin D in their serum. Overall total Ca was normal in 702 (97.4%) cases while only 19 (2.6%) had low serum Ca levels. Whereas, serum phosphate (PO_4) levels was found normal in 338 (93.9%) cases with severe vitamin D deficiency, 194 (98.0%) patients with normal levels of PO_4 had insufficient vitamin D, 152 (95.0%) patients with desirable and 3 (100.0%) cases with toxic levels of vitamin D had normal PO_4 levels. Serum PO_4 levels were on lower side only in 24 (3.3%) patients out of 721 (Table V).

Renal function tests (Urea, creatinine and uric acid) were also determined in serum of studied patients. Out of 721 there were only 51 (7.2%) patients had

Table II: Vitamin D status in different Age Groups

Age Groups	Deficient (%) <20.0	Insufficient (%) 20.1-30	Sufficient (%) 30.1-110	Toxic (%) >110	Total (%)
0-15	7 (46.7)	4 (26.7)	3 (20.0)	1 (6.7)	15 (2.0)
16-25	8 (44.4)	6 (33.3)	4 (22.2)	0 (0.0)	18 (2.5)
26-45	110 (45.8)	69 (28.8)	61 (25.4)	0 (0.0)	240 (33.3)
46-65	139 (44.1)	98 (31.1)	77 (24.4)	1 (0.3)	315 (43.7)
66-85	88 (70.4)	21 (16.8)	15 (12.0)	1 (0.8)	125 (17.4)
>85	8 (100.0)	- -	- -	- -	08 (1.1)
Total	360 (49.9)	198 (27.4)	160 (22.1)	3 (0.41)	721 (100)

Table III: Gender wise distribution of Vitamin D status

Gender	Deficient (%) <20.0	Insufficient (%) 20.1-30	Sufficient (%) 30.1-110	Toxic (%) >110	Total (%)
Male	150 (43.2)	81 (23.3)	113 (32.6)	3 (0.9)	347 (48.1)
Female	210 (56.1)	117 (31.3)	47 (12.6)	0 (0.0)	374 (51.8)
Total	360 (49.9)	198 (27.4)	160 (22.1)	3 (0.41)	721 (100)

Table IV: ANOVA Table

		Sum of Squares	Df	Mean Square	F	Sig.
Age	Between Groups	111210.187	280	397.179	2.539	.000
	Within Groups	68821.399	440	156.412		
	Total	180031.587	720			
Phosphorus	Between Groups	151.051	280	.539	2.099	.000
	Within Groups	113.074	440	.257		
	Total	264.125	720			
Total Calcium	Between Groups	221.886	280	.792	3.684	.000
	Within Groups	94.649	440	.215		
	Total	316.535	720			
Urea	Between Groups	83834.289	280	299.408	1.930	.000
	Within Groups	68273.113	440	155.166		
	Total	152107.401	720			
Creatinine	Between Groups	161.174	280	.576	1.470	.000
	Within Groups	172.258	440	.391		
	Total	333.432	720			
Uric Acid	Between Groups	993.889	280	3.550	2.542	.000
	Within Groups	614.371	440	1.396		
	Total	1608.260	720			

Vitamin D Status	Calcium <8.5 mg/dl Low n(%)	Calcium 8.6- 10.5 mg/dl Normal n(%)	PO ₄ <2.5 mg/dl Low n(%)	PO ₄ 2.5-4.5 mg/dl Normal n(%)	PO ₄ >4.6 mg/dl High n(%)	Urea <45.0 mg/dl Normal n(%)	Urea >46.0 mg/dl High n(%)	Creatinine <1.1 mg/dl Normal n(%)	Creatinine >1.2 mg/dl High n(%)	Uric acid < 7.0 mg/dl Normal n(%)	Uric acid > 7.1 mg/dl High n(%)
Deficient <20 ng/ml	09(2.5)	351(97.5)	14 (3.7)	338(93.9)	08 (2.2)	329(91.4)	31 (8.6)	338 (93.9)	22(6.1)	305(84.7)	55(15.3)
Insufficient 21-30 ng/ml	4 (2.0)	194 (98.0)	9(4.5)	184(92.9)	5(2.5)	188(94.9)	10(5.1)	195 (98.5)	03(1.5)	174 (87.9)	24(12.1)
Sufficient 31-110 ng/ml	6(3.8)	154 (96.2)	01(0.6)	152(95.0)	07 (4.4)	150(93.8)	10(6.2)	153 (95.6)	07 (4.4)	141 (88.1)	19(11.9)
Toxic >110 ng/ml	- -	3 (100.0)	-	3 (100.0)	-	03(100)	-	03(100)	-	2(66.7)	01(33.3)
Total (%)	19(2.6)	702 (97.4)	24 (3.3)	677(93.9)	20 (2.8)	670 (93.0)	51 (7.0)	689 (95.6)	32 (4.4)	622 (86.3)	99 (13.7)
P Value	NS		NS			NS		NS		NS	
NS= Non Significant											

high urea levels, 32 (4.4%) with high serum creatinine levels while high uric acid was seen in 99 (13.7%) patients. Raised urea level was observed in total 31 (8.6%) and 10 (5.1%) cases with vitamin D deficiency and insufficiency status respectively. Serum creatinine was found elevated in only 22 (6.1%) vitamin D deficient people. Uric acid was elevated in 55 (15.3%) study participants with vitamin D deficient status (Table V). We also tried to find out correlation with vitamin D status and estimated laboratory parameters and it was found that there is no significant relationship ($p < 0.05$) of laboratory parameters with any status of vitamin D. (Table V)

Discussion

Vitamin D plays important role in calcium homeostasis, also significantly involve in regulation of cellular differentiation and proliferation, immune functions, reproductive health and lowering the risk of large numbers of chronic illnesses like numerous infectious diseases, cardiovascular disorders, various malignancies and autoimmune diseases²⁹⁻³⁰. It was reported by many studies from all over the world that vitamin D deficiency is very common in all age groups and becoming one of the major public health concerns³⁰. It is therefore, the assessment of vitamin D status in different age groups and gender is needed.

In present study, the status of vitamin D, calcium, phosphorus, urea, creatinine and uric acid were

measured in 721 blood samples of both genders. It was observed in our population that vitamin D was deficient in 49.9% (n=360) population while the 27.4% (n=198) having insufficient vitamin D levels. Comparatively vitamin D deficiency was more commonly observed in elder population and in females as seen in table 2 and 3. In our test population, Vitamin D deficiency was seen in 56.1% (n=210) females while 31.3% (n=117) females were having insufficient vitamin D levels. This showed that females are more deficient to vitamin D than males. However there is no statistical difference was seen in vitamin D deficiency between men and women. Hypervitaminoses noted in total 3 (1.0%) patients and were all males. Sun light is one of the main sources of vitamin D. This study was conducted in twin cities (Islamabad/ Rawalpindi), Islamabad is capital city of Pakistan, although twin cities are shiny cities of Pakistan but direct exposure to sun light is limited especially in female due to wearing of Hijab/ Burkah, full body covered clothes and use of sun blocks due to fear of skin cancer, while the male population

especially working in government and multinational companies are also use to wear long sleeves shirts. Trend of living in full covered houses and in apartments is also increasing. Due to above mentioned reasons vitamin D deficiency is more common in our population.

Our reported results are comparable with previous studies conducted in different cities and neighboring countries of Pakistan. Riaz *et al* conducted a larger study of 4830 participants and reported similar findings that vitamin D was deficient in 53.5% population while 31.20% had insufficient vitamin D levels. Our results are also related to past studies reported from same geographical region³¹. Chaudhary B *et al* gathered the data of 5693 adult patients belongs to Rawalpindi Islamabad and they had reported that 56.5% patients had vitamin D deficient status while 18.2% had insufficient level³². Khan H *et al* also reported vitamin D deficiency in 56.2% individuals³³. Another national study showed 53.5% had Vitamin D deficiency, 31.2% had insufficient Vitamin D and only 15.3% normal Vitamin D³¹. Similar findings were also reported from a study conducted on 1,111 healthy individuals in our neighbor country Iran, they reported vitamin D

deficiency and insufficiency 50.8% and 19.6% individually³⁴.

Our age and gender based results are also comparable with previous studies. In present study, vitamin D deficiency was most common in elder age group while many previous studies also reported same findings³⁵⁻³⁷. According to our reported results vitamin D deficiency and insufficiency were 56.1% and 31.3% respectively while Vitamin D deficiency and insufficiency prevalence reported by Riaz H *et al* was 58.9% and 30.5% respectively³¹, Naqvi KZ *et al* reported 69.6% deficiency in pregnant women³⁸.

In this study we evaluated renal function tests (Urea, creatinine and uric acid), serum calcium and serum phosphorus along with vitamin D in studied patients and tried to find out their relationship with age and gender. It was observed that there is no significant relationship ($p>0.05$) of vitamin D status with age and gender. Different national and international studies also reported that there was no significant correlation of serum calcium and phosphorus with any status of vitamin D^{39, 40}. We found that renal function tests, serum calcium and phosphorus was normal in vitamin D deficient people (both male and female) of all age group.

Conclusion

Vitamin D deficiency in Pakistan is becoming endemic and it is much predominant in elder and female population. Based on our finding renal function tests, serum calcium and phosphorus levels are not the conjecturer of vitamin D deficiency. There is a general agreement that ideal serum vitamin D concentrations should be greater than 40 ng/mL. Judicious exposure to sunlight appears to be the best way to increase the vitamin D concentrations. But when solar exposure is not possible particularly in winter season, intake of 1000-5000 IU/D vitamin D3 is good alternative. For the maintenance of adequate serum vitamin D levels, regular and sufficient amount of foods rich in vitamin D should be taken and spend some time in the sun. But vitamin D3 is probably the best choice if someone takes supplements.

References

1. Holick MF (March 2006). "High prevalence of vitamin D inadequacy and implications for health". *Mayo Clinic Proceedings*. 81 (3): 353–73.

2. McCollum, E. V. (1957) A History of Nutrition Houghton Mifflin Co. Boston, MA
3. Vitamin D Tests". Lab Tests Online (USA). American Association for Clinical Chemistry. Retrieved June 23, 2013.
4. Hollis BW (January 1996). "Assessment of vitamin D nutritional and hormonal status: what to measure and how to do it". *Calcified Tissue International*. 58 (1): 4–5.
5. Lips P, Hosking D, Lippuner K, Norquist JM, Warren L, Maalouf G, et al. The prevalence of vitamin D inadequacy amongst women with osteoporosis: An international epidemiological investigation. *J Intern Med*. 2006; 260:245–54.
6. Lappe JM, Travers-Gustafson D, Davies KM, Recker RR, Heaney RP. Vitamin D and calcium supplementation reduces cancer risk: Results of a randomized trial. *Am J Clin Nutr*. 2007; 85:1586–91
7. Moan J, Porojnicu AC, Dahlback A, Setlow RB. Addressing the health benefits and risks, involving vitamin D or skin cancer, of increased sun exposure. *Proc Natl Acad Sci USA*. 2008; 105:668–73
8. Holick MF, Chen TC. Vitamin D deficiency: A worldwide problem with health consequences. *Am J Clin Nutr*. 2008; 87:1080S–6S.
9. Looker AC, Pfeiffer CM, Lacher DA, Schleicher RL, Picciano MF, Yetley EA. Serum 25-hydroxyvitamin D status of the US population: 1988–1994 compared to 2000–2004. *Am J Clin Nutr*. 2008; 88:1519–27.
10. Calvo MS, Whiting SJ, Barton CN (February 2005). "Vitamin D intake: a global perspective of current status". *The Journal of Nutrition*. 135 (2): 310–6.
11. Norman AW (August 2008). "From vitamin D to hormone D: fundamentals of the vitamin D endocrine system essential for good health". *The American Journal of Clinical Nutrition*. 88 (2): 491S–499S.
12. Dopico XC, Evangelou M, Ferreira RC, Guo H, Pekalski ML et al. Widespread seasonal gene expression reveals annual differences in human immunity and physiology. *Nat Commun*. 2015 May 12; 6:7000.
13. Garland C, Garland F, Gorham E, et al. The role of vitamin D in cancer prevention. *Am J Public Health*. 2006; 96:252–261.
14. Wortsman J, Matsuoka LY, Chen TC, Lu Z, Holick MF. Decreased bioavailability of vitamin D in obesity. *Am J Clin Nutr* 2000; 72:690–3.
15. Yetley EA. Assessing the vitamin D status of the US population. *Am J Clin Nutr*. 2008; 88:558S–564S.
16. Holick MF. Vitamin D deficiency. *N Engl J Med* 2007; 357:266–81.
17. Malabanan AO, Turner AK, Holick MF. Severe generalized bone pain and osteoporosis in a premenopausal black female: effect of vitamin D replacement. *J Clin Densitom* 1998; 1:201–204.
18. Plotnikoff GA, Quigley JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. *Mayo Clin Proc*. 2003; 78: 1463–1470
19. Malabanan AO, Turner AK, Holick MF. Severe generalized bone pain and osteoporosis in a premenopausal black female: effect of vitamin D replacement. *J Clin Densitom* 1998; 1:201–204.
20. Plotnikoff GA, Quigley JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. *Mayo Clin Proc* 2003; 78:1463–70.
21. Holick MF. Resurrection of vitamin D deficiency and rickets *J Clin Invest* 2006; 116:2062–72.
22. Bischoff-Ferrari HA, Giovannucci E, Willett WC, Dietrich T, Dawson-Hughes B: Estimation of optimal serum concentrations of 25-hydroxyvitamin D for multiple health outcomes. *Am J Clin Nutr*. 2006; 84: 18–28
23. Holick MF. Vitamin D: A millennium perspective. *J Cell Biochem* 2003; 88:296–307.
24. Webb AR, Kline L, Holick MF. Influence of season and latitude on the cutaneous synthesis of vitamin D3: exposure to winter sunlight in Boston and Edmonton will not promote vitamin D3 synthesis in human skin. *J Clin Endocrinol Metab* 1988; 67:373–8.
25. Hoel DG, Berwick M, de Gruij FR, Holick MF. The risks and benefits of sun exposure 2016. *Dermatoendocrinol*. 2016 Oct 19; 8(1):
26. Fuleihan GEH, Nabulsi M, Choucair M, et al. Hypovitaminosis D in healthy school children. *Pediatrics* 2001; 107:53–9.
27. Holick MF, Matsuoka LY, Wortsman J. Age, vitamin D, and solar ultraviolet. *Lancet* 1989; 2(8671):1104–5.
28. Tim Newman Vitamin D guidelines may be changed following new study 6 July 2017
29. Al-Ali H, Fuleihan GEH. Nutritional osteomalacia: substantial clinical improvement and gain in bone density post therapy. *J Clin Densitom* 2000; 3:97–101.
30. Shaw NJ, Pal BR. Vitamin D deficiency in UK Asian families: activating a new concern. *Arch Dis Child*. 2002; 86:147–49.
31. Riaz H, Finlayson AE, Bashir S, Hussain S. Prevalence of Vitamin D deficiency in Pakistan and implications for the future. *Expert Rev Clin Pharmacol*. 2016; 9:329–38.
32. Chaudhary B. Vitamin D Deficiency in Rawalpindi–Islamabad Region. *Journal of Rawalpindi Medical College*. 2017 Jun 30; 21(2):169–72.
33. Khan H, Ansari MA, Waheed U, Farooq N. Prevalence of Vitamin D Deficiency in General Population of Islamabad, Pakistan. *Ann Pak Inst Med Sci*. 2013; 9:45–47.
34. Hovsepian S, Amini M, Aminorroaya A, Amini P, Iraj B. Prevalence of vitamin D deficiency among adult population of Isfahan City, Iran. *Journal of health, population, and nutrition*. 2011 Apr; 29(2):149.
35. Omdahl JL, Garry PJ, Hunsaker LA, Hunt WC, Goodwin JS. Nutritional status in a healthy elderly population: vitamin D. *Am J Clin Nutr* 1982; 36:1225–32.
36. Journal of Pakistan Orthopedic Association 2013 VOL.25 (1)18 Vitamin D deficiency in Pakistani Population. Pervaiz Anjum, Naseem Safder, Muhammad Khalid, Irfan Mehboob
37. Heshmat, R., Mohammad, K., Majdzadeh, S.R., Forouzanfar, M.H., Bahrami, A. and Ranjbar Omrani, G.H., 2008. Vitamin D deficiency in Iran: A multi-center study among different urban areas. *Iran J Public Health*, 37(suppl).
38. Naqvi KZ, Ali ST, Thontia S. Prevalence of Vitamin D deficiency in pregnant population attending a tertiary care hospital Karachi. *Pak J Surg*. 2012; 28:122–25.
39. Masood Z, Mahmood Q, Ashraf KT. Vitamin D deficiency—an emerging public health problem in Pakistan. *JUMDC*. 2010; 1(1):4–9.
40. Baloch S, Devrajani BR, Atta-ur-Rahman A. Status of Serum calcium, phosphorus and Vitamin D. *Medical Science*. 2013 Jul; 2(7).