

Strathprints Institutional Repository

Riaz, H. and Finlayson, A. E. and Bashir, S. and Hussain, S. and Mahmood, S. and Malik, F. and Godman, B. (2016) Prevalence of vitamin D deficiency in Pakistan and implications for the future. Expert Review of Clinical Pharmacology, 9 (2). pp. 329-338. ISSN 1751-2433 , http://dx.doi.org/10.1586/17512433.2016.1122519

This version is available at http://strathprints.strath.ac.uk/54813/

Strathprints is designed to allow users to access the research output of the University of Strathclyde. Unless otherwise explicitly stated on the manuscript, Copyright © and Moral Rights for the papers on this site are retained by the individual authors and/or other copyright owners. Please check the manuscript for details of any other licences that may have been applied. You may not engage in further distribution of the material for any profitmaking activities or any commercial gain. You may freely distribute both the url (<u>http://strathprints.strath.ac.uk/</u>) and the content of this paper for research or private study, educational, or not-for-profit purposes without prior permission or charge.

Any correspondence concerning this service should be sent to Strathprints administrator: strathprints@strath.ac.uk

Prevalence of Vitamin D deficiency in Pakistan; implications for the future

Riaz H¹, Finlayson^{2,3}, Bashir S⁴, Hussain S⁵, Mahmood S⁶, Malik F⁷, *Godman B^{8,9}

¹Faculty of Pharmacy, Sargodha University, Sargodha, Pakistan. Email: humayunriaz19@gmail.com ²GreenTempleton College, Oxford University, Oxford, UK. Email: alexanderfinlayson@gmail.com ³Nuffield Department of Primary Care Health Sciences, University of Oxford, 48 Woodstock Road, Oxford OX2 6HG, UK

⁴Faculty of Pharmacy, Sargodha University, Sargodha, Pakistan. Email: sajidpharm@gmail.com ⁵National Institute of Health, Islamabad, Pakistan. Email: shahzadpharmacist1962@gmail.com ⁶International Islamic University, Islamabad, Pakistan. Email: sidrah_mahmood@yahoo.com ⁷National Institute of Health, Islamabad, Pakistan. Email: farnazmalik@yahoo.com ⁸Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska Institute, Karolinska University Hospital Huddinge, SE-141 86, Stockholm, Sweden. Email: Brian.Godman@ki.se

⁹Strathclyde Institute of Pharmacy and Biomedical Sciences, Strathclyde University, Glasgow G4 ORE, UK. Email: brian.godman@strath.ac.uk

Address for correspondence: * Brian Godman Department of Laboratory Medicine, Division of Clinical Pharmacology, Karolinska Institute. Karolinska University Hospital Huddinge, SE-141 86, Stockholm, Sweden. Email: <u>Brian.Godman@ki.se</u>

(Accepted for publication in Expert Review of Clinical Pharmacology – Please keep CONFIDENTIAL)

Abstract

Background and aims: Vitamin D deficiency (25-hydroxyvitamin D - VitDD) affects over one billion people worldwide. VitDD results in progression of osteoporosis as well as other conditions. Previous studies have shown high rates of VitDD in Pakistan despite appreciable levels of sunshine. However, none have assessed VitDD across all age groups, genders, incomes and locations to guide future strategies. Methods: Questionnaire and blood sampling among 4830 randomly selected citizens. Results: High levels of VitDD among all age groups, genders, income levels and locations. 53.5% had VitDD, 31.2% had insufficient Vitamin D and only 15.3% normal Vitamin D. Conclusion: High rates of VitDD in Pakistan despite high levels of sunshine and previous Food Acts asking for food fortification with Vitamin D. Public health strategies are needed to address high VitDD rates, including food fortification, i.e. nurture, alongside increasing exposure to sunlight, i.e. nature. This will involve all key stakeholder groups.

Keywords: 25-hydroxyvitamin-D, epidemiology, food supplements, strategies, risk factors, Pakistan, Vitamin D deficiency

Introduction

It is believed that vitamin D (25-hydroxyvitamin D) deficiency (affects over one billion people worldwide (1, 2). However, VDD appears to be one of the most under-diagnosed and under-treated dietary insufficiencies (3-16). This includes Pakistan (1, 2, 17-21). Vitamin D deficiency has been linked to the progression of osteoporosis and osteoporotic fractures arising from secondary hyperparathyroidism (1, 11, 16, 19, 22-29), and can also lead to rickets and osteomalacia (10, 12, 23, 26, 30) as well as potentially myopathy, helping explain why patients with nonspecific musculoskeletal pain syndrome can be refractory to standard therapies (31-33). Recent studies also suggest vitamin D has a potential role as an immune modulator and tumour suppressor (6, 16, 29, 34-39), although others are less certain (40). Vitamin D may also help prevent a number of other conditions including rheumatoid arthritis, diabetes, coronary heart disease, metabolic syndrome, depression, multiple sclerosis, respiratory diseases and cognitive impairment as well as reduce all-cause mortality (1, 6, 19, 29, 32, 41-49). In addition, Vitamin D deficiency appears to be associated with an increased risk of obesity (20, 50), and Vitamin D supplementation can ameliorate the clinical signs of patients with atopic dermatitis (51). However, others believe there is a less clear link between Vitamin D deficiency and cardiovascular diseases, multiple sclerosis, arterial hypertension or cognitive dysfunction (1, 38,

52). Nevertheless, it is important to address Vitamin D deficiency to reduce the occurrence of osteoporosis, including osteoporotic fractures, as well as rickets and osteomalacia.

Overall, there are three main causes of Vitamin D (25-hydroxyvitamin D) deficiency including dietary insufficiency, problems with absorption as well as lack of exposure to sunlight. Age and the level of physical activity can also affect Vitamin D (25-hydroxyvitamin D) levels (29). This is reflected by body mass index (BMI), skin pigmentation, and environmental factors, i.e. latitude, altitude, and meteorological conditions, influencing circulating vitamin D levels (26, 29, 30). Exposure to sunlight is perhaps the most important factor (2, 20). However, sufficient intake of vitamin D and calcium in a person's diet can also play an appreciable role in the prevention and management of the consequences of Vitamin D deficiency including osteoporosis and reducing the subsequent occurrence of fractures (1, 10, 11, 16, 23, 25). Studies have suggested serum 25(OH)D levels greater than 30 ng/mL (75 nmol/L) may be necessary to maximize intestinal calcium absorption and stop secondary hyperparathyroidism-induced skeletal conditions (1, 33, 53).

Whilst a number of studies of varying sizes have been undertaken in Pakistan to assess Vitamin D status (1, 17-21), there does appear to be a lack of published data on the extent of Vitamin D deficiency across all age groups, genders, income groups and dwellings, e.g. urban and rural locations. We are aware that Khan and colleagues and Iqbal and colleagues assessed the level of Vitamin D deficiency among different localities in Karachi (20, 21), and Sheikh and colleagues assessed VDD among adults in Karachi (1). However to the best of our knowledge, no one has assessed levels of Vitamin D deficiency among all age groups, genders, locations and income levels simultaneously. Consequently, the principal objective of this study is to estimate the prevalence of Vitamin D deficiency. This is because the prevention of osteoporosis as well as other non-communicable diseases, including diabetes and cardiovascular diseases, are seen as high priority areas in Pakistan, and likely to be included in the forthcoming National Health Plan (54, 55). The secondary objective is to assess the potential relationship between serum calcium and phosphate with vitamin D deficiency.

Materials and Methods

This study involved three parts.

The first part was questionnaire based, conducted from January 2010 to December 2012 at five cities (Lahore, Rawalpindi/Islamabad, Sialkot/Gujranwala, Abbotabad and Peshawar) in two provinces of Pakistan (Punjab and Khyber Pakhtonkhwe – KPK). This incorporates almost 50% of the total population of Pakistan.

Each city/town/village was further divided into a number of small compact areas called Enumeration Blocks (EBs) consisting of 200 to 250 households. The populations of the selected areas were further divided into clusters of homogeneous rich and poor, i.e. covering slum and urban areas as well as locations where richer families live, with a random sample of personnel subsequently included in the questionnaire survey. To enhance the robustness of the findings, the survey instruments were developed and pre- tested in other locations than those included in the final sample; however, this data was not included in this study. Overall, a total 4815 people across all age groups, sexes and locations where included in the questionnaire. Selection was undertaken using the convenience sampling technique to ensure true randomisation. The only exclusion criterion was that patients were not currently suffering from any chronic diseases including attending clinics.

The main topics covered in the questionnaire included the age, sex, household income levels and history of fractures. Similar patient characteristics were seen in the study of Sheikh and colleagues (1). All data was captured onto Excel spreadsheets to ascertain the extent of Vitamin D deficiency and its severity among the different sexes and ages in Pakistan.

The second component of the study involved taking approximately 3-5 mls of blood from all the study subjects and collecting this in gel tubes to subsequently analyse their serum vitamin D levels. The serum was separated via centrifugation at 3,000 rpm for 5 minutes. The test principle was based on an Electro-chemiluminescence Immuno Assay (ECLIA). The body mass index (BMI) was calculated

as weight (Kg) divided by height (m) squared (56). The assay uses a vitamin D binding protein (VDBP) as the capture protein in the assay, which binds to vitamin D2 and vitamin D3 (25hydroxylvitamin). The reagents and kit were stored and used according to manufacturer's instructions [Package insert for vitamin D total (25-Hydroxyvitamin D) quantitative determination - Roche Diagnostics GmbH, Sandhofer Strasse 116, D- 68305 Mannheim, July 2012]. All the results were analyzed using MS excel.

Vitamin D deficiency was defined as serum 25-hydroxyvitamin-D levels < 20 ng/mL, with vitamin D insufficiency as 21-29 ng/mL and vitamin D sufficiency as 30 ng/mL or greater for children and adults (1, 15, 16, 57).

The third component was a pilot study involving 173 volunteers sampled in hospitals and private clinics to assess their calcium and phosphorous level as well as a possible relation with vitamin D deficiency. The body mass index (BMI) of the volunteers was calculated based on their weight and height. The sampling of volunteers was opportunistic. The data was captured in Excel, with the Excel statistical package used to determine the standard deviation and the level of significance. A p value of <0.05 was used to demonstrate statistical significance.

All studies were approved by board of studies of Sargodha University, Pakistan, with the study meeting the requirements of the declarations of Helsinki and conducted in accordance with the current Good Clinical Practice (58)

Results

There was appreciable levels of sunshine among all the five selected cities throughout the year (taken from: www.weather-and-climate.com; www.climate-zone.com):

- Lahore: Mean temperature 24.3°C; 3094 sunshine hours
- Sialkot/Gujranwala: Mean temperature 21.1°C; 3044 sunshine hours
- Rawalpindi/Islamabad: Mean temperature 21.3°C; 3050 sunshine hours
- Abbotabad: Mean temperature 18.0°C; 2044 sunshine hours
- Peshawar: Mean temperature 22.7°C; 3110 sunshine hours

Table 1 contains details of the socioeconomic and health status of the 4830 subjects included in the blood sampling. A history of fractures was present in 398 subjects. In Punjab, 1252 males and 2299 females were included and in KPK, 343 males and 936 females were included.

Table 1: Socioeconomic and other parameters (n= 4830) and %s

Male: 1595; Female: 3235							
Monthly Income (Rs)							
<15000	627 (13.0%)						
30000	1729 (35.8%)						
50000 - 99999	2076 (43.0%)						
100000	215 (4.4%)						
Above 100000	183 (3.8%)						
Self-Reported Health							
Good	3099 (64.2%)						
Fair	880 (18.2%)						
Poor	851 (17.6%)						
History of Fracture							
Yes	403 (8.3%)						
No	4427 (91.7%)						

A total of 4830 subjects comprising 1595 males (33.02%) and 3235 (66.98%) females were analysed for their serum 25-hydroxyvitamin-D levels. Figure 1 depicts the percentage of subject in each age group with different levels of 25-hydroxyvitamin-D deficiency, whilst Table 2 gives the absolute levels.





NB: M = Male; F = Female

Overall, there were similar proportions of women and men with vitamin D (25-hydroxyvitamin-D) deficiency (Tables 2 and 3).

Tables 2 and 3. Numbers and prevalence (%) of vitamin D deficiency and its severity among the different sexes and age groups in Pakistan

Age (yrs)	(yrs) Total (Numbe citizens)		Deficient <20 ng/ml		Insufficient 20-30 ng/ml		Normal >30 ng/ml	
	М	F	М	F	М	F	М	F
<20	40	86	27	62	12	21	1	3
21-30	260	537	153	315	75	149	32	73
31-40	363	692	202	365	110	223	51	104
41-50	447	846	231	452	155	246	61	148
51-60	400	867	230	396	101	306	69	165
61-70	40	112	25	50	15	46	0	16
Above 70	45	95	21	56	20	29	4	10
Total	1595	3235	889	1696	488	1020	218	519

Percentages

Age (yrs)	Deficient <20 ng/ml		nsufficient 21-3	30 ng/ml	Normal >30 ng/ml		
	М	F	Μ	F	Μ	F	
<20	67.5	72.1	30.0	24.4	2.5	3.5	
21-30	58.8	58.7	28.8	27.7	12.3	13.6	
31-40	55.6	52.7	30.3	32.2	14.0	15.0	
41-50	51.7	53.4	34.7	29.1	13.6	17.5	
51-60	57.5	45.7	25.3	35.3	17.3	19.0	
61-70	62.5	44.6	37.5	41.1	0.0	14.3	
Above 70	46.7	58.9	44.4	30.5	8.9	10.5	

NB: M = Male; F = Female

There were typically similar characteristics when the figures were broken down for the five cities (Appendix – Tables A1 to A5) although some variations were noted (Figure 2).



Figure 2 – The extent of Vitamin D deficiency by sex and city in Pakistan

Table 4 contains the clinical characteristics of the 173 patients sampled in hospital with 156 subsequently analyzed for serum calcium, phosphate and PTH concentrations. No significant differences were found and consequently, the findings were not taken further.

Variables	Serum 25 OHD levels			
	Insufficient	Sufficient	Normal Value	P-value
	(≤20-30 ng/l)	(>30 ng/ml)		
	(n=156)	(n=17)		
Age group (years) Gender				
Male (n=47) Female (n=129)				
Body mass index (kg/m2)	21.12 +/-2.26	22.83 +/- 1.98	18.5-24.9 kg/m2	0.84
Serum corrected calcium (mmol/L)	2.18 +/- 0.68	2.24 +/ 0.24	2.15–2.5 mmol/L	0.25
Serum iPTH levels (ng/L)	78.24 +/ -24.12	62.82 +/ - 18.75	16–87 ng/L	0.82
Alkaline phosphatase (ng/L)	72.24 +/- 10.82	68.18 +/ - 12.76	female: 29–132 IU/L male: 28–124 IU/L	0.18
Phosphorus (mg/dL)	3.22 +/- 1.24	3.12 +/ 1.69	2.7–4. 9 mg/dL	0.26

Table 4 - Clinical characteristics among the 173 patients sampled in asymptomatic subjects with varying serum 25 OHD levels

Discussion

We believe this is one of the largest studies conducted in Pakistan to assess 25-hydroxyvitamin D levels among an asymptomatic population taken from almost 50% of the Pakistan population. This complements the studies of lqbal and colleagues, Sheikh and colleagues as well as Pakistan's Nutritional Survey in 2011 (1, 20, 21).

A high prevalence of vitamin D deficiency was recorded with 53.5% of citizens sampled having Vitamin D deficiency, 31.2% having insufficient Vitamin D and only 15.3% having normal Vitamin D (25-hydroxyvitamin D) levels (Figure 1, Tables 2 and 3). This appears irrespective of gender, age, location and income with only a minority of patients having a monthly income of RS10,000 or above with just under 50% having an income RS3000 or less (Figures 1 and 2, Tables 1 to 3 and A1 to A5). High rates of Vitamin D deficiency were also seen irrespective of the different levels of sunshine among the five city locations.

The high rates of Vitamin D deficiency seen in our study are similar to previous studies conducted in Pakistan and in the neighbouring countries, as well as studies of Pakistani women conducted in other countries (1, 13, 14, 17-21, 26, 59-62). A high level of Vitamin D deficiency (40%) was also noted among children in the 2011 National Nutrition Survey with limited differences between urban and rural areas, although differences were seen at the provincial level (63). Other authors have shown though higher rates of VDD in postmenopausal women living in town houses and apartments versus those living in small and large bungalows, with similar findings among Saudi Arabian women (20, 64). This was not seen in our study. Other authors have also shown higher prevalence in lower income groups (65). This may be due to differences in sampling or other techniques used. In addition, only a minority of households in our study earned RS10,000 per month or above and we did not break the figures down further into the various income groups outlined in Table 1.

In any event, there is an urgent need to address current high levels of Vitamin D deficiency among the population in Pakistan. Strategies to address this can be divided into nature, e.g. aspects such as sunlight, and nurture, e.g. Vitamin D supplementation. This is because personal, social, and cultural factors are important determinants of Vitamin D availability via their effects on sun exposure to ultraviolet light (UVB) and adequate intake from diet or supplements (2, 20, 26, 29, 66). Pakistan is among the world's most sun rich countries, with the cutaneous production of Vitamin D possible throughout the year (2). However, town dwellers may well be subject to Vitamin D deficiency due to air pollutants (67, 68), with for instance Karachi ranked as one of the most polluted cities reflected by multi pollutant indices (69). This may help explain why a higher incidence of Vitamin D deficiency was found among postmenopausal women dwelling in town houses and apartments in Karachi versus those living in bungalows in the suburbs (20), as well more densely versus less densely populated areas of Karachi in the study by Iqbal and colleagues (21). However, this cannot be the only explanation with high levels of vitamin D deficiency seen across both urban and rural areas in our study and across all age groups. Chronic and acute malnutrition in a country is one of the primary reasons for Vitamin D deficiency, i.e. attributable to poverty, high illiteracy rates among mothers and food insecurity (15, 16). Generally natural food sources have low vitamin D content and consequently require fortification where Vitamin D deficiency rates are high (26). This is especially important in countries where a population has limited knowledge of the level of Vitamin D in foodstuffs as seen in Pakistan (Personal communication Shahazad Hussain).

Vitamin D supplements can be given to prevent Vitamin D deficiency in Pakistan, building on existing appreciable self purchasing of bisphosphonates in Pakistan to prevent fractures in patients with osteoporosis (54). The Institute of Medicine's current recommended intake of vitamin D is 15 micrograms (600 IU) up to age 70 and 20 micrograms (800 IU) after 70 (1, 70).

Overall, a thorough understanding of the association of these factors is important given the extent of Vitamin D deficiency in Pakistan highlighted in our study as well as those of others (1, 17, 18, 20, 21). The Pakistan Food Act of 1965 states that all fats and oils produced in Pakistan should be fortified with Vitamin D (20). However, this is typically not enforced, with currently no labels on these food stuffs indicating any fortification. Presently, there are also no recommended guidelines to address VDD in Pakistan (1). However, this may change with the forthcoming Pakistan National Health Plan aimed at combating high priority non-communicable diseases including cardiovascular diseases and osteoporosis (54, 55, 71).

In view of the demonstrated prevalence of Vitamin D deficiency in Pakistan across all age groups in our study, it is suggested that policy makers urgently develop initiatives to address this (53). These could include firstly strategies involving education and food fortification programs with vitamin D, building on the 1965 Food Act. Secondly, encouraging the ingestion of vitamin D supplements when needed (66). This builds on patient concerns regarding the development of osteoporosis, demonstrated by the current extent of self-purchasing of bisphosphonates and Vitamin D in Pakistan (54), i.e. nurture. Thirdly, increasing citizen exposure to sunlight, i.e. nature, given the extent of sunlight in Pakistan. This is because Asians living in Pakistan typically have serum 25-hydroxycholecalciferol concentrations well within the normal range (72). This could be achieved through for instance involving religious leaders, who in their sermons on Fridays could emphasize the importance of vitamin D deficiency. Initially emphasising diet; however in the longer term reviewing issues such as dress in public since there are limited opportunities for private sub exposure among dwellings in Pakistan. This builds on successful strategies involving religious leaders to help eradicate polio through immunisation programmes.

Future Public Health strategies to reduce appreciable levels of Vitamin D deficiency in Pakistan should also involve all key stakeholder groups working together. This includes government personnel, physicians including those involved with Medical Colleges, clinical pharmacologists and pertinent patient organisations working alongside religious leaders to urgently address the current rates of Vitamin D deficiency in Pakistan. A number of Western countries have Vitamin D food fortification policies (73), serving as an example. Consequently, Pakistan could follow this lead as well as include other strategies in their forthcoming National Health Plan to appreciably reduce the extent of Vitamin D deficiency and its consequences on morbidity and mortality. We will monitor the situation in future research projects.

We acknowledge that there are a number of weaknesses with our research. These include the fact that appreciably more women were recruited than men due to the sampling technique used. Typically, the ratio of men to women in Pakistan is just over 1. We also did not ask questions about current Vitamin D supplementation. Nevertheless, we believe our study is valid and provides a good insight into the current extent of Vitamin D deficiency in Pakistan and potential ways forward.

In conclusion, we believe we have demonstrated that there are appreciable levels of Vitamin D deficiency among all age groups, genders, income levels and locations in Pakistan. This is despite high levels of sunlight throughout the year. A number of strategies have been identified to address this concern including following up previous Food Acts in the forthcoming Pakistan National Health Plan. We will monitor these in future research projects.

Key Issues

- Vitamin D deficiency (VDD) is a prevalent condition affecting over one billion people worldwide, and appears to be one of the most under-diagnosed and under-treated dietary insufficiencies
- VDD has been linked to the progression of osteoporosis and osteoporotic fractures arising from secondary hyperparathyroidism as well as a variety of other conditions. Consequently, VDD needs to be addressed
- Previous studies have shown high rates of VDD in Pakistan despite appreciable levels of sunshine across cities and regions, and previous Food Acts asking for food fortification with Vitamin D. However to date, no published appears to have assessed VDD across all age groups, genders, incomes and locations in Pakistan to guide future strategies.
- Our findings demonstrated high levels of VDD among all age groups, genders, income levels and locations in Pakistan. 38% of patients sampled had severe VDD, 29% moderate VDD and 25% mild VDD using our definitions, with only 8.6% normal Vitamin D levels.
- These findings have considerable implications for the future morbidity and mortality of citizens in Pakistan as well as costs.

- Public health strategies are needed to address high VDD rates. These include food fortification, i.e. nurture, alongside increasing exposure to sunlight, i.e. nature, involving all key stakeholder groups including religious leaders.
- Religious leaders in their sermons on Fridays could emphasize the importance of vitamin D deficiency. Initially emphasising diet; however in the longer term reviewing issues such as dress in public with limited opportunities for exposure in private to overcome high levels of moderate to severe VDD in Pakistan

References

1. Sheikh A, Saeed Z, Jafri SA, Yazdani I, Hussain SA. Vitamin D levels in asymptomatic adultsa population survey in Karachi, Pakistan. PloS one. 2012;7(3):e33452.

*Good paper describing the prevalence of vitamin D generally and in Pakistan

2. Humayun Q, Iqbal R, Azam I, Khan AH, Siddiqui AR, Baig-Ansari N. Development and validation of sunlight exposure measurement questionnaire (SEM-Q) for use in adult population residing in Pakistan. BMC public health. 2012;12:421.

3. Greene-Finestone LS, Berger C, de Groh M, Hanley DA, Hidiroglou N, Sarafin K, et al. 25-Hydroxyvitamin D in Canadian adults: biological, environmental, and behavioral correlates. Osteoporosis international. 2011;22(5):1389-99.

4. Gill TK, Hill CL, Shanahan EM, Taylor AW, Appleton SL, Grant JF, et al. Vitamin D levels in an Australian population. BMC public health. 2014;14(1):1001.

 Ginde AA, Liu MC, Camargo CA, Jr. Demographic differences and trends of vitamin D insufficiency in the US population, 1988-2004. Archives of internal medicine. 2009;169(6):626-32.
 Khaw K-T LR, Wareham N. Serum 25-hydroxyvitamin D, mortality, and incident

cardiovascular disease, respiratory disease, cancers, and fractures: a 13-y prospective population study. Am J Clin Nutr. 2014:1-10.

7. Mithal A, Wahl DA, Bonjour JP, Burckhardt P, Dawson-Hughes B, Eisman JA, et al. Global vitamin D status and determinants of hypovitaminosis D. Osteoporosis international. 2009;20(11):1807-20.

8. van der Meer IM, Middelkoop BJ, Boeke AJ, Lips P. Prevalence of vitamin D deficiency among Turkish, Moroccan, Indian and sub-Sahara African populations in Europe and their countries of origin: an overview. Osteoporosis international. 2011;22(4):1009-21.

9. van Schoor NM, Lips P. Worldwide vitamin D status. Best practice & research Clinical endocrinology & metabolism. 2011;25(4):671-80.

10. Ritu G, Gupta A. Vitamin D deficiency in India: prevalence, causalities and interventions. Nutrients. 2014;6(2):729-75.

11. Hill TR, Aspray TJ, Francis RM. Vitamin D and bone health outcomes in older age. The Proceedings of the Nutrition Society. 2013;72(4):372-80.

12. Holick MF, Chen TC. Vitamin D deficiency: a worldwide problem with health consequences. Am J Clin Nutr. 2008;87(4):1080s-6s.

*Good paper discussing the consequences of VDD

 Meyer HE, Falch JA, Sogaard AJ, Haug E. Vitamin D deficiency and secondary hyperparathyroidism and the association with bone mineral density in persons with Pakistani and Norwegian background living in Oslo, Norway, The Oslo Health Study. Bone. 2004;35(2):412-7.
 Madar AA, Stene LC, Meyer HE. Vitamin D status among immigrant mothers from Pakistan,

Turkey and Somalia and their infants attending child health clinics in Norway. The British journal of nutrition. 2009;101(7):1052-8.

15. Horlick MF. The Vitamin D Deficiency Pandemic: a Forgotten Hormone Important for Health. Public Health Reviews. 2010;32(1):267-83.

16. Hossein-nezhad A, Holick MF. Vitamin D for health: a global perspective. Mayo Clinic proceedings Mayo Clinic. 2013;88(7):720-55.

**Updated paper discussing the implications of VDD

17. Mansoor S, Habib A, Ghani F, Fatmi Z, Badruddin S, Mansoor S, et al. Prevalence and significance of vitamin D deficiency and insufficiency among apparently healthy adults. Clinical biochemistry. 2010;43(18):1431-5.

18. Zuberi LM, Habib A, Haque N, Jabbar A. Vitamin D Deficiency in ambulatory patients. JPMA The Journal of the Pakistan Medical Association. 2008;58(9):482-4.

19. Palacios C, Gonzalez L. Is vitamin D deficiency a major global public health problem? The Journal of steroid biochemistry and molecular biology. 2014;144 Pt A:138-45.

20. Khan AH, Iqbal R, Naureen G, Dar FJ, Ahmed FN. Prevalence of vitamin D deficiency and its correlates: results of a community-based study conducted in Karachi, Pakistan. Archives of osteoporosis. 2012;7(1-2):275-82.

*Study describing the prevalence of Vitamin D in Pakistan as well as the importance of sunlight to address VDD

21. Iqbal R, Jafri L, Haroon A, Habib Khan A. Illuminating the dark side--vitamin D status in different localities of Karachi. Journal of the College of Physicians and Surgeons - Pakistan. 2013;23(8):604-6.

* Recent published paper assessing the level of VDD in Pakistan

22. Kota S, Jammula S, Kota S, Meher L, Modi K. Correlation of vitamin D, bone mineral density and parathyroid hormone levels in adults with low bone density. Indian journal of orthopaedics. 2013;47(4):402-7.

23. Laird E, Ward M, McSorley E, Strain JJ, Wallace J. Vitamin D and bone health: potential mechanisms. Nutrients. 2010;2(7):693-724.

24. Simonelli C. The role of vitamin D deficiency in osteoporosis and fractures. Minnesota medicine. 2005;88(11):34-6.

 Nieves JW. Osteoporosis: the role of micronutrients. Am J Clin Nutr. 2005;81(5):1232s-9s.
 Iqbal R, Khan A. Possible Causes of Vitamin D Deficiency (VDD) in Pakistani Population Residing in Pakistan. The Journal of the Pakistan Medical Association. 2010;60(1):1-2.

Residing in Pakistan. The Journal of the Pakistan Medical Association. 2010;60(1):1-2.
27. Heaney RP, Dowell MS, Hale CA, Bendich A. Calcium absorption varies within the reference range for serum 25-hydroxyvitamin D. Journal of the American College of Nutrition. 2003;22(2):142-6.
28. Gallagher JC, Sai AJ. Vitamin D insufficiency, deficiency, and bone health. The Journal of clinical endocrinology and metabolism. 2010;95(6):2630-3.

29. Thacher TD, Clarke BL. Vitamin D insufficiency. Mayo Clinic proceedings Mayo Clinic. 2011;86(1):50-60.

30. Holick MF. Vitamin D: A millenium perspective. Journal of cellular biochemistry. 2003;88(2):296-307.

31. Ali JMM. Vitamin D deficiency in outpatient department: eastern province of KSA experience. Rawal Med J. 2010;35:221-3.

32. Zhang R, Naughton DP. Vitamin D in health and disease: current perspectives. Nutrition journal. 2010;9:65.

33. Plotnikoff GA, Quigley JM. Prevalence of severe hypovitaminosis D in patients with persistent, nonspecific musculoskeletal pain. Mayo Clinic proceedings Mayo Clinic. 2003;78(12):1463-70.

34. Schwartz GG. Vitamin D, sunlight, and the epidemiology of prostate cancer. Anti-cancer agents in medicinal chemistry. 2013;13(1):45-57.

35. Suba Z. Light deficiency confers breast cancer risk by endocrine disorders. Recent patents on anti-cancer drug discovery. 2012;7(3):337-44.

36. Giovannucci E. The epidemiology of vitamin D and cancer incidence and mortality: a review (United States). Cancer causes & control. 2005;16(2):83-95.

37. Krishnan AV, Trump DL, Johnson CS, Feldman D. The role of vitamin D in cancer prevention and treatment. Rheumatic diseases clinics of North America. 2012;38(1):161-78.

38. Peterlik M. Vitamin D insufficiency and chronic diseases: hype and reality. Food & function. 2012;3(8):784-94.

39. Yin L, Grandi N, Raum E, Haug U, Arndt V, Brenner H. Meta-analysis: longitudinal studies of serum vitamin D and colorectal cancer risk. Alimentary pharmacology & therapeutics. 2009;30(2):113-25.

40. van der Rhee H, Coebergh JW, de Vries E. Sunlight, vitamin D and the prevention of cancer: a systematic review of epidemiological studies. European journal of cancer prevention. 2009;18(6):458-75.

41. Holick MF. Diabetes and the vitamin d connection. Current diabetes reports. 2008;8(5):393-8.

42. Ravani P, Malberti F, Tripepi G, Pecchini P, Cutrupi S, Pizzini P, et al. Vitamin D levels and patient outcome in chronic kidney disease. Kidney international. 2009;75(1):88-95.

43. Zwerina K, Baum W, Axmann R, Heiland GR, Distler JH, Smolen J, et al. Vitamin D receptor regulates TNF-mediated arthritis. Annals of the rheumatic diseases. 2011;70(6):1122-9.

44. Janssens W, Mathieu C, Boonen S, Decramer M. Vitamin D deficiency and chronic obstructive pulmonary disease: a vicious circle. Vitamins and hormones. 2011;86:379-99.

45. Zittermann A, Prokop S. The role of vitamin D for cardiovascular disease and overall mortality. Advances in experimental medicine and biology. 2014;810:106-19.

46. Ryu OH, Chung W, Lee S, Hong KS, Choi MG, Yoo HJ. The effect of high-dose vitamin D supplementation on insulin resistance and arterial stiffness in patients with type 2 diabetes. The Korean journal of internal medicine. 2014;29(5):620-9.

47. Bertone-Johnson ER. Vitamin D and the occurrence of depression: causal association or circumstantial evidence? Nutrition reviews. 2009;67(8):481-92.

48. Pittas AG, Nelson J, Mitri J, Hillmann W, Garganta C, Nathan DM, et al. Plasma 25hydroxyvitamin D and progression to diabetes in patients at risk for diabetes: an ancillary analysis in the Diabetes Prevention Program. Diabetes care. 2012;35(3):565-73.

49. Alvarez JA, Ashraf A. Role of vitamin d in insulin secretion and insulin sensitivity for glucose homeostasis. International journal of endocrinology. 2010;2010:351385.

50. Gonzalez-Molero I, Rojo-Martinez G, Morcillo S, Gutierrez C, Rubio E, Perez-Valero V, et al. Hypovitaminosis D and incidence of obesity: a prospective study. European journal of clinical nutrition. 2013;67(6):680-2.

51. Samochocki Z, Bogaczewicz J, Jeziorkowska R, Sysa-Jedrzejowska A, Glinska O, Karczmarewicz E, et al. Vitamin D effects in atopic dermatitis. Journal of the American Academy of Dermatology. 2013;69(2):238-44.

52. Guessous I, Bochud M. [Effects of calcium and vitamin D supplementations on cardiovascular disease: review article]. Revue medicale suisse. 2012;8(348):1458-63.

53. Grober U, Spitz J, Reichrath J, Kisters K, Holick MF. Vitamin D: Update 2013: From rickets prophylaxis to general preventive healthcare. Dermato-endocrinology. 2013;5(3):331-47.

54. Riaz H, Godman B, Hussain S, Malik F, Mahmood S, Shami A, Bashir S. Prescribing of bisphosphonates and antibiotics in Pakistan: challenges and opportunities for the future. JPHSR 2015. 6: 111–121

*Paper discussing the National Health Plan in Pakistan as well as the extent of self-purchasing of bisphosphonates among the population

55. Godman B, Acurcio F, Guerra Junior AA, Alvarez-Madrazo S, Faridah Aryani MY et al Initiatives among authorities to improve the quality and efficiency of prescribing and the implications. J Pharma Care Health Sys 2014;1(3):1-15.

56. Sheikh SA, Baig JA, Iqbal T, Kazmi T, Baig M, Husain SS. Prevalence of microalbuminuria with relation to glycemic control in type-2 diabetic patients in Karachi. Journal of Ayub Medical College, Abbottabad. 2009;21(3):83-6.

57. Holick MF. Vitamin D deficiency. NEJM. 2007;357(3):266-81.

58. WMA. WMA Decleration of Helsinki - Ethical Principles for Medical Research involving humans. Available at URL: <u>http://www.wma.net/en/30publications/10policies/b3/index.html.pdf?print-media-type&footer-right=[page]/[toPage]</u>.

59. Brunvand L, Haug E. Vitamin D deficiency amongst Pakistani women in Oslo. Acta obstetricia et gynecologica Scandinavica. 1993;72(4):264-8.

60. Atiq M, Suria A, Nizami SQ, Ahmed I. Vitamin D status of breastfed Pakistani infants. Acta paediatrica. 1998;87(7):737-40.

61. Masud F. Vitamin D levels for optimum bone health. Singapore medical journal. 2007;48(3):207-12.

62. Andersen R, Molgaard C, Skovgaard LT, Brot C, Cashman KD, Jakobsen J, et al. Effect of vitamin D supplementation on bone and vitamin D status among Pakistani immigrants in Denmark: a randomised double-blinded placebo-controlled intervention study. The British journal of nutrition. 2008;100(1):197-207.

63. Pakistan Government. National Nutritional Survey - Conducted by Aga Khan University, Pakistan. Available at URL: <u>http://pakresponse.info/LinkClick.aspx?fileticket=Ao4s-</u> rwdFVI%3D&tabid=117&mid=7522011.

**Nutrition survey giving details of the extent of VDD among children in Pakistan

64. Fonseca V, Tongia R, el-Hazmi M, Abu-Aisha H. Exposure to sunlight and vitamin D deficiency in Saudi Arabian women. Postgraduate medical journal. 1984;60(707):589-91.

65. Weaver SP, Passmore C, Collins B, Fung E. Vitamin D, sunlight exposure, and bone density in elderly African American females of low socioeconomic status. Family medicine. 2010;42(1):47-51.
66. Wacker M, Holick MF. Sunlight and Vitamin D: A global perspective for health. Dermato-endocrinology. 2013;5(1):51-108.

67. Hosseinpanah F, Pour SH, Heibatollahi M, Moghbel N, Asefzade S, Azizi F. The effects of air pollution on vitamin D status in healthy women: a cross sectional study. BMC public health. 2010;10:519.

68. Humayun Q, Iqbal R. The impact of atmospheric pollution on vitamin D status. JPMA The Journal of the Pakistan Medical Association. 2011;61(2):197-8.

69. Gurjar BR, Butler T, Lawrence MG, Lelieveld J. Evaluation of emissions and air quality in megacities. Atmos Env 2008;42:1593-606.

70. Institute of Medicine. Institute of Medicine (US) Committee to Review Dietary Reference Intakes for Vitamin D and Calcium; Ross AC, Taylor CL, Yaktine AL, et al., editors. Dietary Reference Intakes for Calcium and Vitamin D. Washington (DC): National Academies Press (US); 2011 . Available at URL: <u>http://www.ncbi.nlm.nih.gov/books/NBK56070/</u>.

71. Godman B, Hussain S, Mahmood S, Malik F. Current challenges to the management of CV diseases in Pakistan; implications for the future. Basic & clinical pharmacology & toxicology. 2014;115 (Suppl 1):16.

72. Rashid A, Mohammed T, Stephens WP, Warrington S, Berry JL, Mawer EB. Vitamin D state of Asians living in Pakistan. British medical journal. 1983;286(6360):182-4.

73. Spiro A BJ. Vitamin D: An overview of vitamin D status and intake in Europe. Nutrition Bulletin. 2014;39:322-50.

*Discussion of food fortification programmes in Europe

* Of importance; ** of considerable importance

Supplementary Tables (Appendix)

	Lahore (Citizen numbers)									
	Та	T . (.)		cient	Insuf	ficient	Nor	mal		
Age (yrs)	Total		<20 ng/ml		21-30 ng/ml		>30 ng/ml			
	М	F	М	F	М	F	М	F		
<20	13	23	9	15	3	6	1	2		
21-30	91	197	59	132	23	34	9	31		
31-40	109	197	79	143	20	38	10	16		
41-50	118	284	77	216	28	46	13	22		
51-60	84	333	57	136	18	132	9	65		
61-70	14	18	9	15	5	3	0	0		
Above 70	7	13	7	13	0	0	0	0		
Total	436	1065	297	670	97	259	42	136		
NR·M - Male	E = Eomolo									

Table A1: Vitamin D deficiency and its severity in males and females in Lahore

NB: M = Male; F = Female

Lahore %

Age (yrs)	Deficient <20 ng/ml		Insufficient	21-30 ng/ml	Normal >30 ng/ml		
	М	F	М	F	М	F	
<20	69.2	65.2	23.1	26.1	7.7	8.7	
21-30	64.8	67.0	25.3	17.3	9.9	15.7	
31-40	72.5	72.6	18.3	19.3	9.2	8.1	
41-50	65.3	76.1	23.7	16.2	11.0	7.7	
51-60	67.9	40.8	21.4	39.6	10.7	19.5	
61-70	64.3	83.3	35.7	16.7	0.0	0.0	
Above 70	100.0	100.0	0.0	0.0	0.0	0.0	
Total	68.1	62.9	22.2	24.3	9.6	12.8	
NB: M = Male; I	F = Female						

Table A2: Vitamin D deficiency and its severity in males and females in Sialkot/Gujranwala (RWP/ISB)

	SKT/GWA (Patient numbers)										
	Tatal		Defi	cient	Insuff	ficient	Nor	mal			
Ages (yrs)	10	lai	<20 ı	ng/ml	21-30	ng/ml	>30 r	ng/ml			
	М	F	М	F	М	F	М	F			
<20	5	13	3	12	2	1	0	0			
21-30	29	44	21	32	6	10	2	2			
31-40	70	111	48	71	14	34	8	6			
41-50	173	187	82	114	65	35	26	38			
51-60	112	252	61	128	25	74	26	50			
61-70	3	5	2	3	1	2	0	0			
Above 70	1	3	1	3	0	0	0	0			
Total	393	615	218	363	113	156	62	96			
NB: M = Male;	F = Female										

Age (yrs)	Defi <20 r	SKT/GWA % Deficient <20 ng/ml		ficient ng/ml	Normal >30 ng/ml		
	Μ	F	М	F	М	F	
<20	60.0	92.3	40.0	7.7	0.0	0.0	
21-30	72.4	72.7	20.7	22.7	6.9	4.5	
31-40	68.6	64.0	20.0	30.6	11.4	5.4	
41-50	47.4	61.0	37.6	18.7	15.0	20.3	
51-60	54.5	50.8	22.3	29.4	23.2	19.8	
61-70	66.7	60.0	33.3	40.0	0.0	0.0	
Above 70	100.0	100.0	0.0	0.0	0.0	0.0	
Total NB: M = Male;	55.5 F = Female	59.0	28.8	25.4	15.8	15.6	

Table A3: Vitamin D deficiency and its severity in males and females in Islamabad/Rawalpindi (RWP/ISB)

	RWP/ISB (Citizen numbers)									
Ago (vrs)	Total		Defi	cient	Insuf	ficient	Nor	mal		
Age (yrs)			<20 ng/ml		20-30 ng/ml		>30 ng/ml			
	Μ	F	Μ	F	М	F	М	F		
<20	5	19	3	15	2	4	0	0		
21-30	66	112	39	63	20	34	7	15		
31-40	111	148	56	82	41	46	14	20		
41-50	101	150	49	62	41	57	11	31		
51-60	126	166	86	94	31	49	9	23		
61-70	7	13	5	11	2	2	0	0		
Above 70	7	11	7	8	0	3	0	0		
Total	423	619	245	335	137	195	41	89		

NB: M = Male; F = Female

		RWP/ISB %				
	Defic	cient	Insuff	ficient	Nor	mal
Age (yrs)	<20 r	ıg/ml	21-30	ng/ml	>30 ng/ml	
	М	F	М	F	М	F
<20	60.0	78.9	40.0	21.1	0.0	0.0
21-30	59.1	56.3	30.3	30.4	10.6	13.4
31-40	50.5	55.4	36.9	31.1	12.6	13.5
41-50	48.5	41.3	40.6	38.0	10.9	20.7
51-60	68.3	56.6	24.6	29.5	7.1	13.9
61-70	71.4	84.6	28.6	15.4	0.0	0.0
Above 70	100.0	72.7	0.0	27.3	0.0	0.0
Total	57.9	54.1	32.4	31.5	9.7	14.4
NB: M = Male;	F = Female					

	То	Total		cient	Insuf	ficient	Nor	mal
Age (JIS)	10			<20 ng/ml		ng/ml	>30 ng/ml	
	М	F	М	F	М	F	М	F
<20	4	7	4	4	0	2	0	1
21-30	26	31	13	19	7	11	6	1
31-40	21	38	7	22	11	12	3	4
41-50	13	33	5	19	7	6	1	8
51-60	36	42	14	13	12	19	10	10
61-70	4	20	3	12	1	5	0	3
Above 70	3	13	1	7	2	3	0	3
Total	107	184	47	96	40	58	20	30

Table A4: Vitamin D deficiency and its severity in males and females in Abbotabad (ABD)

NB: M = Male; F = Female

		ABD %						
	Defie	cient	Insuf	ficient	Nor	mal		
Age (yrs)	<20 r	ng/ml	21-30	ng/ml	>30 ı	>30 ng/ml		
	М	F	М	F	М	F		
<20	100.0	57.1	0.0	28.6	0.0	14.3		
21-30	50.0	61.3	26.9	35.5	23.1	3.2		
31-40	33.3	57.9	52.4	31.6	14.3	10.5		
41-50	38.5	57.6	53.8	18.2	7.7	24.2		
51-60	38.9	31.0	33.3	45.2	27.8	23.8		
61-70	75.0	60.0	25.0	25.0	0.0	15.0		
Above 70	33.3	53.8	66.7	23.1	0.0	23.1		
Total	43.9	52.2	37.4	31.5	18.7	16.3		

NB: M = Male; F = Female

Table A5: Vitamin D deficiency and its severity in males and females in Peshawar (PWR)

				PWR				
Age (yrs)	Total		Deficient <20 ng/ml		Insufficient 20-30 ng/ml		Normal >30 ng/ml	
<20	13	24	8	16	5	8	0	0
21-30	48	153	21	69	19	60	8	24
31-40	52	198	12	47	24	93	16	58
41-50	42	192	18	41	14	102	10	49
51-60	42	74	12	25	15	32	15	17
61-70	12	56	6	9	6	34	0	13
Above 70	27	55	5	25	18	23	4	7
Total	236	752	82	232	101	352	53	168
NB: M = Male;	F = Female							

15

		PWR %				
Age (yrs)	Defi	cient	Insuff	ficient	Normal	
	<20 r	ng/ml	21-30	ng/ml	>30 ng/ml	
	М	F	М	F	М	F
<20	61.5	66.7	38.5	33.3	0.0	0.0
21-30	43.8	45.1	39.6	39.2	16.7	15.7
31-40	23.1	23.7	46.2	47.0	30.8	29.3
41-50	42.9	21.4	33.3	53.1	23.8	25.5
51-60	28.6	33.8	35.7	43.2	35.7	23.0
61-70	50.0	16.1	50.0	60.7	0.0	23.2
Above 70	18.5	45.5	66.7	41.8	14.8	12.7
Total NB: M = Male;	34.7 F = Female	30.9	42.8	46.8	22.5	22.3