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VITAMIN D DEFICIENCY: LESSON FOR PAKISTAN, A META-ANALYSIS

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Abstract
This study highlights the importance of food fortification to reduce the vitamin D deficiency. A systematic review of the published literature on Pakistan reveals that vitamin D deficiency is a major problem in Pakistan, and situation is much worse when it comes to women. Around 70% of the population is either vitamin D deficient or near the borderline value. Despite having good sunny days, Pakistan is one of the vitamin D deficient countries, which is primarily the result of poor nutrition. Food fortification techniques along with some oral vitamin doses can be a possible way to bring the country out of deficiency. This study points to the need of food fortification on a larger scale, to cope with the problems arising from vitamin D deficiency.

Keywords: Vitamin D deficiency, food fortification, meta-analysis

Introduction
Vitamin D deficiency is associated with insufficient exposure to sunlight, certain diseases and aging (Bouillon and carmeliat., 2018, Holick 2017). Vitamin D deficiency is also related with certain non-skeletal diseases such as chronic kidney diseases, cardiovascular diseases, various types of cancer, depression and diabetes. Low concentration of vitamin D in the human body can accommodate bone metabolism and interfere with calcium absorption, lead to increasing the risk of osteoporotic fractures, rickets and osteomalacia. (Jiang *et al*, 2021).
Despite a country having a good number of sunny days, Pakistan is also a victim of Vitamin D3 deficiency. According to data of a nutritional survey, it is estimated that pregnant females are highly deficient of vitamin D3 of around 70 percent. Children are also prone to vitamin D3 deficiency up to an alarming level of 40 percent (Javed *et al*; 2018).

The major reason behind this deficiency is that the daily food consumption of most of the population is not sufficient in vitamin D. Many countries like the UK and Canada have covered the deficiency level of their population through various fortification methods.

Vitamin D is a critical element of the human body. It is a steroid playing a critical role in calcium absorption in intestine. Additionally, it not only helps in maintaining glucose levels in the blood but also regulates the level of different body hormones and cellular growth. A sufficient amount of vitamin D helps in preventing different diseases like cancer, diabetes, psychological disorders, and cardiovascular diseases (Deluca, 2004).

Owing to the evolution of the literature in this area, we can find many studies related to this topic in the context of Pakistan as well. However, the results are scattered, or the impacts vary due to certain reasons, like sampling difference, technique difference, time of the study, region of study, and the main design of different data collection processes. There are studies on the developing countries including Pakistan, studying the impact of vitamin D deficiency on physical and mental health. This study suggests combining the results of these studies and explaining the level of vitamin D deficiency in Pakistan.

This study is designed to do a detailed survey of the published literature to provide a comprehensive review of issues related to vitamin D deficiency. Present study analyzed: the variety of physical and mental issues related to the deficiency of this nutrient. Finally, food fortification techniques for vitamin D will be analyzed by putting them in the context of Pakistan. This study identifies the possible and effective fortification techniques of vitamin D.

Literature Review

Cashman *et al*., in 2017 carried out a systematic literature review of all the data available on vitamin D status the prevalence of vitamin D deficiency in 83 low middle income countries (LMIC's). Vitamin D deficiency was determined as serum 25-hydroxyvitamin D <25/30 nmol/L within > 20% of the entire population or population subgroups at risk i.e., infants, children, women of childbearing age, pregnancy. The systematic literature review revealed that out of 83 low-income countries 54 countries were lacking in published studies with vitamin D data that was suitable for inclusion. However according to the data of the remaining countries they had evidence of excess burden of vitamin D deficiency in one or more population subgroups while some of the low middle income countries (LMIC's) had no evidence of excess burden. The study concluded that Vitamin D deficiency was a major public health issue but not in all the low middle income countries and that strategies should be used to prevent vitamin D deficiency in countries having an excess burden of vitamin D deficiency.

Research was conducted by Tariq *et al*., (2020) among students from two universities regarding vitamin D sources. Result showed that 9% participants were able to recognize the correct food sources of vitamin D, and 36% analyzed sunlight exposure as a factor affecting vitamin D Production, and 33% were conscious about the bone health benefits. 52% female and 40% male students were determined vitamin D deficient. Study concluded that information about vitamin D were limited among university students.

A study was reported by Siddique*et al*.(2021) to access the Prevalence of vitamin D deficiency in children presenting with supracondylar fractures of humerus. The most prevalent age was 18 months-18 years. Out of 227 children contributed with upper limb fracture of which 72 (31.7%) were supracondylar. Among these patients, 40 were females and 32 were males. Low vitamin D level was found to be 34.7%(n=25), the mean vitamin D level will be 32.5 $\pm$9.1 ng/ml, ideal in 5.5% and adequate in 59.7%(n=43). They concluded that out of 227 children the mean vitamin D level for toddlers was 33.5ng/ml, preschool children were 27.66ng/ml, school-age children were 30.4ng/ml and adolescents were 37ng/ml. This study showed that prevalence of vitamin D deficiency in 35% of children with supracondylar fractures, with the lowest mean values in the preschool age groups. After analysis they concluded that vitamin D supplementation can shut out a significant number of fractures in all parts of the globe.

Another study was designed by Siddiqee*et al*., (2021) to determine the high prevalence of vitamin D

deficiency among the South Asian adults. A meta-analysis of the study showed that the highest prevalence of vitamin D deficiency was found in Pakistan 73%, while the Sri Lanka had the lowest percentage 48% of prevalence, as follows Bangladesh 67%, India 67%, and Nepal 57% respectively. From the study they found out that in South Asia, the gender wise analysis suggested that the prevalence of vitamin D deficiency was higher in females than males. They concluded that in 7/10 South Asian adults are suffering with vitamin D deficiency.

A study was conducted by Shamsi *et al*., at Aga khan university Hospital and Karachi Institute of Radiation and Nuclear Medicine Hospital. They diagnosed 57% women out of 784 were vitamin D deficient. Pre- menopausal women were highly vitamin D deficient 64.7% as compared to post-menopausal women 49%. The median serum concentrations of 25-hydroxyvitamin D were 16.7ng/ml. From the result it was found that the prevalence rate was high in younger women belonging to lower socioeconomic status.

Pearce and Cheetham (2010) conductedresearch to determine the vitamin D status in the UK population. This observational study showed that 50% of the adults have inadequate vitamin D level and 16% have severe vitamin D deficiency especially in winter and spring season. According to the findings the most common cause of vitamin D deficiency include obesity, protective clothing, use of antiretroviral drugs and rifampicin, sun blocker, colorant skin, short bowel, malabsorption and people with liver or renal disease are at high risk. The study suggested that the use of vitamin D supplements for 8-12 months (colicalciferol or ergocalciferol) helps to treat Rickets and osteomalacia.

Research was reported by Alam *et al*.,(2020) to determine the Vitamin D levels in diabetic patients with and without painful diabetic neuropathy. In their study they found out that both positive and negative symptoms of diabetic neuropathy were greater in painful diabetic neuropathy PDN as compared with diabetic painless neuropathy DPN respectively. Similarly, they also found that Serum 25(OH)D levels were greater in DPN (34.6$\pm$15.0ng/ml) as compared with PDN (24.0$\pm$14.1ng/ml). whereas the odd ratio of painful diabetic neuropathy was 4.4(P=.03) for vitamin D deficiency (<20ng/ml) and 9.8 (P=.03) for vitamin D insufficiency (<30ng/ml). overall study revealed that vitamin D deficiency and insufficiency are linked with painful diabetic neuropathy.

Another study was designed to check the effect of vitamin D Replacement on Basal Cell carcinoma occurrence and Recurrence Rates in Patients with Vitamin D deficiency. A total of 496 patients were included in the study. The most prevalent age was 69 years. They conclude that in the 1st,2nd and 3rd stage skin lesions in the head and neck were 88%, 79% and 97.4%, The mean Serum 25 (OH) D vitamin D3 level who were diagnosed with BCC was 12.2 ng/ml, 10.1 ng/ml, and 11.8ng/ml respectively. They determine that during summer 92% of the patients had insufficient Sunbathed 5-10 minutes/ day. The result concluded that to sustain 25(OH) vitamin D3 Level greater than 25ng/ml in patients with early diagnosis of Basal Cell Carcinoma can substantially reduce the recurrence rate after Basal Cell Carcinoma (Ince *et al*.,2019).

A study was conducted by Parameswaran *et al*. (2020) at the tertiary care hospital in North-India to check the prevalence of vitamin D deficiency in children with intrathoracic Tuberculosis in Indian children. The most prevalent age was <12 year. They diagnosed that children (28/40) were vitamin D deficient; vitamin D sufficiency and insufficiency were 12.5% and 17.5% respectively. This observational study also demonstrates that vitamin D deficiency in Primary Pulmonary Complex (PPC) cases were 33.3%, Progressive Primary Disease (PPD) cases were 74.3% and in pleural Effusion (PE) 50% cases was noted. After analysis they concluded that there was no relation between vitamin D deficiency and type of intrathoracic TB, but the prevalence of vitamin D deficiency was high in children with intrathoracic Tuberculosis.

Another study was reported to determine the Correlation between maternal and neonatal blood vitamin D level in Pakistan. The samples include 213 pregnant women. They measured height, weight, length and blood samples were collected from both mother and their neonatal for 25(OH)D level. The mean maternal age of the participants was 27.8 (4.1) years, and the mean gestational age was 38(1.7) weeks. They analyzed that 16% mothers have less than 10 minutes sun exposure and 84% had daily sun exposure was more than 10 minutes. The prevalence of 25-hydroxyvitamin D deficiency in mothers was 61.5% and their newborn infants was 99.5%. The result show that there

was a high prevalence of vitamin D deficiency in pregnant mothers and their newborns.(Robbani *et al*.,2021).

Inamo*et al*., (2011) performed a study on Serum vitamin D concentrations and associated severity of acute lower respiratory tract infections in Japanese hospitalized children. Out of 28 children's, 2 were diagnosed as having pneumonia and 26 were having bronchitis. 7 breast fed children with Acute Lower Respiratory Tract Infections (ALRI) showed plasma 25(OH)D level was <37.5 nmol/l, 6 bottle fed children with ALRI showed 28.9± 6.9 nmol/l, and 15 children who were on a regular diet the mean 25(OH)D level was 24.6±8.8 nmol/l. After analysis they concluded that there was significant correlation between low vitamin D status (<15ng/l) and the need for supplementary oxygen and ventilator management.

A study was conducted to determine the effects of vitamin D supplementation on blood vitamin D level, union time pain relief and functional outcome in patients with osteoporotic vertebral compression fracture and vitamin D deficiency. A sample size of 130 patients were taken, in which (36 males and 94 were females) and analyzed using a questionnaire to assess the quality of life and radiographs were taken to evaluate fractures unit. Out of 130 patients 47 were taken in the supplementation group and 83 were taken in the non-supplementation group (control group). Age between the two groups is significantly same (p=0.458).In their study they found out that severity of low back pain were statistically same between the two groups (p=0.08), time by group interaction between the supplementation and non-supplementation group (p=0.194), no consequential differences were found in functional outcomes, initial severity of low back pain, and quality of life between the deficient group and insufficient group (all p value were >0.05). The result concluded that fracture union was accomplish in all patients of serum vitamin D level and there were great improvements in functional outcomes, severity of low back pain and patients with osteoporotic vertebral compression fracture quality of life would be improved over 12 months (Ko*et al*., 2021).

Another study was reported by Nath *et al*. (2019) to check the prevalence and clinical outcomes of vitamin D deficiency in myeloma patients in tropical Australia. Out of 41 patients 27% were vitamin D deficient. The angle of sunlight reaching to earth and the seasonal changes should be measured. vitamin D deficient patients have higher peripheral neuropathy 73% compared with non-vitamin D counterparts 33% (P=0.03). lower 25-hydroxyvitamin D was associated with poor performance status p=0.003.

A study was conducted by Kay Khaei *et al*. (2011) to find out the prevalence of vitamin D in a sunny city Zahedan, Iran. 993 subjects were included in the study and their calcium, phosphorus, parathyroid hormone, 25-hydroxy vitamin D levels were measured. Final assessment of the results showed that prevalence of vitamin D was more prevalent among women and younger age group

Maryam *et al*. (2021) carried out a cross-sectional study in Gilgit Baltistan, which included 575 participants. The subjects were assessed through a questionnaire to determine the knowledge, attitude, practices and medical conditions related to vitamin D deficiency. During the study they found that out of 575 participants 306(53.2%) were known to have symptoms of vitamin D deficiency with bone weakness, tiredness and fatigue, the vitamin D knowledge of participants was 64.8%, large association between knowledge and attitude were found (p=o.oo1) and minimal relationship were determined between knowledge and practices (p=0.1). After analysis they concluded that adequate micronutrient knowledge, attitude and practices are related with better education. This study prescribed that awareness campaign on micronutrients deficiency start in both rural and urban regions of Pakistan, concentrating on destitute financial settings.

Pettifor and John (2014) analyzed that vitamin D deficiency has been clearly established as the cause, coming about from social traditions which limit skin exposure to day light and UV radiation of both the mother and her breastfed newborn child, and of adolescent girls. They stated that rickets remain an open wellbeing issue among newborn children and young people in many developing countries.From the result it was found that vitamin D deficiency and low dietary calcium intake act synergistically in increasing the predominance of rickets in communities where both issues are present.

A study was reported by Hovsepian *et al*., (2011) in order to find out the prevalence of vitamin D

deficiency among the adult population of Isfahan, Iran. A total of 1111 subjects were included in the study and their levels of 25-hydroxy vitamin D, parathyroid hormone, calcium, and phosphorus were measured. Mild, moderate, and severe deficiency of vitamin D were defined as value of 30-20ng/ml, 20-10ng/ml, and <10ng/ml. From the result it was found that prevalence of Vitamin D deficiency was more common in autumn and winter as compared to spring and summer. Similarly, the deficiency of Vitamin D was more prevalent among women and younger age group in Isfahan.

Another study was designed to determine the dietary calcium intake, bone health, and vitamin D status in post-menopausal women in Nahaqi, rural Pakistan. A total of 140 postmenopausal women were included in the study. The subjects were assessed using a 24-hour dietary recall questionnaire, Quantitative Ultrasound Index (QUI) and 25(OH)D was scaled in fasting blood samples. After analysis they found that 42% and 29% of the women demonstrated of osteopenia and osteoporosis, mean calcium intake were 346mg/dL. They concluded that low calcium intake and vitamin D deficiency are the key variables contributing to destitute bone health in this population (Lowe *et al.*, 2011).

Kozgar *et al*.,(2020) evaluated Serum 25(OH)D and calcium concentration in 600 samples that were collected through venous test to begin with 24 hours after birth over a period of 12 months. This study shows there were high insufficiency of vitamin D (27.6%) and deficiency (21.3%). from higher risk mother's group.

Al Zarooni *et al*., (2019) performed a study to explore the prevalence of vitamin D deficiency and its associated comorbidities among the people of Abu Dhabi living in urban and sub urban areas. The study sample consisted of 12,346 participants out of which 36.9% were male and 63.1% were female. The study revealed that deficiency of Vitamin D was common among both males and females and that low levels of vitamin D were positively associated with central obesity, high body mass index, high blood pressure, high cholesterol level, imbalanced levels of glucose, and high Framingham risk score.

A meta-analysis was designed to check the prevalence of vitamin D deficiency in Asia in relation with Age, Gender, Region, Altitude and Disease. A total of 746,564 participants were included with 472 studies. The prevalence of vitamin D in males was higher 51.62nmol/L than that of females 45.58nol/L. While the serum level of 25(OH)D in infants were 37.17nmol/L, children (50.46nmol/L), adults (49.97nmol/L) and pregnant women were 43.20nmol/L. They found out that people in central Asia had the lowest level of 25- hydroxyvitamin D (45.03nmol/L) as compared with southeast Asia had the highest level of 25- hydroxyvitaminD (60.71). They demonstrated a link between 25(OH)D levels and diseases and revealed that many diseases were associated with vitamin D deficiency. From the result they found that vitamin D deficiency was common in Asia (Jiang*et al.,* 2021).

A research was conducted by Ebrahimzadeh *et al*.,(2021) in order to find the association between the vitamin D deficiency and its higher risk to covid-19 susceptibility .A total of 13 observational studies were collected .out of 9 studies show positive relationship between vitamin D deficiency and its mortality in hospital patients, and 5 studies show inverse relationship between vitamin D deficiency and its association between susceptibility to covid-19 patients mortality in hospital

Methodology

Extracting a single conclusion about a common point of study but different narratives is a much difficult and confusing job. In such a case, meta-analysis helps in a way to summarize many published results through an objective methodology. Given this strength of meta-analysis, this study is utilized the methodology to present a systematic review on vitamin D deficiency and its implication.

For this study, Meta-analysis is based on the search method from google scholar search based on the words "vitamin D", "vitamin D deficiency", "lack of vitamin D", "nutritional deficiency", "vitamin D supplements", "vitamin D status", and "vitamin D fortified food". Initially, this study analyzed all the studies containing these words in the title or abstract from google scholar. As a second step, this study extracted similar studies from the references of the published articles to increase the pool of our studies and not to leave out relevant studies.

Finally, after creating a comprehensive data set of studies exploring different effects of vitamin D,

weutilized the Hunter-Schmidt Meta-analysis Programs (Hunter and Schmidt, 2004) to find results. This methodology was conducted in three stages. First, the study corrects for sample error in the first stage of analysis. Second, additional corrections measures are taken by restricting the ranges of the results in a repetitive analysis. Third, if there are remaining variances after the first two error correction stages, a moderator analysis based on study characteristics is conducted to avoid any potential sample or other characteristics bias. Statistical software STATA was used for the final analysis and extraction of results.

identified multiple studies on the vitamin D level in Pakistan and restricted sample of studies to around 16 based on the availability of quantitative results, and calculation of ranges of the vitamin D level in the sampled population. Details of the studies are given in Table 1.

After determining that the level of vitamin D is low in Pakistan, this study aimed to find what can be the best fortified practice in context of Pakistan. Unfortunately, there is no strong study on Pakistani based data to show the impact of fortified food on vitamin D level in Pakistan. Therefore, this study utilized the published research on different international countries, to pen down the standard food fortification practices and its beneficial impacts. Present study mostlyrelied on the studies published with a large samplesize and conducted in a control environment. Special focus was on the quantitative findings of the study, where we can find that how the specific food fortification was effective in altering the vitamin D level in the blood of the target sample. For details see Table

Table 1: Selected Studies on Vitamin D level in Pakistan

	Sample size (N)	Average level of vitamin D (ng /mL)	Standard deviation (S.D)
Mubashir *et al*., (2017)	345	16.1	11.7
Roomi *et al*., (2015)	88	8.44	0.49
Junaid *et al*., (2015)	215	16.6	13.8
Mehboob *et al.*, (2015)	858	20.63	7.25
Mansoor *et al*. (2010)	123	16.44	3.78
Mustafa *et al.*, (2018)	67	8.35	3.076
Rehman *et al.*, (2018)	313	30.42	5.83
Khan *et al*., (2019)	167	12.1	8.81
Nadeem *et al.*, (2018)	221	9.8	8.7
Iqbal *et al.*, (2019)	226	15	10.7
Mahmood *et al.*, (2009)	244	15.65	9.91
Afsar *et al.*, (2014)	376	22.11	8.56
Kumar*et al.*, (2016)	160	32	6.6
Sharif *et al.*, (2013)	60	11.04	8.44
Khan *et al.*, (2012)	305	8.708	8.664
Dar *et al.*, (2012)	174	15.32	6.16

Table 2: Studies on Vitamin D fortification (Systematic Review)			
Studies	**Country**	**Focus of investigations**	**Results**
Hirvonen *et al.,* (2007)	Finland	Analyze the impact of fortified food in Finland	Fortified food like milk, yogurt, margarine, fruit juices, bread, cheese, dessert, jam, sweets and others are proved to be efficient and safe.
Brown *et al.,* (2013)	Germany	Estimating the impact of sunlight exposure, and fortified food on the level of 25(OH)D serum concentrations in Germany	Fortified bread is found to be a good source of vitamin D. Ingestion of 11.3 µg vitamin D per 100 g of bread raises the level of serum to 75 nmol/L
Allen *et al.,*(2015)	United Kingdom	Impact of fortified food and vitamin D concentration in UK population.	Fortified milk or wheat flour were found to be effective. Intake of 10 µg vitamin D raises the vitamin D level from 20 to 27 nmol/L.
Grønborg *et al.,* (2019)	Denmark	Vitamin D fortification and impact on Danish women aged 18–50 years.	Safe levels of vitamin D intake (<100 µg/day) were observed with the four fortified foods (vitamin D contribution of 20 µg/day) and 40 µg/day of a vitamin- D supplement
Moyersoen *et al.,* (2019)	Belgium	Analyzing the impact of vitamin D fortified milk and bread	Milk, milk beverage and bread were found to be beneficial for vitamin D level within an optimal level of intake.
McCourt*et al.,* (2020)	Ireland	Focused on elderly Irish population aged more than 50 years.	Fortified milk or bread increase the level of vitamin D in a range of 30 to 50 percent of the original level. A combination of both raises the level by around 70 percent.
Mostafai *et al.*, (2018)	Iran	Impact of Vitamin D intake in the fortified yogurt (25 µg) vitamin D	Increase the 25(OH)D level in a range of 30 to 40 nmol/L
Bonjour *et al.,* (2015)	United Kingdom	Impact of fortified yogurt in Aged white women over age of 60 years	Intake of 10 g of vitamin D3 increase the level of serum by 6.3 nmol/L.
Piirainen *et al.,* (2007)	Finland	Fortified milk and margarines impact in 4 years old Children	A significant increase of 25(OH)D serum was observed because of fortified food. The increase was in a range of 8.7-11.7 nmol/L
Tangpricha *et al.,* (2003)	United States	Fortification of milk with 1000 IU of vitamin D3 and its impact on 25(OH)D level.	Around 45% increase in the serum level. The serum concentration increased by 23 nmol/L on average with the consumption of fortified milk.
Nikooyeh *et al.,* (2016)	Iran	Fortified bread impact on the serum level	9.2 nmol/L on average increase in the 25(OH)D serum concentration because of fortified bread.
Jaaskelainen*et al.,* (2017)	Finland	Impact of daily consumption of fortified fluid milk on the serum concentration.	An increase of 6 nmol/L was observed.

Results and Discussion tamin D level in Pakistan

Present study selected a total of 16 studies for this analysis to determine the level of vitamin D in Pakistan (Table 1 reports these studies and key results). These studies gathered data from different cities, gender, and social groups on their level of Serum concentration of 25(OH)D which is considered as a measure of vitamin D level in human body. According to Institute of Medicine, an individual is considered to be deficient in Vitamin D if the level of 25(OH)D is less than 20 nmol/L. Most of these studies shows that on average the Pakistani population is facing the problem of vitamin D deficiency. The deficiency is particularly higher in females, as most of these studies conducted for female population. The only study for male is Rahman *et al.*, (2018), which shows that the vitamin D level in males' participants is high. In another study by Ali *et al.*, (2021) provided the results showed that female participants of their sample were vitamin D deficient having low level compared to the male participants.

In this meta-analysis sample, 16 studies with 3942 participants were analyzed. The weighted average of vitamin D was around 18 percent with a S.D of around 7.6 %. This meta-analysis results showed that the weighted mean deficiency with around 70 percent of the whole sample selected. This can be explained that 7 out of 10 people were facing the deficiency. These results are in line with the

literature where most of the studies reported the deficiency prevails in around 50-75 percent of the total sample, where the proportion was higher when the studies were particularly conducted for females.

Effectiveness of Food fortification

After a systematic review of literature provided above, Meta-analysis of food fortification techniques was done that are used widely around the world. The main purpose of this analysis was to pen down the effectiveness of the different fortification methods which helped in reducing the vitamin D deficiency among the population.

There are two clinically approved methods to treat for the vitamin D deficiency. One is the direct intake through IOM (Institute of Medicine). While the other is the recently modified food fortification processes. Many studies have been conducted to find the effect of the fortified food items on total intake, the level of 25(OH)D serum concentration in blood and the relative effectiveness of each method. For this study, I have selected 12 studies out of a panel of 60 studies, based on the criteria of randomized trial, and measured change in some quantitative measure. The overall conclusion is that any type of food fortification is more or less effective compared to other methods of vitamin D. The daily sunlight is not the sole or sufficient source of vitamin D, and the population of vitamin D deficient countries need to have fortified food. Of all the fortified foods, milk and bread are two widely used food and are most effective when use together. McCourt *et al*. (2020) analyzed the impact of fortified milk and bread and find that both have an enhancing impact on serum 25(OH)D around 30-50 percent. And when used together, they boost the level by around 70 percent.

Likewise, fortified food in other products like yogurts, orange juice, eggs and margarine are also found to be effective. Mostafai *et al*., (2018) found that impact fortified yogurt increases the level of vitamin D in the range of 30 to 40 nmol/L. Hirvonen *et al*., (2007) find that other fortified food like cultured milk, cheese, milk dessert, ice cream, ready-to-eat breakfast cereals, jam, sweets, soft drinks, biscuits, mineral water, salad dressing, and snacks are also effective in raising the level of vitamin D in the blood.

Analyzing the studies reported in Table 2, it was revealed that milk and bread are two of the most widely used fortified products around the world. It increases the level of vitamin D in the blood by around 30 to 50 percent, and when used together by around 70 percent. From meta-analysis we know that average level of a vitamin D deficient Pakistani is around 18 nmol/L which is 2 nmol less than the minimum level of vitamin D. So, even a minimal effort of fortifying the wheat or milk, can bring significant improvement.Khan *et al* (2022) and many other studies show that fortification of wheat seed can be a revolutionary change in the intake of vitamin-D through daily food. Likewise, milk and other items fortification can be coupled with bread/wheat, to bring the population out of the deficiency as well as low level of vitamin D, into a stable range of above 30 nmol/L.

Figure 01 represents Vitamin D deficiency status in Pakistan across the existing literature. The vertical axis measures Vitamin D level while the horizontal axis shows different studies conducted to measure / analyze the vitamin D deficiency in Pakistan. All the points on the blue line represents the level of Vitamin D in ng/ml being calculated in the study or used from some external source against different literature. The red line shows the threshold level of vitamin D deficiency. As can be seen clearly in the graph the threshold level of vitamin D Deficiency is set at 20 ng/ml which states that the level of vitamin D below the threshold level is declared as Vitamin D deficiency while the level of Vitamin D above the threshold level of 20 ng/ml is considered as vitamin d sufficiency. As can be seen clearly from the graph that almost all the studies, except the two studies i.e., Afsar et all (2009) and Mehboob et al (2015), indicates a deficient level of Vitamin D in Pakistan. Even in these two studies, indicating Vitamin D sufficiency, the level of vitamin D is not significantly high and is slightly above the threshold level.

Figure: 01

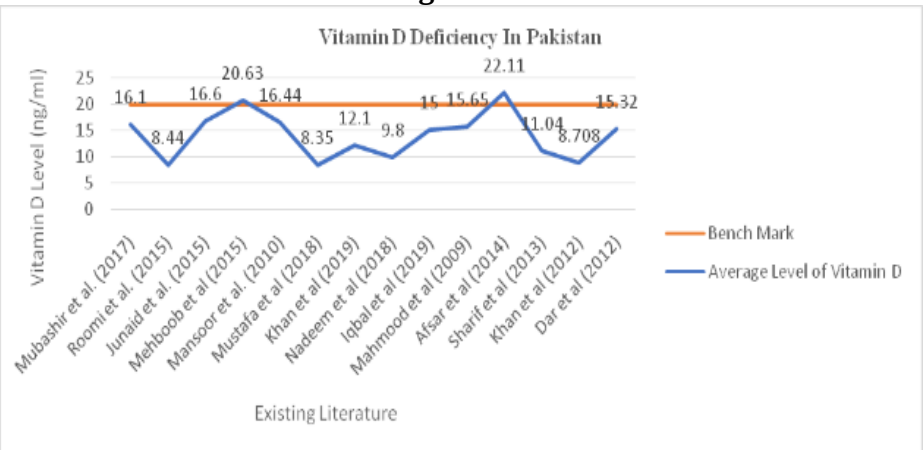

Figure: 02

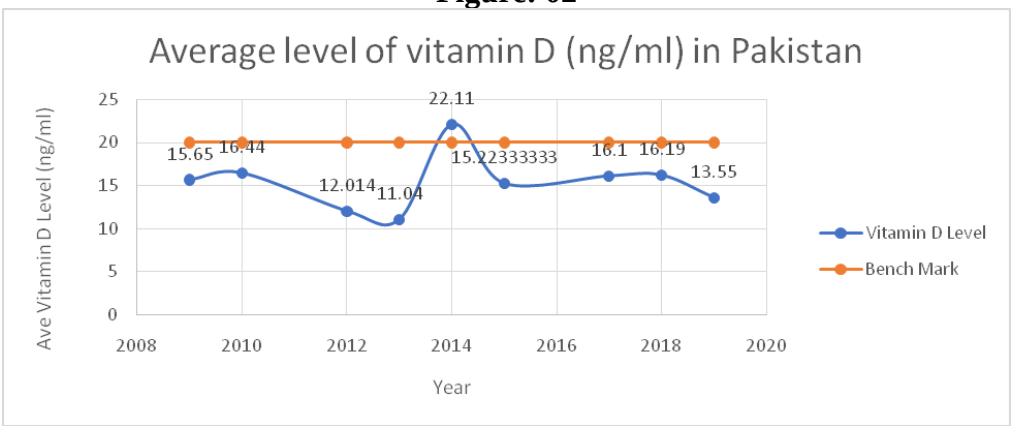

Figure 02 explains the status of Vitamin D deficiency in Pakistan across time. As can be seen in the graph, the vertical axis in the figure above measures the average level of Vitamin D in ng/ml while the horizontal axis represents years in ascending order in which different studies are conducted regarding the Vitamin D deficiency in Pakistan. The blue line is obtained from joining the points of average level of vitamin D in different years, showing the overall status of Vitamin D deficiency across time in Pakistan. Every point on the blue line is an average of Vitamin D level used / calculated in different studies for every year of the study period.The red line is the threshold level of Vitamin D which is set at 20 ng/ml. If the level of Vitamin D is below the threshold level of 20 ng/ml it is considered to be a deficient level of Vitamin D. The level of Vitamin D above the threshold level of 20 ng/ml represents Vitamin D sufficiency. As can be seen in Figure 02 above, the average level of Vitamin D in Pakistan is below the threshold level of 20 ng/ml across all the time period for the study conducted except for the exemption of 2014 where the level of Vitamin D is slightly above the threshold level. It can be seen from the graph above that there is not any significant change in Vitamin D deficiency status in Pakistan in the last decade.

Conclusion and recommendations

This study highlights the key finding in the literature and provides a systematic review. Vitamin D deficiency is related to several health problems like diabetes, osteoporosis and heart-related issues. In addition, some studies also highlighted the mental issues related to the deficiency of this nutrient. This study tried to analyze the results of studies related to Pakistan in a systematic way to calculate a weighted average representation of all studies after controlling for variations in samples and studies. The analysis reveals that vitamin D deficiency is a major problem in Pakistan, with around 70 percent of the population facing the deficiency. The level of an average deficient person is around 18 nmol/L again a minimum threshold of 20nmol/L. This shows that despite having plenty of sunny days,

Pakistanis have less intake of vitamin D. Therefore, there is a need to increase the vitamin D intake through some other means like direct intake. A systematic review of the literature on fortified food and its effectiveness shows that different fortified food items are widely used to cope with the situation of vitamin D low levels in the population. Wheat and milk fortification are widely used in addition to other items like cheese, snacks, juices, yogurt, jam, dessert, etc. The fortified bread and milk are found to increase the level of vitamin D by around 30 to 50 percent, and together they can increase the level by up to 70 percent. Other studies also show similar patterns, with a minimum increase of 25(OH)D of 6 nmol/L to a maximum of 23 nmol/L. A report by USDA (2017) quoted that wheat covers more than 70 percent of the calories intake of an average Pakistani. From here, it can be concluded that a minimum effort of effective strategies of only wheat fortification can bring promising results. However, there are more evidence and studies needed on how the wheat or wheat products are effective specifically in the Pakistan sample and across different age and regional group. Therefore, this study is calling for future research on how the international strategies can be effective in Pakistan, and what product fortification can be more effective given the specific characteristics of Pakistan.

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