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FREQUENCY OF SEVERE VITAMIN D DEFECIENCY IN PATIENTS PRESENTED TO BENAZIR BHUTTO HOSPITAL OVER 3 YEARS

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ABSTRACT

Background: Vitamin D deficiency (25-hydroxyvitamin D) affects over one billion people worldwide. Vitamin D deficiency results in progression of osteoporosis as well as other conditions. Previous studies have shown high rates of vitamin D deficiency in Pakistan despite appreciable levels of sunshine. However, none have assessed vitamin D deficiency across all age groups, genders, incomes, and locations to guide future strategies.

Objective: To determine the frequency of vitamin D deficiency in opd patients presenting in a tertiary care hospital in Rawalpindi.

Methodology: The cross-sectional study involving 1075 consecutive patients was conducted at the out-patient department of Benazir Bhutto Hospital Rawalpindi, from January 2020 to December 2022.Patients of all age groups were included in the study, while those having renal or liver failure and using drugs that could potentially cause a decrease in vitamin D3 were excluded. SPSS 25 was used for statistical analyses.

Results:A total of 1075 patients visits Benazir Bhutto Hospital Rawalpindi in the selected time span. Out of those about 1075 patients presented with signs and symptoms of Vitamin D deficiency were referred for the Vitamin D assessment. I collected data of 1075 patients.According to my study out of 1075 patients during my research period ,332 were males and 743 were females with Vitamin D deficiency. This shows that Vitamin D deficiency was more prevelant in the females as compare to males.Percentage of females was 69% and of males was 31%.My research study shows that Vitamin D deficiency was most common in age group of 26 -52 out of 5 groups . 26-52 age group contain 515 patients.After calculating frequency for all five stages of Vitamin D deficiency it was observed that stage 2 was most frequent among all patients diagnosed with Vitamin D deficiency.

Conclusion: The study revealed a high percentage of vitamin D deficient individuals. The frequency of vitamin D deficiency increased considerably with age and most commonly affected age group is 26-52. According to our analysis, there is a high prevalence of VDD, especially among females than males due to less exposed to sunlight and not taking Vitamin D rich supplements or diet. Prevelance of Vitamin D defeciency in females can be lowered by maintaining the normal levels of Vitamin D . There is a need to take immediate measures to challenge this growing public health problem, including food fortification, i.e. nurture, alongside increasing exposure to sunlight, i.e. nature. There is also a need for yearly comprehensive nation-wide analyses on vitamin D levels.

1. INTRODUCTION

1.1 VITAMIN D:

Vitamin D (sunshine vitamin) belongs to the class of secosteroids which are fat-soluble and performs the important function of intestinal absorption of calcium and phosphate(1). The vitamin, first identified in 1921, has two main types namely Vitamin D3 and vitamin D2. These two vitamins are also called cholecalciferol and ergocalciferol respectively. Both these types are collectively termed as calciferol(2).

1.2 Sources of Vitamin D: The major source of vitamin D for most humans is exposure to sunlight(3). As shown in Figure 1, seasonal variation is found in the major circulating form of vitamin D, 25-hydroxyvitamin D [25(OH)D]. Few foods naturally contain vitamin D, including oily fish such as salmon, mackerel, and herring and oils from fish, including cod liver oil. We recently conducted a study and observed that wild-caught salmon had on average 500–1000 IU vitamin D in 100 g (3.5 ounces), whereas farmed salmon contained ≈100–250 IU vitamin D per 100-g serving . The most likely reason is that vitamin D is plentiful in the food chain but is not plentiful in the pelleted diet fed to farmed salmon. In the United States, milk, some juice products, some breads, yogurts, and cheeses are fortified with vitamin D. Multivitamins that contain 400 IU vitamin D and supplements containing vitamin D only are now available in various amounts including 400, 1000, 2000, 4000, 5000 and 50 000 IU vitamin D_3. The pharmaceutical form of vitamin D in the United States is vitamin D_2 and is available as 50 000 IU vitamin D_2 in a capsule or 8000 IU vitamin D_2/mL . In Canada, Europe, Japan, and India, vitamin D_3 is available as a pharmaceutic.

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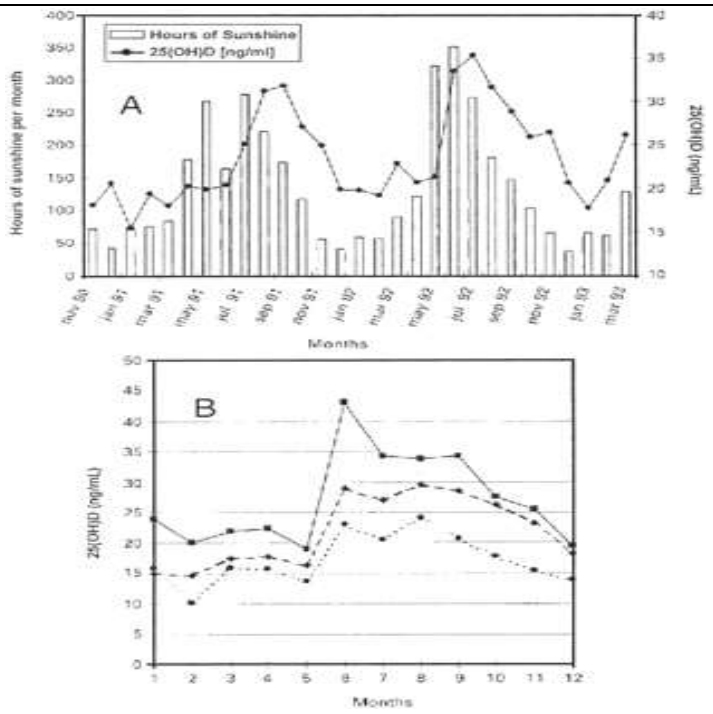

Fig 1 Vitamin D

A: Relation between hours of sunshine and serum 25-hydroxyvitamin D [25(OH)D] concentrations. ▪, hours of sunshine; •, 25(OH)D. **B:** Seasonal fluctuation in serum 25(OH)D according to frequency of sun exposure. ▪, regular sun exposure; ♦, occasional sun exposure; •, avoiding direct sun exposure.(Brot C, Vestergaard P, Kolthoff N, Gram J, Hermann AP, Sørensen OH. 2001 Aug;86(S1):S97-103).

1.3 Snthesis and Metabolism of Vitamin D:

1.3.1 Absorption

Owing to its fat-soluble nature, dietary vitamin D (either D_2 or D_3) is absorbed with other dietary fats in the small intestine(4). The efficient absorption of vitamin D is dependent upon the presence of fat in the lumen, which triggers the release of bile acids and pancreatic lipase (5). In turn, bile acids initiate the emulsification of lipids, pancreatic lipase hydrolyzes the triglycerides into monoglycerides and free fatty acids, and bile acids support the formation of lipid-containing micelles, which diffuse into enterocytes. Early studies demonstrated that radiolabeled vitamin D_3 appeared almost exclusively in the lymphatics and in the chylomicron fraction of plasma; as well, subjects with impaired bile acid release or pancreatic insufficiency both demonstrated significantly reduced absorption of vitamin D(6). Subsequently, other clinical and experimental animal studies confirmed that vitamin D is most efficiently absorbed when consumed with foods containing fat (7).And, conversely, that a weight-loss agent that blocks fat absorption also impairs the absorption of vitamin D(8). The optimal amount of fat required for maximal absorption of vitamin D has not been determined.

Within the intestinal wall, vitamin D, cholesterol, triglycerides, lipoproteins, and other lipids are packaged together into chylomicrons. Importantly, while a fraction of newly absorbed intestinal vitamin D is also transported along with amino acids and carbohydrates into the portal system to reach the liver directly, the main pathway of vitamin D uptake is incorporation into chylomicrons that reach the systemic circulation via the lymphatics. Chylomicron lipids are metabolized in peripheral tissues that express lipoprotein lipase, but particularly in adipose tissue and skeletal muscle, which are rich in this enzyme. During hydrolysis of the chylomicron triglycerides, a fraction of the vitamin D contained in the chylomicron can be taken up by these tissues. Uptake into adipose tissue and skeletal muscle accounts for the rapid postprandial disappearance of vitamin D from plasma and probably also explains why increased adiposity causes sequestering of vitamin D and is associated with lower 25OHD levels(9). What remains of the original chylomicron after lipolysis is a chylomicron remnant, a cholesterol-enriched, triglyceride-depleted particle that still contains a fraction of its vitamin D content.

1.3.2 Synthesis: During exposure to sunlight, 7-dehydrocholesterol (7-DHC) in the skin is converted to previtamin D_3 (preD_3) and then by a heat-dependent process to vitamin D_3 (10). Vitamin D (D represents D_2 or D_3) made in the skin or ingested in the diet is converted by the vitamin D-25-hydroxylase (25-OHase) to 25-hydroxyvitamin D [25(OH)D]. 25(OH)D is converted in the kidneys by the 25-hydroxyvitamin D-1α-hydroxylase (1-OHase) to its

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biologically active form 1,25-dihydroxyvitamin D [1,25(OH)$_2$D]. 1,25(OH)$_2$D increases the expression of the 25-hydroxyvitamin D-24-hydroxylase (24-OHase) to catabolize 1,25(OH)$_2$D and 25(OH)D to the water-soluble biologically inactive calcitroic acid. 1,25(OH)$_2$D enhances intestinal calcium absorption in the small intestine. 1,25(OH)$_2$D is recognized by its receptor in osteoblasts, causing an increase in the expression of receptor activator of NFκB ligand (RANKL). CaBP, calcium binding protein; ECaC, epithelial channel calcium; FGF23, fibroblast growth factor 23; OJ,orange juice; Pi, inorganic phosphate; PTH, parathyroid hormone; UVB, ultraviolet B radiation.

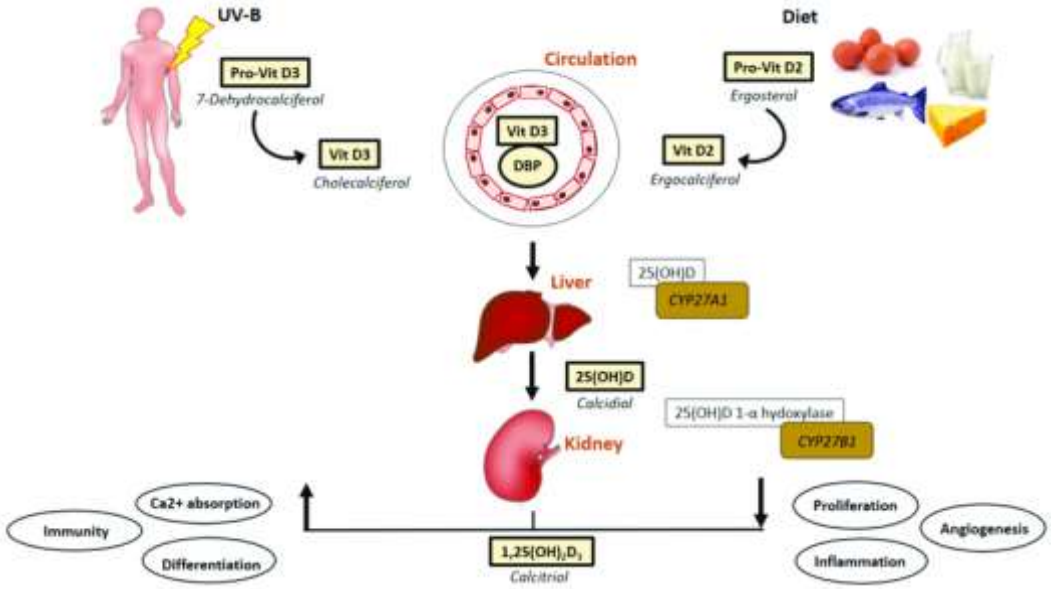

Fig 2 Synthesis of Vitamin D

1.3.3 Storage

Adipose tissue stores of vitamin D probably represent “non-specific” stores sequestered because of the hydrophobic nature of vitamin D, but the extent to which the processes of accumulation or mobilization are regulated by normal physiological mechanisms remains unknown at this time. Rosenstreich SJ first identified adipose tissue as the primary site of vitamin D accumulation from experiments in which radiolabeled vitamin D was administered to vitamin D–deficient rats(11). Tissue levels of radioactivity measured during vitamin D repletion and during a subsequent period of deprivation showed that adipose tissue acquired the greatest quantity of radioactive compound and had the slowest rate of release. Work by Liel suggested that there was enhanced uptake and clearance of vitamin D by adipose tissue in obese subjects compared with those of normal weights(12). Similarly, Wortsman concluded that in obese subjects, vitamin D was stored in adipose tissue and not released when needed (13). Finally,Blum found that, in elderly subjects supplemented with 700 IU of vitamin D per day, for every additional 15 kg of weight above “normal” at baseline, the mean adjusted change in 25OHD level was approximately 10 nmol/L lower after 1 year of supplementation (14). The authors estimated that in order for subjects with body mass indexes (BMIs) above the normal range to obtain an increase in serum 25OHD level similar to that of subjects with weight in the normal range, an additional 17 percent increase in vitamin D above the administered dose of 700 IU/day would be needed for every 10 kg increase in body weight above baseline in their study population.

The implication of these studies is that vitamin D deposited in fat tissue is not readily available, and obese individuals may require larger than usual doses of vitamin D supplements to achieve a serum 25OHD level comparable to that of their normal weight counterparts. In support of the hypothesis that vitamin D is stored in adipose tissues, weight reduction studies show that serum 25OHD levels rise when obese individuals lose body fat. Conclusive statements regarding changes in serum 25OHD levels after gastric bypass surgery cannot be made, as a result of confounding factors, such as weight change, possible malabsorption, and diet. There is evidence of a rise in serum 25OHD levels after surgery, as well as evidence that there is no change after surgery indicated that serum 25OHD levels decrease after gastric bypass surgery, although the quality of the methods used is questionable(15).

1.3.4 Excretion

As described previously, the products of vitamin D metabolism are excreted through the bile into the feces, and very little is eliminated through the urine. This is in part due to renal reuptake of vitamin D metabolites bound to DBP, as mediated by the cubilin–megalin receptor system(16).

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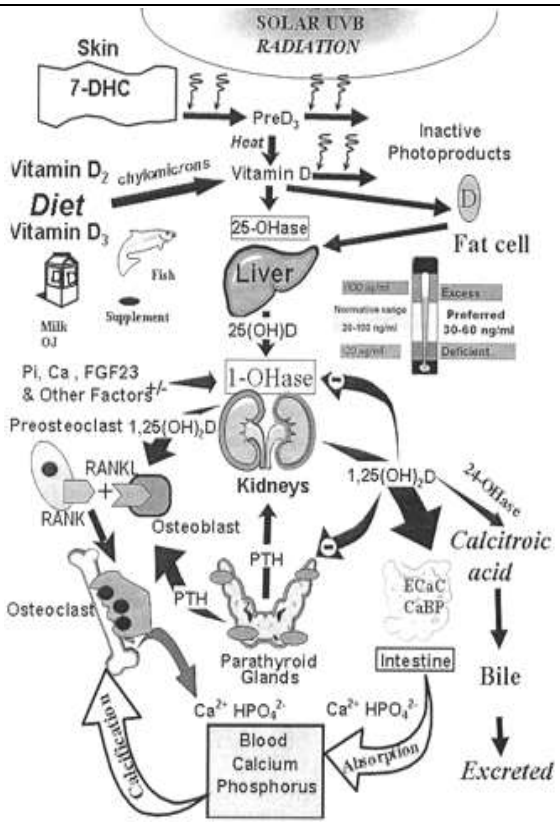

Fig 3 Metabolism of Vitamin D

1.3.5 Excess Intake

Excess intake of vitamin D—but not sun exposure, which is associated with a series of thermal and photoisomerization reactions can lead to a state of vitamin D "intoxication" or "hypervitaminosis D." Chemically synthesized vitamin D became available late in the third decade of the 20th century; reports of vitamin D intoxication were first found from 1928 to 1932 and continued throughout most of the 20th century (17). The condition of hypervitaminosis D leads to hypercalcemia and eventually to soft tissue calcification and resultant renal and cardiovascular damage(18). In the case of animal models, at necropsy, vitamin D–intoxicated rats show widespread calcification of organs and tissues. The form of the vitamin implicated in the intoxication is 25OHD(19). In fact, it has been shown in dietary supplementation studies using the CYP27B1 knockout mouse, which is incapable of making calcitriol, sufficiently high concentrations of serum levels of 25OHD can cause changes in vitamin D–dependent general expression even in the absence of calcitriol (20).

1.4 Vitamin D deficiency, Prevalence: VDD has been historically defined and recently recommended by the Institute of Medicine (IOM) as a 25(OH)D of less than 0.8 IU. Vitamin D insufficiency has been defined as a 25(OH) D of 21–29 ng/mL(21).Children and young- and middle aged adults are at equally high risk for VDD and insufficiency worldwide. VDD is common in Australia, the Middle East, India, Africa, and South America. Pregnant and lactating women who take a prenatal vitamin and a calcium supplement with vitamin D remain at high risk for VDD.

1.5 Vitamin D deficiency, Causes:

The major source of vitamin D for children and adults is exposure to natural sunlight. Thus, the major cause of VDD is inadequate exposure to sunlight(22). Wearing a sunscreen with a sun protection factor of 30 reduces vitamin D synthesis in the skin by more than 95%. People with a naturally dark skin tone have natural sun protection and require at least three to five times longer exposure to make the same amount of vitamin D as a person with a white skin tone. There is an inverse association of serum 25(OH)D and body mass index (BMI) greater than 30 kg/m2 , and thus, obesity is associated with VDD(23). Patients with one of the fat malabsorption syndromes and bariatric patients are often unable to absorb the fat soluble vitamin D, and patients with nephritic syndrome lose 25(OH)D bound to the vitamin D-binding protein in the urine.Patients on a wide variety of medications, including anticonvulsants and medications to treat AIDS/ HIV, are at risk because these drugs enhance the catabolism of 25(OH)D and 1,25(OH)2D. Patients with chronic granuloma-forming disorders (sarcoidosis, tuberculosis, and chronic fungal infections), some lymphomas, and primary hyperparathyroidism who have increased metabolism of 25(OH)D to 1,25(OH)2D are also at high risk for VDD.

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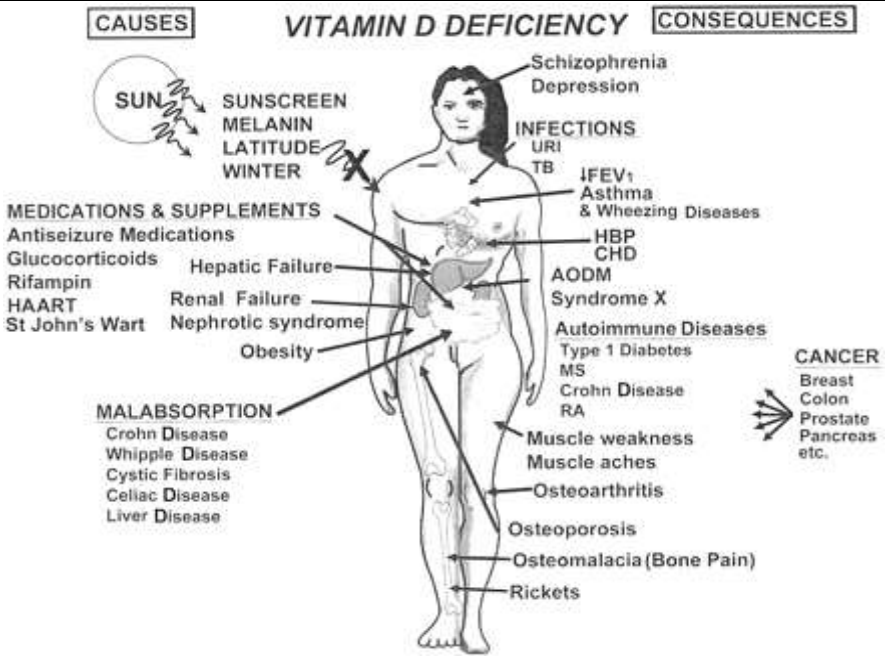

Fig 4 Causes and Consequences of Vit.D defeciency

A schematic representation of the major causes of vitamin D deficiency and potential health consequences. AODM, adult onset diabetes mellitus; CHD, coronary heart disease; FEV_1, forced expiratory volume in 1 s; HAART, highly active antiretroviral therapy; HBP, high blood pressure; MS, multiple sclerosis; RA, rheumatoid arthritis; TB, tuberculosis; URI, urinary tract infection.

1.6 Vitamin D deficiency, Consequences:

VDD results in abnormalities in calcium, phosphorus, and bone metabolism. VDD causes a decrease in the absorption of dietary calcium and phosphorus, resulting in an increase in PTH levels(24).The PTH-mediated increase in osteoclastic activity creates local foci of bone weakness and causes a generalized decrease in bone mineral density (BMD), resulting in osteopenia and osteoporosis. An inadequate calcium–phosphorus product causes a mineralization defect in the skeleton. VDD also causes muscle weakness; affected children have difficulty in standing and walking, whereas the elderly have increasing sway and more frequent falls,] thereby increasing their risk of fracture(25).

1.6.1 Rickets: Rickets, a childhood disease, is characterized by impeded growth and soft, weak, deformed long bones that bend and bow under their weight as children start to walk(26). Rickets typically appears between 3 and 18 months of age. Cases continue to be reported in North American and other Western Countries and is primarily seen in breastfed infants and those with darker skin complexions. This condition is characterized by bow legs, which can be caused by calcium or phosphorus deficiency, as well as a lack of vitamin D; in the 21st century, it is largely found in low-income countries in Africa, Asia, or the Middle East and in those with genetic disorders such as pseudovitamin D deficiency rickets. Maternal vitamin D deficiency may cause overt bone disease from before birth and impairment of bone quality after birth. Nutritional rickets exists in countries with intense year-round sunlight such as Nigeria and can occur without vitamin D deficiency. Although rickets and osteomalacia are now rare in the UK, outbreaks have happened in some immigrant communities in which osteomalacia patients included women with seemingly adequate daylight outdoor exposure wearing Western clothing. Having darker skin and reduced exposure to sunshine did not produce rickets unless the diet deviated from a Western omnivore pattern characterized by high intakes of meat, fish, and eggs. The dietary risk factors for rickets include abstaining from animal foods. Vitamin D deficiency remains the main cause of rickets among young infants in most countries because breast milk is low in vitamin D and social customs and climatic conditions can prevent adequate sun exposure. In sunny countries such as Nigeria, South Africa, and Bangladesh, where rickets occurs among older toddlers and children, it has been attributed to low dietary calcium intakes, which are characteristic of cereal-based diets with limited access to dairy products. Rickets was formerly a major public health problem among the US population; in Denver, where ultraviolet rays are about 20% stronger than at sea level on the same latitude, almost two-thirds of 500 children had mild rickets in the late 1920s. An increase in the proportion of animal protein in the 20th century American diet coupled with increased consumption of milk fortified with relatively small quantities of vitamin D coincided with a dramatic decline in the number of rickets cases. Also, in the United States and Canada, vitamin D-fortified milk, infant vitamin supplements, and vitamin supplements have helped to eradicate the majority of cases of rickets for children with fat malabsorption conditions.

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1.6.2 Osteomalacia is a disease in adults that results from vitamin D deficiency(27). Characteristics of this disease are softening of the bones, leading to bending of the spine, bowing of the legs, proximal muscle weakness, bone fragility, and increased risk for fractures. Osteomalacia reduces calcium absorption and increases calcium loss from bone, which increases the risk for bone fractures. Osteomalacia is usually present when 25-hydroxyvitamin D levels are less than about 10 ng/mL. Although the effects of osteomalacia are thought to contribute to chronic musculoskeletal pain, there is no persuasive evidence of lower vitamin D levels in chronic pain sufferers or that supplementation alleviates chronic nonspecific musculoskeletal pain.

1.6.3 Osteoporosis is a condition of reduced bone mineral density with increased bone fragility and risk of bone fractures(28). Osteoporosis can be a long-term effect of calcium and/or vitamin D insufficiency, at least in part. This may result from inadequate calcium intake, with insufficient vitamin D contributing by reducing calcium absorption.

1.6.4 Cancer: Vitamin D decreases cell proliferation and increases cell differentiation, stops the growth of new blood vessels, and has significant anti-inflammatory effects. Many studies have suggested a link between low vitamin D levels and an increased risk of cancer, with the strongest evidence for colorectal cancer. In the Health Professionals Follow-up Study (HPFS), subjects with high vitamin D concentrations were half as likely to be diagnosed with colon cancer as those with low concentrations. A definitive conclusion cannot yet be made about the association between vitamin D concentration and cancer risk, but results from many studies are promising. There is some evidence linking higher vitamin D intake to a lower risk for breast cancer. The effect of menopausal status on this association is still unclear.

1.6.5 Heart disease: Several studies are providing evidence that the protective effect of vitamin D on the heart could be via the renin–angiotensin hormone system, through the suppression of inflammation, or directly on the cells of the heart and blood-vessel walls. In the Framingham Heart Study, patients with low vitamin D concentrations (30 ng/mL).

1.6.6 Hypertension: The third National Health and Nutrition Examination Survey (NHANES-III), which is representative of the noninstitutionalized US civilian population, showed that systolic blood pressure and pulse pressure were inversely and significantly correlated with 25(OH)D levels among 12,644 participants. Age-associated increase in systolic blood pressure was significantly lower in individuals with vitamin D sufficiency. The prevalence of arterial hypertension was also associated with reduced serum 25(OH)D levels in 4030 participants of the German National Interview and Examination Survey, in 6810 participants of the 1958 British Birth Cohort, and in other study populations. The antihypertensive effects of vitamin D are mediated by renoprotective effects, suppression of the RAAS, by beneficial effects on calcium homeostasis, including the prevention of secondary hyperparathyroidism, and by vasculoprotection.

1.6.7 Obesity: Low concentrations of circulating vitamin D are common with obesity and may represent a potential mechanism explaining the elevated risk of certain cancers and cardiovascular outcomes. Levels of 25(OH)D are inversely associated with BMI, waist circumference, and body fat but are positively associated with age, lean body mass, and vitamin D intake. The prevalence of VDD is higher in black versus white children regardless of season predictors of VDD in children include black race, female sex, pre-pubertal status, and winter/ spring season. Weight loss is associated with an increase in 25(OH)D levels among postmenopausal overweight or obese women.

1.6.8 Type 2 diabetes: A trial of nondiabetic patients aged 65 years and older found that those who received 700 IU of vitamin D (plus calcium) had a smaller rise in fasting plasma glucose over 3 years versus those who received placebo. A correlation between vitamin D and the risk diabetes can be ruled in from the results.

1.6.9 Depression: A Norwegian trial of overweight subjects showed that those receiving a high dose of vitamin D (20,000 or 40,000 IU weekly) had a significant improvement in depressive symptom scale scores after 1 year versus those receiving placebo. The result determines a correlation between vitamin D and the risk of depression.

1.6.10 Cognitive impairment: In the Invecchiare in Chianti (InCHIANTI) Italian population based study, low levels of vitamin D were associated with substantial cognitive decline in the elderly population studied during a 6-year period. Low levels of 25(OH)D may be especially harmful to executive functions, whereas memory and other cognitive domains may be relatively preserved.

1.6.11 Parkinson's disease: Parkinson's disease is a major cause of disability in the elderly population. Unfortunately, risk factors for this disease are relatively unknown. Recently, it has been suggested that chronically inadequate vitamin D intake may play a significant role in the pathogenesis of Parkinson's disease. A cohort study based on the Mini-Finland Health Survey demonstrated that low vitamin D levels may predict the development of Parkinson's disease.

1.6.12 Fractures and falls: Vitamin D is known to help the body absorb calcium, and it plays a role in bone health. In addition, VDRs are located on the fast-twitch muscle fibers, which are the first to respond in a fall. It is theorized that vitamin D may increase muscle strength, thereby preventing falls. Many studies have shown an association between low vitamin D concentrations and an increased risk of fractures and falls in older adults. A combined analysis of 12 fracture-prevention trials found that supplementation with about 800 IU of vitamin D per day reduced hip and nonspinal fractures by about 20%, and that supplementation with about 400 IU per day showed no benefit. Researchers at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University have examined the best trials of vitamin D versus placebo for falls. Their conclusion is that "fall risk reduction begins at 700 IU and increases progressively with higher doses."

1.6.13 Autoimmune diseases: VDD can contribute to autoimmune diseases such as multiple sclerosis (MS), type 1 diabetes, rheumatoid arthritis, and autoimmune thyroid disease. A prospective study of white subjects found that those with the highest vitamin D concentrations had a 62% lower risk of developing MS versus those with the lowest concentrations. A Finnish study that followed children from birth noted that those given vitamin D supplements during infancy had a nearly 90% lower risk of developing type 1 diabetes compared with children who did not receive supplements.

1.6.14 Influenza: VDD in the winter months may be the seasonal stimulus that triggers influenza outbreaks in the winter. In a Japanese randomized, controlled trial, children given a daily vitamin D supplement of 1200 IU had a 40% lower rate of influenza type A compared with those given placebo; there was no significant difference in rates of influenza type B.

1.6.15 Bacterial vaginosis: An analysis of data from the National Health and Nutrition Examination Survey showed that in pregnant women, VDD was associated with nearly a 3-fold increased risk for Bacterial Vaginosis (BV). In non-pregnant women, VDD modulated the association between smoking and BV.

1.7 Groups at risk of vitamin-D inadequacy: Obtaining sufficient vitamin D from natural food sources alone is difficult. Consumption of vitamin D-fortified foods and exposure to some sunlight are essential for maintaining a healthy vitamin D status. Dietary supplements might be required to meet the daily need for vitamin D in some group of people.

1.7.1 Breastfed infants: Vitamin D requirements cannot ordinarily be met by human milk alone, which provides <25 IU/L to 78 IU/L.Vitamin D content of human milk is related to the mother's vitamin D status; therefore mothers who supplement with high doses of vitamin D may have high levels of vitamin D in their milk. American Association of Paediatricians (AAP) recommends that exclusively and partially breastfed infants must be supplemented with 400 IU of vitamin D per day, the recommended daily allowance for this nutrient during infancy.

1.7.2 Older adults: Older adults are at high risk of developing vitamin D insufficiency because of aging. Their skin cannot synthesize vitamin D as efficiently, they are likely to spend more time indoors, and they may have inadequate intakes of the vitamin.

1.7.3 People with limited sun exposure: Homebound individuals, women who wear long robes and head coverings for religious reasons, and people with occupations that limit sun exposure are unlikely to obtain adequate vitamin D from sunlight. The significance of the role that sunscreen may play in reducing vitamin D synthesis is still unclear. Intake of RDA levels of vitamin D from foods and/or supplements will provide adequate amounts of this nutrient to these individuals.

1.7.4 People with dark skin: Larger amounts of the pigment melanin in the epidermal layer result in darker skin and reduce the skin's ability to produce vitamin D from sunlight. It is not sure that lower levels of 25(OH)D for persons with dark skin have significant health consequences. Intake of RDA levels of vitamin D from foods and/or supplements will provide adequate amounts of this nutrient to these individuals.

1.7.5 People with fat malabsorption: Vitamin D is fat soluble, therefore it requires some dietary fat in the gut for absorption. Individuals with reduced ability to absorb dietary fat might require vitamin D supplements.Fat malabsorption is associated with a variety of medical conditions including some forms of liver disease, cystic fibrosis, and Crohn's disease.

1.7.6 People who are obese or who have undergone gastric bypass surgery: A BMI value of ≥30 is associated with lower serum 25(OH)D levels compared with nonobese individuals. Obese people may need larger than usual intakes of vitamin D to achieve 25(OH)D levels comparable to those of normal weight.Greater amounts of subcutaneous fat sequester (captivate) more of the vitamin and alter its release into the circulation. Individuals who have undergone gastric bypass surgery may become vitamin D deficient over time without a sufficient intake of vitamin D from food or supplements; moreover part of the upper small intestine where vitamin D is absorbed is bypassed.

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- Research indicates that VDD has become a global epidemic irrespective of the region's developmental status, health facilities and the amount of UV-B radiation that it receives(29). Currently, about one billion people have been diagnosed with VDD and belong to various races, age groups and geographical regions. However, South Asian, Southeast Asian, Central African and Andean regions are particularly susceptible to suffer from VDD . Current statistics reveal that more than half of the Pakistani population (53.5 per cent) is affected by VDD. In Pakistan, population sub-groups that are most affected by the epidemic include elderly, neonates and pregnant women . Geographical location, however, does not seem to greatly impact vitamin D levels; rather they are governed by socio-cultural conditions. These include the confinement of women, children and elderly within the homes coupled with a nutrient-poor diet . VDD is an emerging issue for Pakistan and its causes need to be identified to determine the best course of action for its eradication. The current paper is focused on the issue of VDD in Pakistan with respect to prevalence, possible causes and measures that can be taken to reduce the issue. The number of publications regarding VDD in Pakistan is steadily rising, and here we have attempted to systematically review the publications as per different measurement parameters and influence of external variables such as age, gender, geography and pregnancy.
- This study aims to determine the frequency of vitamin D deficiency among elderly adults.

Aims And Objectives:

- To measure the serum vitamin D levels in all the subjects visiting tertiary care hospitals.
- To evaluate the epidemiology of Vitamin D deficiency in the sample population.

2. REVIEW OF LITERATURE

The serum vitamin D level consider exogenous intake in diet as well as endogenous production from exposure to sunlight. VDD is presently on the rise in Pakistan; thus, it is an emerging health threat(30). The current study was done to define the frequency of vitamin D in healthy population of Rawalpindi, Pakistan and focused on reviewing 3 years' worth of literature from various publications in Pakistan. Statistics that were reported in the studies were examined for prevalence of VDD and insufficiency, possible causes and several predisposing factors that lead to the condition(31). In this review, external variables such as age, gender, year, geography and pregnancy were also considered when analysing the results. The data obtained from each publication were subject to a qualitative meta-analysis that gave a bigger picture of the current VDD epidemic surrounding the country. We enrolled a total of 1075 cases and majority were living in urban areas. An alarmingly very high prevalence of vitamin deficiency in females than men was found in this study. In women, on an average, 69 percent were vitamin D-deficient respectively. Whereas, in men, the average percentages amounted to 31 per cent being vitamin D-deficient. The reason for women having a higher prevalence of VDD may be because of the lifestyle and cultural norms that limit their exposure to sun, such as spending most of the time indoors and using purdah (veil) when going outside, which covers most of the body(Okonofua et al., 1986; Sachan et al., 2005). Childbirth and breastfeeding may also be contributing factors for higher prevalence of VDD in women, as a pre-existing VDD can further aggravate because of a pregnancy.

A previous local report on the prevalence of vitamin D levels showed deficiency in (41.1%) children(32). Another study on healthy Pakistani population found a high prevalence (76%) of vitamin D deficiency. Some previous studies conducted on general population show high level of deficiency of vitamin D. These reports on vitamin D levels are comparable to the current study findings. In South Asia the prevalence of vitamin D deficiency is quite high. A similar study from India reported 90% prevalence. In another study in North India, 96% of neonates, 91% of healthy school girls, 78% of healthy hospital staff and 84% of pregnant women were found to have vitamin D deficiency(33). Studies carried across different countries in South and South East Asia showed a similar trend. In a study from Afghanistan, 73% of 107 preschool children randomly sampled in winter had low 25-hydroxyvitamin D levels(34). A trial in Bangladesh noted that serum 25-hydroxyvitamin D levels were low in 78% of 36 university students and in 83% of 30 veiled women(35). It has been observed that South Asian region has UVB radiation levels that are sufficient for vitamin D synthesis for almost whole year(36), However, low serum25-hydroxyvitamin D levels have been reported in more than 50% of the infants, children and women studied. Many previous studies have found comparable data on gender distribution of vitamin D levels. In the current study males and females were equally affected by vitamin deficiency (58.0% and 60.5%) respectively. Comparatively a study from Lebanon found (56.0%) females to be deficient of vitamin D. A very recent study from Islamabad by Khan et al reported prevalence of vitamin D deficiency as high as 71% with more females being affected (56%). However, there are contrary results as well; one study from Iran reported a high prevalence of severe and moderate vitamin D deficiency in men. As most of the reports on vitamin D deficiency have revealed female preponderance. The current findings of high vitamin D deficiency and insufficiency show a deteriorated health condition of general population in Northern Pakistan. This could be due to the

indoor living conditions of the urban community and also the working hours and environment of people. Moreover, increased indoor activities like mobile games, internet and TV could also be a source of deranged vitamin D levels. It is well known that vitamin D deficiency is a frequent presentation in developed countries and is not limited to the under developed regions of the world. In the United States, it is equally affecting the children and adults. There is scientific evidence which confirms the association of vitamin D deficiency with high possibility of other morbidities such as osteoporosis, heart disease, diabetes, some cancers, and multiple sclerosis, as well as infectious diseases, such as tuberculosis and even the seasonal flu(37). The results of vitamin D levels are also used as an aid for the assessment of bone metabolism. In cases where the level of vitamin D falls below 10 mg/ml, intraventricular or oral management is opted. Pakistan is a developing country where health services and resources are limited. It is very difficult to address the health needs of every individual due to poor patient doctor and patient health facility ratio in Pakistan. The issue of vitamin D deficiency can be curtailed using preventive measures by education and awareness. There is a need to aware the population to get reasonable exposure from sun and also to adopt a life style which in turn could balance the natural life cycle. The infants born to vitamin D deficient mothers are at risk of severe morbidity, along with general vet D deficient population they should be investigated.

Definitions of terms:

Severe Vitamin D deficieny:defined as level of Vitamin D <5ng/ml.

Moderately Vitamin D deficiency:defined as level of Vitamin D <10ng/ml.

Mildly Vitamin D deficiency:defined as level of Vitamin D <20ng/ml

Sufficient level of Vitamin D:defined as level of Vitamin D >20ng/ml.

Toxic level of Vitamin D:defined as level of Vitamin D >150ng/ml

3. MATERIALS AND METHODS

3.1 STUDY DESIGN:

Cross sectional descriptive study

3.2 STUDY PERIOD:

06 months after the approval of Research proposal

3.3 SAMPLING TECHNIQUE:

Consecutive Non-Probability Sampling

3.4 SAMPLE SIZE:

A total of 1075 samples were included in the study

3.5 SETTING:

Special Chemistry laboratory of Benazir Bhutto Hospital, Rawalpindi

3.6 SAMPLE COLLECTION AND EXAMINATION:

Vitamin D data of 2020-2022 was collected from Benazir Bhutto Hospital whose serum Vitamin D test was conducted at the Special Chemistry Laboratory of Benazir Bhutto Hospital.

3.7 SAMPLE SELECTION:

3.7.1 Inclusion Criteria:

- The inclusion criteria were to enrol those subjects who have Vitamin D deficiency diagnosed.
- Vitamin D deficiency defined as serum levels of 25hydroxyvitamin D 25(OH) less than 20ng/ml.

3.7.2 Exclusion Criteria:

- All the patient visiting tertiary care hospital for Vitamin D deficiency examination.
- Patients with any signs and symptoms of Vitamin D deficiency such as unexplained musculoskeletal pain and aches, generalized bone pain, bony deformities/tenderness, proximal myopathy.

3.8 Data Collection Procedure: After taking approval from the ethical review board of my institute, a total of 1075 subjects meeting inclusion criteria were included in the study from the Department of Pathology, Benazir Bhutto Hospital,Rawalpindi.Informed written consent was obtained .Vitamin D level estimation was performed on samples at room temperature 25-degree centigrade.

3.9 MATERIALS REQUIRED FOR SAMPLING:

- Gloves
- Mask
- Antiseptic Solution

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- Cotton
- Tourniquet
- 5ml Disposable Syringe
- Gel and Clot Activator Tube

3.10 Sample Collection:

- First of all, I introduced myself to patient and asked for full name of the patient and checked these against requisition form.
- Checked the requisition form for requested tests, other patient information and any special draw requirements.
- Gathered the tubes and supplies that were needed for the draw.
- Positioned the patient in a chair or sitting or lying on a bed.
- Washed my hands and put mask and gloves on.
- Selected a suitable site for venepuncture, by placing a tourniquet 3 to 4 inches above the puncture site on the patient.
- Did not put the tourniquet on too tightly or left it on the patient longer than 1 minute.
- Disinfected the sampling site on the patient with 70% alcohol and allowed it to dry.
- Inserted the needle into the vein and withdrew blood until required quantity of blood was obtained. Did not withdraw piston too forcefully as it could collapse the vein.
- Release tourniquet once the needle has entered the vein.
- Place cotton immediately on the puncture site. Applied and held adequate pressure to avoid formation of hematoma.
- Then poured 3-5 ml blood in gel tube for the estimation of Vitamin D . Label the sample accordingly and delivered it to the lab.

3.11 Sample Transportation:

- Sample was transported immediately to the lab without dalay.In case of delay; serum was stored at -20ºC.

3.12 Sample Examination: About 3 ml blood from all the study subjects was collected in gel tubes and allowed it to clot and serum was separated from clotted sample via centrifugation at 4,000 rpm for 5 minutes. Vitamin D concentrations in serum were measured by using a special chemistry analyzer, Mindray BS-480 which works on the principle of a photoelectric colorimeter. Samples were first centrifuged and then run in an automated analyzer and results were noted. Serum sample used for estimation.

Fig showing Mindray BS 480

3.12 Statistical Analysis:

Statistical analysis of data was performed by using Statistical Package for Social Sciences (SPSS) version 25. Frequency and percentage were calculated for categorical data.

3.13 Ethical Consideration:

Institutional consent was taken before the start of research work.

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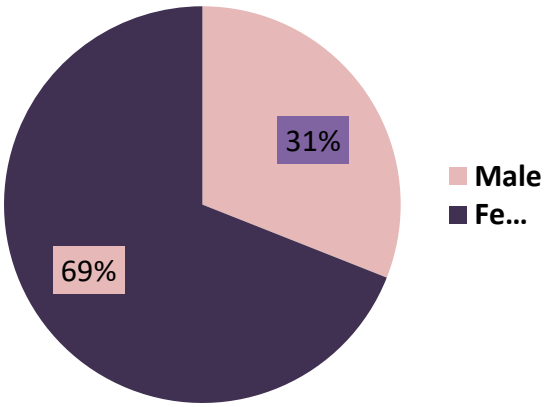
4. RESULTS

1075 patients were observed in the entire duration of research work .Most of the patients were female presented with vitamin D deficiency .Results are drawn for Frequency of severe Vitamin D deficiency among patients of all age group.

4.1.1 Gender Wise Distribution(n=1075)

This table shows gender wise distribution of Vitamin D deficient patients. There were total 1075 patients with Vitamin D deficiency out of which n=743 are females and n=332 are males.

Gender	Frequency	Percentage
Male	332	31
Female	743	69
Total	1075	100

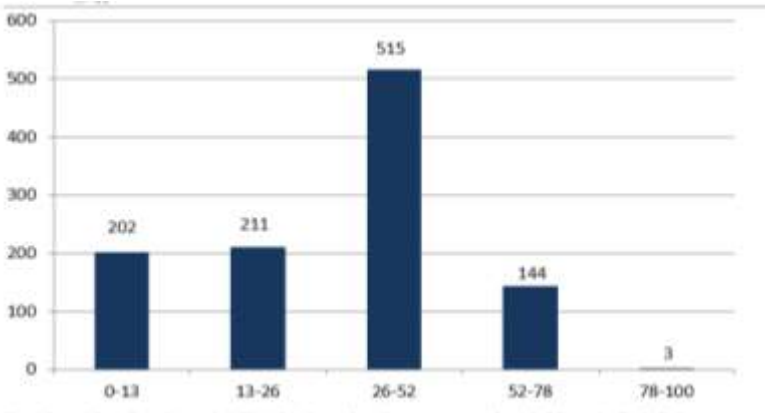


Pie chart showing Gender wise distribution of patients presented with Vitamin D deficiency

4.1.2 Age Wise Distribution:(n=1075)

Out of 1075 patients n= 202 were from age group 0-13, 211 from 13-26, 515 from 26-52, 144 from 52-78, 3 from 78-100.

Age	Frequency	Percentage
0-13	202	18.8
13-26	211	19.6
26-52	515	47.9
52-78	144	13.4
78 -100	03	0.3
Total	1075	100



Bar chart showing Age wise distribution of patient presented with Vitamin D deficiency

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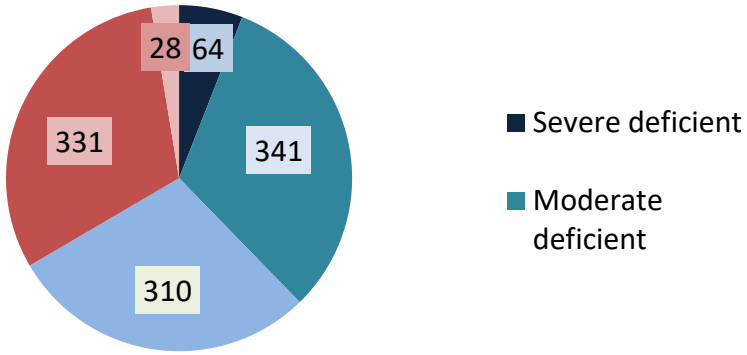
4.1.3 The frequency and percentage distribution of stages of Vitamin D deficiency:(n=1075)

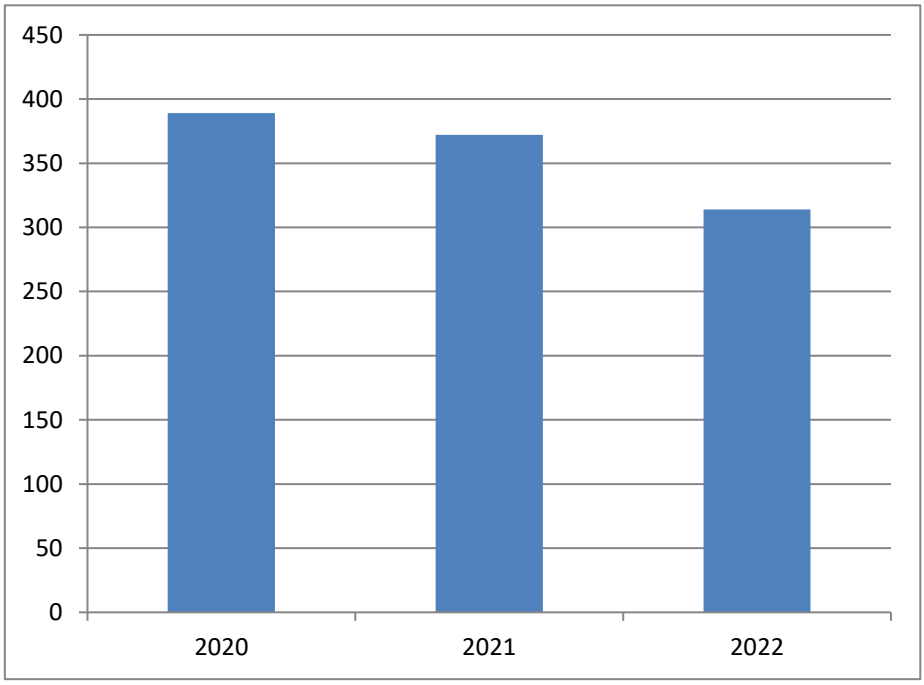
Stages of Vat D deficiency	Frequency	Percentage
Severe deficient	64	6.0
Moderate deficient	341	31.8
Mildly deficient	310	28.9
Sufficient	331	30.8
Toxic	28	2.6
Total	1075	100



Pie chart showing different stages of Vitamin D deficiency

4.1.4 Frequency and percentage distribution of year wise analysis of Vitamin D deficiency:(n=1075)

Year of analysis	Frequency	Percentage
2020	389	36.2
2021	372	34.6
2022	314	29.2
Total	1075	100



Bar chart showing year wise analysis of vitamin D deficiency

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4.1.5 The hypothesis of non-parametric test summary, Hypothesis Test Summary

	Null Hypothesis	Test	Sig.	Decision
1	The distribution of Vit D is the same across categories of Gender	Independent-Samples Mann-Whitney U Test	.011	Reject the null hypothesis

5. DISCUSSION

There are many features which contribute to the vitamin D deficiency in general population worldwide(38). These factors comprise reduced exposure to sunlight, age-linked reduction in cutaneous synthesis, and ingestion of food with a reduced vitamin D level. Vitamin D deficiency is a new global epidemic among both children and adults. There are several indications which confirm the relationship of vitamin D deficiency with high risk of other diseases such as diabetes mellitus (both types 1 and 2), cardiovascular illness, and malignancy, mainly of the intestine and prostate. Vitamin D deficiency also remains a common cause of secondary hyperparathyroidism and there are almost 200 genes whose expression has been changed with vitamin D levels. The effects of vitamin D levels are also used as an aid for the valuation of bone metabolism. Vitamin D supplements are given to make the muscle strength better and lessen the fall percentage by around 50%. In cases, where the levels of vitamin D decreases below 10 mg/ml, the clinicians prescribe a loading dose which contain a 50,000 IU of vitamin D orally once a week for 60-90 days, or 3 times a week for 30 days. According to my study out of 1075 patients during my research period, 332 were males and 743 were females with Vitamin D deficiency. Percentage of females was 69% and of males was 31%. In our findings, the prevalence of vitamin D deficiency were found to be similar by Studies from Kashmir and other areas show a high prevalence in females which is most likely due to less sun exposure, less outdoor activities, and decreased body area exposure than the male counterparts(39). My results was also supported by a national data reported from Faisalabad, Punjab in which 77.5% of the sample showed Vitamin D deficiency whereas 18% were in the category of Vitamin D insufficiency (40). Another study from the same city (Faisalabad) reported the highest prevalence of vitamin D deficiency in females. The study showed that 87% pregnant women were having Vitamin D deficiency, 10% were having Vitamin D insufficiency while only 3% had normal levels (41). Another study from Karachi in 305 premenopausal females, showed 90.1 % vitamin D deficiency (42). My research study shows that Vitamin D deficiency was most common in age group of 26-52 out of 5 groups. 26-52 age groups contain 515 patients. Frequency and percentage of Vitamin D deficient patients in different age groups was 0-13(202, 18.8%), 13-26(211, 19.6%) 26-52(515, 47.9%), 52-78(144, 13.4%), 78-100(03, 0.3%).This shows that frequency of Vitamin D deficiency was most common in 26-52 age group. In my study Patients were further categorized into 5 stages according to different concentration of 25(OH) D, 6% were severe deficient, 31.8% were moderate deficient, 28.9% were mildly deficient, 30.8% had sufficient level of Vitamin D, and 2.6% had toxic level of Vitamin D. My study shows the year wise analysis of Vitamin D deficiency .2020 contain 389 subjects, 2021 contain 372 patients, 2022 contain 314 subjects. Frequency and percentage distribution of Vitamin D deficiency was more in 2020 . According to non-parametric test summary, the distribution of Vitamin D was different across the categories of gender thus we reject null hypothesis.

6. CONCLUSION

The study revealed a high percentage of vitamin D deficient individuals. The frequency of vitamin D deficiency increased considerably with age and most commonly affected age group is 26-52. According to our analysis, there is a high prevalence of VDD, especially among females than males due to less exposed to sunlight and not taking Vitamin D rich supplements or diet. Prevelance of Vitamin D defeciency in females can be lowered by maintaining the normal levels of Vitamin D . There is a need to take immediate measures to challenge this growing public health problem, including food fortification, i.e. nurture, alongside increasing exposure to sunlight, i.e. nature. There is also a need for yearly comprehensive nation-wide analyses on vitamin D levels.

7. LIMITATIONS

1. Sample size of current study was just 1075.So there is need to conduct this study with large sample size.
2. Short duration of study
3. This study was conducted only in single Tertiary Care Hospital Rawalpindi. There is need to conduct this study all across Pakistan.

8. RECOMMENDATION

As this study shows that Vitamin D deficiency should not be neglected as its consequences can be severe. Future Public Health strategies involving education and food fortification programs with vitamin D to reduce appreciable levels of Vitamin D deficiency in Pakistan and should also involve all key stakeholder groups working together. This includes government personnel, physicians including those involved with Medical Colleges, clinical pharmacologists and relevant patient organisations working urgently to address the current rates of Vitamin D deficiency in Pakistan. A number of Western countries have Vitamin D food fortification policies 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. Secondly, A national program on the supplementation of vitamin D urgently needed. 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 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.

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