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Research Article

The Prevalence of Vitamin A, D, E and K Deficiency in Cystic Fibrosis Patients in a Tertiary Care Center

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Abstract

Introduction: Cystic fibrosis (CF) Individuals are at risk for deficiency of all lipid-soluble vitamins.

Objectives: To obtain the prevalence of the Vitamins A, D, E, and K deficiency in CF children in a tertiary care center.

Methodology: A retrospective chart review of all confirmed CF patients from the period 1984- 2018. Vitamins ADEK levels were done at presentation and at follow up periods.

Results: A total of 291 patients had their vitamins A, D, E, K levels done during routine assessment and at last follow up visits while on regular Vitamins (ADEK supplement). One hundred and forty-three of 199 (72%) who had first sample Vit A level measured were Vit A deficient with a mean of 214.73 ug/L (87.9). Two-hundred and thirty-six of 291 (81%) patients who had vitamin D measured were deficient with a mean level of 33.9 (18.6) nmol/l. Sixty-five of 213 (31%) who had Vit E measured were deficient with a mean of 3.5 mg/L (1.3). Thirty-five of 151 patients (23%) who had Vit K measured were deficient with a mean of 0.019 ng/ml (0.02). Follow up samples of ADEK vitamins showed persistence of deficiency between 36 - 81% despite supplementation for 4 - 7 years' period.

Conclusion: The result of deficiency of one or more fat-soluble vitamins was present in 36 - 81% of CF patients despite of being on regular vitamins supplements over 4 - 7 years' period. Routine monitoring of these vitamins is recommended to prevent such deficiency. Higher dosages should be considered in the treatment of the CF population.

Keywords: *Fat Soluble Vitamins Deficiency; Vitamins A, D, E, K Deficiency; CFTR; Arab*

Introduction

Cystic fibrosis (CF) is dynamic ailment that influences numerous systems, for example, respiratory, gastrointestinal, urogenital and sweat glands. CF is a genetic hereditary disease acquired as autosomal recessive because of transformation of CF transmembrane conductance regulator (CFTR) gene [1]. It is the most widely recognized life-shortening autosomal recessive illness among Caucasian, with a recurrence of 1 out of 2000 to 3000 live births [2-4]. Anomalous CFTR works in the pancreas causes pancreatic ductular obstruction which leads to pancreatic deficiency [5]. In many patients, pancreatic inadequacy prompts malabsorption of lipid, protein, and carbohydrates [6] and consequently lead to fat soluble vitamins (Vitamin A, D, E and K) deficiency.

Vitamins are basic natural exacerbates that are required in exceptionally limited quantities (micronutrients) and are engaged with basic capacities in the body, for example: development, upkeep of wellbeing, and metabolism. A vitamin may have a few functions. Since our bodies can't biosynthesize vitamins, they should be provided by the eating regimen or as supplements [7].

Fat-soluble vitamin deficiency is common in patients with CF due to pancreatic inadequacy and hepatobiliary dysfunction or one of them. A prospective study of patients recognized by infant screening showed that Insufficiency of at least one vitamin was available in 44 (45.8%) of 96 patients at age 4 to 8 weeks as follows: vitamin A 29.0%, vitamin D 22.5%, and vitamin E 22.8% [8].

The fat-soluble vitamins (A, D, E and K) have important functions in the body. Many diseases that lead to lipid malabsorption result in deficiency of one or more of fat-soluble vitamins which harm the body. The functions, daily requirement and deficiency effect are summarized in table 1 [9-13].

Fat soluble vitamins	Functions	Deficiency syndrome	Daily requirement	CF Foundation recommendations for Daily requirement of fat-soluble vitamins	KFSH preparations	
					Liquid Amount per 1 ml	Chewable tablets Amount per 2 Tablets
Vitamin A (IU) (retinol, retinal, retinoic acid)*	Vision integrity of epithelial cells role in embryonic development maintenance of immune function [9].	Night blindness Xerophthalmia Bitot's spot Follicular hyperkeratosis [9].	1-3 years (300 mcg/day) [9] 4-8 years (400 mcg/day) 9-13 years (600 mcg/day) 14-18 years Boys (900 mcg/day) 14-18 years Girls (700 mcg/day)	0-12 months: 1500 1-3 years: 5000 4-8 years: 5,000-10,0000 > 8 years: 10,000	Vitamin A (As 87% beta-carotene and 13% palmitate): 5751 IU (3002 mcg of beta-carotene)	Vitamin A (As 92% beta-carotene and 8% palmitate): 18167 IU (10028.2 mcg of beta-carotene)
Vitamin D (IU) (calciferol)**	Regulation of calcium and phosphate homeostasis [11].	Rickets Osteomalacia [11].	1-3 years (400-600 IU/day) [11]. 4-8 years (400-600 IU/day) 9-13 years (400-600 IU/day) 14-18 years (400-600 IU/day)	0-12 months: 400 - 500 12 months - 10 yrs: 800 - 1000 10 yrs - 18 yrs: 800 - 2000	Vitamin D3 (As cholecalciferol): 600 IU (15 mcg)	Vitamin D3 (As cholecalciferol): 1200 IU (30 mcg)

Vitamin E (IU) (tocopherols)***	Antioxidant [10].	Peripheral neuropathy Spinocerebellar ataxia Skeletal myopathy Pigmented retinopathy Hemolytic anemia [10].	1-3 years (6 mg/day) [10] 4-8 years (7 mg/day) 9-13 years (11 mg/day) 14-18 years (15 mg/day)	0-12 months: 40-50 1-3 years: 80-150 4-8 years: 100-200 > 8 years: 200-400	-Vitamin E (As d-alpha-tocopherol): 50 IU (33.5 mg) -Vitamin E (As other mixed tocopherols): 15 mg	-Vitamin E (As d-alpha-tocopherol): 100 IU (67 mg) -Vitamin E (As other mixed tocopherols): 30 mg
Vitamin K (mg) (phylloquinone, menaquinone, menadione)	Blood coagulation and bone metabolism [9].	Hemorrhagic disease [9].	1-3 years (30 mcg/day) [9]. 4-8 years (55 mcg/day) 9-13 years (60 mcg/day) 14-18 years (75 mcg/day)	0-12 months: 0.3-0.5 1-3 years: 0.3-0.5 4-8 years: 0.3-0.5 > 8 years: 0.3-0.5	Vitamin K1 (As phytonadione): 400 mcg	Vitamin K1 (As phytonadione): 700 mcg

Table 1: *Vitamins ADEK requirements and the complications of their deficiencies. Legend table 1: *: Vitamins conversion units: 1 IU Vitamin A equals the biological equivalent of 0.3 mcg retinol, or of 0.6 mcg beta-carotene. **: 1 IU Vitamin D equals the biological equivalent of 0.025 mcg cholecalciferol/ergocalciferol. ***: 1 IU Vitamin E equals the biological equivalent of about 0.667 mg d-alpha-tocopherol (2/3 mg exactly), or of 1 mg of dl-alpha-tocopherol acetate.*

In our study we aim to obtain the prevalence of the Vitamins A, D, E and K deficiency in CF children in a Tertiary Care Center in Saudi Arabia.

Definitions

- **Cystic fibrosis:** Was diagnosed on typical clinical picture of cough, sputum production and high sweat chloride test in two subsequent testing samples > 60 mmol/L by the Wescor quantitative method, USA. In this study, routine evaluation of all patients included: detailed medical and family history, physical examination, laboratory investigations and *CFTR* mutation testing [14-20].
- **Pancreatic insufficiency:** Was diagnosed based on stool elastase measurement of > 100 - 200 ug E1/g stool, or a 72-hour fecal fat estimation using the van de Kamer method; of which a positive result corresponded to a fecal fat content > 7 g/24 hours [5,6].
- **Bronchiectasis:** Is identified as dilated bronchi through radiological studies like chest X-rays or Computed Topography (CT) [2,5].

Methodology

A retrospective chart review of all confirmed CF patient's data from the period 1st January 1984 - December 2018. All the age group that have Vitamins A, D, E, and K levels were done "at presentation and at follow up" were reported. The laboratory requests for the assessment of body fat-soluble vitamin (FSV) status included: vitamin A (retinol), vitamin D (25-OHD), vitamin E (α-tocopherol) and vitamin K1. There were considerable difficulties to accurate FSV measurement in blood. These difficulties included their physical and chemical properties, incomplete standardization of measurement and limitations in the techniques that are currently used for quantification [21]. However, the present study included the clear results that used the same analytical methodology (e.g. protein precipitation with high-performance liquid chromatography (HPLC) and ultraviolet detection; radioimmunoassay; solid phase extraction with HPLC and fluorescence detection). Vitamins deficiency are defined as: Vitamin A deficiency (< 20 Mcg/dL), Vitamin D deficiency (< 10 ng/ml), Vitamin E deficiency (< 0.5 mg/dL), and vitamin K deficiency (< 0.2 - 3.2 mg/ml) [7-13].

Vitamin A serum levels were performed using protein precipitation with high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection (in-house method) and the normal range was 343 -838 g/L. Serum retinol was considered low based on National Health and Nutrition Examination Survey (NHANES) 1999 - 2002 data for the 5th percentile (< 300 g/L) [22].

Vitamin D serum levels were measured by competitive binding methods, high-performance liquid chromatography (HPLC), and radioimmunoassay (RIA). A commonly used RIA kit, developed by DiaSorin S.p.A (Saluggia, Italy) was the method used by many reference laboratories and is considered the gold standard [23]. The most accurate way to measure how much vitamin D is in your body is the 25-hydroxy vitamin D blood test. A level of 20 nanograms/milliliter to 50 ng/mL is considered adequate for healthy people. A level less than 12 ng/mL indicates vitamin D deficiency [24].

Vitamin E serum levels were assessed by quantitative high-performance liquid chromatography (HPLC) and the normal range was 5.50 -15.5 mg/L. Low levels of vitamin E (α-tocopherol) were defined from clinical laboratory reference ranges as < 3 mg/L for children ages 1 to 12 yrs and < 6 mg/L for 13 to 19 yrs (Soldin., *et al*. 2011). In the present study serum α-tocopherol was considered low based on NHANES 1999 - 2002 data for the 5th percentile (< 5 mg/L) [25].

Vitamin K1 levels were performed by solid phase extraction with quantitative high-performance liquid chromatography (HPLC) and fluorescence detection. The reference range for vitamin K was 0.3 - 2.6 nmol/L. Deficiency of vitamin K1 was defined as < 0.3 nmol/L [26].

BMD measurement: Bone density scanning, also named dual-energy x-ray absorptiometry (DXA) or bone densitometry, it's a developed form of the x-ray technology that can be used to measure the loss of the bone. DXA is the standard establishment for measuring bone mineral density (BMD). DXA is most generally operated on the hips and lower spine [16].

A bone mineral density (BMD) test measures the amount of calcium and other variety of minerals in the bones. BMD test is used to help health care provider to distinguish Osteoporosis disease and anticipate the risk for bone fracture [17].

DEXA scan has two types which are: Central DEXA which depend upon lying on a soft table, then the scanner passes by the lower spine and hips. This scan is the outstanding test for predicting the risk of fractures, mainly of the hip. Peripheral DEXA (p-DEXA) is the second type, which measure the density of the bone at the wrist, fingers, leg or heel [16]. The results of the test are commonly recorded as a T-score and Z-score: T-score states the differences of the bone density with a healthy person. Z-score states the differences of the bone density with other people of similar age, gender, and race [27].

In both scores, a negative number means having thinner bones compared to average. The risk for a bone fracture increases as more the negative the number. A T-score normal range is -1.0 or above. Bone mineral density testing helps predicting the risk for having a bone

fracture in the future, however not diagnosing fractures. T-score between -1 and -2.5, indicates early bone loss (osteopenia), and scores below -2.5, primarily refer to osteoporosis [16]. The bone density scan was invented by the late John R. Cameron (1922 - 2005), professor emeritus at the University of Wisconsin at Madison [27]. He earned a PhD in physics. He invented bone densitometry in the late 1960s. Bone densitometry, which uses precise, very small radiation measurements to determine the mineral content of bone, was one of his many important contributions to medical physics [27].

Inclusion criteria: All confirmed CF the patients of all age group that have Vitamin A, D, E, K measurement during their follow up period in CF clinic form the period January 1984 - December 2018.

CFTR identification: As described before in previous publication [28].

Ethical considerations

1. All data were stored in Pediatric Research Unit, accessed only by the Principal Investigator and the assigned Assistant Clinical Research Coordinators.

2. The entire patient's information was kept strictly confidential. Each patient was given a study number, and all patient data were entered in to the designated data sheet (EXCEL) without any patient's identifiers.

3. The declaration of Helsinki and GCP guidelines were followed.

Statistical statement: The T-Test was used to calculate the continuous variables, median, mean, and standard deviation. All values were expressed in mean ± standard deviation (SD) and results were presented at a level of significance of $p < 0.05$.

Results

The result of deficiency of one or more fat-soluble vitamins was present in 23% - 81% of CF patients on their follow up vitamin despite of being on regular vitamins ADEK supplements. Data for Fat-soluble vitamin (FSV) measurements was available for 291 Saudi children with confirmed cystic fibrosis.

There was no significant difference between boys and girls in the level of all vitamins. Most of the studied CF children showed no obvious signs of fat-soluble vitamins deficiency, such as: Bitot spots and night blindness (vitamin A), rickets (vitamin D), peripheral neuropathies and spinocerebellar degeneration with ataxia (vitamin E), or coagulopathy (vitamin K).

Vitamin A: The serum level for vitamin A (Retinol) was collected from 199 CF patients; 95 (48%) were males and 104 (52%) were females.

The mean age of the 199 children at first Vitamin A level measurement was 8 (6.5) years with a mean serum level of 277.73 ug/L (136.8). The serum level of retinol in the first time after diagnosis was low (less than 300 ug/L) in 143 (72%) with a mean vitamin level of 214 (87.9) ug/L. The second measurement after vitamin supplements was recorded from 141 patients (Mean age 11 (7.7) years) and vitamin A serum level was low in 118 (84%) with mean vitamin level of 230 (108.4) ug/L. The third measurement after vitamin supplements was recorded from 103 patients (Mean age 12.9 (7.8) years) and vitamin A serum level was low in 75 (73%) with a mean vitamin level of 226 (114.4) ug/L. The percentage of CF patients with vitamin A deficiency was not improved in the second and third measurements despite of vitamin supplementation (Table 3) ($P = < 0.05$).

Demographic data	Vitamin A (n = 199)	Vitamin D (n = 291)	Vitamin E (n = 213)	Vitamin K (n =151)	BMD (n=154)
Gender Male	95 (48%)	140 (48%)	105 (49%)	70 (46%)	76 (49%)
Female	104 (52%)	151 (52%)	108 (51%)	81 (54%)	78 (51%)
Region East	84 (42%)	108 (37.2%)	85 (40%)	57 (39%)	56 (36%)
West	22 (11%)	37 (12.7%)	25 (12%)	14 (9%)	10 (6.4%)
Central	41 (20%)	75 (25.7%)	47 (22%)	32 (21%)	42 (27%)
North	26 (13%)	29 (13.4%)	28 (13%)	26 (17%)	30 (19%)
South	26 (13%)	32 (11%)	28 (13%)	22 (14%)	16 (10%)
Patient situation Active	159 (80%)	230 (79%)	162 (76%)	133 (88%)	135 (87%)
Died/Lost follow up	40 (20%)	61 (21%)	51 (24%)	18 (12%)	19 (13%)

Table 2: *Demographic data of CF and vitamins ADEK deficiencies. Legend table 2:*

n = Number.

Demographic data	Vitamin A (n = 199)	Vitamin D (n = 291)	Vitamin E (n = 213)	Vitamin K (n = 151)
Normal level	343 - 838 ug/L	> 75 nmol/l	5.5 - 15.5 mg/L	0.1 - 2.2 ng/ml
1st sample Mean Age (year)	8 (6.5)	6.845 (6.1)	7.8 (6.7)	10.35 (6.4)
Mean	277.73 ug/L (136.8)	46.77 nmol/L (33.7)	8.8 mg/L (6.2)	1.22 (2.6) ng/ml
Median	248 ug/L	38 nmol/L	8 mg/L	0.2 ng/ml
Range	49 -845ug/L	3-224 nmol/l	0.7-59 mg/L	0.03-14.1 ng/ml
1st sample Vits Deficient patients # (%)	143 (72%)	236/291 (81%)	65/213 (31%)	35/151 (23%)
1st sample vits Deficient mean (SD)	214 (87.9) ug/L	33.9 (18.6) mmol/l	3.5 (1.3) mg/L	0.019 (0.02) ng/ml
2nd sample at FU #	n=141	n=226	n=141	n=79
Mean (SD) Age (year)	11 (7.7)	10.04 (6.52)	10.7 (7.3)	13.9 (6.7)
Mean (SD)	265.21 (129.29) ug/L	48.66 (29.97) nmol/l	9 (5.3) mg/L	1.02 (1.9) ng/ml
Median	250.0 ug/L	42 nmol/l	8.3 mg/L	0.23 ng/ml
range	95-1086 ug/L	3-134 nmol/l	0.37-0.47 mg/L	0.03-9.8 ng/ml
2nd sample Vits Deficient patients # (%)	118 (84%)	181/226 (80%)	35/141 (25%)	24/79 (30%)
2nd sample vits Deficient mean (SD) level	230 (108.4) ug/L	37 (19.5) nmol/l	3.5 (1.1) mg/L	0.022 (0.02) ng/ml
Total last FU sample#	n= 103	n= 177	n= 102	n= 44
Mean Age (SD)in years	12.9 (7.87)	12.62 (6.997)	12.26 (7.6)	16.9 (6.7)
Mean (SD)	286.89 (165.2) ug/L	53.22 (76.28) nmol/l	9.5 (4.8) mg/L	1.2 (2.5) ng/ml
Median	260 ug/L	42 nmol/l	8.15 mg/L	0.2 ng/ml
range	42-1000 ug/L	8-986 nmol/l	2.2-31 mg/L	0.03-13.65 ng/ml
Last FU Vits Deficient patients # (%)	75 (73%)	143 /177 (81%)	18 /102 (18%)	16/44 (36%)
Last FU Vits Deficient mean (SD) level	226 (114.4) ug/L	36.8 (18) nmol/l	3.8 (1.0) mg/L	0.018 (0.02) ng/ml

Table 3: *Comparisons of CF and Vitamins ADEK levels at First sample measurement and at follow-up.*

Legend table 3:

n = Number; 1st = First; 2nd = Second; 3rd = Third; SD = Standard Deviation; Vits = Vitamin; FU = Follow Up.

Vitamin D: The serum level for vitamin D (25OHD) was extracted from 291 CF patients'; 140 (48%) were males and 151 (52%) were females. The mean age of the 291 CF children at Vitamin D level measurement was 6.8 (6.1) years. Two-hundred and thirty-six of 291 (81%) patients were deficient with mean vitamin level 33.9 (18.6) nmol/l. While in the second period, Vitamin D level was low in 181 (80%) of patients, with a mean vitamin level of 37 (19.5) nmol/l. The last follow-up period, Vitamin D was extracted at a mean age of 12.6 (6.9) years and it was low in 143/177 (81%) of patients, with a mean vitamin level of 36.8 (18) nmol/l. All the 3 measurements were below the normal range ($p < 0.05$) (Table 3).

Vitamin E: The serum level for vitamin E (α-Tocopherol) was collected from 213 CF patients; 105 (48%) were males and 108 (52%) were females. The mean age of the 213 CF children at first sample was 7.8 (6.7) years with a mean serum level of 8.8 mg/L (6.2). The serum level of α-tocopherol in the first sample was low in 65 (31%) with a mean level of 3.5 (1.3) mg/L. The second measurement after vitamin supplements was recorded from 141 patients (Mean age 10.7 (7.3) years and the vitamin E serum level was low in 35 (25%) with a mean vitamin level of 3.5 (1.1) mg/L. The third measurement after vitamin supplements was recorded from 102 patients (Mean age 12.2 (7.6) years) and the level was low in 18 (18%) with a mean level of 3.8 (1.0) mg/L. ($p < 0.05$) (Table 3).

Vitamin K1: The serum level for vitamin K1 was obtained from 151 CF patients'; 70 (46%) were males and 81 (54%) were females. The serum level of vitamin K in the first ample was low (< 0.3 nmol/L) in 35 (23%) with a mean level of 0.019 (0.02) ng/ml. The second sample was recorded from 79 patients (Mean age 13.9 (6.7) years) and the vitamin K1 serum level was low in 24 (30%) with a mean level of 0.022 (0.02) ng/ml. The third sample was recorded from 44 patients at a mean age 16.9 (6.7) years) and the vitamin K1 serum level was low in 16 (36%) with a mean vitamin level of 0.018 (0.02) ng/ml ($p < 0.05$) (Table 3).

Bone densitometry analysis: BMD analysis was done for 147 patients initially; 60 (40.8%) were normal ,61 (41%) mildly decreased, 8 (5.4%) moderately decreased, 18 (12.2%) severely decreased. The Z score for lumber BMD was ranging from 2.3 to -4.7 (with a T score =13). Meanwhile, Middle stage BMD was done on 50 patients; 15 (30%) were normal ,22 (40%) mildly decreased, 1 (2%) moderately decreased, 12 (24%) severely decreased. The z score for lumber BMD for the middle stage was ranging from 1.5 to -5.8 (with a T score = 8) At the third stage, 23 patients had done a third BMD; 7 (30.4%) were normal ,15 (65%) mildly decreased, 1 (4.3%) severely decrease with a z score of lumber BMD ranging 1 to-5 (Table 4). The BMD score continued to decrease with time despite regular vitamin D supplements over 6 years' period.

Variable	Total 1st BMD (n = 147)	Total middle BMD (n = 50)	Total last BMD (n = 23)
Normal	60 (40.8%)	15 (30%)	7 (30.4%)
Mildly decreased	61 (41%)	22 (40%)	15 (65%)
Moderately decreased	8 (5.4%)	1 (2%)	0 (0%)
Sever decrease/osteopenia	18 (12.2%)	12 (24%)	1 (4.3%)
BMD lumber	147	50	23
Total Z score lumber	Total: 152 Maximum: 2.3 Minimum: -4.7 SD: (0.25)	Total: 49 Maximum: 1.5 Minimum: -5.8 SD: (1.5)	Total: 21 Maximum: 1 Minimum: -5 SD: (1.6)
Total T score lumber	13	8	8

Table 4: *Bone marrow densitometry results during follow up period (total 147 patients).*

Legend table 4:

n = Number; 1st = First; SD = Standard Deviation.

Discussion

Rana., *et al*. [30] performed a retrospective analysis of fat-soluble vitamin levels in children aged ≤ 18 years who lived in (North-south Wales, Australia) NSW and attended any of the three pediatric CF centers from 2007 to 2010. She found that the deficiency of one or more fat-soluble vitamins was present in 240/530 children (45%) on their first vitamin level test in the study period. The prevalence of vitamin A deficiency increased from 11.17% to 13.13%. The prevalence of vitamins D and E deficiency fell from 22.11% in 2007 to 15.54% in 2010, and 20.22% to 13.89%, respectively. Low vitamin K was present in 29% in 2007, and the prevalence of prolonged prothrombin time increased from 19.21% to 22.62%. Fat-soluble vitamin deficiency is present in 10% - 35% of children with pancreatic insufficiency, but only a very small proportion of children who are pancreatic-sufficient. She recommended that: Fat soluble vitamin testing is essential to identify deficiency in pancreatic-insufficient children who may be noncompliant to supplementation or require a higher supplement dose, and pancreatic-sufficient children who may be progressing to insufficiency, in addition, vitamin K dependent factors need to be considered in follow-up of these patients [30].

In another study by the Dietitians Association of Australia Cystic Fibrosis Interest Group [31], they have observed that the deficiencies of fat-soluble vitamins were found in infants younger than 3 months of age [31].

In a prospective study by Feranchak., *et al*. [32] of 127 infants with CF in USA, they found that 27/93 (29.1%) were deficient in vitamin A [32].

In our study, we found no difference between males and females in fat ADEK deficiencies and the Eastern province had the highest prevalence of all ADEK vitamins ranges from 37 - 42% compared to 12 - 27% in other provinces (Table 2). For vitamin A, regular supplement over 4 years, still maintained deficient level in 73% despite vitamin A supplement and proper compliance (Table 3).

Regarding vitamin D Rovner., *et al*. [33] found that Vitamin D (25-OHD) deficiency in American children with CF was present in 22.5% of infants (< 14 ng/ml; < 37.5 nmol/L), and 90% in older children (< 30 ng/ml; < 70 nmol/L) despite routine oral supplementation [33]. The median daily vitamin D supplementation was 800 IU. The mean (SD) serum concentrations of 25 (OH)D were 20.7 (6.5) ng/mL [33].

The goal of vitamin D supplementation in the pediatric CF population is to modify the potential risk for the developing CF bone disease. There is an ongoing debate about the optimal dosing regime and the formula of vitamin D which is most efficacious. Many studies have demonstrated a lack of success in maintaining an increased 25-OHD level despite supplementation [34]. They also showed that in adult CF patients, treatment regimens based on US CF Foundation guidelines for bone health, such as 50,000 IU of ergocalciferol (vitamin D2) weekly or twice weekly respectively, over 8 weeks, did not significantly increase 25-OHD levels. In contrast, single high-dose vitamin D3 replacement therapy (100,000 IU - 600,000 IU (2500 - 15,000 µg), known as stoss therapy (from the German "to push"), is an effective method for treating vitamin D deficiency [34]. It involves a single oral or intramuscular dose of vitamin D based on the age and vitamin D level of the patient [34].

Shepherd D., *et al*. performed a study to determine the safety and efficacy of stoss therapy on vitamin D levels over a 12-month period in children with cystic fibrosis and vitamin D deficiency (b75 nmol/L) during the period 2007 - 2011. Thirty-eight children received stoss therapy and 37 children with vitamin D deficiency were not treated and served as a control group. The stoss treated group had a significant and sustained increase in 25-hydroxyvitamin D levels measured at 1, 3, 6 and 12 months' post treatment compared to controls (94.82 ± 41.0 nmol/L, p = 0.001; 81.54 ± 24.6 nmol/L, p = 0.001; 92.18 ± 36.5 nmol/L, p = 0.008 and 64.6 ± 20.0 nmol/L, p = 0.006 respectively). At 12 months' post intervention, the mean difference in vitamin D levels from baseline between the stoss treated group and controls was significant at 15 nmol/L compared to 5 nmol/L (p = 0.038). Stoss therapy effectively achieved and maintained levels of 25-hydroxyvitamin D greater than 75 nmol/L over 12 months' period [34].

For our patients Vitamin D with regular supplement remained deficient over 6 years' follow–up period in 81% of CF patients (Table 3).

Regarding vitamin E its deficiency can cause a host of conditions such as hemolytic anemia, cerebellar ataxia and cognitive difficulties [35]. Vitamin E supplementation is widely recommended in cystic fibrosis and aims to ameliorate this deficiency.

To determine the effects of any level of vitamin E supplementation on the frequency of vitamin E deficiency disorders in people with cystic fibrosis [35]. A Cochrane study was performed from Cochrane Group's Cystic Fibrosis Trials Register and also from the international trial registers including randomized controlled trials and quasi-randomized controlled trials comparing any preparation of vitamin E supplementation to placebo or no supplement, regardless of dosage or duration [35]. The author found that: Four studies with a total of 141 participants were included in the review, two of these were in children (aged six months to 14.5 years), and the other two did not specify participants' age [35]. All studies used different formulations and doses of vitamin E for various durations of treatment (10 days to six months). Two studies compared the supplementation of fat-soluble as well as water-soluble formulations to no supplementation in different arms of the same study. A third study compared a water-soluble formulation to a placebo and in the fourth study a fat-soluble formulation of vitamin E was assessed against placebo [35].

At one month, three months and six months, water-soluble vitamin E significantly improved serum vitamin E levels compared with control: at one month, two studies, mean difference 17.66 (95% confidence interval 10.59 to 24.74); at three months, one study, mean difference 11.61 (95% confidence interval 4.77 to 18.45); and at six months, one study, mean difference 19.74 (95% confidence interval 13.48 to 26.00). At one-month fat-soluble vitamin E significantly improved serum vitamin E levels compared with control: one month, two studies, mean difference 13.59 (95% CI 9.52 to 17.66). The findings at three months were imprecise; one study; mean difference 6.40 (95% confidence interval -1.45 to 14.25) [35].

None of the studies report the review's primary outcomes of vitamin E total lipid ratio or the incidence of vitamin E-specific deficiency disorders, or the secondary outcomes lung function or quality of life. Only one study, comparing water-soluble vitamin E with placebo, reported the secondary outcome of growth and nutritional status (weight), but the results are uncertain due to imprecision around the effect estimate [35].

There was limited detail about randomization and blinding in the included studies which compromises the quality of the evidence base for the review. The heterogeneous mix of the formulations with differing bioavailability among these studies also limits the generalizability of the data to the wider cystic fibrosis population [35].

Their conclusion is that vitamin E supplementation led to an improvement in vitamin E levels in people with cystic fibrosis, although the studies may have been at risk of bias. No data on other outcomes of interest were available to allow conclusions about any other benefits of this therapy [35].

In our study, for vitamin E deficiency, it remained deficient in 18% over 4-5 years' period (Table 3).

Regarding vitamin K it is a cofactor for the carboxylation reaction that transforms gamma-glutamyl residuals (Glu) residues to gamma carboxyglutamate (Gla) residues. In subclinical vitamin K deficiency, the undercarboxylated species of the vitamin K dependent proteins are released into the circulation. The percent undercarboxylated osteocalcin (%Glu-OC) rises when vitamin K status is suboptimal and has been found to be inversely correlated with measures of bone quality, bone quantity, and fracture risk [36].

Drury., *et al*. [36] studied the efficacy of high dose phylloquinone in correcting vitamin K deficiency in cystic fibrosis and found that; Of the 50% of subjects who were below the optimal serum vitamin K1 at baseline, all rose into the normal range with supplementation. Supplementation also significantly reduced the overall % Glu-OC from a median of 46.8 to 29.1%. His results suggested that both 1 mg and 5 mg of vitamin K1, given over a 1 m period in PI pediatric CF patients improve vitamin K status [36].

A Canadian study of 14 children found that seven (50%) had suboptimal vitamin K (< 0.3 nmol/L) while 65/93 (70%) of children in a British study had suboptimal vitamin K based on low serum vitamin K, elevated prothrombin time (PT), or both. Indeed, only 18% of CF centers in the UK supplemented vitamin K1 [30].

Children with CF are frequently on antibiotics and are malnourished, which reduces the contribution of bacterial vitamin K synthesis to the overall vitamin K body pool. PT is often used as an indirect measure of vitamin K status, however, vitamin K stores must fall below 50% before PT becomes prolonged [30].

Osteocalcin γ-carboxylation and proteins induced by vitamin K absence (PIVKA-II) are direct measures of vitamin K status but were not performed at these CF centers. Measurement of vitamin K and dependent factors may have a significant role to play in bone assessment in the near future [30].

Wilson., *et al*. found that in his CF population, before supplementation 58 (81%) of 72 patients had abnormal PIVKA-II levels (> 2.9 ng/mL). After supplementation 29 (40%) had abnormal PIVKA-II levels. All 6 patients with advanced CF liver disease (CFLD) had abnormal PIVKA-II levels (median, range of 20.8, 5.5 to 55 ng/mL) before treatment, which corrected to normal in 50%. Four patients, 2 with CFLD, had a prolonged PT (> 13.5 seconds) at both time periods. An oral fat-soluble vitamin combination with a modest amount of vitamin K can, as a daily supplement, improve the PIVKA-II levels in patients with PI and CF [37].

Kleinman., *et al*. found that only those children with CF and PI who received >1000 mcg/d of vitamin K achieved a status similar to healthy subjects [38].

Rashid., *et al*. concluded that Vitamin K deficiency is common in non-supplemented patients with CF and PI and routine supplementation should be considered in all of these patients) [39].

In our study, for vitamin K deficiency remained low in 16 (36%) despite supplementation for 6 - 7 years (Table 3).

Overall, our study showed that the follow up samples of ADEK vitamins showed persistence of deficiency between 36 - 81% despite regular supplementation and proper compliance for 4 - 7 years' period.

Factors that may have contributed this persistence of deficiency could be related to low dietary intake, un-reported poor compliance, intestinal poor absorption to ADEK in certain Genotype, vitamin preparation, and low dosage that is inadequate to this type of disease. Further study is needed to evaluate such factors.

Conclusion

The result of deficiency of one or more fat-soluble vitamins was present in 36 - 81% of CF patients despite of being on regular vitamins supplements over 4 - 7 years' period. Routine monitoring of these vitamins is recommended to prevent such deficiency. Higher dosages should be considered in the treatment of the CF population.

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