http:// ijp.mums.ac.ir
Review Article (Pages: 9701-9710)

Bone Mineral Density and Cystic Fibrosis: A Review

Maryam Hassanzad[1], *Poopak Farnia[2,3], Ali Akbar Samadani[4], Seyed Javad Sayedi[5], Ali Akbar Velayati[2]

[1]Pediatric Respiratory Diseases Research Center, National Research Institute of Tuberculosis and Lung Diseases (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[2]Mycobacteriology Research Centre (MRC), National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[3]Department of Biotechnology, School of Advanced Technologies in Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran.
[4]Gastrointestinal and Liver Disease Research Center (GLDRC), Guilan University of Medical Sciences, Rasht, Iran.
[5]Department of Pediatrics, Faculty of Medicine, Mashhad University of Medical Sciences, Mashhad, Iran.

Abstract

Cystic fibrosis (CF) is a common progressive genetic disorder among children which involves lungs, kidneys, intestine and liver. Apart from the significance of genetic factors, various environmental factors particularly bone mineral density are directly associated with CF. Remarkably, bone disease is appeared as a routine and common trait in long term CF survivors which implies that environmental parameters including calcium and vitamin D intake as well as drug inducement are the most important risk factors causing low bone mineral density.

Conspicuously, absolute and notable treatment of cystic fibrosis associated to bone disorder must involve investigating the risk factors including the reduced intake of certain vitamins and minerals due to pancreatic inadequacy, modified hormone production, severe and chronic lung infection with increased ranges of bone function cytokines for a weak bone health situation. In this review, focus is on these considerable factors alongside the genetic factors in cystic fibrosis.

Key Words: Cystic Fibrosis, Low Bone Mineral Density, Genetic Factors, Vitamin D.

*Please cite this article as: Hassanzad M, Farnia P, Samadani AA, Sayedi SJ, Velayati AA. Bone Mineral Density and Cystic Fibrosis: A Review. Int J Pediatr 2019; 7(7): 9701-10. DOI: **10.22038/ijp.2019.38975.3325**

Corresponding Author:

Poopak Farnia (M.D), Mycobacteriology Research Centre (MRC), National Research Institute of Tuberculosis and Lung Disease (NRITLD), Shahid Beheshti University of Medical Sciences, Tehran, Iran.

Email: p.farnia@sbmu.ac.ir

Received date: Feb.14, 2019; Accepted date: Apr. 22, 2019

1- INTRODUCTION

Cystic fibrosis (CF) is one of the common lethal autosomal recessive situations affecting the children's population worldwide, and it is inherited in an autosomal recessive function. It results mainly from the presence of certain mutations in both copies of the *CFTR* gene (1, 2). The defective CF regulator gene is evident in some clinical traits like chronic pulmonary disease, biliary cirrhosis and pancreatic insufficiency (3, 4). Notably, improved treatment age is different in eclectic population and it is approximately about 31 years (5-7). Consequently, different complications of CF disease are emerging and comprise low bone mineral density (**Figure.1**).

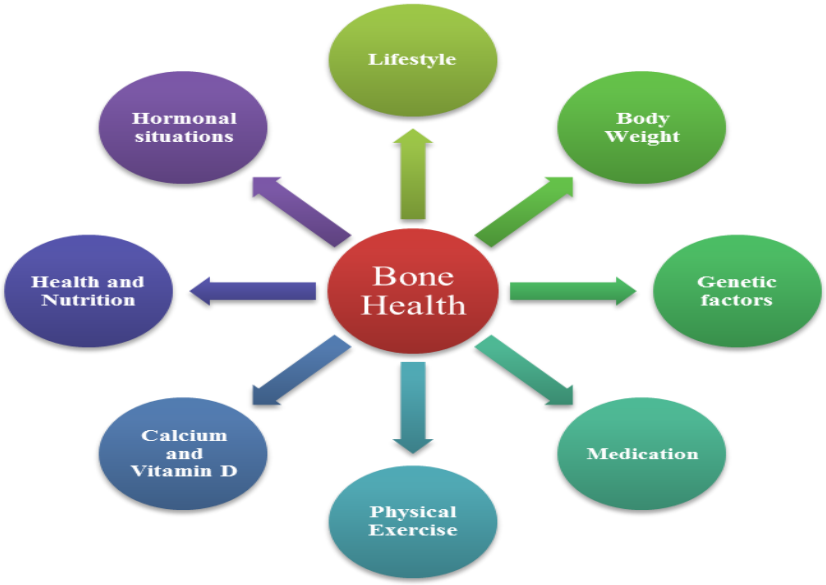

Fig.1: Main factors that can affect bone density and its chemical combinations.

Patients suffering from CF have many notable risk factors for the progression of low bone mineral density (BMD) including low vitamin D intake, pulmonary disease, glucocorticoid therapy, hypogonadism with diabetes, delayed puberty, decreased levels of physical function and malnutrition. The most important clinical consequence of low bone mineral density is fracture, and patients with CF have increased fracture rate compared to normal people (8-10).

Generally, when the bone density is lower than normal, it may mean that there is a greater chance of getting osteoporosis in the future. There are many factors in CF that may affect bone mineral density and it is important to evaluate these factors. The aim of this investigation was to discuss the most influential parameters by which CF affects different aspects of bone mineral density. First, we talk over clinical qualifications and the prevalence of bone mineral density in cystic fibrosis and the related factors. Then, we discuss the relationship between bone histomorphometry and CF. Also, density fluctuation and intake fluctuation of bone are taken into account, the pathophysiology of bone disease is elaborated in CF and finally, the inheritance and genetic factors are discussed.

2- MATERIALS AND METHODS

In order to find the documents related to the review article, the keywords including "cystic fibrosis, bone mineral

density, vitamin D, fracture, children, and pediatric" were searched on PubMed, Scopus and Science Direct databases from 1975 until now. Over this period, more than 500 documents were examined and the articles were selected according to the following criteria: first, the titles were examined for thematic relationship. After reviewing the titles of the articles, they were evaluated at the next stage in terms of abstract communication with the intended purpose. Selected items were thoroughly studied and the important notes were extracted. Then, the collected materials were classified into two main areas: "environmental factors", and "genetic factors" which were subsequently summarized. In cases where it was required, additional comments and descriptions for the content were provided by the author for better clarity.

3- RESULTS

3-1. Role of clinical qualifications of bone mineral density in cystic fibrosis

Many studies and researches have indicated the role of low bone mineral density in cystic fibrosis patients (11-16). Diminished bone mineral density is quite routine in post pubertal kids and adults with cystic fibrosis (17). As a result, the prevalence of bone mineral density is very common. Correspondingly, improved methods can be made for bone evaluation including bone age or size by applying bone mineral density, but, some additional problems will be encountered by the new methods used to evaluate bone size with X-ray absorptiometry. Many studies have focused on the density of bone structure and have indicated the importance of low bone mass fixed after fitting for these factors. This means that some of the alterations in these searches could be illustrated by the patients' situation including age (for instance, children have a better volumetric bone density than adults) (18, 19).

The prevalence of bone disorder tends to increase with the severity of lung problems and malnutrition. Younger people may have normal status of bone mineral density, suggesting that the bone disorder is not originally associated to the *CFTR* gene mutation and remarkably, some mutations in this gene can happen, and diverse mutations cause many structural defects in the *CFTR* protein and, in some situations, diseases (20-24). Clinically, patients with acute pulmonary problem sometimes have a serious and severe bone disease, with a high rate of fractures of vertebrae, ribs and bones and kyphosis (**Figure.2**).

3-2. Role of histomorphometry in cystic fibrosis

Recent findings of bone histomorphometry in adults with cystic fibrosis and low bone mineral density illustrate decreased bone volume and a trend towards reduced connective tissues (25, 26). Notably, in this situation the indexes of bone formation were also significantly decreased. Evaluation of the intake cavities indicated tiny regions, depths and reconstructed lengths of the cavities in CF patients, and this causes the amount of bone formation to be reduced by approximately 50% (27-29). Conclusively, these findings explain that the major reason for low cancellous bone volume seems to be low bone concentration in tissues. Many rare case studies about the autopsy of bone specimens from a mixture with transplantation activity in people with cystic fibrosis illustrated acute osteopenia in bone (3, 30). There was reduced osteoblastic function and increased osteoclastic function at the cellular level. The decrease in osteoblastic function was due to the reduction in osteoblast and also the biosynthetic potential of these cells (13, 31, 32). Remarkably, cellular and cortical bone mass amounts would be lower after transplantation. Moreover,

none of the cystic fibrosis biopsies indicated osteoid items traits of osteomalacia due to vitamin D deficiency (18, 33, 34).

3-3. Density fluctuation of bone in cystic fibrosis

There are many important factors for lifetime bone health, which are established during infancy, childhood, and adolescence and require sufficient nutrition, normal body mass, physical exercise and normal hormone production (22, 35). Puberty is a specially required process when growth and mineral accrual are advanced (12, 19). These important factors may be compromised in cystic fibrosis patients who suffer from decreased pubertal growth and delayed puberty. In this account, a study of pubertal children with cystic fibrosis confirmed that insufficient bone mineral amount was found to be lower than the expected bone mineral density and volumetric bone mineral density (36). One study acknowledged this result by illustrating femur, total body, wrist mineral density and spine bone mineral density in children aged 5–10 years with cystic fibrosis, but total body and distal wrist mineral density in middle-aged people was significantly decreased (11, 37). In fact, these remarkable findings and results must be considered because there is restricted normative bone mineral density data during primary period of life (38, 39). Moreover, the majority of patients with low bone mineral density of cystic fibrosis are adults. The obtained data from the adult and pediatric searches concluded that low bone mineral density results from insufficient bone deposition during puberty stage (16, 40).

3-4. Intake fluctuation of bone in cystic fibrosis

Investigations and searches of biochemical markers for bone situation recommend an imbalanced bone intake in cystic fibrosis cases that are clinically stable (41). Accelerated bone intake was initially reported and also the high urinary hydroxyproline levels were also reported (42, 43) (**Figure.2**). The results indicated lower levels of serum osteocalcin in pubertal children and also young adults. Remarkably, there are different formation markers in this case (44-46).

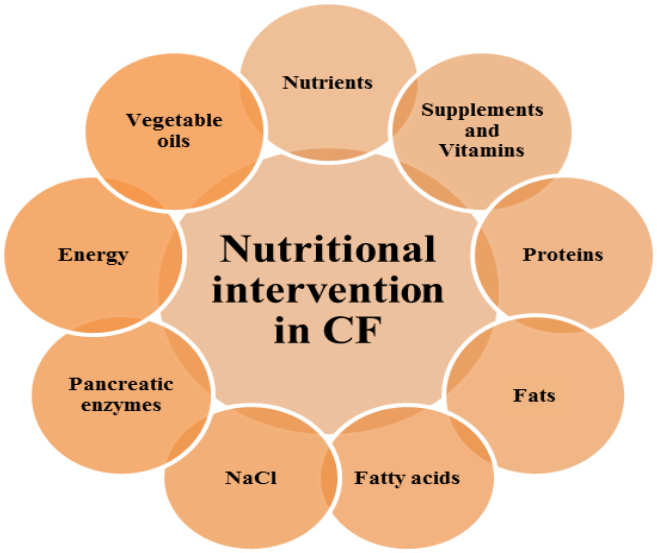

Fig.2: Nutritional main factors can also show their effects in cystic fibrosis.

3-5. Pathophysiology of bone disease in CF

Besides the genetic factors, major factors that are involved in pathobiology of cystic fibrosis can be environmental factors like pancreatic exocrine insufficiency and also malabsorption, vitamin D insufficiency, pancreatic endocrine insufficiency, physical activity, delayed puberty or early gonadal failure, chronic infection, hormonal alterations like glucocorticoids and lung transplantation and immunosuppressant therapy (47, 48). In pancreatic exocrine insufficiency and malabsorption, optimal nutrient intake is necessary to maintain sufficient body and bone mass health. Evidently, malnutrition is a common complication of CF (49). This implies that malabsorption is associated with the catabolic process occurring with severe lung infections (25, 50). In this way, one of the most important risk factors that increases the severity of the disease and its complications is BMI. Interestingly, many researches have indicated a correlation between malnutrition and low bone mineral density in cystic fibrosis (**Figure.3**).

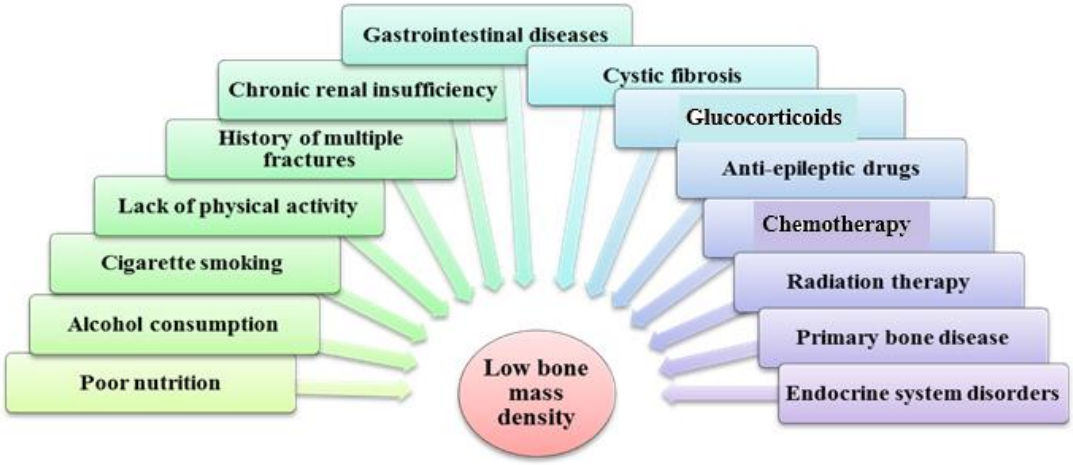

Fig.3: Risk factors that are involved directly in low bone mass density.

There is considerable evidence that acknowledges and confirms vitamin D insufficiency, both low or low normal 25hydroxyvitamin D levels, is a common event among people with cystic fibrosis (51). Compared to normal people in whom vitamin D concentration is approximately 20 ng ml^{-1}, most people with cystic fibrosis have low concentration levels of vitamin D. Evidently, at the final stage of cystic fibrosis, vitamin D deficiency is the most common clinical trait and may happen in 25–35% of patients (52). Nevertheless, the cause of vitamin D insufficiency in patients with cystic fibrosis has not been sufficiently investigated and it seems essential to study the causes and factors responsible for this symptom. For pancreatic endocrine insufficiency, we can focus on diabetes function. Diabetes mellitus has been associated with decreased trabecular bone mass and it is developed in 10% of people with cystic fibrosis (53). Cystic fibrosis associated diabetes may differ in type I and II diabetes. The abnormalities in glucose metabolism result from chronic infection and the inadequate formation of islet cells. In addition, chronic infection leading to insulin resistance may play a key role in reducing the bone mineral density. Besides the main mentioned factors, physical inactivity is of great importance. Cystic fibrosis may lead to a sedentary life style because of decreased lung function and chronic pulmonary infection (54).

Correspondingly, steroid deficiency due to delayed puberty or early hypogonadism certainly supports cystic fibrosis associated bone disease (55). Pubertal delay in cystic fibrosis was identified many years ago and is associated with disease status. For chronic infection, bone remodeling is under the impact of normal hormones, cytokines, and also localized main growth factors. Many factors in the serum and also respiratory system of people with cystic fibrosis can provoke osteoclastic bone intake, comprising parathyroid hormone (PTH), tumor necrosis factor α (TNF-α) and growth factor (10). Apparently, many inflammatory cytokines exist in very high concentrations in infected cystic fibrosis lungs and also serum. For glucocorticoids, a measured range of about 25-50% of people with cystic fibrosis are treated and cured with exogenous glucocorticoids for the progress of pulmonary activity (56). Interestingly, many researches and studies have discovered glucocorticoid therapy to be a main risk factor for low bone mass in cystic fibrosis (57). The relationship between corticosteroid application and bone mineral density is confused with disease severity. Lung transplantation and Immunosuppressive therapy are also of great significance. They are used in people with cystic fibrosis in order to extend the survival rate and improve the quality of life (58). Further, immunosuppression is obligatory after transplantation and will aggravate the former low bone mineral density.

3-6. Genetic approach to cystic fibrosis

The specific gene in cystic fibrosis is *CFTR* which was localized to 7q21-34 on the long arm of chromosome 7 (Genomic location) (59-61). The gene encodes a 1480 amino acid protein which has been called the cystic fibrosis transmembrane conductance regulator or, *CFTR* gene (62-64). Remarkably, many cystic fibrosis mutations have been reported (approximately, 1500) and many are rare, while some may not have any clinical symptoms. Different mutations influence the function and activity of *CFTR* gene in different pathways. This implies that some influence the abnormal *CFTR* gene proliferation and others influence the intracellular processing of *CFTR* gene and cellular activity (65-67). According to the above explanation, there are different mutations in *CFTR* gene and the most common mutation is delta F508 which occurs in approximately 75% of people with cystic fibrosis (68-70). In this way, a mutant *CFTR* protein, which is not able to fold into its proper form, is produced (71-73).

3-7. Inheritance and risk of passing on the cystic fibrosis

Cystic fibrosis has a simple Mendelian autosomal recessive inheritance. This implies that people with cystic fibrosis have duplicates of the mutant *CFTR* gene. Noticeably, carriers have different situations (one normal and one mutant *CFTR* gene), and have no health problems, because the *CFTR* gene in normal position produces sufficient protein to permit normal cellular activity. Carriers have a 50% chance of transmitting the mutant *CFTR* gene to their children (74). When parents are carriers, there is about 25% chance in every normal pregnancy that the child will have cystic fibrosis and also a 25% chance that the child will have normal *CFTR* genes and 50% chance that the child will be a carrier.

4- CONCLUSION

According to many obtained results and findings, the direct correlation of low mineral bone density in cystic fibrosis is recognized. Low bone mineral density kyphosis and also fragility fractures are the most common and routine complications in adults which are extended from childhood. Inflammation, inactivity, malnutrition, medications, and hormone

deficits have all been implicated as the most common risk factors for decreased bone mineral loss. The presence of these main factors makes it difficult to realize the assistance of each variable. Correspondingly, bone mineral density is significantly decreased in adult patients with CF and is associated with markers of disease severity. Moreover, the level of vitamin D supplementation seems to be insufficient and the differences in bone fluctuation between delta F508 homozygotes and non-homozygotes suggest that there may be a genetic correlation to the cause of low bone mineral density in adults with CF. New studies are required to better define the compatible nutritional supplementation and practical programs necessary to avoid poor bone health condition in CF patients, particularly for people with nutritional deficits and also severe pulmonary problems, who obviously need to decrease the risk of bone problems and also reduce fragility fractures.

5- CONFLICT OF INTEREST: None.

6- REFERENCES

1. Shale D. Predicting survival in cystic fibrosis. Thorax. 1997;52(4):309.

2. Haworth C, Selby P, Webb A, Adams J. Osteoporosis in adults with cystic fibrosis. Journal of the Royal Society of Medicine. 1998;91(34_suppl):14-8.

3. Aris RM, Renner JB, Winders AD, Buell HE, Riggs DB, Lester GE, et al. Increased rate of fractures and severe kyphosis: sequelae of living into adulthood with cystic fibrosis. Annals of Internal Medicine. 1998;128(3):186-93.

4. Henderson RC, Specter BB. Kyphosis and fractures in children and young adults with cystic fibrosis. The Journal of pediatrics. 1994;125(2):208-12.

5. Ratjen F, Klingel M, Black P, Powers MR, Grasemann H, Solomon M, et al. Changes in Lung Clearance Index in Preschool Cystic Fibrosis Treated with Ivacaftor (GOAL): A Clinical Trial. Am J Respir Crit Care Med. 2018; 198(4):526-528.

6. Cirilli N, Raia V, Rocco I, De Gregorio F, Tosco A, Salvadori L, et al. Intra-individual biological variation in sweat chloride concentrations in CF, CFTR dysfunction, and healthy pediatric subjects. Pediatric pulmonology. 2018;53(6):728-34.

7. Crowley EM, Bosslet GT, Khan B, Ciccarelli M, Brown CD. Impact of social complexity on outcomes in cystic fibrosis after transfer to adult care. Pediatric pulmonology. 2018;53(6):735-40.

8. Everitt BS. Statistical methods for medical investigations: Edward Arnold London; 1994.

9. Cummings SR, Browner W, Black D, Nevitt M, Genant H, Cauley J, et al. Bone density at various sites for prediction of hip fractures. The Lancet. 1993;341(8837):72-5.

10. Donovan Jr DS, Papadopoulos A, Staron RB, Addesso V, Schulman L, McGREGOR C, et al. Bone mass and vitamin D deficiency in adults with advanced cystic fibrosis lung disease. American journal of respiratory and critical care medicine. 1998;157(6):1892-99.

11. Bhudhikanok GS, Lim J, Marcus R, Harkins A, Moss RB, Bachrach LK. Correlates of osteopenia in patients with cystic fibrosis. Pediatrics. 1996;97(1):103-11.

12. Grey A, Ames R, Matthews R, Reid I. Bone mineral density and body composition in adult patients with cystic fibrosis. Thorax. 1993;48(6):589-93.

13. Haworth C, Selby P, Webb A, Dodd M, Musson H, Niven RM, et al. Low bone mineral density in adults with cystic fibrosis. Thorax. 1999;54(11):961-7.

14. Gaudelus IS, Souberbielle JC, Ruiz JC, Vrielynck S, Heuillon B, Azhar I, et al. Low Bone Mineral Density in Young Children with Cystic Fibrosis. American Journal of Respiratory and Critical Care Medicine. 2007;175:951–7.

15. Wahab AA, Hammoudeh M, Allangawi M, Al-Khalaf F, Chandra P. Bone Mineral Density in Cystic Fibrosis Patients with the CFTR I1234V Mutation in a Large Kindred Family Is Associated with Pancreatic

Sufficiency. International Journal of Rheumatology. 2014;2014:1-6.

16. Hardin D, Arumugam R, Seilheimer D, LeBlanc A, Ellis K. Normal bone mineral density in cystic fibrosis. Archives of disease in childhood. 2001;84(4):363-8.

17. Selby PL, Davies M, Adams JE, Mawer EB. Bone loss in celiac disease is related to secondary hyperparathyroidism. Journal of Bone and Mineral Research. 1999;14(4):652-7.

18. Gibbens DT, Gilsanz V, Boechat MI, Dufer D, Carison ME, Wang C-I. Osteoporosis in cystic fibrosis. The Journal of pediatrics. 1988;113(2):295-300.

19. Shaw N, Bedford C, Heaf D, Carty H, Dutton J. Osteopenia in adults with cystic fibrosis. The American journal of medicine. 1995;99(6):690-1.

20. Corey M, McLaughlin F, Williams M, Levison H. A comparison of survival, growth, and pulmonary function in patients with cystic fibrosis in Boston and Toronto. Journal of clinical epidemiology. 1988;41(6):583-91.

21. Thomas MK, Lloyd-Jones DM, Thadhani RI, Shaw AC, Deraska DJ, Kitch BT, et al. Hypovitaminosis D in medical inpatients. New England Journal of Medicine. 1998;338(12):777-83.

22. Bachrach LK, Loutit CW, Moss RB, Marcus R. Osteopenia in adults with cystic fibrosis. The American journal of medicine. 1994;96(1):27-34.

23. Stead R, Houlder S, Agnew J, Thomas M, Hodson M, Batten J, et al. Vitamin D and parathyroid hormone and bone mineralisation in adults with cystic fibrosis. Thorax. 1988;43(3):190-4.

24. Hanly J, McKenna M, Quigley C, Freaney R, Muldowney F, FitzGerald M. Hypovitaminosis D and response to supplementation in older patients with cystic fibrosis. QJM: An International Journal of Medicine. 1985;56(1):377-85.

25. Baroncelli GI, De Luca F, Magazzú G, Arrigo T, Sferlazzas C, Catena C, et al. Bone demineralization in cystic fibrosis: evidence of imbalance between bone formation and degradation. Pediatric Research. 1997;41(3):397.

26. Haworth CS, Selby PL, Webb AK, Mawer EB, Adams JE, Freemont TJ. Severe bone pain after intravenous pamidronate in adult patients with cystic fibrosis. The Lancet. 1998;352(9142):1753-54.

27. Bethesda M. Cystic fibrosis foundation registry data annual report. Cystic fibrosis foundation national cystic fibrosis patient registry 2001. Annual data report. 2002.

28. Mischler EH, Chesney PJ, Chesney RW, Mazess RB. Demineralization in cystic fibrosis: Detected by direct photon absorptiometry. American Journal of Diseases of Children. 1979;133(6):632-5.

29. Hahn TJ, Squires AE, Halstead LR, Strominger DB. Reduced serum 25-hydroxyvitamin D concentration and disordered mineral metabolism in patients with cystic fibrosis. The Journal of pediatrics. 1979;94(1):38-42.

30. Elkin S, Fairney A, Burnett S, Kemp M, Kyd P, Burgess J, et al. Vertebral deformities and low bone mineral density in adults with cystic fibrosis: a cross-sectional study. Osteoporosis International. 2001;12(5):366-72.

31. Henderson RC, Madsen CD. Bone mineral content and body composition in children and young adults with cystic fibrosis. Pediatric pulmonology. 1999;27(2):80-4.

32. Moran C, Sosa E, Martinez S, Geldren P, Messina D, Russo A, et al. Bone mineral density in patients with pancreatic insufficiency and steatorrhea. American Journal of Gastroenterology. 1997;92(5).

33. Shane E, Silverberg SJ, Donovan D, Papadopoulos A, Staron RB, Addesso V, et al. Osteoporosis in lung transplantation candidates with end-stage pulmonary disease. The American Journal of Medicine. 1996;101(3):262-9.

34. Tschopp O, Boehler A, Speich R, Weder W, Seifert B, Russi EW, et al. Osteoporosis before lung transplantation: association with low body mass index, but not with underlying disease. American journal of transplantation. 2002;2(2):167-72.

35. Conway S, Morton A, Oldroyd B, Truscott J, White H, Smith A, et al. Osteoporosis and osteopenia in adults and

adolescents with cystic fibrosis: prevalence and associated factors. Thorax. 2000;55(9):798-804.

36. Rochat T, Slosman DO, Pichard C, Belli DC. Body composition analysis by dual-energy x-ray absorptiometry in adults with cystic fibrosis. Chest. 1994;106(3):800-5.

37. Henderson RC, Madsen CD. Bone density in children and adolescents with cystic fibrosis. The Journal of pediatrics. 1996;128(1):28-34.

38. Bhudhikanok GS, Wang M-C, Marcus R, Harkins A, Moss RB, Bachrach LK. Bone acquisition and loss in children and adults with cystic fibrosis: a longitudinal study. The Journal of pediatrics. 1998;133(1):18-27.

39. Salamoni F, Roulet M, Gudinchet F, Pilet M, Thiebaud D, Burckhardt P. Bone mineral content in cystic fibrosis patients: correlation with fat-free mass. Archives of disease in childhood. 1996;74(4):314-8.

40. Humphries I, Allen J, Waters D, Howman-Giles R, Gaskin K. Volumetric bone mineral density in children with cystic fibrosis. Applied radiation and isotopes: including data, instrumentation and methods for use in agriculture, industry and medicine. 1998;49(5-6):593-5.

41. Laursen E, Mølgaard C, Michaelsen K, Koch C, Müller J. Bone mineral status in 134 patients with cystic fibrosis. Archives of disease in childhood. 1999;81(3):235-40.

42. Sood M, Hambleton G, Super M, Fraser W, Adams J, Mughal M. Bone status in cystic fibrosis. Archives of disease in childhood. 2001;84(6):516-20.

43. Elkin SL, Vedi S, Bord S, Garrahan NJ, Hodson ME, Compston JE. Histomorphometric analysis of bone biopsies from the iliac crest of adults with cystic fibrosis. American journal of respiratory and critical care medicine. 2002;166(11):1470-74.

44. Haworth CS, Webb AK, Egan JJ, Selby PL, Hasleton PS, Bishop PW, et al. Bone histomorphometry in adult patients with cystic fibrosis. Chest. 2000;118(2):434-9.

45. Bachrach LK. Acquisition of optimal bone mass in childhood and adolescence. Trends in Endocrinology & Metabolism. 2001;12(1):22-8.

46. Bailey D, McKay H, Mirwald R, Crocker P, Faulkner R. A six-year longitudinal study of the relationship of physical activity to bone mineral accrual in growing children: the university of Saskatchewan bone mineral accrual study. Journal of bone and mineral research. 1999;14(10):1672-79.

47. Mortensen LA, Chan GM, Alder SC, Marshall BC. Bone mineral status in prepubertal children with cystic fibrosis. The Journal of pediatrics. 2000;136(5):648-52.

48. Buntain H, Greer RM, Schluter P, Wong J, Batch J, Potter J, et al. Bone mineral density in Australian children, adolescents and adults with cystic fibrosis: a controlled cross sectional study. Thorax. 2004;59(2):149-55.

49. Haworth C, Selby P, Horrocks A, Mawer E, Adams J, Webb A. A prospective study of change in bone mineral density over one year in adults with cystic fibrosis. Thorax. 2002;57(8):719-23.

50. Aris R, Lester G, Camaniti M, Hensler M, Lark R, Blackwood A, et al. Alendronate for cystic fibrosis adults with low bone density: results of a randomized, controlled trial. Am J Respir Crit Care Med. 2004;169:77-82.

51. Aris R, Ontjes D, Buell H, Blackwood A, Lark R, Caminiti M, et al. Abnormal bone turnover in cystic fibrosis adults. Osteoporosis International. 2002;13(2):151-7.

52. De Schepper J, Smitz J, Dab I, Piepsz A, Jonckheer M, Bergmann P. Low serum bone gamma-carboxyglutamic acid protein concentrations in patients with cystic fibrosis: correlation with hormonal parameters and bone mineral density. Hormone Research in Paediatrics. 1993;39(5-6):197-201.

53. Schulze KJ, O'brien KO, Germain-Lee EL, Booth SL, Leonard A, Rosenstein BJ. Calcium kinetics are altered in clinically stable girls with cystic fibrosis. The Journal of Clinical Endocrinology & Metabolism. 2004;89(7):3385-91.

54. Borowitz D, Baker RD, Stallings V. Consensus report on nutrition for pediatric patients with cystic fibrosis. Journal of pediatric gastroenterology and nutrition. 2002;35(3):246-59.

55. Ott SM, Aitken ML. Osteoporosis in patients with cystic fibrosis. Clinics in chest medicine. 1998;19(3):555-67.

56. Lark RK, Lester GE, Ontjes DA, Blackwood AD, Hollis BW, Hensler MM, et al. Diminished and erratic absorption of ergocalciferol in adult cystic fibrosis patients–. The American journal of clinical nutrition. 2001;73(3):602-6.

57. Cohn JA, Friedman KJ, Noone PG, Knowles MR, Silverman LM, Jowell PS. Relation between mutations of the cystic fibrosis gene and idiopathic pancreatitis. New England Journal of Medicine. 1998;339(10):653-8.

58. Davies P, Drumm M, Konstan M. State of the art: Cystic fibrosis. Am J Respir Crit Care Med. 1996;154:1229-56.

59. Dumur V, Gervais R, Rigot J-M, Lafitte J-J, Manouvrier S, Biserte J, et al. Abnormal distribution of CF ΔF508 allele in azoospermic men with congenital aplasia of epididymis and vas deferens. The Lancet. 1990;336(8713):512.

60. Gairdner D. Heterozygote advantage in cystic fibrosis. The Lancet. 1975;305(7901):279.

61. Gan K-H, Veeze HJ, van den Ouweland A, Halley D, Scheffer H, van der Hout A, et al. A cystic fibrosis mutation associated with mild lung disease. New England Journal of Medicine. 1995;333(2):95-9.

62. Jorde L, Lathrop G. A test of the heterozygote-advantage hypothesis in cystic fibrosis carriers. American journal of human genetics. 1988;42(6):808.

63. Kalman YM, Kerem E, Darvasi A, DeMarchi J, Kerem B. Difference in frequencies of the cystic fibrosis alleles,Δ F508 and W1282X, between carriers and patients. European Journal of Human Genetics. 1994;2:77-82.

64. Kerem B-s, Rommens JM, Buchanan JA, Markiewicz D, Cox TK, Chakravarti A, et al. Identification of the cystic fibrosis gene: genetic analysis. Science. 1989;245(4922):1073-80.

65. Kiesewetter S, Macek M, Davis C, Curristin S, Chu C-S, Graham C, et al. A mutation in CFTR produces different phenotypes depending on chromosomal background. Nature genetics. 1993;5(3):274-8.

66. Massie R, Poplawski N, Wilcken B, Goldblatt J, Byrnes C, Robertson C. Intron-8 polythymidine sequence in Australasian individuals with CF mutations R117H and R117C. European Respiratory Journal. 2001;17(6):1195-200.

67. Peckham D, Conway S, Morton A, Jones A, Webb K. Delayed diagnosis of cystic fibrosis associated with R117H on a background of 7T polythymidine tract at intron 8. Journal of Cystic Fibrosis. 2006;5(1):63-5.

68. Pritchard DJ. Cystic fibrosis allele frequency, sex ratio anomalies and fertility: a new theory for the dissemination of mutant alleles. Human genetics. 1991;87(6):671-6.

69. Rave-Harel N, Kerem E, Nissim-Rafinia M, Madjar I, Goshen R, Augarten A, et al. The molecular basis of partial penetrance of splicing mutations in cystic fibrosis. American journal of human genetics. 1997;60(1):87.

70. Riordan JR, Rommens JM, Kerem B-s, Alon N, Rozmahel R, Grzelczak Z, et al. Identification of the cystic fibrosis gene: cloning and characterization of complementary DNA. Science. 1989;245(4922):1066-73.

71. Rommens JM, Iannuzzi MC, Kerem B-s, Drumm ML, Melmer G, Dean M, et al. Identification of the cystic fibrosis gene: chromosome walking and jumping. Science. 1989;245(4922):1059-65.

72. Rosenstein BJ, Cutting GR. The diagnosis of cystic fibrosis: a consensus statement. The Journal of pediatrics. 1998;132(4):589-95.

73. Wainwright BJ, Scambler PJ, Schmidtke J, Watson EA, Law H-Y, Farrall M, et al. Localization of cystic fibrosis locus to human chromosome 7cen–q22. Nature. 1985;318(6044):384.

74. Zielenski J, Tsui L-C. Cystic fibrosis: genotypic and phenotypic variations. Annual review of genetics. 1995;29(1):777-807.