Fibroblast Growth Factor-23 (FGF-23)
In Late Rickets

Thesis Submitted for

Partial Fulfillment of Master Degree in Pediatrics

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Introduction

Vitamin D deficiency is highly prevalent among children and adolescents worldwide. The high rates of vitamin D deficiency during childhood are of major puplic health relevance, given the growing evidence that vitamin D deficiency may play a key role in the pathophysiology of many chronic diseases beyond rickets. Identification, treatment, and prevention of vitamin D deficiency in childhood may therefore have profound health effects throughout the life span ***(Huh and Gordon, 2008)***.

Fibroblast growth factor-23(FGF-23), a novel member of the FGF family, is the product of the gene mutated in autosomal dominant hypophosphatemic rickets (ADHR). The tissue with the highest level of FGF-23 expression is bone (osteocytes and osteoblasts), where it is highly expressed during phases of active bone remodeling ***(Riminucci et al., 2003*)**.

Endo et al., (2008) reported that FGF-23 plays important roles in the development of hypophosphatemic diseases such as tumor–induced osteomalacia (TIO) and X-linked hypophosphatemic rickets/osteomalacia (XLH), relationship between phosphate and FGF-23 indicated that TIO and XLH are diseases with high FGF-23 and hypophosphatemia judged by age-dependant reference ranges for serum phosphate. High FGF23 with low phosphate judged by age-dependent reference range for phosphate establishes the diagnosis of diseases caused by excess actions of FGF-23.

Aim of the work

This study was designed to assess the FGF-23 level in patients with late rickets and correlate any changes found with the clinical and laboratory parameters of the patients.

Patients and methods

The study will be conducted on 35 patients suffering from vitamin D deficiency rickets recruited from the pediatric outpatient clinic of the National Institute of Neuromuscular System, according to the following inclusion criteria:-

1- Age above two years.

2- Free of any chronic liver or kidney diseases and not on any medication.

3- No previous history of vitamin D supplementation whether prophylactic or therapeutic.

After obtaining a verbal consent from the parents, each patient will be subjected to the following:

a) Full history taking with special emphasis on dietetic history, sunlight exposure, history of previous infections and developmental history.

b) Clinical examination laying stress on the signs and complications of rickets as well as the anthropometric measurement.

c) Plain X-ray of the knees & ankles joints (A. P) view.

d) Laboratory investigations including:-

S. calcium

S. phosphorus

S. alkaline phosphatase

complete blood count (CBC)

S. creatinine

S. ALT

e) Serum FGF23 assay by Western Blot technique.

The patients will be compared to a cohort of fifteen healthy age &sex matched controls.

The data of the patient will be collected, analyzed using the appropriate statistical methods. The results will be tabulated and the demonstrative figures will be drawn.

References

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VITAMIN D

Vitamins are chemicals other than proteins, carbohydrates, fats and mineral salts that are essential constituents of the food. Small amounts of it are essential for the regulation of all body processes, with the exception of vitamin D. The human body cannot make its own vitamins and some cannot be stored, therefore a person's diet must provide all the necessary vitamins. Sunlight activates the metabolisim of vitamin D in the body ***(Harrison, 1997).***

Vitamin D is a group of fat-soluble prohormones, meaning that it has no hormone activity itself, but as converted to the active hormone 1,25 dihydroxy vitamin D ($1,25(OH)_2D$) through a tightly regulated synthesis mechanism ***(Norman,, 1998).***

Biochemistry:

Chemically, the various forms of vitamin D are secosteroids i.e. broken open steroids. Vitamin D refers to two biologically inactive precursors, D3 also known as cholicalciferol and D2 also known as ergocalciferol. The structural difference between vitamin D2 and vitamin D3 is in their side chains. The side chain of vitamin D2 contains a double bond between carbons 22 and 23 and a methyl group on carbon 24. Vitamin D2 is derived from fungal and plant sources. It

only enters the body via diet from consumption of foods such as oily fish, egg yolk and liver. Vitamin D3 is derived from animal sources and made in skin on exposure to UV-B radiation (290-320).Both D3 and D2 precursors are hydroxylated in the liver to form 25(OH) D (the non active storage form), then goes to the kidney to form $1,25(OH)_2$ D, the biologically active form of vitamin D which is tightly controlled by the body ***(Botella-Carretero et al., 2007).***

The two forms have traditionally been regarded as equivalent based on their ability to cure rickets, but evidence has been offered that they are metabolized differently. Vitamin D3 could be more than three times as effective as vitamin D2 in raising serum 25(OH) D concentrations and maintaining those levels for a longer time and its metabolites have superior affinity for vitamin D-binding proteins (VDBP) in plasma ***(Armas et al., 2004).***

Both forms (as well as vitamin D in foods and from cutaneous synthesis) effectively raise serum 25(OH) D levels ***(Cranney et al., 2007).***

Many supplements are being reformulated to contain vitamin D3 instead of vitamin D2 ***(Houghton and Vieth, 2006).***

Several forms (vitamers) of vitamin D other than vitamin D2 and vitamin D3 have been discovered and they are:

- Vitamin D1: molecular compound of ergocalciferol with lumisterol 1:1.
- Vitamin D4: 22-dihydroergocalciferol.
- Vitamin D5: sitocalciferol (made from 7-dihydrositosterol)

(Dorland, 2007)

Structure and Synthesis:

The term vitamin D is, unfortunately, an imprecise term referring to one or more members of a group of steroid molecules. Vitamin D3, also known as cholecalciferol is generated in the skin of animals when light energy is absorbed by a precursor molecule 7-dehydrocholesterol. Vitamin D is thus not a true vitamin, because individuals with adequate exposure to sunlight do not require dietary supplementation. Vitamin D, as either D3 or D2, does not have significant biological activity. Rather, it must be metabolized within the body to the hormonally-active form known as $1,25(OH)_2D$ **(Fig. 1).** This transformation occurs in two steps:

1. Within the liver, cholecalciferal is hydroxylated to 25(OH) D by the enzyme 25-hydroxylase.

2. Within the kidney, 25(OH) D serves as a substrate for 1-alpha-hydroxylase, yielding $1,25(OH)_2D3$, the biologically active form ***(Bowen, 2007).***

Each of the forms of vitamin D is hydrophobic, and is transported in blood bound to carrier proteins. The major carrier is called appropriately VDBP. The half life of 25 (OH) D is several weeks, while that of $1,25(OH)_2$ D is only a few hours. Hepatic synthesis of 25(OH) D is only loosely regulated, and blood levels of this molecule largely reflect the amount of vitamin D produced in the skin or ingested. In contrast, the activity of 1-alpha-hydroxylase in the kidney is tightly regulated and serves as the major control point in production of the active hormone. The major inducer of 1-alpha-hydroxylase is parathyroid hormone (PTH); it is also induced by low blood levels of phosphate ***(Bowen, 2007).***

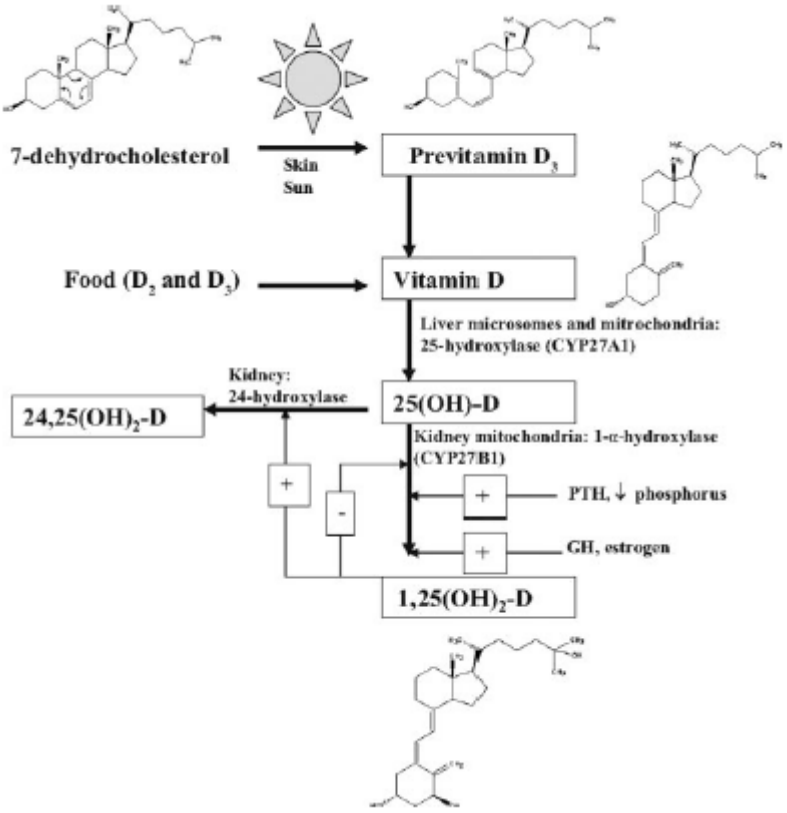

Fig (1): Vitamin D synthesis and processing ***(Misra al., 2008).***

Mechanism of action:

The physiologically active form of vitamin D is then released in the circulation and by binding to a carrier protein in the plasma VDBP; it is transported to various target organs. The hormonally active form of vitamin D mediates its biological effects by binding to the vitamin D receptor (VDR), which is principally located in the nuclei of target cells. The binding of active form of vitamin D to the VDR allows the

VDR to act as a transcription factor that modulates the gene expression of transport proteins (Such as TRPV6 and calbindin), which is involved in calcium absorption in the intestine. The vitamin D receptor belongs to the nuclear receptor super family of steroid/thyroid hormone receptors and VDR are expressed by cells in most of the organs including brain, heart, skin, gonads, prostate and breast. VDR activation in the intestine, bone, kidney and parathyroid gland leads to the maintenance of calcium and phosphorous levels in the blood with the assistance of PTH and calcitonin and to the maintenance of bone content ***(Holick, 2004).***

Biological actions of vitamin D (Fig. 2):

1. The vitamin D endocrine system is an essential component of the interactions among the kidney, bone, parathyroid gland and intestine that maintain extracellular calcium levels within narrow limits, a process vital for normal cellular physiology and skeletal integrity. 1,25$(OH)_2$D3 produced in the kidneys induces intestinal calcium absorption, controls bone remodeling, suppresses parathyroid function (PTH synthesis and cell growth) and induces renal calcium reabsorption to maintain calcium in the extracellular fluid within the narrow limits essential for normal cell physiology and skeletal integrity. Vitamin D is essential to enhance the efficiency of the small intestine to absorb dietary calcium and phosphate ***(Bouillon et al., 2003).***

2. It inhibits PTH secretion from parathyroid gland, as vitamin D deficiency results in parathyroid hyperplasia and increased PTH synthesis and secretion ***(Dusso et al., 2004).***

3. The most important endocrine effect of $1,25(OH)_2D3$ in the kidney is a tight control of its own homeostasis through simultaneous suppression of 1-alfa-hydroxylase and stimulation of 24-hydroxylase ***(Li et al., 1998).***

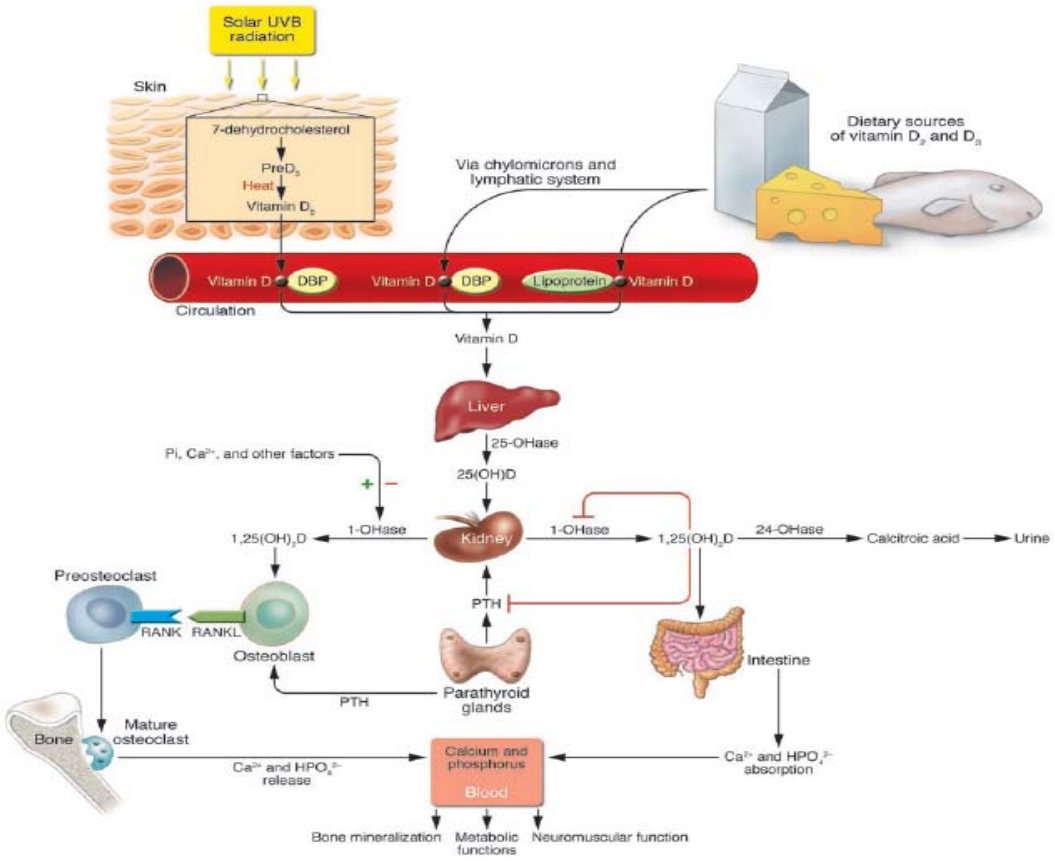

Fig (2): The various biologic effects of 1,25(OH)2D on calcium, phosphorus, and bone metabolism ***(Holick, 2006)***.

Non classic actions (Fig. 3):

1. Vitamin D affects the immune system by promoting phagocytosis, anti-tumor activity and immunomodulatory functions ***(Johnson, 2007).***

2. Vitamin D regulates the expression of genes associated with cancers and autoimmune disease by controlling the activation of the VDR, a type 1 nuclear receptor and DNA transcription factor ***(Marshall, 2008).***

Research has indicated that vitamin D deficiency is linked to colon cancer and more recently to breast cancer. Conflicting evidence links vitamin D deficiency to other forms of cancers (***The American Society of Clinical Oncology's annual meeting, 2008).***

3. Control of differentiation and function in the skin: Vitamin D was used to treat a variety of skin diseases including psoriasis. A dramatic improvement was seen in psoriatic lesions in a patient receiving oral $1,25(OH)_2$ D3 to treat severe osteoporosis ***(Morimoto and Kumahara, 1985).***

4. As $1,25(OH)_2$ D3 has antiproliferative properties in psoriatic keractinocytes over expressing tumor growth factor by inhibiting mitogenic signals coming from it. The immunosuppressive properties of $1,25(OH)_2$ D3 on langerhans cells, the antigen-presenting cells of the epidermis, could also mediate efficacy of sterol in the treating of