The Endocrine System

PHARMACOLOGY

☑ Sheet ☐ Slide ☐ Handout

Number:	6
Subject:	Agents that Affect Bone & Mineral Homeostasis
Done By:	Shahd Rihan.
Corrected by:	Mohammad Qussay Al-Sabbagh
Doctor:	Yacoub Irshaid.

Date: 00/0/2016

Price:

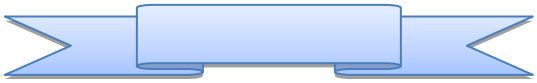
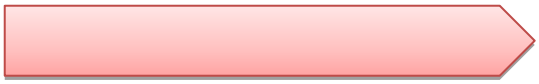
Before starting

1. Watch this video : https://www.youtube.com/watch?v=0vDAEX1LCbY ; in order to rise your energy , it is very beautiful video ☺
   (start from 0:15 & skip this part (3:31 – 4:41))
   Sorry for non-Arabic students; the video in Arabic!!

2. In this sheet I am going to give my feedback about previously written sheets , simply by using certain characteristics that I like it in others sheets & improving certain characteristics according to my point of view .

3. Be aware while studying this sheet & if you find any mistake , **please** correct it.

* I used **specific characteristics** to give specific meanings as presented in this table:

	**Main topic**
	**Subtopic**
	**related to the figures**
	**Note**
	**Drug**
Brown color	**Extra information that I added to facilitate understanding of the topic**
Blue color	**The information that are not included in the exam , but the doctor explained them (Dr. advised us to study them for our knowledge)**

* **References:**

- Record section 2.
- Slide_4 : Bone and Mineral Homeostasis … (from slide # 1 to slide # 32).
- Basic & Clinical Pharmacology - BG Katzung, SB Masters, AJ Trevor - 13th edition pages (747-752).
- Different websites.

* **Topics** :

1. General introduction & explanation of figures
2. Principal hormonal regulators of bone mineral homeostasis :
   >> PTH
   >>Vitamin D
   >> FGF23
3. Interaction of PTH, FGF23 & Vitamin D

Sorry for this long introduction .. Let's start ☺

* **Abbreviations** used in this sheet :

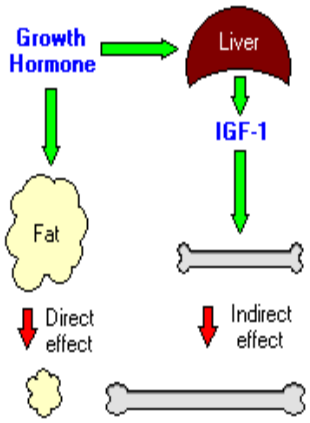
PTH	Parathyroid hormone
FGF23	Fibroblast growth factor 23
CT	Calcitonin
OPG	Osteoprotegrin
RANK	Receptor for Activation of Nuclear factor Kappa B ($_{K}$B)
RANKL	RANK Ligand
CaSR	Calcium-Sensing Receptor
VDR	Vitamin D receptor
DBP	Vitamin D-binding protein
GH	Growth hormone
IGF-1	Insulin-like growth factor-1
DMP	dentin matrix protein

Note : this page I add it to facilitate the understanding of certain ideas.

❖ Direct vs indirect effect

Let's take GH as an example :

Direct effects are the result of growth hormone binding its receptor on target cells. Fat cells (adipocytes), for example, have growth hormone receptors, and growth hormone stimulates them to break down triglyceride and suppresses their ability to take up and accumulate circulating lipids.



Indirect effects are mediated primarily by an insulin-like growth factor-I (IGF-I), a hormone that is secreted from the liver and other tissues in response to growth hormone.

❖ Vitamin D (Hormone OR vitamin !)

Vitamin D isn't like most other vitamins. Your body can make its own vitamin D when you expose your skin to sunlight. But your body can't make other vitamins. You need to get other vitamins from the diet. For example, you need to get vitamin C from fruits and vegetables.

Also what makes vitamin D unique compared to other vitamins, is that when your body gets its vitamin D, it turns vitamin D into a **hormone**. This hormone is sometimes called "activated vitamin D" or "calcitriol."

* To know more about Vitamin D as a hormone read this article :

http://www.hormone.org/hormones-and-health/what-do-hormones-do/vitamin-d

* To know the differences between vitamin & hormone read this article :

http://www.preservearticles.com/201101103021/difference-between-vitamin-and-hormone.html

Let's start the record ☺

This lecture & the next one is about the homeostasis of Ca & P

This subject is complex and interrelated , so don't confuse that there are oppositions in it.

There is a **direct** & **indirect** effect of the hormone , the indirect effect may be in the same direction of the direct effect or opposite to it but through other type of mechanism , the most important thing to be obvious is the net effect .

Regulation of these minerals happens by 2 kinds of regulators :

- Primary (1^{o}) >> the main hormones that affects the minerals.
- Secondary (2o)
  - many regulators
  - their effect is **minor**
  - only important at **pharmacological doses** so they can be used for treatment of certain diseases.

The 3 **principle regulators** are :

PTH Vitamin D FGF23

need to be in **active form** which is :
1,25-dihydroxyvitamin D ($1,25(OH)_2D$)

- These 3 hormones has **distinct** effects & **similar** effects which means there are similarities & differences between them and there is **interrelation** between them (i.e . each one of them affect the other 2 regulators)

Dr.Yacoub advise us to pay attention to this topic , read it slowly with understanding & if we find any opposition we should look for the causations.

The next 2 graphs summarize the lecture so you have 2 options: either study them first then go to the lecture OR study the lecture first then study them, so there will be a lot of repetition ☺

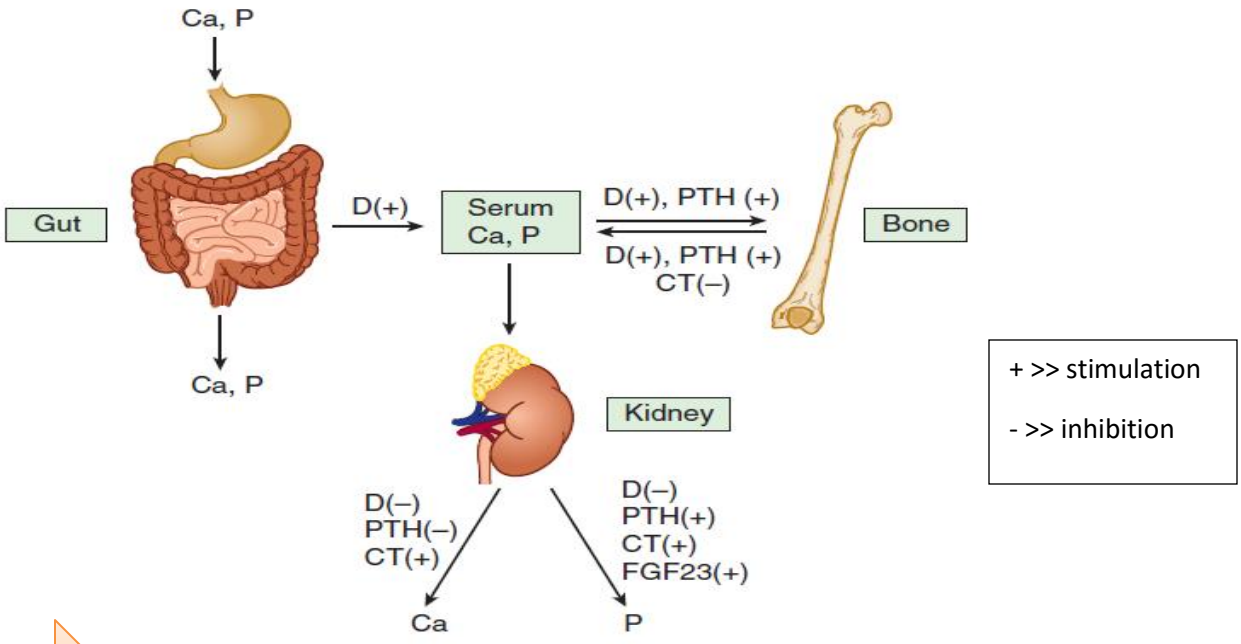


Figure 1

Regulation of bone minerals (Ca & P) need 3 organs in our body work together: GIT , Bone & Kidney

GIT >> the main site for absorption of Ca & P under the influence of vitamin D , so vitamin D enhances the absorption of Ca & P & increases their serum concentration.

When we say Vitamin D OR D we mean 1,25$(OH)_2$D , if we mean other forms we will explain them (NOT to said vitamin D).

Bone >> first keep CT away as its rule very minimum , then look to vitamin D & PTH they stimulate the mineralization of bone by deposition of Ca & P , BUT at the same time the dissolution of these minerals are stimulated by these 2 hormones so they also stimulate resorption of bone (look to the figure there is stimulation in both direction "deposition + resorption).

Vitamin D here is a hormone NOT a vitamin & we will see why later on.

Kidney >> it has 2 functions: metabolic & excretory.

Metabolic function : the formation of $1,25(OH)_2D$. (see below)

Excretory function : kidney excrete Ca & P , Vitamin D inhibits the excretion of both, so the serum level of Ca & P will increase, while PTH inhibits the excretion of Ca, so increase its serum level , but stimulate the excretion of P . (This is distinct effect). FGF23 enhances P excretion .

NOW there are 2 important information :

1. Vitamin D **inhibits** PTH secretion which lead to Ca lose (as PTH inhibit its excretion so inhibition of inhibition lead to stimulation) , but there is NO lose of P (this is indirect effect) .

2. Vitamin D **stimulates** FGF23 (which enhances P excretion) , so vitamin D indirectly enhancee P excretion .

At the end of this sheet there is a table to summarize all these effects.

Formation of vitamin D :

The 1st step happen in the skin under the influence of UV light, which converts 7-Dehydrocholesterol that already present in the skin to Pre-vitamin D_3

Note that 7-Dehydrocholesterol come from cholesterol.

Then in the liver it will be converted to 25(OH)D which transported to the kidney to undergo another hydroxylation & become $1,25(OH)_2D$ which is the active form of vitamin D.

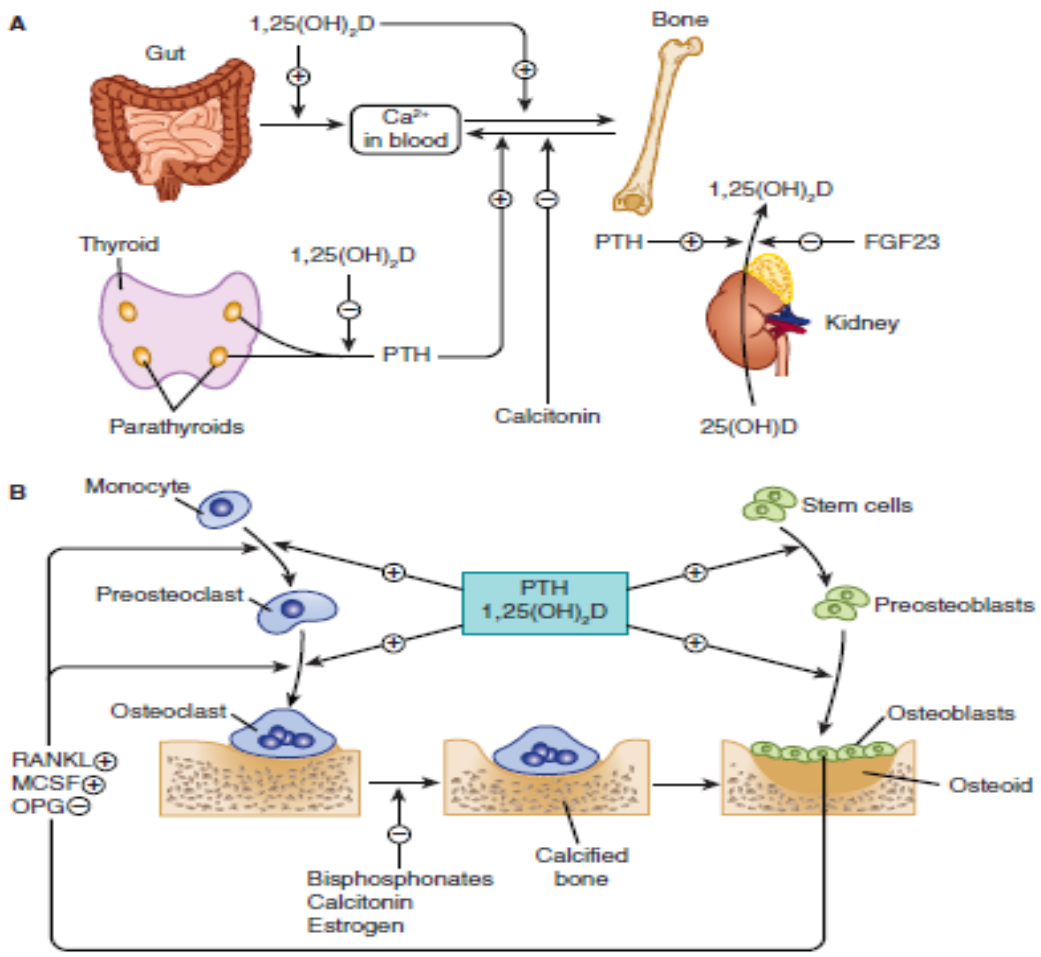


Figure 2

Part A

- ✓ Vitamin D inhibits PTH release.
- ✓ The formation of $1,25(OH)_2D$ by kidney enzymes is stimulated by PTH & inhibited by FGF23 (so these hormones : PTH & FGF23 produce indirect effects secondary to their effects on the formation of $1,25(OH)_2D$)

The effect of PTH & FGF23 on the vitamin D is opposite to the effect of Vitamin D on them (as Vitamin D inhibits PTH secretion, while PTH stimulates Vitamin D formation, & FGF23 inhibits Vitamin D formation while Vitamin D stimulates FGF23).

Part B The effect of PTH & 1,25$(OH)_2$D on the bone :

We said before that they stimulate bone formation & bone resorption .

Bone formation mean that they affect the osteoblast cells while bone resorption affect the osteoclast cells .

So **How** they do that ?

These hormones lead to deposition of Ca & P on the bone to stimulate the stem cell to be converted to preosteoblast which further converted into osteoblast for bone formation (make osteoid tissue) >>> this is the direct effect.

These hormones also stimulate monocyte to be converted to preosteoclast , then they activate the conversion of it into osteoclast for bone resorption >>> this is the direct effect .

Now there is other effects which is <u>important</u> :

PTH & 1,25$(OH)_2$D stimulate osteoblasts to produce RANKL (protein) which activate the receptors on osteoclast & this lead to bone resorption >> this is indirect effect .

RANK >> is the receptor that its action done by activation of nuclear factor kappa-B – the effector for the action of certain drugs & hormones

<u>PTH</u> also stimulates osteoblasts formation by inhibiting osteocyte's production of **sclerostin** which blocks osteoblast proliferation, so block the block lead to stimulation >> this is the indirect effect .

Now there is another protein which is the **OPG** :

OPG **blocks** RANKL action, so lead to inhibition of bone resorption .

What about FGF23 ?

FGF23 in excess amount inhibits Vitamin D production and lowers P levels (as it increase the excretion of P) , so this lead to decrease the deposition of Calcium

phosphate or hydroxyapatite $(Ca_5(PO_4)_3(OH)$ in bone >> resulting in the a condition known as **Osteomalacia.**

What are the differences between Osteomalacia & Osteoporosis ??

Osteomalacia	Osteoporosis
Only the amount of minerals decrease.	The amount of osteoid itself is decreased.
Generalized in all bone.	There are defected areas & other normal areas.

Osteomalacia counterpart of rickets which arise from vitamin D deficiency that characterized by soft bone due to decrease of mineralization process while in adult the bone is already mineralized & now it is losing the minerals (so Osteomalacia)

Dr. didn't mention slide # 7 so read it from the slide ☺

Principal hormonal regulators of bone minerals homeostasis

We will talk about 3 hormones : PTH , Vitamin D , FGF23

PTH

It is a single-chain peptide hormone composed of 84 amino acids BUT its activity found in the first 34 amino acids from the N-terminal direction (from the left) & the rest 50 amino acids are not necessary , so they prepared a drug from these 34 amino acids & they found that it is fully active , but if the 1st two amino acids from the N-terminal removed then all the activity will be lost !!
So it is critical for these 1st two amino acids to be the same & intact , otherwise you will lose the activity of this drug . (this is available as drug)

Ca is the main controller of the PTH , by 2 mechanisms:

1. Ca is needed for protease which destroys PTH , so if you don't have adequate Ca then the PTH will NOT be destroyed , instead you will have excess (Hyperparathyroidism).

2. There is a receptor on the parathyroid gland known as CaSR , which inhibits the formation & the release of PTH when it is equipped by Ca .

There is also another controller mechanism : (By **Vitamin D**)

The parathyroid gland contain VDR , which will inhibit the production of PTH once it is stimulated by $1,25(OH)_2D$.

So vitamin D inhibits the production of PTH as well as it also induces the CaSR .

Induction of the receptor means more synthesis of the receptor (more gene transcription and translation>> so more receptors).
So induction >> increase the number of active form of the molecule.

Teriparatide

- It is the drug that composed from the first 34 amino acids in the PTH.
- Used in the treatment of osteoporosis.
- Given daily by subcutaneous injection.
- Adequate intake of calcium and vitamin D must be maintained (This apply to **all drugs** used to treat **osteoporosis**, so the first step in treatment of osteoporosis is vitamin D & calcium supplements).

In the **bone**, PTH increases the activity and number of osteoclasts (the cells responsible for bone resorption) indirectly by acting on the osteoblast (the bone-

forming cell) to induce membrane-bound and secreted soluble forms of a protein called RANK ligand (RANKL).

Ligand >> is what bound to the receptor, it is a protein.
The direct effect is through the formation of osteoblast while the indirect effect is through other substances.

Osteoporosis happens due to excessive osteoclast, so they use this idea to create drugs that treat the osteoporosis.

Denosumab.

- monoclonal antibody (remember mab : Denosu**mab**)
- inhibit RANKL so it is prevent bone resorption
- used in the treatment of osteoporosis

Monoclonal vs Polyclonal:

**monoclonal >> from one source of cell so they form identical cells & the antibodies will be identical (the same molecule).

**polyclonal >> different cells, so the antibodies will be different, so they form mixture of molecules.

monoclonal antibodies more specific than polyclonal, so they use them as a drug under the name of **biologics** .

There is another protein which is **sclerostin ,** it acts to block osteoblast proliferation , so it prevents the formation of osteoblast . NOW , PTH comes & inhibits the production and secretion of sclerostin from osteocytes , so it inhibit the formation of bone >> another indirect effect of PTH.

We said before that PTH & Vitamin D cause bone resorption as well as bone formation !!! HOW ??

Simply it is **dose dependent** ☺

Let's talk about the endogenous PTH in which there is hyperparathyroidism (**High** amount of PTH) , which lead to increase bone **resorption** .

But when we give exogenous PTH in **low & intermittent** doses , then this lead to increase bone **formation** .

So we can generalized it as this :

- ✓ High PTH lead to resorption of bone.
- ✓ Low PTH lead to formation of the bone.

In certain conditions that result from disturbances in PTH & Vitamin D , bone have excess formation & deficiency at the same time so in certain areas there is excess amount of bone while in other areas there is derangement in the bone which mean the shape of the bone is irregular !!
>>> this usually happen in <u>Chronic kidney disease.</u>

Vitamin D deficiency could reach 30% in the population , don't think there is a lot of sunshine, because as we said before that ONLY the 1st step requires UV , so what about the other steps which require enzymes ??

If there is a deficiency in any enzyme then you will not have Vitamin D ☹

Vitamin D has receptors in almost all cells , it has a role in immunity , CV function , neurological diseases , diabetes pathogeneses …many diseases will be developed if there is a deficiency in Vitamin D &\or its receptors, if there is no stimulation , many defects will happen >> so for this reason the Dr. told us at the beginning of the lecture we consider Vitamin D as a hormone NOT a vitamin alone >> Vitamin D have multiple functions .

Vitamin D

- ✓ It must be in the form of 1,25(OH)2D , in order to do its function .
- ✓ Dietary Vitamin D supplement is not deficient , it present in both animal & plant origins.

>>>So the source of Vitamin D deficiency is NOT food , NOT sunlight , something else (The activation process ; there is deficiency in it)

- ✓ Vitamin D has 2 forms :

1. In **animals** → cholecalciferol OR vitamin D3
2. In **plants** → ergocalciferol OR vitamin D2

But both forms are effective and they require 1,25 dihydroxylation to become fully active.

The hydroxylation process happen in 2 organs :

1. **liver** which form 25(OH)D
2. **Kidney** which form 1,25(OH)D

When we have a kidney disease like renal failure , then we will have Vitamin D deficiency , why ??
because the 1,25(OH) hydroxylation will not produced !!

So HOW to solve this problem ?!
we have 2 options :
either give the patient 1,25(OH)D OR 1(OH)D (as the liver make the 25 hydroxylation)
in renal unit they prefer to use 1(OH)D.

- ✓ **Regulation** of Vitamin D :

It is involve Ca , P , PTH & FGF23 .

- PTH **stimulate** the production of 1,25(OH)D.
- FGF23 **inhibit** the production of 1,25(OH)D.

- ✓ Vitamin D have binding proteins (VBP) , which carry it in the circulation .
- ✓ Excess vitamin D is stored in adipose tissue (fat cells).
- ✓ $1,25(OH)_2D$ is well established as the most potent stimulant of intestinal calcium and phosphate absorption.

If there is a patient that have Vitamin D deficiency & you have to give him Vitamin D , then you should tell him to eat dairy products which contain high concentration of Ca . (Remember Vitamin D increase the absorption of Ca)

- ✓ Vitamin D **analogs** :

We talked before that Vitamin D have different receptors in different cells .
Now we will talk about the analogs of 1,25(OH)D

The analogs differ from 1,25(OH)D in potency at different locations .

Calcipotriene

- Used for the treatment of **psoriasis** الصدفية , HOW ??
  The psoriasis is autoimmune disease, immune cells have receptors for vitamin D , But the potency of Calcipotriene on Ca elevation = 1\1000 of 1,25(OH)D, so Calcipotriene will not raise Ca (i.e. no hypercalcemia will result) ; however we still consider hypercalcemia as a side effect of Calcipotriene .

Recall Potency meaning by reading this article :
https://en.wikipedia.org/wiki/Potency_(pharmacology)

Doxercalciferol & paricalcitol

- For secondary hyperparathyroidism in patients with chronic kidney diseases, chronic kidney diseases affect parathyroid gland indirectly, so we call it secondary hyperparathyroidism , and the treatment is by giving vitamin D analogs OR alpha hydroxy vitamin D (1(OH)D) .

There is also investigational drugs still used for many reasons :
** osteoporosis treatment
**many receptors present in different tissues & produce different diseases that we need to treat them

>>> so a lot of research is doing up to develop new drugs which are analogs OR antagonists for vitamin D .

Don't forget that vitamin D have receptors in almost all cells in the body , affecting many functions .

Eldecalcitol .. not mentioned through the lecture, only from the slides.

- is in phase 3 clinical trials for the treatment of osteoporosis.

Some of Indirect effect of vitamin D :

- Induction of RANKL on osteoblast , so bone resorption will happen.
- Induction of another protein which is **osteocalcin** , that may regulate the mineralization process (it is still under the investigation).

Sometimes there is reduced intestinal calcium absorption In osteoporosis which is opposite to what we should expect, as in osteoporosis Ca & P levels should go up !!!

So what we should do in this cases ??

Simply by giving the patient Vitamin D with calcium supplementation. (Remember what we said in **Teriparatide** drug).

FGF23

- ✓ is a single-chain protein with 251 amino acids.
- ✓ Produced by osteoid tissue , osteoblast & osteoclast .
- ✓ It inhibits 1,25(OH)2D production and phosphate reabsorption (so it increase the excretion of P) in the kidney >> so it can lead to both **hypophosphatemia** and **low levels of 1,25(OH)2D**. ..... This is the main effect of FGF23.

==========

NOW the next 2 slides (# 28 & 29) are not required in the exam, Dr. explained them for better understanding.

FGF23 in order to become active, it needs **O-glycosylation** which mediated by *the glycosyl* transferase GALNT3 .

now if there is a mutation in GALNT3 , this will lead to abnormal effect of FGF23 , so P level will increase in the body – remember that FGF23 excrete the P – when the P increase it will interact with Ca >> so Calcium phosphate will formed which is insoluble , that's mean it will precipitate in predisposed area (joint , around joint , ligament .. etc) resulting in the formation of calcinosis with elevated phosphate and $1,25(OH)_2D$.
>> so FGF23 have a relation in certain diseases .

FGF23 is normally inactivated by proteolytic cleavage at amino acids 176–179. Now if there is a mutation in its gene lead to differences in these amino acids (from 176 to 179) this will result in no cleavage !! and this is the underlying cause of **autosomal dominant hypophosphatemic rickets .**

>> we said **hypophosphatemic rickets** because the mutation present in the gene affect the composition of the protein as it can't undergo cleavage process & the P excretion will increase, resulting in hypophosphatemia, which means poor deposition of calciphosphate in bone & this lead to rickets in the children.

We didn't talk about the adult because the mutation will appear in the childhood period.

>> we said **autosomal dominant;** because hypophosphatemic rickets have many varieties (like X-linked) , but in this situation is due to a mutation in FGF23 gene. Our Dr. didn't agree with this " autosomal dominant " name.

In order for FGF23 to do its action and bind to its receptors which are **1 and 3c** , it need an accessory receptor ,so in the absence of this accessory receptor , the FGF23 will not act !!

So if there is a mutation in accessory receptor , FGF23 will not act resulting in elevated phosphate and 1,25$(OH)_2$D levels.

Slide # 30

FGF23 production is **stimulated** by 1,25(OH)2D and phosphate and directly or indirectly **inhibited** by the dentin matrix protein DMP1 found in osteocytes. >> so DMP is an inhibitor for FGF23.

Mutations in DMP1 lead to increased FGF23 levels and as a result osteomalacia, because it decreases the P level & in order to compensate this decrease the body will take the P from the bone .

Interaction of PTH, FGF23, & Vitamin D

- The net effect of PTH is to **raise serum calcium** and **reduce serum phosphate**.
- The net effect of FGF23 is to **decrease serum phosphate**.
- The net effect of vitamin D is to **raise both.**

See Table in slide # 32.
Please refer to slides .

Please send me your feedback for this sheet by filling this form :

https://docs.google.com/forms/d/e/1FAIpQLSeH9QW58Lz5aHmW5JhJWjlSrqt-qiCq8Spg64Lnd1LcAFWjGw/viewform

Special thanks to : Sara Zayadneh

Sorry for any mistake.
Shahd Rihan.