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FINAL THESIS FOR
THE DIPLOMA OF DOCTOR OF PHARMACY

TITLE:

Analytical methods for quantification of vitamin D and implications for diagnosis of vitamin D deficiency among children and adolescents

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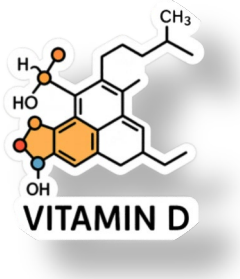
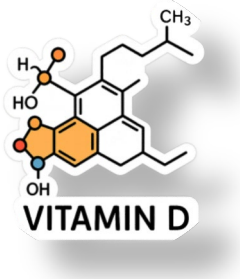


Dedications



When I was a little girl of six years old, while my mother was taking me with her for shopping, we stopped on our way at the neighborhood pharmacy, I spontaneously told her that when I grow up I will become a doctor; this childish memory was the beginning of a love for this distinctive and vast field.

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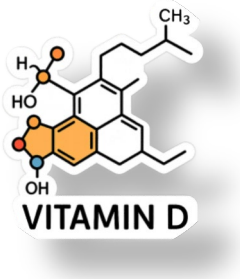
Finally to **the childhood dream** that was established, struggled, persevered and did not stop until it was realized, it is today lived with joy and pride, so praise be to God,many, good and blessed.



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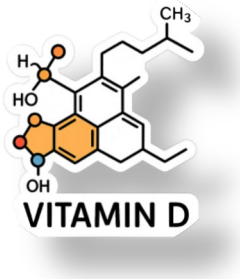

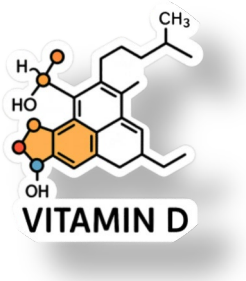

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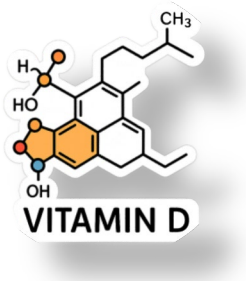

I dedicate this thesis to the dearest people in my life, who have given me so much affection, love, and courage throughout my pharmacy pathway.

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No dedication could ever express the respect, admiration, and deep feelings I have for you. You are the pillars of my life. Your love, sacrifices, patience, and unwavering faith in me have been my greatest strength.

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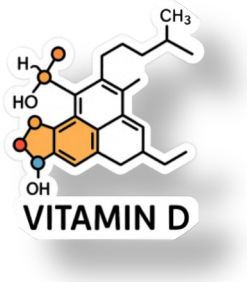

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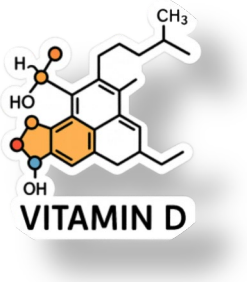

Table of contents

List of abbreviations

1, 25(OH) $_2$D: 1, 25-dihydroxyvitamin D (Calcitriol)

25(OH) D2:25-hydroxy-ergocalciferol

25(OH) D3:25-hydroxy-cholecalciferol

25(OH) D:25-hydroxyvitamin D (Calcidiol)

AAbs: Auto antibodies

Ac: Antibody (Anticorps)

Ag: Antigen

ALP: Alkaline Phosphatases

BMI: Body Mass Index

CL: Chemiluminescence

CLIA: ChemiluminescentImmunoAssay

CYP: Cytochrome P450

CYP24A1: Cytochrome P450 family 24 subfamily A member 1

CYP27A1: Cytochrome P450 family 27 subfamily A member 1

CYP27B1: Cytochrome P450 family 27 subfamily B member 1

D2: Ergocalciferol (Vitamin D2)

D3: Cholecalciferol (Vitamin D3)

ELISA: Enzyme-Linked Immuno Sorbent Assay

ESPGHAN: European Society for Pediatric Gastroenterology, Hepatology and Nutrition

FGF23 : Fibroblastic Growth Factor 23

FOPH: Federal Office of Public Health (Switzerland)

HPLC-UV: High-Performance Liquid Chromatography with Ultraviolet Detection

IU: International Unit

LC-MS/MS: Liquid Chromatography – Tandem Mass Spectrometry

LLE: Liquid–Liquid Extraction

LOD: Limit of detection

m/z: Mass-to-Charge Ratio

NAM: National Academy of Medicine (USA)

NIH: National Institutes of Health (USA)

NIST: National Institute of Standards and Technology

ODS: Office of Dietary Supplements (NIH)

PTH: Parathyroid Hormone

R:Radioactivity

RIA: Radioimmunoassay

RLU: Relative light units

SAD: Seasonal Affective Disorder

SPE: Solid-Phase Extraction

UH-HPLC: Ultra-High Performance Liquid Chromatography

UV: Ultraviolet

UV-B: Ultraviolet B rays

VDBA14: Vitamin D Binding Aptamer 14

VDBP: Vitamin D Binding Protein

VDD: Vitamin D deficiency

VDI: Vitamin DInsufficiency

VDR: Vitamin D Receptor

WHO: World Health Organization

List of tables

List of figures

Introduction

Introduction

A classic hormone become the subject of extensive research: vitamin D (1). For a long time considered solely for its role in bone health, vitamin D is now recognized for its major influence on immunity, metabolism, cardiovascular function, even certain neurological processes and the control of anti-inflammatory mechanism (2), due to the association of multiple genes carrying vitamin D receptors in various cells of the human body (1). Its deficiency is now associated with a wide range of chronic pathologies: type 2 diabetes, autoimmune diseases, cancers, musculoskeletal disorders, depression and others (3–5), which explains its major importance for human health, and this makes hypovitaminosis D a worldwide public health problem (1).

In Algeria, the prevalence of hypovitaminosis D according to certain studies is very high: 92.6% among 945 subjects in the Blida region (6), 76.6% had levels below 30 ng/mL in a study in the Wilaya of Msila (7), and 81.2% with 27.4% insufficiency and 53,6% deficiency in children of both sexes, aged between 11 and 15, living in the town of Ali Mendjeli in Constantine(8), although Algeria is a very sunny country, and the average number of hours of sunshine per year is 2,600 in the north and 3,500 in the south (9).

Faced to this alarming situation, the need for reliable and accurate detection is becoming essential. This is where vitamin D assay techniques come into their own: combining immunological and non-immunological methods, they represent a major step forward in the diagnosis and management of hypovitaminosis D. An appreciation of these techniques, their foundations and constraints, represents crucial progress in terms of improved prevention and more specific therapy.

In this context, the aim of this work is twofold: the main objective is to gain knowledge of the different methods used in public and private medical laboratories for the determination of vitamin D in the blood, their principles, advantages and limitations.

The second main objective is to assess the frequency of hypovitaminosis D in the general population, and principally in children and adolescents (aged between 2 and 17 years) in the year 2024 in the Wilaya of Tlemcen.

The secondary objective is to find out about the clinical manifestations associated with hypovitaminosis D and the risk factors in children and adolescents, in order to understand the

causes of this phenomenon and help raise awareness of the importance of dosage to avoid the damage caused by low vitamin D levels.

This dissertation is in two parts:

The first part (literature review), subdivided into three chapters: the first concerns a general overview of vitamin D, its physical and chemical properties, its origin, metabolism, catabolism, and its main role in human health. The second chapter concerns hypovitaminosis D, its definition, risk factors, consequences, prevention and treatment. The third chapter is devoted to the different methods of vitamin D dosage, with their advantages and limitations.

The second part, known as the practical part, gives the tails of the materials and methods used for this study, as well as the results obtained, and finishes with a discussion explaining the previous results, followed by a general conclusion to this work.

LITERATURE REVIEW

Chapter I

Vitamin D

1. Generalities on vitamin D

1.1 Historical aspects of vitamin D

The history of vitamin D is a rich and storied subject and is now over 350 years old (10), It has been closely linked to the prevention and treatment of rickets, at the end of the 18th century, Dale Perceval(an English physician)advocated for the administration of cod liver oil as a preventive and curative treatment of rickets, which had become widespread in Europe and North American due to the industrial revolution (11).

In 1822, Dr. Sniadecki suggested that children suffering from rickets are insufficiently exposed to sunlight. Ten years later, Palm recognized that the lack of sunlight exposure is the common denominator for the increase in rickets in these age groups (11).

In 1919, Sir Edward Mellanby experimentally demonstrated the existence of vitamin D in beagle dogs suffering from rickets. He believed that the recovery of the dogs was due to the vitamin A present in cod liver oil, which had been discovered by Elmer McCollum (11).

In 1922, he found that this oil retained antirachitic properties even after the destruction of vitamin A, and he showed that this oil contained an antirachitic substance responsible for the mineralization of the skeleton and consequently the healing of rickets. He named vitamin D (11).

Alfred F. Hess., a North American pediatrician, and Adolph Otto Windaus, a German chemist, defined the chemical structure of the active principle of vitamin D during the 1920s (12).

In 1919, another Polish pediatrician, Kurt Huldschinsky (1883-1940), achieved a ‘’spectacular” cure by exposing children suffering from rickets to the radiation emitted by a mercury lamp that generated UV-B rays. Whether the children were exposed directly to sunlight or to UV radiation, the success was consistent, rickets was indeed due to a deficiency of sunlight(12).

1.2 Definition and Structure

Vitamin D is a group of fat-soluble vitamins which are responsible for mineral absorption (calcium, magnesium, phosphate and zinc) and multiple biochemical functions in humans (13). It is also considered a steroid hormone, which plays an important role in its biologically

active form (calcitriol) in the regulation of numerous cellular processes, having effects on the growth of both normal and malignant cells and their differentiation, on the immune system, and on cardiovascular functions (14).

The name of vitamin D refers to two steroids (secosteroids the bond between carbons 9 and 10 of the B ring is broken) that have antirachitic activity: ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3). These are derivatives of the cyclophenanthrene nucleus and differ by their side chain fixed at C17: saturated in the case of vitamin D3, unsaturated between C22 and C23, and methylated at C24 in the case of D2(11), Figure (1) shows the structure of ergocalciferol (vitamin D2) and cholecalciferol (vitamin D3).

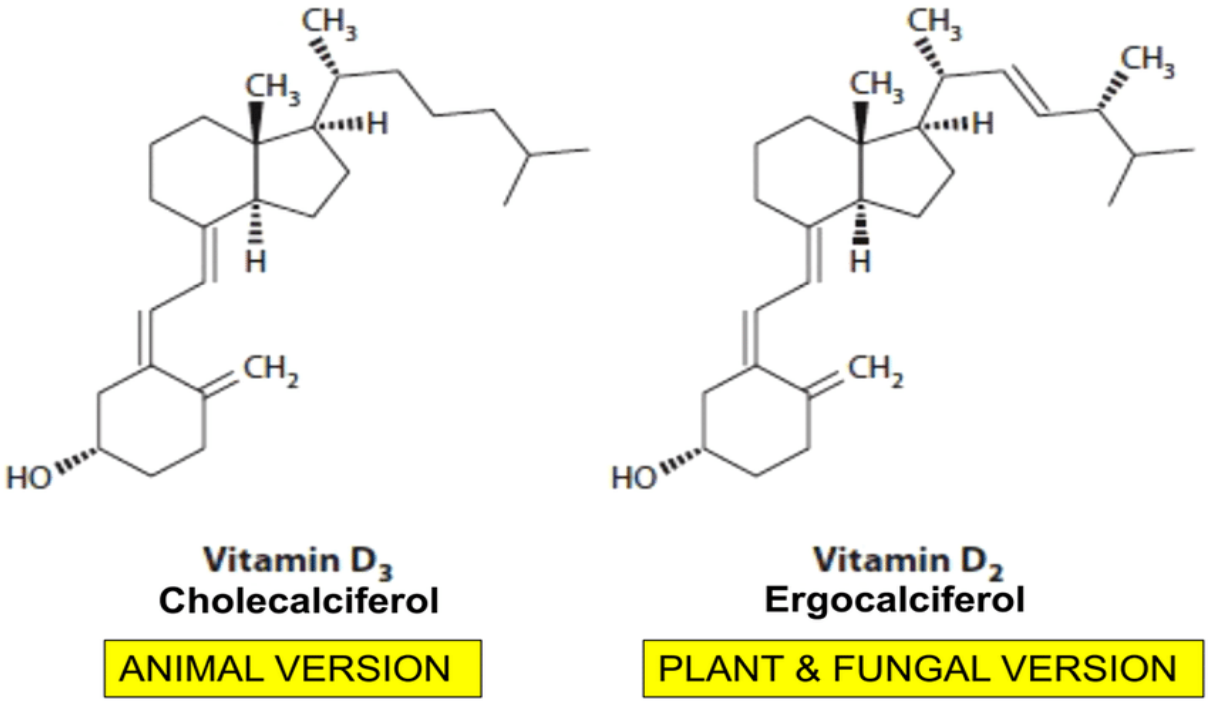

Figure 1: Chemicals structures of cholecalciferol (D3) and ergocalciferol (D2) (10)

1.3 Physicochemical properties

- Vitamin D is a hydrophobic vitamin (15), it is soluble only in fats and organic solvents such as methanol, Acetonitrile, and chloroform, insoluble in water and is stable up to 38°C(7,8).
- Vitamin D2 ($C_{28}H_{44}O$) molar mass = 396.7, melting point equals 113-118 °C (11).

- Vitamin D3 ($C_{27}H_{44}O$) molar mass = 384.6, melting point equals 82-88°C (11).
- Both forms exhibit a maximum absorption at 265 nm in alcoholic solutions, are fairly stable to heat but are relatively quickly degraded by light and to a lesser extent by oxygen and acids (11).
- Vitamin D2 is less stable than vitamin D3, especially when exposed to light and oxygen, which can affect its bioavailability (18).

2. Origin

Unlike other vitamins that are exclusively obtained from food, vitamin D has a dual origin: exogenous, which corresponds to dietary intake, but also endogenous, resulting from a neo synthesis occurring at the level of the epidermis (19). Vitamin D2 can only be procured through diet and supplementation, and parts of vitamin D3 can be synthesized through sun exposure in the skin of mammals (13).

➢ Exogenous origin

•Vitamin D2, or ergocalciferol, is primarily produced by plants and fungi, very few sources; the only significant ones are sun-dried mushrooms, such as dried shiitake mushrooms, which provide approximately 20–25 µg (800–1000 IU) per 100 g (20).

•Animal origin of vitamin D3 or cholecalciferol is explained in the table I.

Table I : The main dietary sources of vitamin D (1µg=40IU).

FOOD	µg/100g (IU/100g)
Cod liver oil	Approximately 500 µg (20,000 IU) per 100 ml.
Wild salmon, herring, or tuna	15–25 µg (600–1,000 IU) per 100 g.
Farmed salmon	7–10 µg (280–400 IU) per 100 g.
Canned sardines in oil	Approximately 7.5 µg (300 IU) per 100 g.
Oysters	Approximately 10 µg (400 IU) per 100 g.
Trout	Approximately 5 µg (200 IU) per 100 g.
Sole	Approximately 2 µg (80 IU) per 100 g
Pike	Approximately 2 µg (80 IU) per 100 g.
Egg yolk	Approximately 2–3 µg (80–120 IU) per 100 g.
Veal liver	Approximately 0.5 µg (20 IU) per 100 g.
Dairy products or cereals fortified with vitamin	1.25g (50 IU) per 100 g or 100 ml.

(20)

➢ **Endogenous origin**

The main source of vitamin D3 is an endogenous synthesis that occurs in the epidermis after exposure to ultraviolet B (UVB) radiation provided by sunlight, it is produced from 7-dehydrocholesterol, an intermediate in the synthesis of cholesterol, which is present in the membranes of dermal and epidermal cells. The energy provided by UVB rays enables its transformation into previtamin D3, which is then rapidly converted under the influence of heat into vitamin D3, released into the circulation (21).

3. Metabolism

Vitamin D (D2 or D3) must be transformed in the liver and then in the kidneys to become fully active by binding to a receptor present in target tissues that it reaches via the bloodstream. Vitamin D is absorbed slowly (on average, over 3 days) in the small intestine, incorporated into bile salts and free fatty acids. Once synthesized or absorbed, a portion of vitamin D is stored in adipose tissue. The other portion enters the bloodstream (16).

Vitamin D2 and vitamin D3 are transported in the blood by DBP (vitamin D binding protein). They are hydroxylated in the liver to form 25-hydroxyvitamine D through 1-alpha hydroxylase(CYP27B1), which is the vitamin D activating enzyme (13), this hepatic hydroxylation is minimally regulated, meaning that the more vitamin D is ingested or synthesized, the more 25OHD is produced. This 25OHD is then hydroxylated again to produce 1, 25(OH) $_2$ D, the active metabolite of vitamin D. This second hydroxylation can occur either in renal proximal tubular cells or in many other tissues. Renal hydroxylation, which is tightly regulated by hormones involved in phosphate calcium metabolism such as PTH (parathyroid hormone) or FGF23 (fibroblast growth factor 23), produces 1, 25(OH) $_2$D "hormone" (which means it enters the bloodstream and acts on target tissues where it binds to the VDR –coetaneous vitamin D receptor), while "peripheral" hydroxylation is independent of phosphate-calcium regulation and produces 1,25(OH)$_2$D that acts locally (in an "intracrine" manner) and does not participate in phosphate-calcium metabolism(20), figure (2) showing vitamin D metabolism.

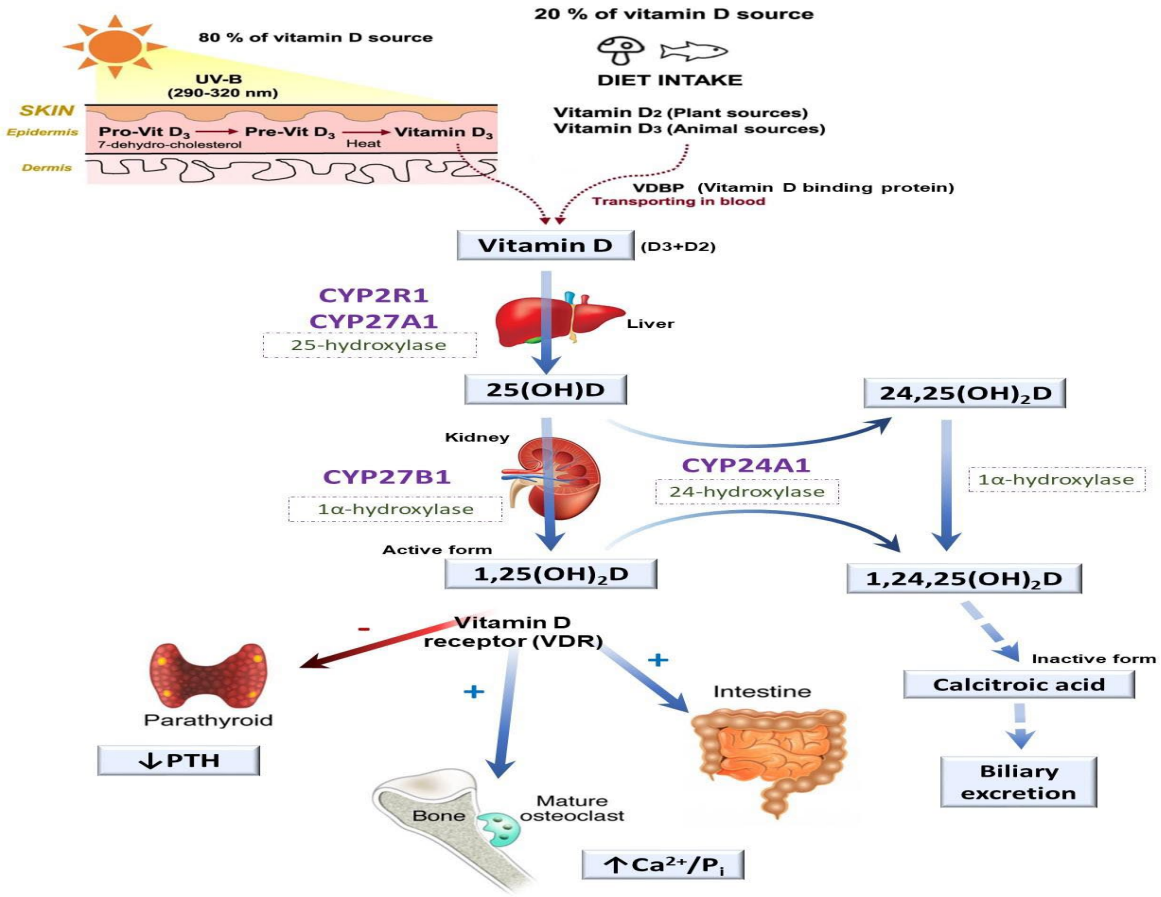

Figure 2 : Molecular pathway of vitamin D metabolism and function (22).

3.1 Vitamin D metabolites of interest

In human blood over 50 vitamin D metabolites circulate with variable activity although 25(OH)D3 and 1, 25(OH)$_2$D3 are the only compounds that are routinely measured in clinical laboratories (23).

- **25(OH) D:** measurement of 25(OH) D is accepted as a reliable clinical indicator of vitamin D status in humans. The steady circulating serum level and long half-life of approximately 2 to 3 weeks make 25(OH) D an ideal diagnostic indicator of vitamin D deficiency and monitoring target in supplementation therapy. Two forms of 25(OH)D exist:25(OH)D3 and 25(OH)D2, when measuring 25(OH)D, it is important that the assay methodology has co-specificity for 25(OH)D3 and 25(OH)D2, as both metabolites are metabolically active, more so for 25(OH)D3. The serum concentration of 25(OH) D3 is higher than 25(OH) D2 due to the coetaneous synthesis by UVB radiation and broader sources of vitamin D3 available in foods and supplements derived from animal origin.

25(OH)D2 is less prevalent, though can be present in individuals taking vitamin D2 supplements derived from plant products (22).

- **1α, 25-dihydroxyvitaminD (1, 25(OH) 2D) calcitriol:** is the physiologically active form of vitamin D. It is a product of the enzymatic hydroxylation of 25(OH) D by 1α-hydroxylase that takes place primarily in the kidneys. The physiological regulation of 1, 25(OH) $_2$D is more tightly regulated than 25(OH) D. When serum 25(OH) D stores decline, 1, 25(OH) $_2$D is maintained by increasing PTH stimulation of 1α-hydroxylation. Measurement of serum 1,25(OH) $_2$D should be considered upon suspicion of deficiency or excess of 1, 25(OH)$_2$D leading to hypo-or hypercalcemia, hypoparathyroidism and hypomagnesaemia(22). It is not the ideal form for measuring vitamin D because its circulating half-life is only 4 to 6 hours. The circulating levels of 1, 25(OH) D are a thousand times lower than those of 25(OH) D. As a patient becomes deficient in vitamin D, there is a decrease in intestinal calcium absorption, which temporarily reduces ionized calcium. This signal is recognized by the calcium sensor in the parathyroid glands, leading to an increase in the production and secretion of parathyroid hormone (PTH). PTH regulates calcium metabolism by increasing tubular reabsorption of calcium in the kidneys, enhancing calcium mobilization from the skeleton, and increasing renal production of 1,25(OH)D. Therefore, the rise in PTH levels results in normal or elevated levels of 1, 25(OH) D. This renders the analysis of 1, 25(OH)D unnecessary as a measure of vitamin D status (24).

- **24, 25(OH) 2D:** is formed via C24-hydroxylation of 25(OH) D by cytochrome P450 24-hydroxylase enzyme CYP24A1.However, the biological function of 24, 25(OH) 2D remains unclear besides being the apparent inactive catabolite of 25(OH) D. A recent publication suggested the measurement of serum 24, 25(OH) $_2$D in conjunction with 25(OH) D shows promise as a novel marker of 25(OH)D catabolism and predictor of serum 25(OH) D response to vitamin D supplementation. 24, 25(OH) $_2$D is quantitatively most abundant circulating vitamin D metabolite besides 25(OH)D. Studies have shown serum 24,25(OH) $_2$D correlated positively with serum 25(OH) D levels (22).

- **C3-Epimer 25-hydroxyvitamin D:** 25(OH) D3 and 25(OH) D2 can be metabolized through an alternate C3 epimerization pathway, into C3-epi-25(OH) D3 and C3-epi-25(OH) D2 and further into their respective C3-epi-1, 25(OH) 2D forms. Reports had shown that C3-

epi-1, 25(OH) $_2$D3 is nearly as potent as 1, 25(OH) $_2$D3 in suppressing PTH secretion, but has reduced calcemic properties. The biological relevance of C3-epi-25(OH) D remains elucidated. The 3-epi-25(OH) D3 metabolite is identical in chemical structure to 25(OH) D3 except for molecular asymmetry at the OH position on C3. As both forms have the same mass precursor to product ion transitions, insufficient separation of the C3-epimer could lead to overestimation of 25(OH)D3 (22).

4. Role and function of vitamin D in the body

- Bone role, phosphocalcic metabolism

Vitamin D is involved in the absorption of calcium and phosphorus from the intestinal mucosa, and promotes the binding of calcium to bones, thus contributing to the mineralization of bones, cartilage and teeth. Calcium and phosphorus influence muscular contractibility (particularly myocardial contractibility) and hormone regulation (25).

- An extra-bone role: as many cells and organs have vitamin D receptors, they may be protected from various types of cancer and other pathologies such as diabetes, cardiovascular disease, metabolic disorders… (25).
- Role of the immune modulator, is becoming increasingly well established in science, and it also helps control the anti-inflammatory process by restraining the overproduction of cytokines (25).

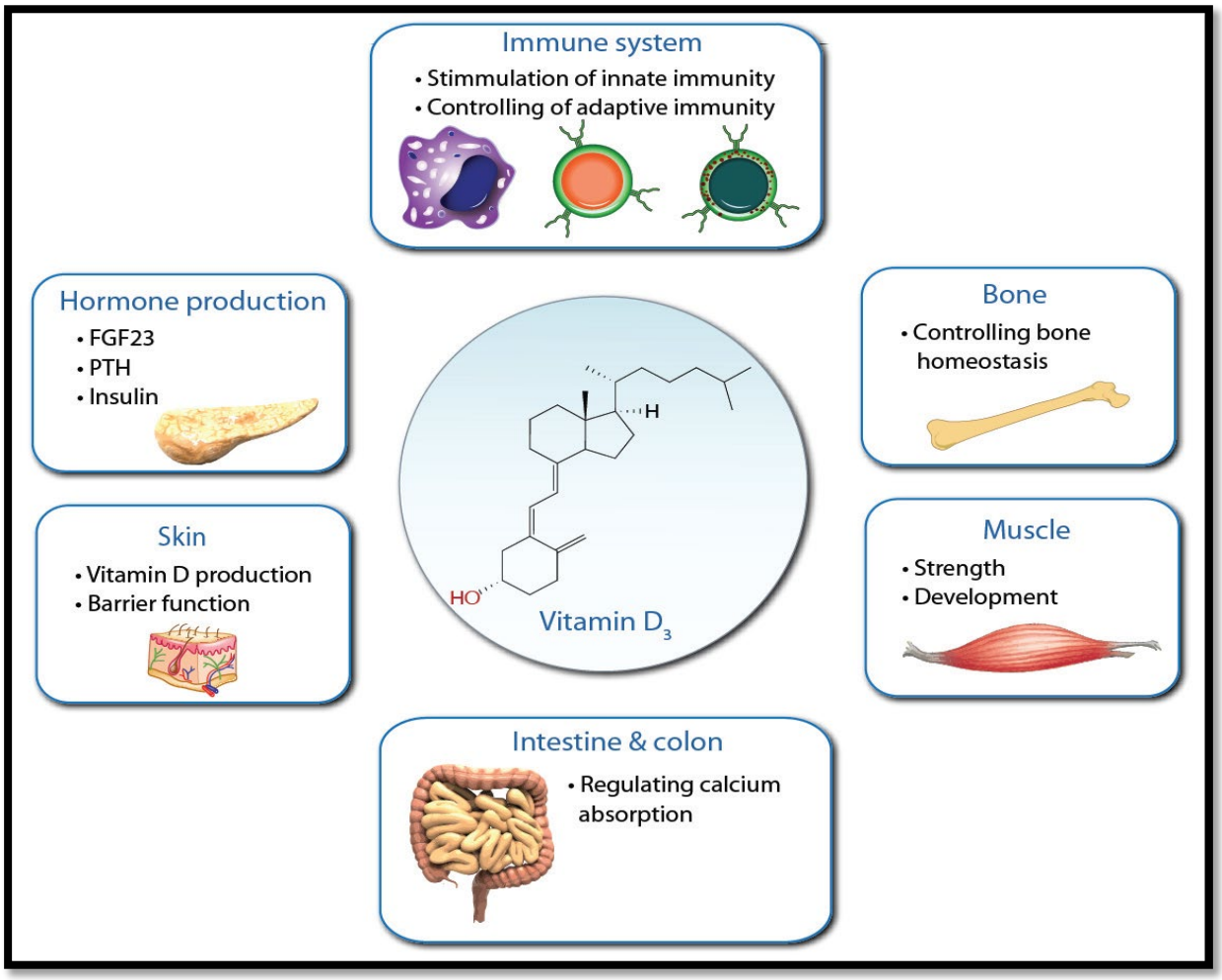

Figure 3 : Pleotropic physiological functions of vitamin D (26).

5. Catabolism of vitamin D

The circulating concentration of 1, 25(OH) $_2$D (active vitamin D) also depends on its catabolism occurring in target cells. CYP24A1 catalyzes the conversion of 1, 25(OH) $_2$D3 into 1, 24, 25-trihydroxyvitamin D3 (1, 24, 25(OH) $_3$D3), which is the first step in the degradation pathway of vitamin D, leading to an inactive form, calcitroic acid. Unlike CYP27A1 and CYP27B1, which are primarily located in the liver and the kidney respectively, CYP24A1 is ubiquitous, thereby regulating the levels of active vitamin D3 throughout the organism(27).

ChapterII

Vitamin D deficiency

Vitamin D deficiency is a global health problem affecting one billion children and adults worldwide. This increase in prevalence is certainly a link to changes in lifestyle habits over the last few decades (use sunscreen, and reduce exposure to the sun and outdoor activities)(28). Various studies on the subject report an association between vitamin D deficiency and an increased risk of numerous diseases and abnormalities(29).

1. Definition

‘’Vitamin D deficiency’’, we read the most recent journal articles on this subject(30),is an a significant health issue that affects both developed and developing countries(31), with a 30-80% global prevalence in adults and children(32). People might acquire VDD when their typical intakes are lower over time than recommended levels(5). 25(OH) D serum concentrations of less than 50 nmol/l(< 20 ng/ml)are considered as indicative of vitamin D deficiency(33).According to the World Health Organization (WHO), 25(OH)D levels below 30 nanomoles per liter(nmol/L)of serum indicate a high risk of vitamin D deficiency, whereas healthy infants are thought to be 50 nmol/L or higher(34).

In terms of biological diagnostic criteria, most international experts agree on defining minimum serum concentration thresholds for 25-OHD:

- At 20 ng/mL (50 nmol/L) to define vitamin D deficiency, and a limit of 30 ng/mL (75 nmol/L) to define vitamin D insufficiency in adults(35).
- In children, the consensus is less clear, but it is considered that a minimum serum concentration of 20 ng/mL is necessary(35).

The table (II) outlines the different levels of vitamin D Status, expressed in both nmol/ml and ng/ml along their clinical interpretation in relation to bone and overall health, as defined byNational Institutes of Health (NIH), Office of Dietary Supplements (ODS)(5).

Table II : Serum 25-Hydroxyvitamin D (25(OH) D) Concentrations and health.

nmol/L	ng/mL	Heath statu
<30	<12	Associated with vitamin D deficiency, which can lead to rickets in infants and children and osteomalacia in adults

	12 to<20	Generally considered inadequate for bone and overall health in healthy individuals
≥50	≥20	Generally considered adequate for bone and overall health in healthy individuals
>125	>50	Linked to potential adverse effects, particularly at >150 nmol/L (>60 ng/mL)

(5)

25(OH) D serum concentrations are expressed in nanogrammes per milliliter (ng/mL) as well as nanomoles per liter (nmol/L). One ng/mL = 2.5 nmol/L, and one nmol/L = 0.4 ng/Ml (5).

The table (III) compares vitamin D status thresholds as defined by three major health organizations (3).

Table III : Interpretations of the 25 (OH) D.

Organization/Country	Status		
	Sufficient	Insufficient	Deficient
The National Academy of Medicine (NAM) in the United States	> 50 nmol/L	50-30 nmol/L	< 30 nmol/L
European Society for Pediatric Gastroenterology, Hepatology and Nutrition (ESPGHAN)	> 50 nmol/L	50-25 nmol/L	< 25 nmol/L
American Endocrine Society	> 75 nmol/	50-75 nmol/L	< 50 nmol/L

(3)

2. Groups at Risk of Vitamin D Inadequacy

It is challenging to get enough vitamin D from natural (no fortified) dietary sources alone. Maintaining a good vitamin D status for many people requires eating foods fortified with vitamin D and getting some sunshine. To achieve their vitamin D needs, some groups,

however, may require dietary supplementation. Those who are most prone to have insufficient vitamin D status include the following groups (5).

2.1 Breastfed infants

Infants typically cannot meet their vitamin D needs by consuming human milk alone since it contains less than 0.6 to 2.0 mcg/L (25 to 78 IU/L).Human milk's vitamin D concentration is correlated with the mother's vitamin D status; research indicates that moms who take daily supplements that contain at least 50 mcg (2,000 IU) of vitamin D3 have higher amounts of the mineral in their breast milk (5).

2.2 Older adults

Older persons are more likely to develop vitamin D deficiency, which is caused in part by a reduction in the skin's ability to synthesize vitamin D. Furthermore, older people are prone to spend more time indoors than younger ones, and they may have inadequate dietary intakes of the vitamin (5).

2.3 People with dark skin

Higher levels of the pigment melanin in the epidermal layer of the skin cause darker skin and impair the skin's capacity to synthesis vitamin D from sunlight(5).Skin pigmentation absorbs UVB radiation; therefore those with darker skin have reduced UVB absorption(36).People of African American descent have lower rates of bone fractures and osteoporosis compared to white individuals (5).

2.4 People with conditions that limit fat absorption

Vitamin D is fat soluble, therefore its absorption is dependent on the gut's ability to absorb dietary fat. Fat malabsorption is linked to a variety of medical problems, including liver disease, cystic fibrosis, celiac disease and ulcerative colitis. In addition to having an elevated risk of vitamin D deficiency, people with certain illnesses may not eat specific foods, such as dairy products (many of which are fortified with vitamin D), or consume very modest amount of these items (5).

2.5 People with obesity or who have undergone gastric bypass surgery

Individuals with a BMI (body mass index) of 30 or higher have lower serum 25(OH) D levels than non-obese individuals. Obese adults may require higher vitamin D doses to achieve 25(OH) D levels comparable to normal-weight people (5).

Obese people who have had gastric bypass surgery can develop vitamin D deficiency. This technique bypasses a portion of the upper small intestine, where vitamin D is absorbed, and vitamin D mobilized into the bloodstream from fat storage may not elevate 25(OH)D to appropriate levels over time (37).

3. Causes of vitamin D deficiency

Vitamin D deficiency usually occurs in people who are not exposed to sunlight and who do not consume enough vitamin D in their diet. Certain disorders can also lead to this deficiency (37).

3.1 Inadequate exposure to sunlight

Limited sun exposure can result from several variables, including: high latitude and season; where UV-B photons reaching the skin surface are reduced by more than 80% during the winter months above 40° latitude, elevated melanin content in skin pigment; whole-body attire based on climate or cultural traditions, such as Muslim women (34,35), homebound people, and those with jobs that limit sun exposure are among those who are unlikely to get enough vitamin D from sunlight. The use of sunscreen also reduces vitamin D production from sunshine (5).Because breast milk contains only trace levels of vitamin D, breastfed children who are not exposed to adequate sunlight are in danger of deficiency and rickets. Some experts advise that arms and legs, or the face, arms, and hands, should be exposed to direct sunshine for a duration of 5 to 15 minutes at least three times weekly (37).

3.2 Abnormal metabolism

Vitamin D deficiency may result from defects in the synthesis of 25-hydroxyvitamin D or 1, 25-dihydroxyvitamin D. Chronic kidney diseases often lead to reduced production of 1, 25-dihydroxyvitamin D and elevated phosphate, which can cause rickets or osteomalacia. Additionally, hepatic dysfunction can interfere with the production of active vitamin D metabolites (38).

3.3 Reduced absorption

Malabsorption can deprive the body of dietary vitamin D; only a small amount of 25(OH)D is recirculated enter hepatic ally (37).Malabsorption of vitamin D, and the resulting deficiency, can be a consequence of gastrointestinal disorders (39).

3.4 Deficit in intake

People who follow a vegetarian or vegan diet may be more susceptible to vitamin D deficiency because the majority of foods that contain this nutrient come from animals.
Furthermore, the fetus receives vitamin D through the placenta during pregnancy, so both the mother and the fetus must consume more of it to avoid deficiencies (39).

3.5 Medication

Certain medications could change how vitamin D is metabolized, which results in vitamin D deficiency. Phenytoin, carbamazepine, isoniazid, and rifampicin can cause vitamin D deficiency by inducing CYP enzymes and increasing catabolism of 25(OH) D and calcitriol. Orlistat and other medications that inhibit fat absorption, such as cholesterol, may also inhibit the absorption of vitamin D. Furthermore, greater dosages of vitamin D must be administered concurrently with ketoconazole in order to produce appropriate plasma levels of the active metabolite since ketoconazole inhibits the kidneys' ability to activate 25(OH)D (39).

Prednisone and other corticosteroid drugs can decrease calcium absorption and affect the metabolism of vitamin D (5).

4. Consequences of Vitamin D deficiency

The symptoms of a vitamin D deficiency are variable and can be general. Their manifestation depends on the severity of the deficiency, as well as the age of onset. These symptoms may be vague or poorly defined, as seen in adolescents, or conversely, may present as specific bone signs of rickets in younger children (28).

Vitamin D deficiency can manifest as fatigue and/or irritability. The more severe the deficiency, the more pronounced the symptoms. Hypovitaminosis D manifests as rickets in children and osteomalacia in adults (28).

4.1 Skeletal

- **Rickets**

Rickets is a syndrome resulting from a defect in bone mineralization. There are several etiological forms of rickets, the main one being related to a deficiency in vitamin D. Nutritional rickets can occur when serum concentrations of 25(OH) D are below 25 nmol/L (40).

- **Osteomalacia**

Osteomalacia is a metabolic bone disorder that affects the entire skeleton. It is characterized by a defect in bone mineralization, leading to an accumulation of osteoid tissue. The bone tissue becomes fragile, significantly increasing the risk of fractures. Osteomalacia manifests as diffuse bone pain and difficulty walking due to muscle weakness. The most common etiology of osteomalacia is vitamin D deficiency (41).

- **Osteoporosis and Bone Fractures**

Vitamin D plays an important role in calcium-phosphate metabolism. When there is a deficiency of vitamin D, intestinal calcium absorption is reduced, and the tendency towards induced hypocalcaemia stimulates the secretion of parathyroid hormone (PTH), promoting bone remodeling and fragility, which, in the long term, contributes to the development of osteoporosis (42), and an increased risk of fractures (43).

4.2 Non-skeletal

- **Falls**

Osteomalacia and rickets are associated with muscle weakness, contributing to reduced physical performance. A possible association between vitamin D levels and physical performance and/or falls has been suggested, supported by the fact that the vitamin D receptor (VDR) is present in muscle cells (4).

- **Cancer**

Epidemiological studies have also suggested possible associations between low concentrations of vitamin D and the occurrence of certain cancers (colorectal, prostate,

pancreas, lungs, etc.). The antitumor effect is thought to be related to the active form of vitamin D [1, 25 (OH) 2D] regulating genes involved in cell proliferation (4).

- **Cardiovascular disease**

Vitamin D controls the renin-angiotensin-aldosterone system, the growth of vascular cells, and the pathways for inflammation and scarring. Vascular dysfunction, arterial stiffness, left ventricular hypertrophy, and hyperlipidemia are all linked to vitamin D deficiency. Because of these factors, vitamin D has been connected to heart health and cardiovascular disease risk (44).

- **Depression**

Individuals who experience depression (sadness or anxiety) during the same season every year are frequently diagnosed with seasonal affective disorder (SAD), which is linked to VDD (44).

Vitamin D also seems to alter brain function, and it has been established that there are associations between low vitamin D status and numerous neurological illnesses, including schizophrenia, Parkinson's disease, Alzheimer's disease, and impaired cognitive function (45).

- **Type 2 diabetes**

The metabolism of glucose is influenced by vitamin D. Through vitamin D receptors in the muscles and liver, it lowers peripheral insulin resistance and increases insulin production via the vitamin D receptor on pancreatic beta cells. Because of its effects on insulin signaling and glucose metabolism, as well as its capacity to lower inflammation and enhance pancreatic beta-cell function, vitamin D may have a role in the pathogenesis of type 2 diabetes(5).

4.3 . Biological signs

-Normal or low calcium levels depending on the degree of deficiency (46).

- Hypophosphatemia (46).

-Significantly reduced calciuria (46).

- Secondary hyperpara-thyroidism: elevated PTH (46).

- Elevated alkaline phosphatases: the level of alkaline phosphatases must be interpreted in relation to the age and sex of the patient; an increase in alkaline phosphatases is a cardinal sign of rickets (46).

5. Prevention of vitamin D deficiency

The preventive strategy is based on one hand, on the establishment of the recommended daily intake that relies on natural sources of vitamin D (diet and sun exposure) and on the other hand, on preventive supplementation(47).

Research conducted in various ethnic and geographic areas indicates a variable duration of sun exposure required to achieve sufficient serum 25(OH)D levels (48).

According to Holick., sun exposure of 5 to 30 minutes on the arms and legs, twice a week between 10 a.m. and 3 p.m., significantly increases the level of 25(OH)D. It is considered that, with an exposure of about 12 minutes, one obtains the equivalent of an oral intake of 3,000 IU of vitamin D3. Furthermore, vitamin D synthesized by the skin has a half-life approximately twice as long as that of vitamin D absorbed through ingestion. Indeed, the recommended exposure time should be adjusted according to the season, latitude, time of day, age, and skin type (47).

During the winter, the low exposure to sunlight makes it crucial to consume foods rich in vitamin D to prevent a potential vitamin deficiency. Indeed, vitamin D is found in cod liver oil, certain types of fish (such as herring, sardines, and mackerel), veal liver, and eggs. To meet daily needs, it is necessary to consume one and a half tablespoons of cod liver oil, or 20 sardines, or 22 eggs. Therefore, one should not rely solely on nutrition to meet vitamin D needs. About 90% of vitamin D is produced through skin synthesis, while only 10% comes from dietary sources (47).

6. Treatment of vitamin D deficiency

Since 2012, the FOPH's recommendations have made it possible to better recognize and treat the population at risk of vitamin D deficiency(28).Vitamin D2 and D3 are the two most commonly used forms for the prevention and treatment of VDD and VDI (49).

Vitamin D deficiency can be effectively treated by injecting or taking vitamin D orally (50).

High dosages of vitamin D, often taken orally every day for around one month, are used to treat vitamin D insufficiency. The dose is often progressively decreased to the standard suggested level after one month (37). Calcium supplements are also administered if there are muscle spasms or if it is believed that the patient is lacking in calcium. Phosphate supplements are administered in cases of phosphate deficiency. This treatment typically results in full healing (37).

According to the majority of experts, the daily maintenance dose of vitamin D varies depending on the age of the person; both adults and children need 800–1000 IU (20–25 µg) of vitamin D3 to meet their body's vitamin D needs when sunlight cannot supply it. Since there aren't many natural dietary sources of vitamin D, it's critical to get vitamin D replacement from supplements (51).

- Recommendations for vitamin D intake and supplementation

  ✓ Units and conversions

To convert vitamin D concentration from IU to ng, multiply by 25 (43).

1 IU = 25 ng = 0.025 µg i.e. 400 IU = 10 µg (43).

  ✓ Supplementation for children

The Scientific Advisory Board recommends general supplementation of all children, from the first days of life to age 18, at a rate of 400-500 IU/day (i.e. 10-12 µg/day)(43).

  ✓ Intake and supplementation in adults

The Scientific Advisory Board recommends supplementation for at-risk populations(43) :

- ✓ 600 - 800 UI/d (i.e. 15 - 20 µg/d) from 18 to 64 years of age.
- ✓ 800 - 1500 UI/d (i.e. 20 - 37 µg/d) over 65 years of age.

7. Role of the pharmacist

The pharmacist plays a central role in health prevention, in order to prevent the onset of the disease, focusing on the habits and lifestyle of patients, it is essential to educate the

population and address hypovitaminosis to avoid its reappearance. This includes the role of the pharmacist, which encompasses prescription analysis, pharmaceutical counseling, and information about the treatment, whether preventive or curative (47).

ChapterIII

Methods for quantification of vitamin D

We now have the ability to measure a number of different vitamin D metabolites with very accurate methods (52). In humans, vitamin D2 (ergocalciferol) and vitamin D3 (cholecalciferol) are the major compounds in this group. Many clinical studies have shown that 25(OH) D3 is the major component of 25(OH) D in the blood, accounting for more than 95% of the relative abundance. Deficiency of vitamin D can lead to growth retardation in children, rickets, osteoporosis, low calcium levels, bone deformities. Serum levels of 25(OH) D2 and D3 are considered as the go-to measurement for vitamin D status (2). Measurement of serum 25(OH) D is recommended for the diagnosis of vitamin D deficiency (VDD) (53). A literature review identified approximately 90 publications on the measurement of vitamin D within the period 2008-2012. A wide variety of methodologies have been developed for the quantification of vitamin D metabolites in serum/plasma samples, each with its own advantage and limitations (51).

Based on the existing techniques, the detection methods for 25(OH) D are classified into three broad categories. The first category comprises immunoassays, which are further segregated into radioimmunoassay (RIAs), enzyme-linked immunosorbent assays (ELISA), chemiluminescent immunoassays (CLIAs), lateral flow immunoassays, and assays for clinical chemistry analyzers. The second set of methods includes the physical detection methods, which comprise of HPLC and LC-MS/MS. The third category includes sensors based on electrochemical, colorimetric and fluorescence detection methods (2).

1. Dosage of vitamin D

The measurement of vitamin D is a blood test (plasma or serum) that allows for the assessment of the level of 25(OH) D3 or 25(OH) D2, they were assumed as the main vitamin D status biomarkers due to their longer half-life in plasma, better stability, and relatively high concentration compared to other vitamin D metabolites. Other biological fluids, including saliva, urine, cerebrospinal fluid, milk, and tissues can also be used for vitamin D analysis, but they are not typically used in clinical practice. Despite calcitriol being the active metabolite of vitamin D, it is not recommended to be used as a biomarker due to its short half-life and tight physiological regulation of its concentration (39).

2. The importance of vitamin D testing

The dosage of vitamin D allows for the diagnosis of deficiency states, intoxication, monitoring of treatments, and evaluation of risks, following a recommendation from a physician. This dosage is necessary in the presence of symptoms indicative of a deficiency: rickets, which shows up in children as incorrect growth patterns, weakness in muscles, pain in bones and deformities in joints, fatigue, mood changes (54), especially in at-risk individuals (55).

3. Pre-analytical steps for vitamin D measurement

They concern all the manipulations performed on the blood sample before its analysis in the laboratory:

- The serum should preferably be collected while fasting, except for certain therapeutic treatments, in a dry tube (56).
- The volume of blood collected must be sufficient to perform the analysis (57).

The collection must be carried out following aseptic rules, transported at room temperature, and stored after centrifugation at -20°C until analysis (57).

Due to the lipophilicity of vitamin D, regardless of the dosing method employed, a complete dissociation step of 25(OH) D from carrier proteins is necessary to accurately quantify it. Their varying concentrations under physiological conditions can influence the dissociation kinetics of the molecule (55).

4. Analytical methods for dosage of Vitamin D

For today, different analytical methods are available to quantify vitamin D in human plasma or serum samples (58). Currently, two types of methods are used: non immunological, direct detection, Separative, chromatographic methods and immunological methods(immunoassays) (4). Because they can produce results quickly and are automated, the second are utilized more frequently in clinical practice. Contrary to immunoassay techniques, chromatographic methods enable the identification of vitamin D metabolites; however, they are more complicated because of their technical apparatus, lengthy sample preparation, and evaluation (49).

4.1 Non immunological methods

Methods based on chromatographic separation followed by non-immunological direct detection (HPLC-UV and LC-MS/MS) (51).

4.1.1 High Performance Liquid Chromatography-UV for measurement of 25(OH) D2 and D3

- **Definition**

High Performance Liquid Chromatography which is known as High Pressure Liquid Chromatography. It is a widely used analytical method for separating, identifying, and quantifying each mixture component (59). HPLC is one of the most adaptable techniques that have been used for the analysis of 25(OH) D2 and D3 (60). The most popular techniques combine UV detection with liquid-liquid or liquid-solid pre-sample cleanup following column separation (61).

The first direct UV detection-based HPLC assays for 25OHD were published in 1977 (62). The preferred technique for 25(OH) D is now HPLC techniques with UV detection (63).

- **Principle**

With High performance liquid chromatography (HPLC), a simple sample preparation process of protein precipitation followed by liquid-liquid extraction assures effective release of 25(OH) D from the binding protein (62).

Laborious chloroform-methanol extraction was succeeded by chromatography on Sephadex/silica gel columns, followed by high performance liquid chromatography (HPLC) with ultraviolet (UV) detection (64), which can applied in plasma and serum (65).

HPLC coupled with UV detection is a widely used analytical method for quantifying vitamin D metabolites, including 25-Hydroxyvitamin D2 and 25-Hydroxyvitamin D3, in biological samples (66).

Vitamin D metabolites absorb UV light at specific wavelengths, allowing their detection without derivatisation (58). These compounds are separated based on their interactions with the stationary phase, typically a C18 reversed-phase column (67), and eluted using a suitable mobile phase (68).

Accurate quantification requires efficient extraction of vitamin D metabolites from complex biological matrices. Common sample preparation steps include (58):

✓ Protein precipitation using solvent like Acetonitrile (64), to precipitate proteins release bound vitamin D metabolites (58).
✓ Liquid-Liquid extraction employing non-polar solvents such as n-hexane to extract the hydrophobic vitamin D metabolites (58).
✓ Solid-phase extraction (SPE) to further purify and concentrate the analytes (58).

Reversed-phase HPLC, particularly with C18 columns, represents a key advancement in chromatographic techniques for vitamin D analysis (64).

The mobile phase is a mixture of organic solvents (e.g., methanol, Acetonitrile) and water, optimized for effective separation (62–66). Isocratic elution mode, with a constant flow rate, is often used to ensure reproducibility (62,65–68).Detection is carried out at approximately 264 nm, the wavelength corresponding to the maximum absorbance of vitamin D metabolites (68). The software used retention time for peak identification and peak height ratio for quantification (62).

All samples preparations including extractions, dilutions, and measurements are performed in darkness, with the tubes properly covered from light (61).

The determination of 25OHD by HPLC with UV detection can be considered the gold standard method (61).

To quantify 25(OH) D 2 and D 3 and other metabolites from plasma samples, researchers have created an automated ultra-high-performance liquid chromatography (UH-PLC) system based on diode-array detection. An ultimate 3,000 UHPLC dual gradient systems was used to create this technique. It has an online solid-phase extraction-concentration column connected to the analytical column via a six-port switching valve (2).

- **Advantages**

The low bias and variability, the ability to analyze D2 and D3 metabolites independently, and less reagents costs are some benefits of using the HPLC method to measure vitamin status (58). The low analysis time makes it ideal for typical clinical laboratory practice. HPLC procedures would be the ideal techniques for quantifying these two metabolites due to their

great selectivity and superior analytical performance (62). It is an inexpensive, high-capacity method (2).

HPLC has gained popularity for the detection of vitamins in a variety of matrices because of its quick separation, high sensitivity, and precise quantification (62) .

The use of commercial serum calibrators and controls reduces the variability of results obtained using various chromatographic techniques and improves traceability (69).

- **Limitations**

HPLC is insufficiently sensitive for identifying trace quantities of 25(OH) D2 and D3.It requires complex pre-treatment procedure, which needs a skilled person (2).

Lack of specificity in UV detection and high limits of detection (LOD) necessitated huge sample quantities circulating vitamin D metabolites present at low concentration (51).HPLC makes it difficult to separate vitamin D analogues because of their similar structure and chemical characteristics (2).

4.1.2 Liquid chromatography–Tandem Mass Spectrometry(LC-MS/MS) for measurement of vitamin D

- **Definition**

Liquid Chromatography-tandem mass spectrometry (LC-MS/MS) is the most effective instrument available for analyzing vitamin D molecules in bodily fluids at the moment (51). LC-MS/MS is thought to be highly accurate because of tandem mass spectrometry's selectivity (70).The method employs molecular mass as a detection technique following simple extraction and chromatography procedures (71). The development of LC-MS interfaces to separate solvent from solutes and more recent methods to ionize lipid-soluble compounds, like vitamin D metabolites, have enabled the emergence of liquid chromatography-tandem mass spectrometry (LC-MS/MS), the most recent development in HPLC based technologies (71). LC-MS/MS techniques may differentiate between the D3 and D2 forms of vitamin D based on their mass-to-charge (m/z) ratio, facilitating the identification of the vitamin source (64).

- **Principle**

The NIST reference method outlines three steps for LC-MS/MS analysis of serum 25(OH) D: extraction, chromatography and mass spectrometry detection (71).

Vitamin D and its metabolites are tightly bound to VDBP, albumin, and lipoproteins in serum and plasma; for example, about 90% of circulating 25(OH) D is protein bound and must be released and purified before analysis. A variety of extraction techniques have been described in the literature; all of these methods use strong organic solvents to precipitate proteins and release vitamin D metabolites from carrier compounds, followed by an additional extraction step to increase purity and minimize interferences during analysis. Basically, two types of extraction techniques can be distinguished: solid phase extraction and liquid –liquid extraction (LLE) (23), as summarized in table (IV):

Table IV: List of solvents used for protein precipitation, for liquid-extraction and for solid phase extraction

Protein precipitation	Liquid-Liquid extraction	Solid-phase extraction
Methanol(MeOH)	Acetone	Oasis HLB 24 µL 20 mm × 2.1 mm
Acetonitrile	Heptane	POROS R1/10
MeCN/MeOH	Hexane	Zorbax Eclipse XDB-CN 5 µm, 50 mm × 2.0 mm
MeOH/Isopropanol	Ethyl acetate	
Zinc sulphate/MeOH	Methyltert-butylether	

(23)

To account for the low ionization efficiency of vitamin D and its analogues, studies suggest an additional chemical derivatisation is recommended to move the analytes m/z ratios to a higher mass range, where the chemical noise is smaller (2). Derivatisation is necessary for 25(OH)D due to its relative abundance, while low concentration metabolites like 24, 25(OH)2D or 1, 25(OH)2D rely on signal amplification through derivatisation (23).

The separation of substances in a complicated mixture is the aim of liquid chromatography. A hydrophobic stationary phase is necessary for the separation of hydrophobic substances, such as vitamin D metabolites (23). LC-MS/MS often uses reverse-phase chromatography with bonded phase and methanol-water gradient solvent systems, which are more compatible with mass spectrometry processes (71). Successful chromatographic separation relies heavily on the composition of mobile phase. Vitamin D testing requires both an organic and aqueous

mobile phase. The aqueous phase which is commonly water stabilized with formic acid, is utilized to apply the sample to the column. Organic mobile phase is required for eluting hydrophobic analytes from reversed phase columns (23). After separation by chromatography, samples are vaporized and ionized in a mass spectrometer. Ion pass through three electrically charged quadrupoles that filter them based on their mass to charge ratio (m/z). The first and third quadrupoles only allow specified m/z ions to pass. All other ions are removed and do not enter the detector. After successfully passing the first quadruple, ions are fragmented in the second quadruple by colliding with an inert gas like nitrogen, helium, or argon. In the collision cell, each parent ion generates a distinct spectrum of fragment ions, which are then filtered in the third quadrupole before reaching the detector (72). One of these ions serves as a qualifier, while the other is employed for quantification. The qualifier confirms the presence of the correct parent ion in the second quadrupole. The quantifier calculates the concentration of analytes in a sample (23). This process is illustrated in figure 4:

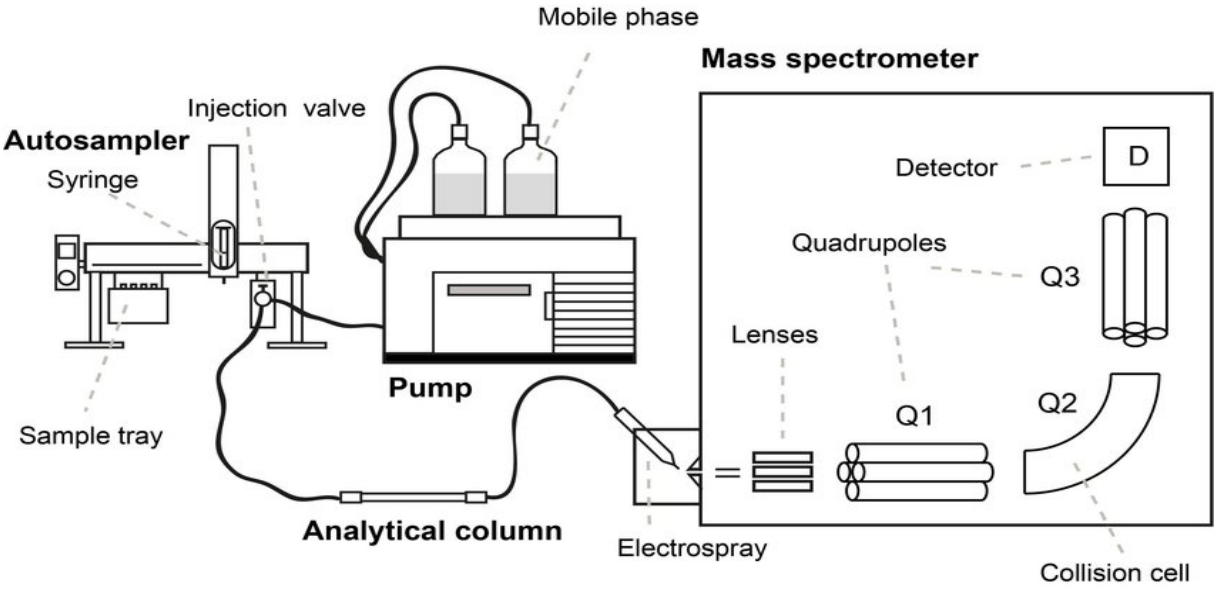

Figure 4 : Schematic-overview-of-LC-MS-MS-system-with a triple-quadrupole-mass-analyzer (73).

- **Advantages**

LC-MS/MS is considered the gold standard for quantifying vitamin D and its metabolites (74,75). It is commonly used in clinical laboratories to analyze low molecular weight analytes

due to its higher specificity, sensitivity and dynamic range (76). LC-MS/MS for vitamin D analysis permit the quantification at high throughput levels, with excellent reproducibility and accuracy in different laboratories (77). Furthermore, LC-MS/MS has shorter run times, can measure D2 and D3 individually, and total 25-OHD (sum of D2 and D3) (70).

LC-MS/MS vitamin D assays improve accuracy in medical decision making by correctly classifying patients as vitamin-D deficient and sufficient (76). Previous studies indicate that this method can quantify 25(OH) D3, 25(OH) D2, and C3-epi 25(OH) D in a single analysis (78). LC-MS/MS can quickly and easily analyze some of the rare vitamin D metabolites in clinical samples, such as 24, 25-$(OH)_2D_3$, 24, 25-$(OH)_2D_2$, and 25-OH-D_3-26, 23-lactone (71).

- **Limitations**

LC-MS/MS have limitations due to complexity and interference from sample matrices (2). This procedure requires experienced staff and costly equipment, which are not commonly available (75). Vitamin D epimers may have similar chromatography to 25-OHD, overlapping peaks and forming the same masses after ionization (70) .

4.2 Sensors for measurement of vitamin D

Analytical sensors have gained popularity recently because of their high sensitivity, portability, low environmental impact, small size and lack of need for trained labor. Several sensors have been investigated for 25(OH) D3. These sensors have been listed in electrochemical and colorimetric sensors (39).

Lee et al, created a gold nanoparticle-based immunoassay for the colorimetric measurement of 25(OH) D2 and D3, utilizing a smart phone. The system includes a Smartphone attachment, an app, and a test strip. The technology can test physiological levels of 25-hydroxyvitamin D with an accuracy of more than 15nM and precision of 10 nM (79).

Bang Hyun Lee Et al., used an aptamer's-based approach to analyze 25(OH) D3 in cases of vitamin D deficiency. The aptamer VDBA14, which was created using the SELEX process, showed a higher affinity than the others. Using an isothermal titration calorimetry method and a colorimetric assay mediated by gold nanoparticle, the specificity and affinity of this aptamer were verified (80). In a study published by Alsager ET al., the aptamer VDBA14 was used to detect vitamin D from the sample solution. Following target recognition, an extra

centrifugation step was performed to remove any remaining binding between the aptamer's non-binding sequences and the gold nanoparticle surface. After vitamin D detection, the aptamer sequences that were still attached to the particle surface were able to completely dissolve through centrifugation and resuspension (2).

Sensor-based 25(OH) D detection has several drawbacks despite its great sensitivity, including low selectivity between vitamins 25(OH) D2 and D3, instability and poor reproducibility. Only one type of vitamin D 25(OH) D2 or D3 can be detected in standard solution by the sensors that are currently in use. Therefore, future studies can concentrate on more sensitive and accurate detection of several vitamins from clinical samples (81).

4.3 Immunological methods (Immunoassays)

An immunoassay consists of any technique that utilizes the antigen-antibody reaction for the detection and quantification of antigens, antibodies, or related substances, are rapid and accurate tests that can be used on-site and in the laboratory to detect specific molecules in a blood sample(82). Most tests measure the total amount of 25(OH) D and cannot distinguish between the D3 and D2 forms, which can lead to an overestimation or underestimation of the results. In contrast, immunoassays differ from one another in their cross-reactivity with various D3 metabolites such as 25(OH)D3, calcitriol, 24,25(OH)2D3, as well as the C3 epimers (39).

The immunoassays analytical methods used for the measurement of vitamin D are: Radioimmunoassay (RIA), Chemiluminescent Immunoassay (CLIA), Enzyme-Linked Immunosorbent Assay (ELISA) (2).

4.3.1 Radioimmunoassay (RIA)

- **Definition**

It is The first immunoassay enabling the measurement of a total of 25(OH)D (39). It allows for the quantification of specific antigens in the patient's serum. This technique uses antigens labeled with radioisotopes, typically I^{125}, which necessitates specific safety measures (83).

- **Principles**

•Biological principle: are highly specific antibody-antigen reactions, capable of identifying certain substances (84).

• Physical principle: this involves labeling these substances by introducing radioactive atoms into their molecules (84).

•The radioimmunoassay techniques are based on the phenomenon of competition between a radioisotope-labeled antigen (Ag) and the unlabeled antigen that one wishes to measure. The labeled antigen produces a labeled Ag-Ac complex, and the free unlabeled antigen preferentially binds to Ac in a manner inversely proportional to the amount of labeled free Ag added to the reaction medium (85).

The higher the concentration of the antigen to be measured, the more competition there will be with the radioactive antigen to bind to the antibody, and the less radioactive antigen bound to the antibody, the lower the measured radioactivity will be, it is a decreasing curve of radioactivity. The decay curve of radioactivity is formed by increasing the amount of unmarked free Ag added to the reaction medium and looks like this in the figure 5 (84).

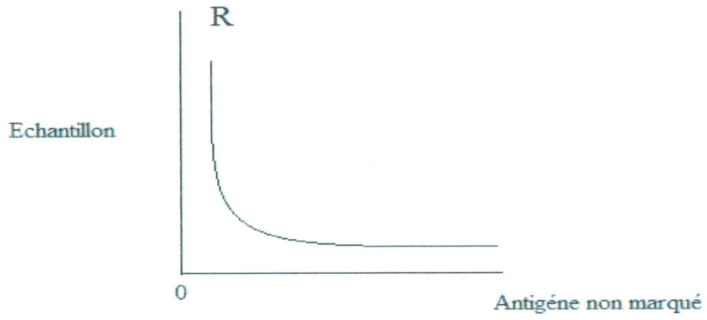

Figure 5 : The decreasing curve of R (84).

The radioactivity of the two fractions can be measured separately: marked Ag-Ac and unmarked Ag-Ac, and the following ratio can be calculated:

$$R = \frac{Linkedradioactivity}{Freeradioactivity} (84)$$

a) Competition method

A fixed concentration of labeled antibodies and antigens is maintained, while simultaneously increasing the concentration of the antigen to be measured, which leads to an increase in the concentration of the antibody-antigen complex at the expense of the labeled antigen-antibody complex (86), as shown in figure 6.

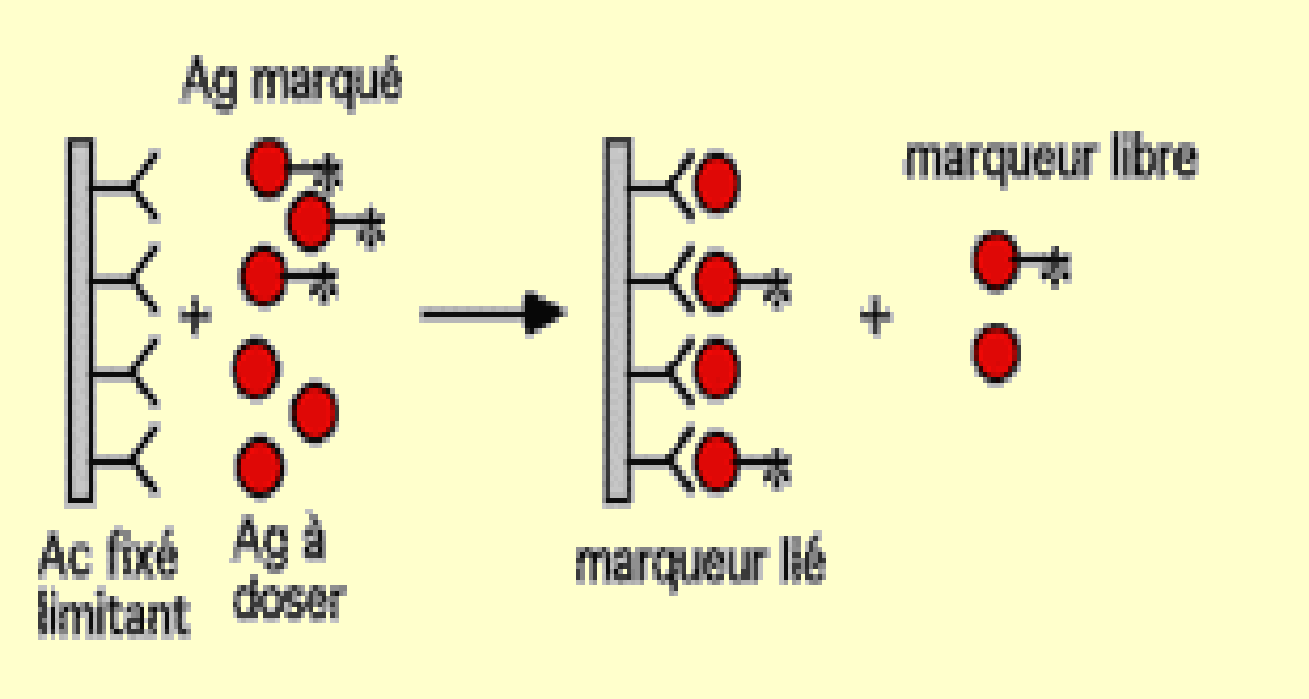

Figure 6 : Radio immunological techniques : competition method (86).

b) The dosing of 25OHD by Radioimmunoassay

There are two steps involved in the dosing of 25(OH) D by RIA, in the first step, 25(OH) D and other hydroxylase metabolites are quickly extracted from serum or plasma using Acetonitrile. Following extraction, RIA competitively doses the treated sample using an antibody with 25(OH) D specificity. The tracer, antibodies, and sample are incubated at 20–25 °C for 90 minutes. After 20 minutes of incubation at 20–25 °C, the phases are separated using a second antibody that precipitates the complex. After this incubation, a tampon is added before centrifugation to reduce the nonspecific interaction (87).

- **Advantages**

- The isotope allows for easy labeling (85).

- The signal is direct, emitted by the marker itself (85).

- The signal is spontaneous, not requiring an external energy source (85).

- Simple, accurate, cost-effective (39).

- **Limitations**

- Require a high affinity of the antibody (Ac) for the antigen (Ag) (85).

- A constant number of antibody sites in each reaction tube is necessary to achieve good accuracy (85).

- Precautions and monitoring are essential during handling (85).

- Manual, radioactive waste, cross-reactivity with vitamin D metabolites (39).

4.2.3 Chemiluminescent Immunoassay (CLIA)

- **Definition**

CLIA is a technique used to determine the concentrations of samples based on the intensity of light emitted by a chemical and biological reaction. CLIA combines chemiluminescence (CL) systems and immunoreactions; certain chemicals have been used as CL labels, and the system produces chemiluminescence when the CL substrate is added, allowing for the measurement of samples. The most common CL substrates include luminol and its derivatives, alkaline phosphatases (ALP), peroxidase, and acridinium ester compounds. The enzyme is also utilized in CLIA for the labeling of the target protein, with ALP and black radish peroxidase being widely used for enzymatic labeling. Clinical diagnostics, environmental monitoring, pharmaceutical analysis, and food safety are just a few of the areas in which CLIA has been utilized. CLIA offers the benefits of high clinical sensitivity and immunoreactions specificity (88).

- **Principle**

The quantitative method for determining 25OHD is a direct competitive chemiluminescent immunoassay (CLIA) conducted on an automated platform. A specific antibody for vitamin D is utilized on a solid phase, and vitamin D is conjugated to an isoluminol derivative. During incubation, 25(OH) D dissociates from its binding protein and competes with the labeled vitamin D for binding sites on the antibody. After incubation, unbound material is removed through a washing cycle. Subsequently, trigger reagents are added, and an instantaneous chemiluminescent reaction is initiated. The light signal is measured by a photomultiplier in relative light units (RLU) and is inversely proportional to the concentration of 25(OH) vitamin D present in the standards, controls, or samples (87), as shown in the following figure 7:

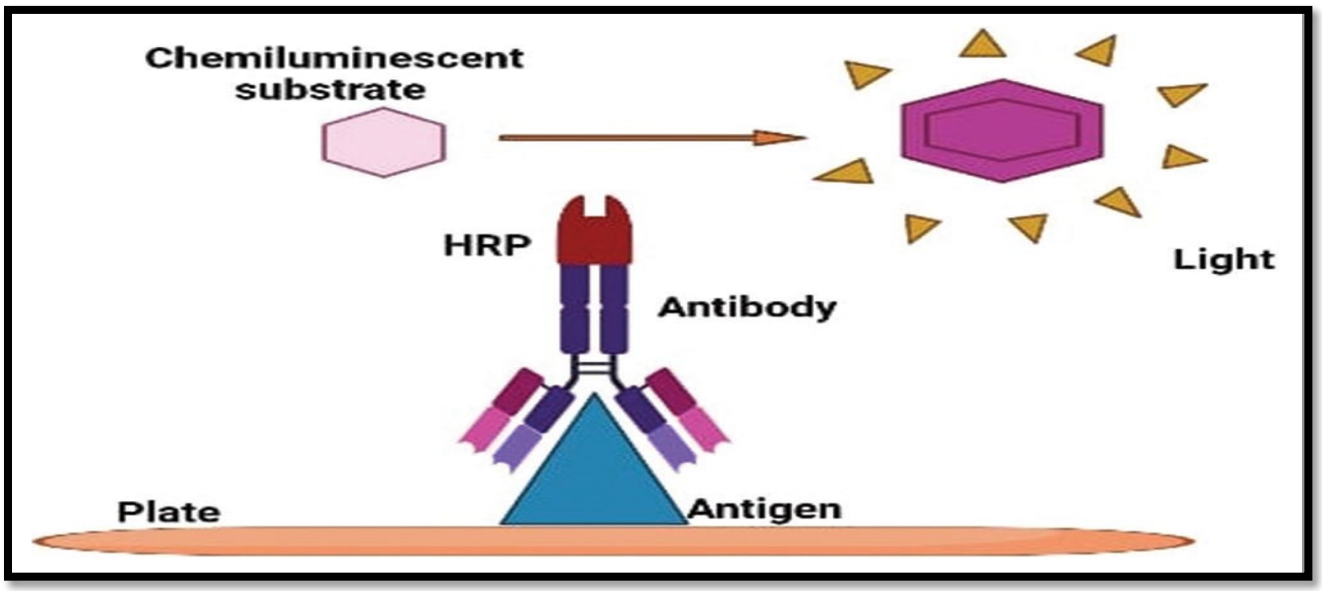

Figure 7: Chemiluminescence immunoassay and its principle (88).

- **Advantages**

-Automated (39).

-High throughput (39).

-Simple (39).

-Fast (39).

- **Limitations**

-Cross-reactivity with vitamin D metabolites (39).

-Some assays detect only 25(OH) D3 (39).

-Affected by VDBP from sample (39).

4.2.4 Enzyme-Linked Immunosorbent Assay (ELISA)

- **Definition**

The ELISA (enzyme-linked immunosorbent assay), also known as EIA (enzyme immunoassay) (89), is a competitive assay in which the 25-hydroxyvitamin D (25OHD) in the sample competes with labeled 25OHD (90). It's an enzyme detection method that allows for the characterization of the majority of antibodies (AAbs). They are now widely used due to their sensitivity (89).

- **Principle**

The samples were incubated with the release agent to separate 25(OH) D molecules from the DBP at a temperature of 37°C for 1 hour. Subsequently, the samples and anti-25(OH) D were added to micro titration plates coated with 25(OH) D. During an overnight incubation (18-22 hours), the 25(OH) D molecules in the sample and those immobilized on the plates competitively interacted with the available anti-25(OH) D antibody. The plates were washed to remove any unbound anti-25(OH) D, and a horseradish peroxidase-conjugated antibody was added to form the 25(OH) D─anti-25(OH)D─peroxidase antibody complex at the surface of the plate. After 1 hour of incubation, the plates were washed, and tetramethylbenzidine (TMB) substrates were added, resulting in a reaction that caused a color change in the solution. After 20 minutes, the reaction was stopped with an acidic solution, and the absorbance of the solution was measured using a spectramax 384 at 450 nm (91). The developed colorimetric intensity is inversely proportional to the concentration of 25-hydroxyvitamin D (25OHD) (90), the method is explained in the figure 8.

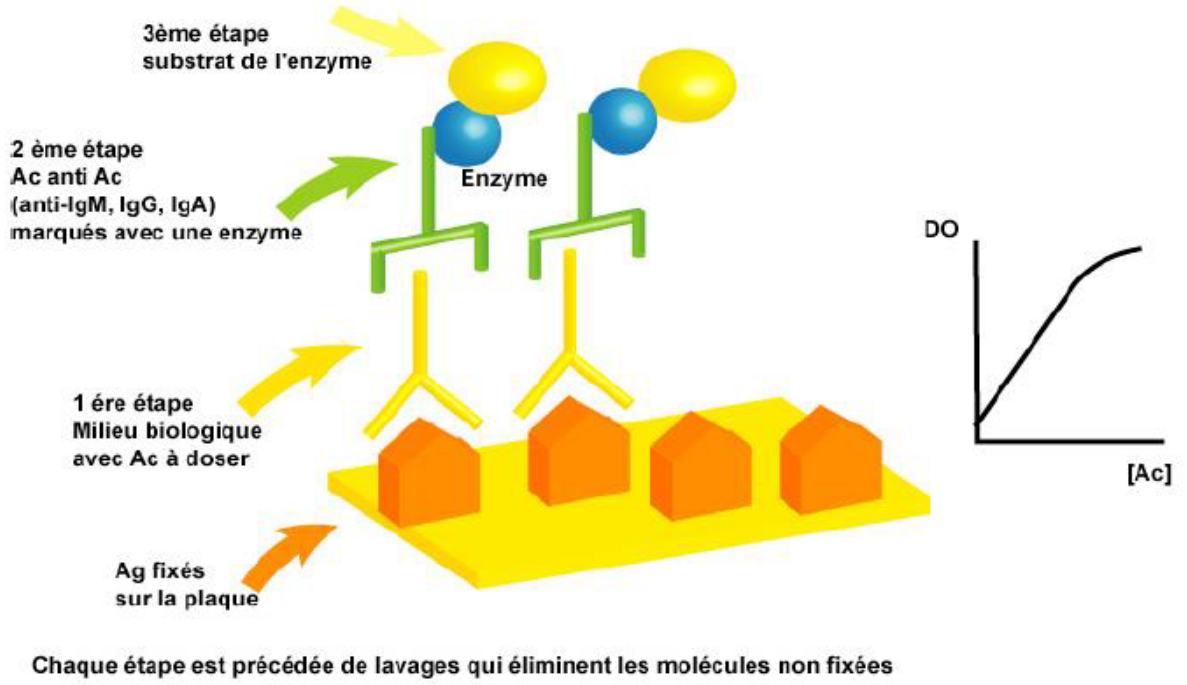

Figure 8: Principle of the direct ELISA test (89).

- **Advantages**

- A simple and highly sensitive technique exhibiting high specificity (91).
- An automated technique allows for the rapid processing of large batches of analyses, thereby saving time (91).
- A reproducibility that often exceeds that of a manual method, and is accessible to all biologists (91).
- Computerized management of results (absence of subjectivity) (91).
- Accurately quantify the antibodies (AC) (91).

- **Limitations**

- There is a risk of receiving falsely positive results. (The nonspecific binding of plasma proteins to the support may be due to inadequate saturation of the free sites on the support) (91).

- They are often not standardized, leading to significant variability in the results among the various commercial kits available (91).
- This method is sensitive to temperature, pH, and light due to the enzymatic reaction (91).
- Depends on the nature and quality of the antigen (91).
- Influenced by the effectiveness of Ag (silver) adsorption on the solid support (91).

PRACTICAL PART

Vitamin D plays an essential role in the health of children and adolescents, and its deficiency is a global health problem with potentially serious consequences. However, quantifying vitamin D can be complicated by the variety of analytical methods, each with its own advantages and limitations in terms of accuracy, sensitivity, and cost. It is therefore necessary to evaluate and compare these methods to ensure a reliable and accurate diagnosis of vitamin D deficiency. In this study, two questionnaires were prepared, based on the information obtained in the theoretical part, one addressed to medical analysis laboratories and the other to different doctors.

1. Study Objectives

1.1 The main objectives

- Identification of analytical techniques for quantification of Vitamin D.
- Assessment of hypovitaminosis D in children and adolescents.

1.2 Secondary objective

- Identify risk factors for vitamin D deficiency, and the importance of dosage in avoiding the consequences of hypovitaminosis D.

2. Materials and methods

2.1. Part one : questionnaire for laboratories

2.1.1. Type of Study

It is a retrospective cross-sectional study carried out in medical analysis laboratories, using data from the year 2024 (01 January, 2024– 31December, 2024).

2.1.2. Location and duration of the Study

It's being carried out in various medical analysis laboratories in and around the Wilaya of Tlemcen, as well as in the Wilaya of Algiers and Adrar.

Data are collected over 4 months: January, February, March and April 2025.

2.1.3. Study Population

13 private laboratories and 03 state laboratories were selected on the basis of inclusion and exclusion criteria.

-For private laboratories: 11 in and around Tlemcen, 2 in Adrar.

-For state laboratories: 2 in Tlemcen and 1 in Algiers.

2.1.3.1. Inclusion Criteria

Any laboratory that performs vitamin D analysis and accepts the request to participate in the study by agreeing to complete the questionnaire.

2.1.3.4. Non-inclusion Criteria

Laboratories that do not perform vitamin D analysis and do not accept requests to participate.

2.1.4. Data collection and study process

Data was collected using a questionnaire that was developed and validated before collecting the necessary information.

The content of the questionnaire focused on 2 main areas:

- **Vitamin D Dosage Methods**

Made up of several headings

1. General information about the laboratory (I.1 to I.2) : It allows describing the questioned laboratories: the type of laboratories and the specialties covered by the laboratories.

2. Pre-analytical (II.1 to II.5) : Studied the pre-analytical stages of vitamin D assay (sample and tube used for assay, storage conditions, storage time and factors influencing assay).

3. Methods of analysis (III.1 to III.13) : Collected information about: the machine and method used for vitamin D dosage, the reasons for choosing the method, Difficulties faced, reported errors and their main sources.

4. Quality and performance of the method (IV.1 to IV.7): Evaluated the quality and performance checks carried out, their frequency and limit of detection of the method used.

5. Results and interpretation (V.1 to V.7) : Contains the unit of measurement, reference intervals for interpreting vitamin D results and reference values used for the diagnosis of deficiency or insufficiency in the general population and in children and adolescents.

6. Data collection (VI.1 to VI.8) : Contains frequency of vitamin D assays, data collection tools and frequency of deficiencies observed.

- **Assessment of Hypovitaminosis D in Children and Adolescents**

This part explored (1 to 11) : Frequency of receipt of vitamin D analyses, the evolution and frequency of hypovitaminosis D in children and adolescents and even the general population during 2024, the sex most implicated in this hypovitaminosis.

The questionnaire was accompanied by an explanatory letter that described the study's aims, as well as the approach's anonymity, confidentiality, and non-commercial nature.

The collection time for a laboratory questionnaire ranged from 1 to 3 weeks and a few of them did not respond due to lack of time, or the absence of a laboratory manager.

The questionnaire is composed of closed answers and open questions, check boxes are used.

An “other” item has been inserted to add any responses we might not have thought of.

The questionnaire was distributed via online platforms (Google Forms), by e-mail, or manually through contact with the participants.

The total number of laboratories selected in this study is 24, 16 laboratories agreed to fill out the questionnaire and provide access to obtain the necessary information, 4 of them responded only to the first part.

The remaining 08 laboratories did not accept the request.

2.2. Part Two : questionnaire for doctors

2.2.1. Type of study

It is a transversal study, carried out with doctors from different specialties.

2.2.2. Location and duration of the study

It was conducted in the pediatrics department of CHU Tlemcen, Boudghen, as well as in private clinics in and around the Wilaya of Tlemcen, for 03 months: from February 2025 to April 2025.

2.2.3. Study population

A group of doctors with different specialties.

2.2.3.1. Inclusion criteria

All doctors who provide regular consultations for children and adolescents (2-17 years old).

2.2.3.2. Non-inclusion criteria

All doctors who do not provide medical consultations for children and adolescents.

2.2.4 Data collection and study process

Data was collected using a questionnaire addressed to the general practitioners, pediatricians, endocrinologists and other specialists treating children and adolescents (2–17 years old), who work in public or private structures.

Contents:

-General information (specialty……)

-Clinical situations demanding dosages (stunted growth, fatigue, etc).

-Frequency of prescription of vitamin D dosage.

-Thresholds used to diagnose a deficiency.

The questionnaire contained an explanatory introduction describing the aim of the work in addition to the anonymity and confidentiality of the questionnaire.

An “other” item has been inserted to add any responses we might not have thought of.

The questionnaire was distributed via online platforms (Google Forms), by e-mail, or manually through contact with the participants.

The time for answering and collecting the questionnaire ranged from 15 to 30 minutes.

2.3 Data analysis

The data from the two questionnaires were entered into an Excel spreadsheet using Microsoft Excel 365(Version 2504) and Microsoft Office Excel 2007.

3. Results

This section presents the results obtained from the analysis of the data collected. It highlights the essential elements in line with the research objectives.

The results are presented in the form of graphs described with percentages and fractions produced by Excel.

3.1 Part One: Data collected on the analytical methods for vitamin D measurement

3.1.1 Type of laboratories participating in the study

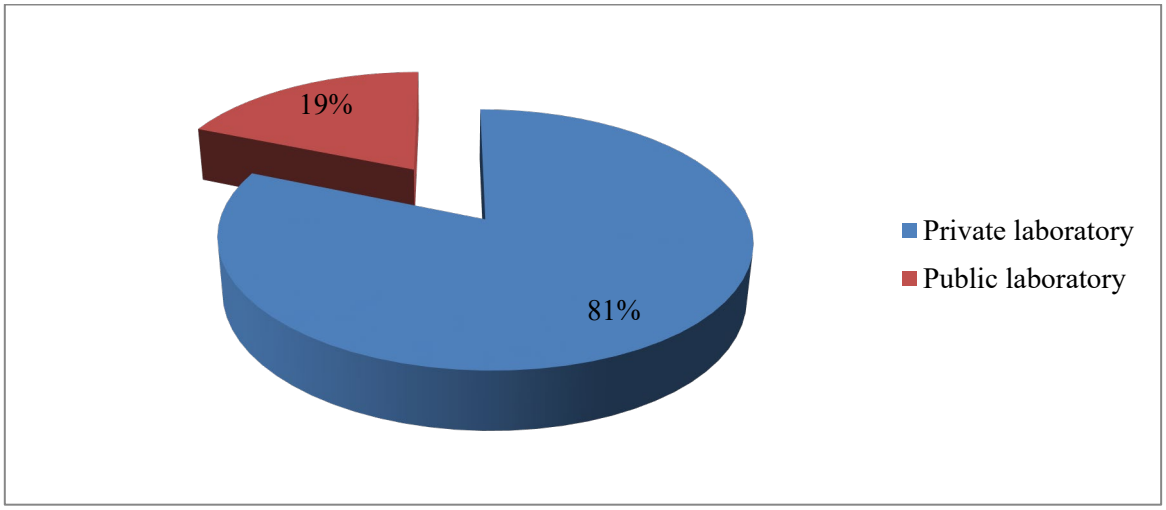

Figure 9 : Breakdown of laboratories types**.**

The number of laboratories participating in the study is 16. The majority of them are private with a percentage of 81%, compared with 19% in the public sector.

3.1.2 Specialties of the laboratories surveyed

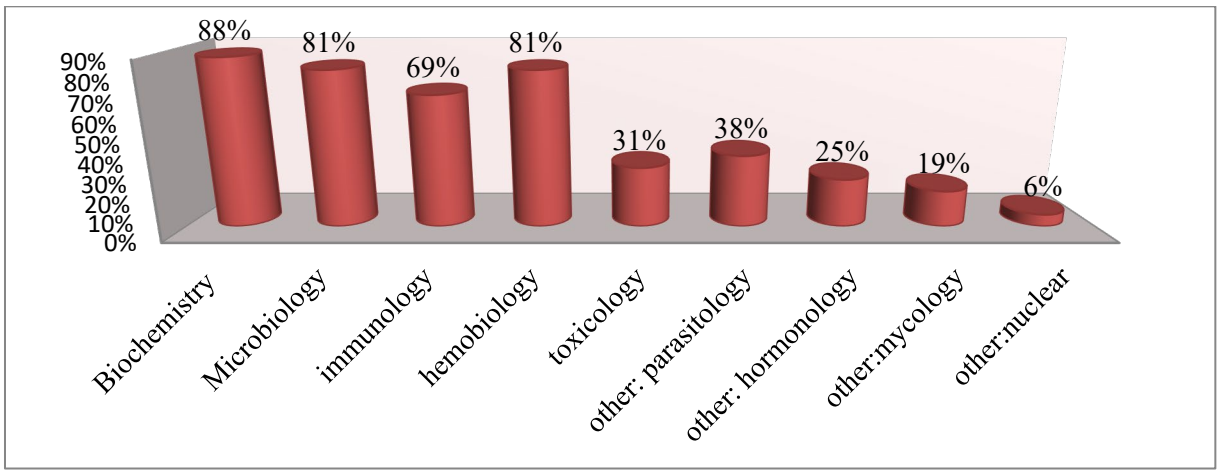

Figure 10 : Distribution of laboratory specialties.

The graph above shows the breakdown of specialties of the laboratories that took part in the study. There is a considerable diversity of disciplines, with a predominance of the following specialties: Biochemistry (88%), microbiology (81%), hemobiology (81%) and immunology (69%).

Other specialties represented include: Parasitology (38%), Toxicology (31%), hormonology (25%), mycology (19%) and nuclear (6%).

3.1.3 Type of sample used for vitamin D dosage

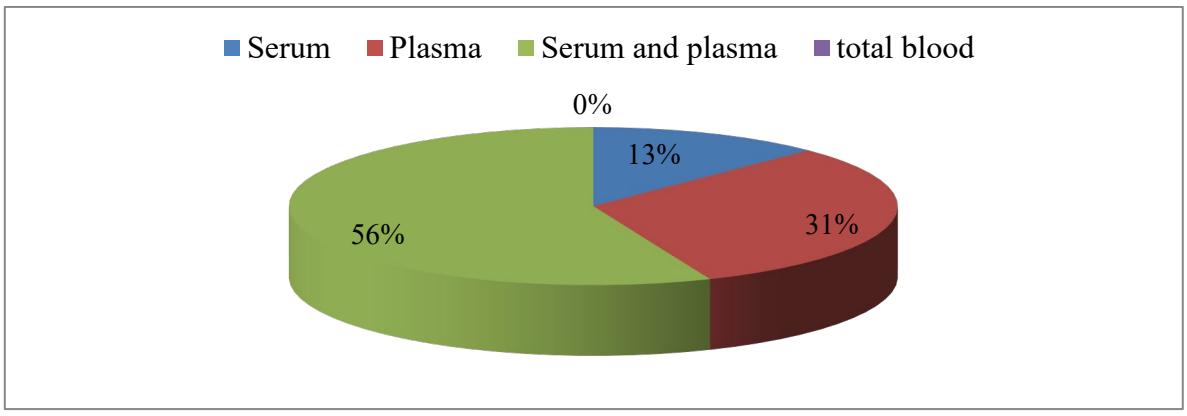

Figure 11 : Type of samples used for vitamin D dosage**.**

The chart illustrates the distribution of biological sample types used for vitamin D analysis in the surveyed laboratories.

It shows that 56% of laboratories used both serum and plasma, which represents the majority. In contrast, 31% used plasma only, and 13% used serum only. No laboratory reported using whole blood for this type of analysis (0%).

3.1.4 Type of tube used for vitamin D dosage

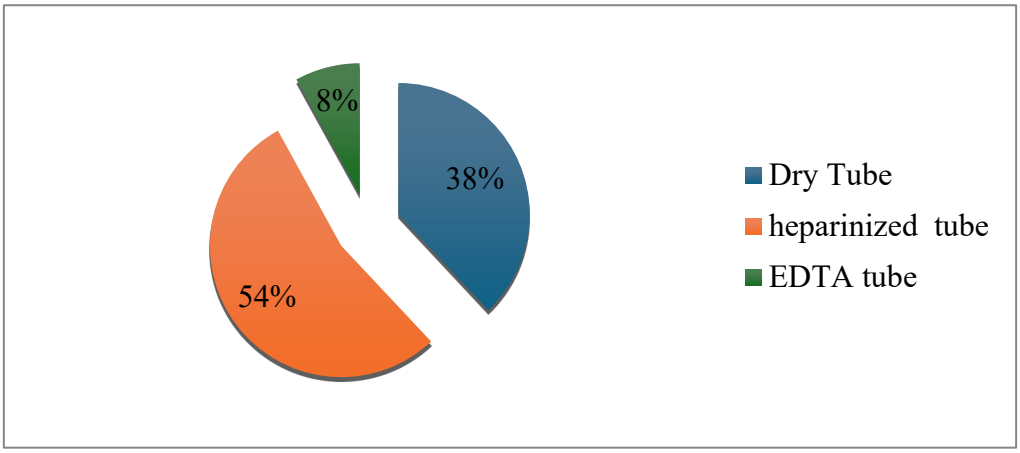

Figure 12 : Type of tube used for vitamin D dosage.

This figure illustrates the distribution of the types of tubes used for sample collection in the context of vitamin D dosage.

The majority of laboratories (54%) used heparinzed tubes, followed by 38% who prefer dry tubes. Only 8% of laboratories used tubes containing EDTA.

3.1.5 Storage conditions of sample before analysis

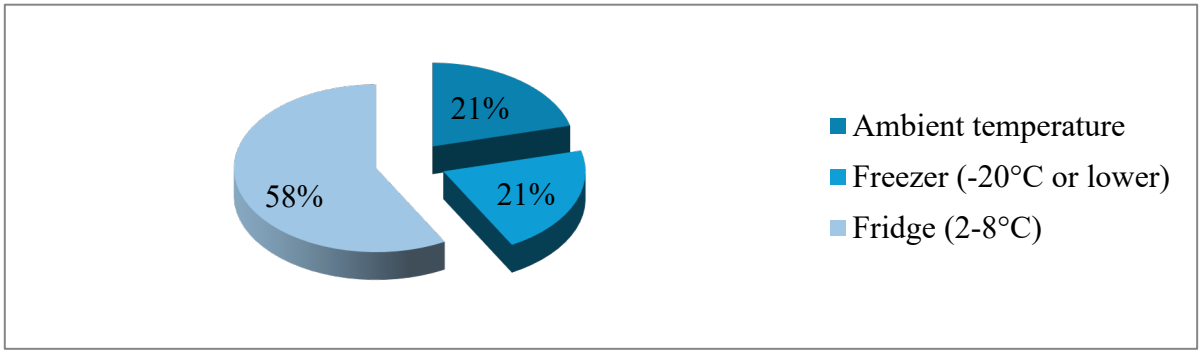

Figure 13 : Distribution of sample storage conditions prior vitamin D analysis.

This chart highlights the storage conditions of samples before vitamin D dosage. 58% of laboratories store samples in a refrigerator (2-8°C).An equal proportion (21%) use ambient temperature or freezers (-20°C or lower).

3.1.6 Maximum storage time for samples before analysis

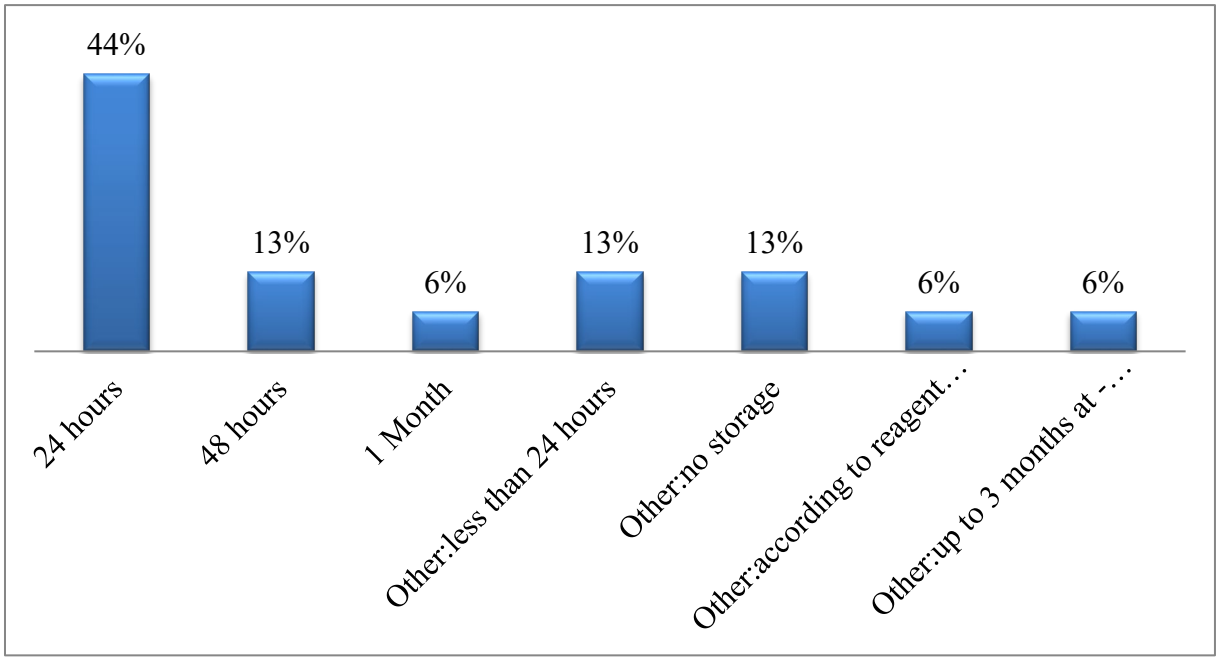

Figure 14 : Maximum storage time for samples before analysis.

The chart above summarizes the maximum storage durations adopted by laboratories for samples intended for vitamin D analysis.

The majority of surveyed laboratories (44%) reported storing samples for a maximum of 24 hours prior to testing. A smaller proportion (13%) reported storage up to 48 hours, while 6% indicated a maximum duration of 1 month.

Several responses categorized as “other” reveal additional variability:

- Less than 24 hours (13%)
- No storage at all (13%)
- Storage depending on reagent availability (6%)
- Storage for up to 3 months at -20°C (6%)

3.1.7 Pre-analytical factors affecting vitamin D assay results

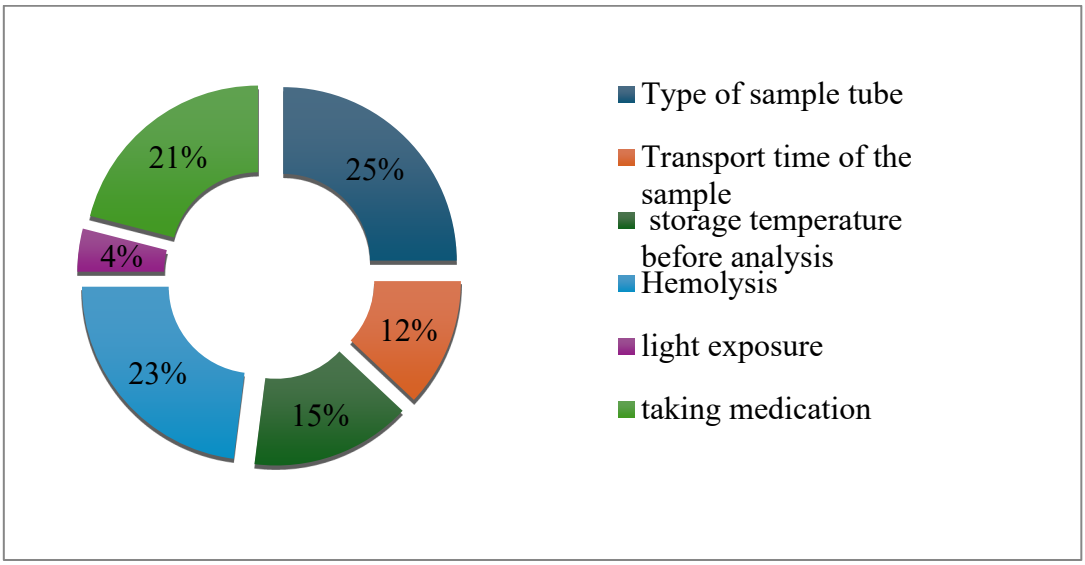

Figure 15 : Distribution of key pre-analytical factors influencing vitamin D assay accuracy.

This graph presents the main pre-analytical factors reported by laboratories as influencing the accuracy of vitamin D assays.

The type of sample tube was cited most frequently (25%), followed closely by hemolysis (23%) and medication intake (21%). Storage temperature prior to analysis accounted for 15%, while sample transport time represented 12% of responses. Less commonly reported was light exposure, noted by only 4% of participants.

3.1.8 Length of laboratory experience in performing vitamin D assays

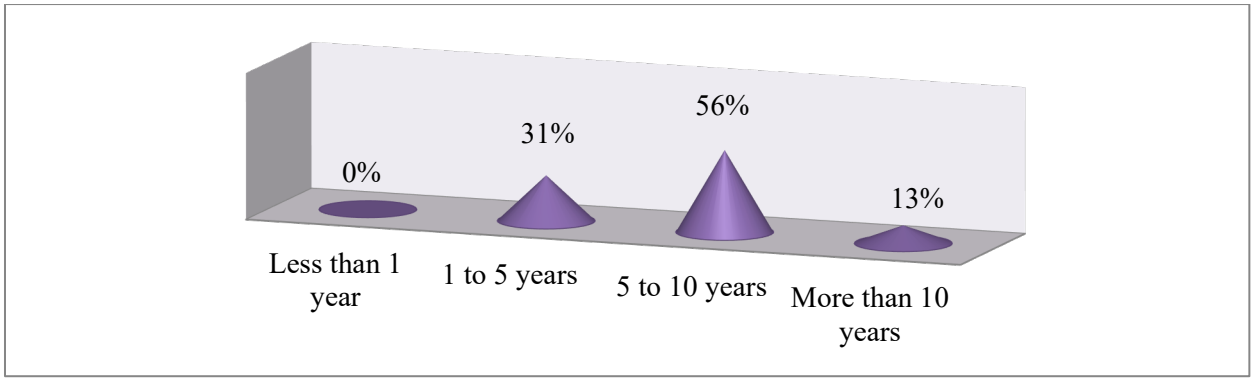

Figure 16 : Distribution of laboratories by experience in vitamin D analysis.

The majority of laboratories (56%) had between 5 and 10 years of experience followed by those with 1 to 5 years (31%). Only 13% had over 10 years of experience, while none reported less than one year.

3.1.9 The Automated system used for vitamin D assay

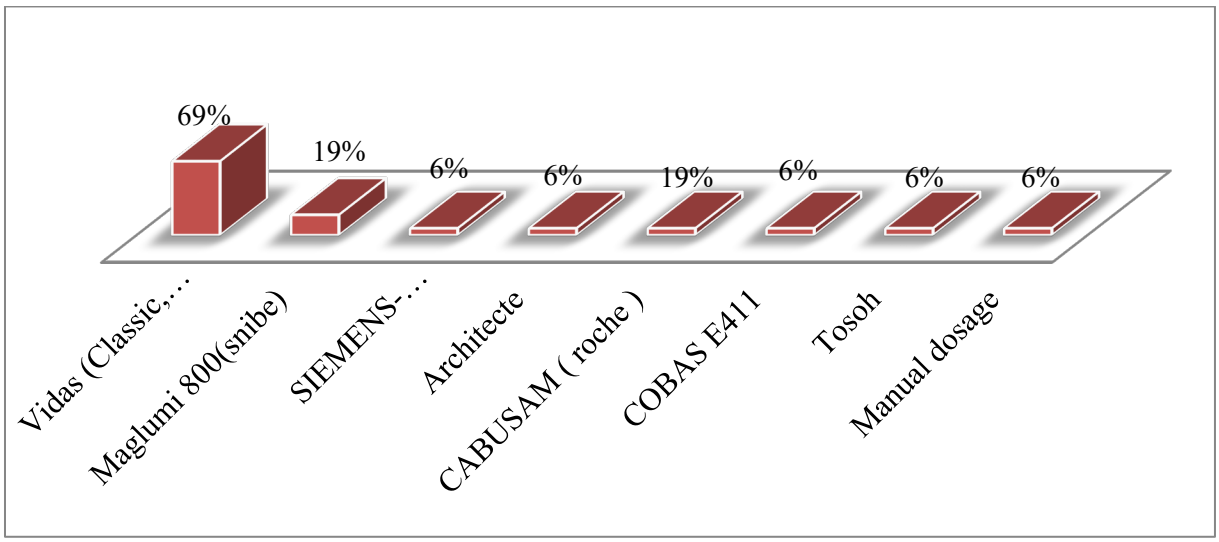

Figure 17 : The automated system used for vitamin D assay.

The graph presents the analytical platforms used by laboratories to perform vitamin D assays. The majority of respondents (69%) reported using the Vidas system with different version, 19% used Maglumi 800(Snibe). Other systems were reported with equal frequency (6%), including SIEMENS-Dimension EXL, Architect, CABUSAM (Roche), COBAS E411, Tosoh and manual assay method.

3.1.10 Period of use of equipment by laboratories

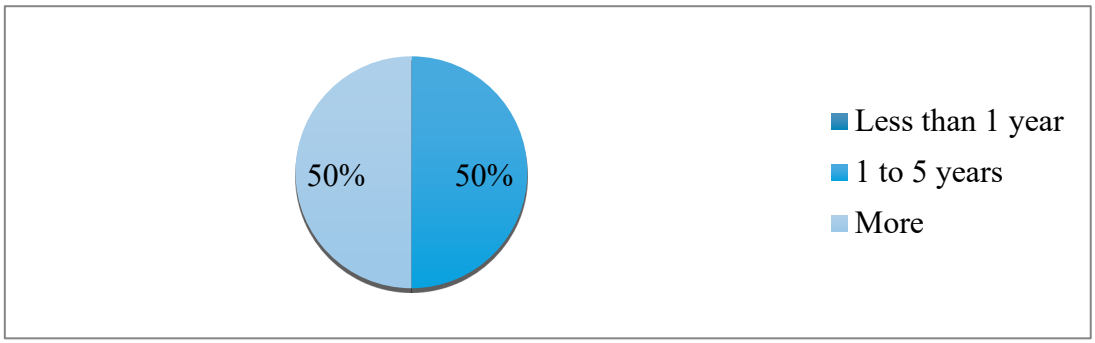

Figure 18 : Period of use of equipment by the laboratories**.**

This chart illustrates the duration for which laboratories have been utilizing their current equipment for vitamin D assays. Half of laboratories (50%) had been using their equipment for a period ranging from 1 to 5 years, while the remaining half (50%) had been operating with their current systems for more than 5 years. Notably, none of laboratories reported equipment use for less than 1 year.

3.1.11 Analytical methods used for vitamin D assay

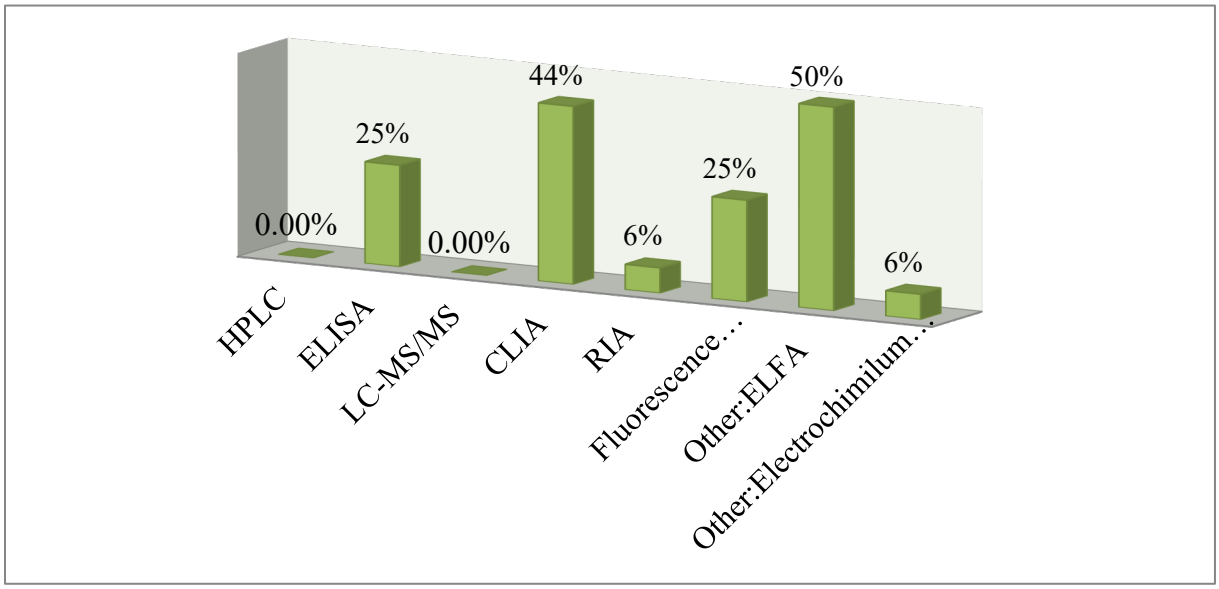

Figure 19 : Analytical methods used for vitamin D assay.

The data reveal a diversity of analytical methods used for the dosage of vitamin D across the surveyed laboratories. The most commonly used technique is ELFA (Enzyme-linked Fluorescent Assay), reported by 50% of the laboratories. This is followed by CLIA (Chemiluminescent Immunoassay), used in 44% of the cases. ELISA and fluorescence immunoassay are each employed by 25% of the laboratories. Less commonly used methods include RIA (Radioimmunoassay) and Electrochimiluminescence, each reported by 6% of laboratories. Notably, none of the laboratories reported using HPLC or LC-MS/MS.

3.1.12 Classification of methods used to measure vitamin D

Table V : Classification of methods used to measure vitamin D

Type of method	Method	(%)
Immunological methods	ELISA	25%
	CLIA	44%

	RIA	6%
	Fluorescence immunoassay	25%
	ELFA	50%
	Electrochimiluminescence	6%
Non-immunological methods	HPLC	0%
	LC-MS/MS	0%

The results show a clear predominance of immunological methods over non-immunological methods.

Immunological methods used in over 90% of cases, while no non-immunological methods (HPLC or LC-MS/MS) were reported by the participating laboratories.

3.1.13 Automated systems and analytical methods employed in vitamin D quantification across participating laboratories

Table VI : Automated systems and analytical methods employed in vitamin D quantification across participating laboratories

Laboratory	Type of laboratory	The automated system used for vitamin D assay	Analytical method used for the dosage of vitamin D
Laboratory 1	Private	Maglumi 800(Snibe) VIDAS	CLIA ELFA
Laboratory 2	Private	Mini VIDAS Maglumi 800(Snibe)	ELFA CLIA
Laboratory 3	Private	VIDAS	ELFA
Laboratory 4	State	SIEMENS-Dimension EXL	CLIA
Laboratory 5	Private	VIDAS	ELFA
Laboratory 6	Private	VIDAS Kube	Fluorescence immunoassay
Laboratory 7	Private	VIDAS	Fluorescence immunoassay
Laboratory 8	Private	VIDAS Maglumi800 (Snibe)	ELISA CLIA Fluorescence immunoassay
Laboratory 9	Private	VIDAS Tosoh	Fluorescence immunoassay
Laboratory 10	State	ARCHITECT	CLIA

		Manual dosage	RIA
Laboratory 11	Private	VIDAS PC	ELISA
Laboratory 12	Private	Maglumi VIDAS	CLIA ELISA
Laboratory 13	Private	VIDAS	ELFA
Laboratory 14	State	CABUSAM (roche)	Electrochimiluminescence
Laboratory 15	Private	CABAS E411	CLIA
Laboratory 16	Private	VIDAS	ELISA

Among the 16 participating laboratories, the two categories (private and state) reveal a notable difference in the automated systems used and the analytical methods applied for vitamin D dosage. The majority of laboratories in the study group (12out of 16) used automated system from the Vida range (Classic, Kube, Pc, Mini), as well as other platforms such as the Maglumi 800(Snibe), Tosoh, and COBAS E411.

In contrast, state laboratories (3out of 16) had more limited equipment, relying on systems such as the SIEMENS-Dimension EXL, ARCHITECT and CABUSAM (Roche).

3.1.14 Existence of gold standard method according to laboratories

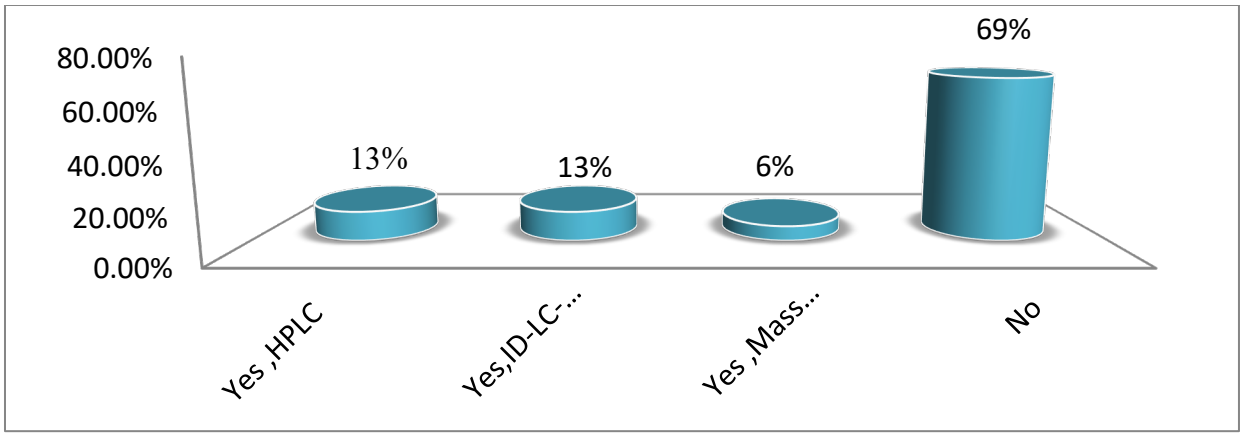

Figure 20 : Laboratory opinions on the existence of a gold standard method for vitamin D assay.

Regarding the existence of a Gold Standard method for vitamin D assay, 69% of laboratories surveyed indicated that, in their opinion, no such reference method exists. Conversely, 13 % identified HPLC as the Gold standard, 13% mentioned ID-LC-MS/MS, and 6% referred to mass spectrometry.

3.1.15 Type(s) of vitamin D measured by laboratories

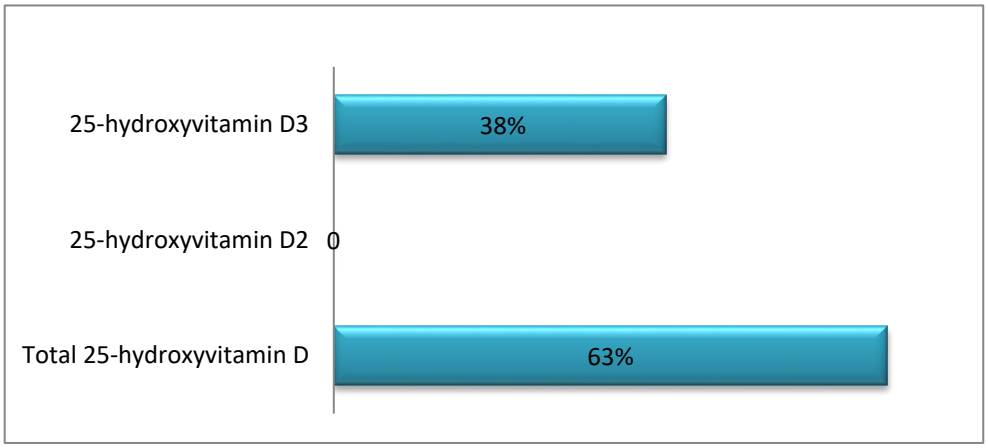

Figure 21 : Types of vitamin D measured by laboratories in the analysis.

Among 16 laboratories surveyed, the majority (63%) reported measuring total 25-hydroxyvitamin D, which includes both D2 and D3 forms. 38% of the laboratories indicated they measure 25-hydroxyvitamin D3, while no laboratory has reported the exclusive measurement of 25-hydroxyvitamin D2.

3.1.16 Main reasons for choosing the vitamin D assay method

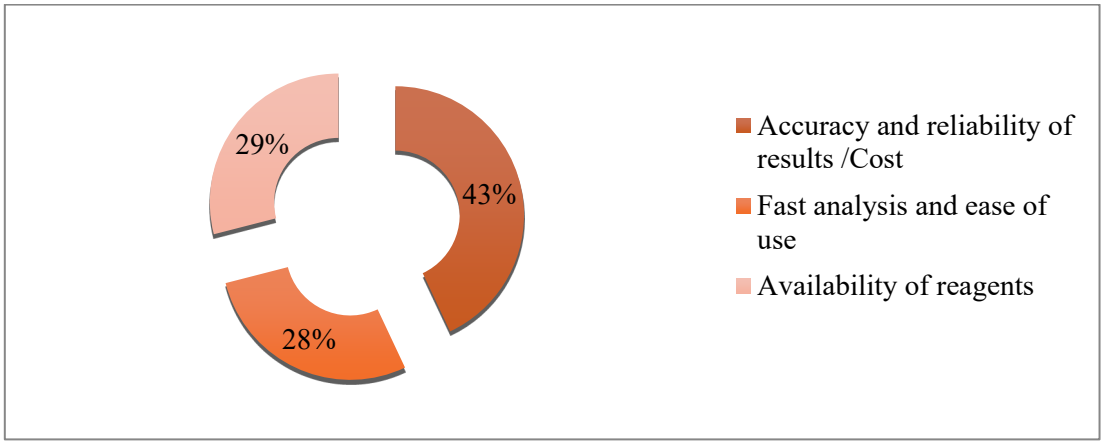

Figure 22: Main reasons behind the selection of vitamin D analytical method by laboratories**.**

The results indicate that the accuracy and reliability of results, along with cost are the criteria most frequently cited by laboratories when selecting a vitamin D assay method (43%). Fast analysis and ease of use follow at 28%, while reagent availability is cited by 29%.

3.1.17 Laboratory satisfaction with accuracy of their current vitamin D assay method

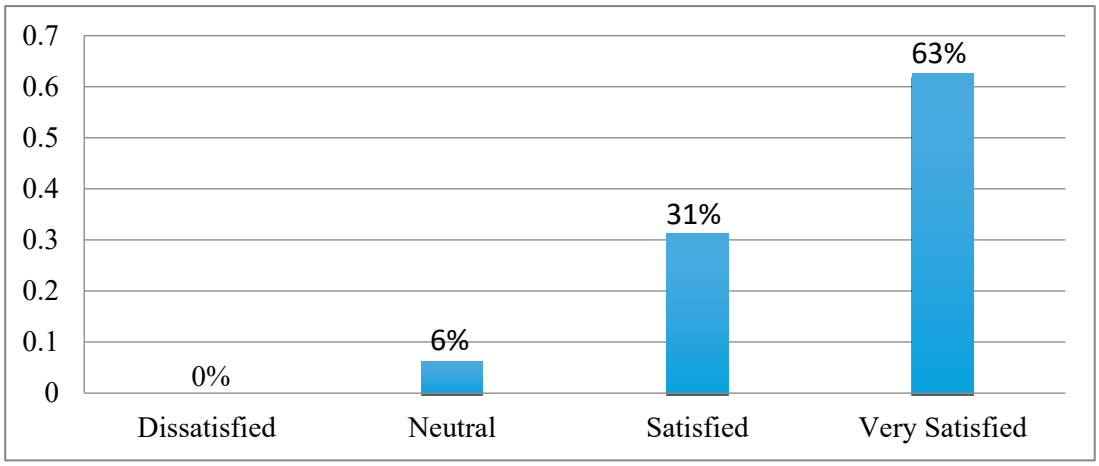

Figure 23 : Distribution of opinions on analytical accuracy of current vitamin D testing method among laboratories.

In terms of laboratory satisfaction with the accuracy of their current vitamin D assay method, (63%) of laboratories reported being very satisfied and (31%) were satisfied, while only one laboratory (6%) expressed a neutral opinion. Notably, none of the laboratories reported dissatisfaction.

3.1.18 The main challenges encountered laboratories in measuring vitamin D

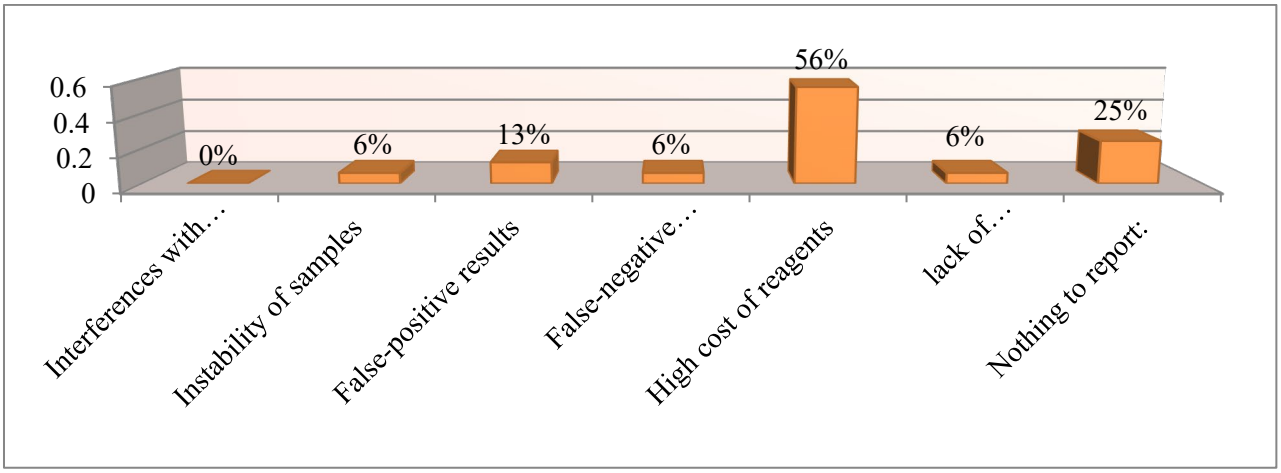

Figure 24 : The main challenges encountered laboratories in measuring vitamin D**.**

Analysis of the laboratory responses shows that the high cost of reagents was the most frequent reported challenge, cited by 56% of laboratories. False -positive results were mentioned by of respondents (13%), while instability of samples, false-negative results and lack of standardization were each cited by (6%). No laboratory reported interferences with other substances. In addition, of laboratories (25%) indicated that they encounter no particular difficulties with vitamin D testing.

3.1.19 Reported errors by laboratories during vitamin D assay

Table VII : Distribution of errors reported by laboratories during vitamin D assay

Reported errors	Number of laboratories	%
No errors reported	9	56%
Calibration problems	2	13%
Presence of fibrin may give false results	1	6%
Determination of vitamin D in Maglumi 800(CLIA)on heparinzed plasma gives values in excess of 150 ng/ml.	1	6%
Hemolysis serum/plasma	1	6%
control problems	1	6%
Taking medications	1	6%
False-positive results	1	6%
Very rare errors	1	6%

This table displays the distribution of various types of errors reported by laboratories when performing vitamin D assays.

A majority of laboratories (56%) reported no errors in performing vitamin D assay. Among the issues cited were calibration problems (13%), false results due to fibrin presence (6%), interferences from hemolysis samples (%) and control problems (6%).One laboratory observed systemically elevated values (above 150ng/ml) when using Maglumi 800 with CLIA on heparinzed plasma. Additionally, the influence of medications was also noted as a contributing factor. Reported errors were generally rare (6%).

3.1.20 Frequency of errors encountered laboratories during vitamin D assay

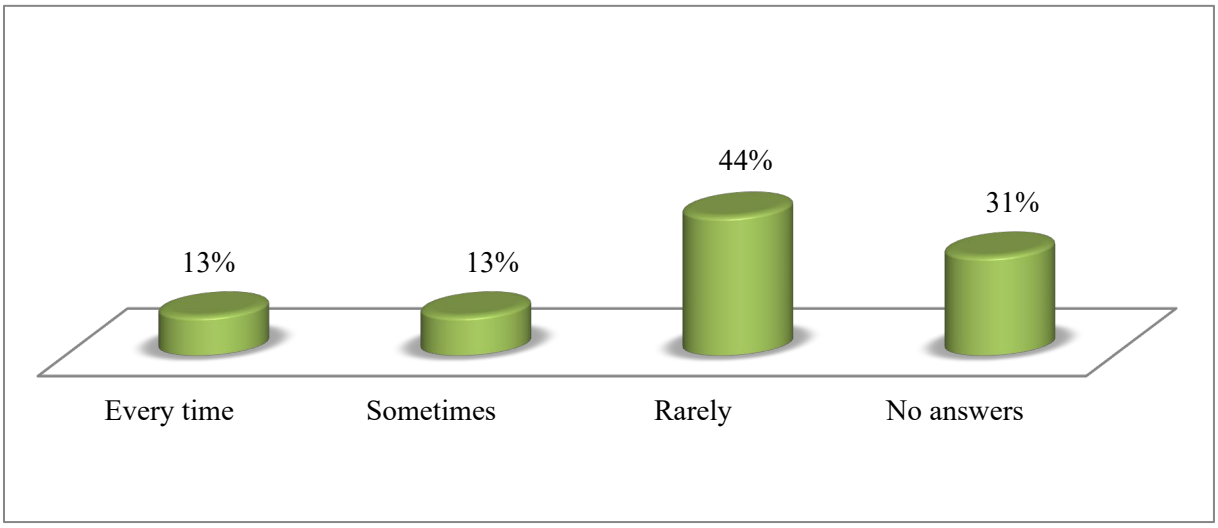

Figure 25 : Distribution of errors frequency encountered laboratories during vitamin D assay.

This chart illustrates how frequently laboratories encounter errors during vitamin D dosage procedures, 44% of laboratories reported that such issues occur rarely, while 13% reported facing errors every time or sometimes. Notably, 31% of respondents did not provide answers.

3.1.21 Main sources of errors in vitamin D dosage

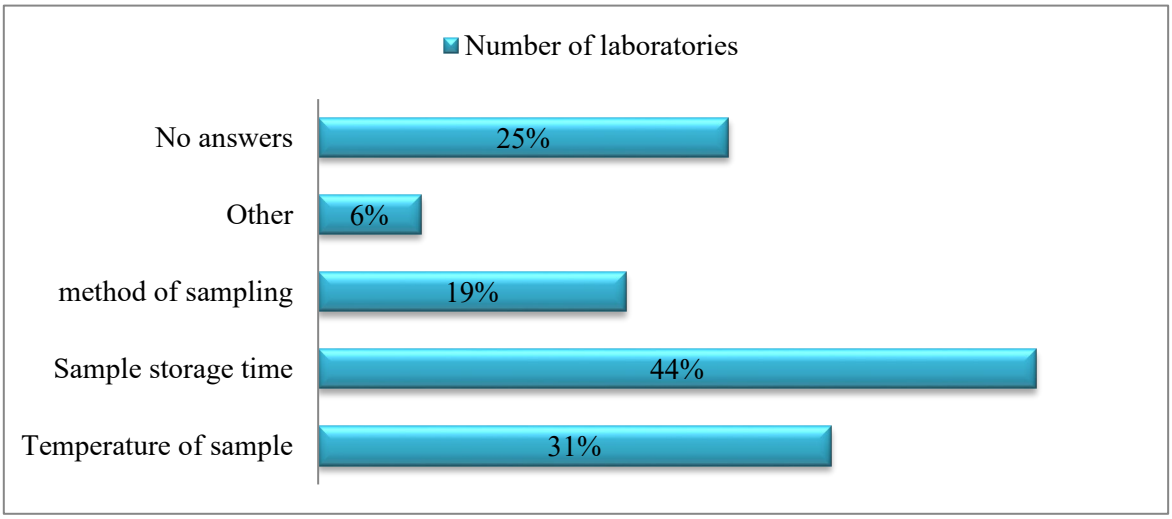

Figure 26 : Distribution of reported sources of errors encountered by laboratories during vitamin D dosage.

Among the laboratories surveyed, the most frequently cited sources of error in vitamin D testing, was sample storage time, reported by44% of participants. This was followed by issues

related to sample temperature (31%) and sampling method (19%).In addition, 6% of laboratories mentioned other unspecified factors, while 25% did not respond to the question.

3.1.22 Management of Dosing Errors

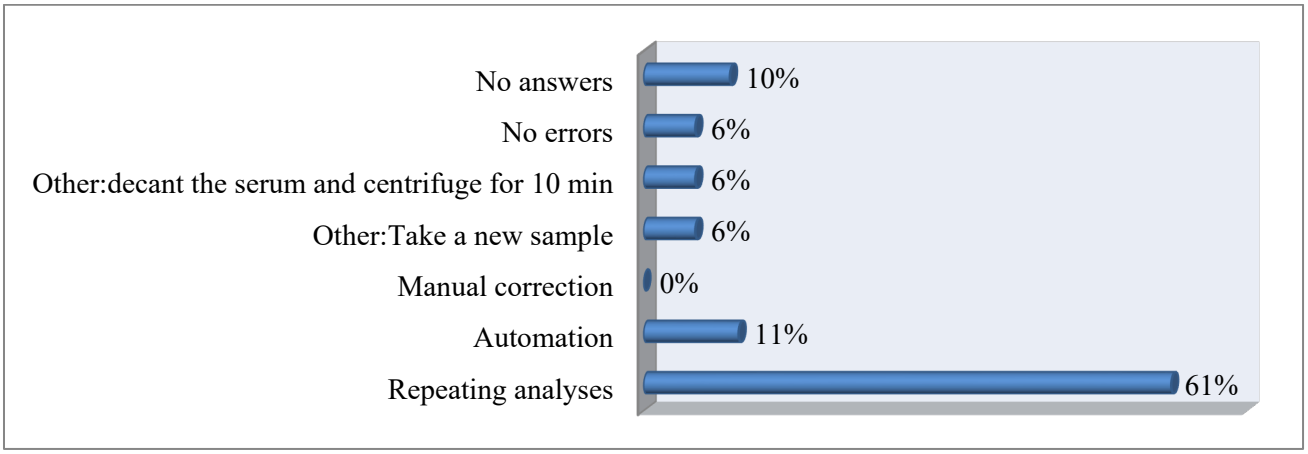

Figure 27 : Distribution of error management's strategies in vitamin D assays.

The figure illustrate the strategies employed by laboratories to manage errors encountered during vitamin D dosage, the most common response among laboratories was to repeat the analysis, cited by 61% of participants. Automation was used by 11% of laboratories, while manual correction was not reported by any. Other measures included taking a new sample and decanting and centrifuging the serum, each mentioned by 6%.Additionally, 6% reported no errors, and 10% of laboratories did not respond.

3.1.23 Calibration method and frequencies

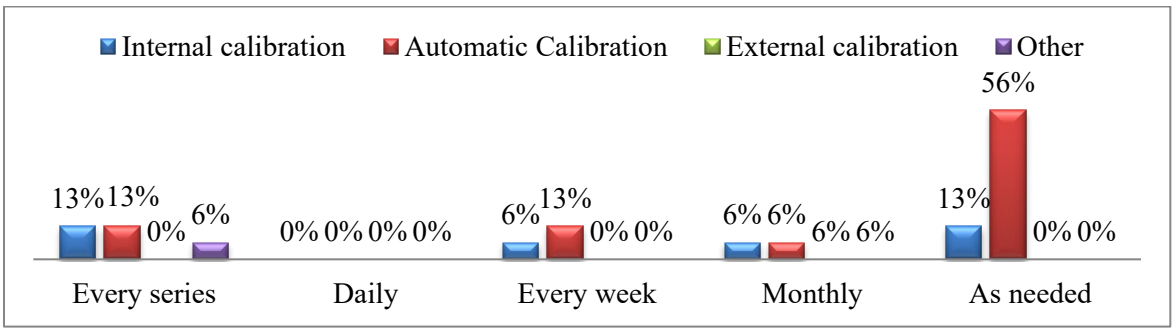

Figure 28 : Distribution of calibration methods and their frequencies in vitamin D assays.

The chart summarizes the different calibration methods reported by laboratories, as well as the frequency with which these methods are applied when conducting vitamin D assays.

Automatic calibration was the most reported method; especially when performed on an as needed (56%).Internal calibration is also employed but shows more variability in its applications. Some laboratories perform it with every series (13%), while others apply it weekly (6%), monthly (6%), or as needed (13%). No laboratory reported using external calibration, and 6% mentioned another method (unspecified).

3.1.24 Types of controls applied in vitamin D assay methods

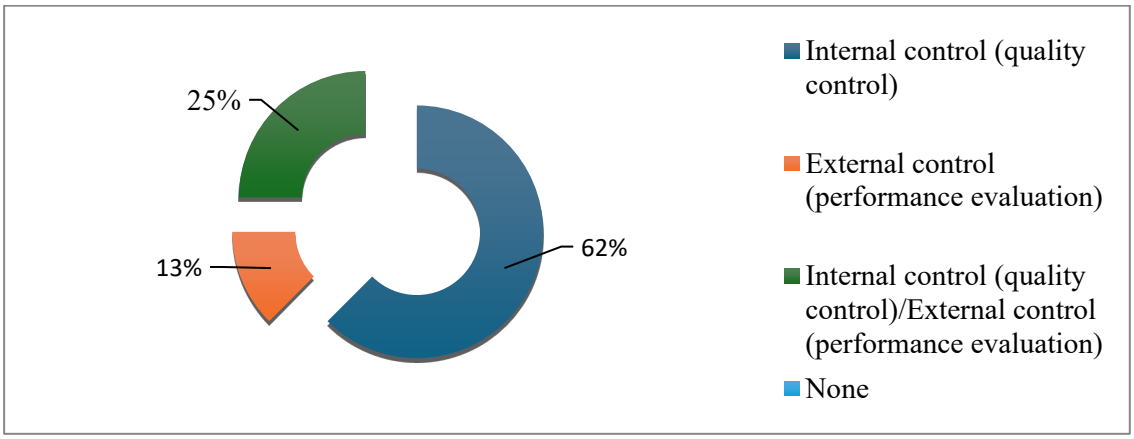

Figure 29 : Distribution of control types by laboratories in vitamin D assay methods.

The results indicate that the majority of laboratories (62%) are mainly based on internal control (quality control).Notably, 25% of laboratories apply both internal and external controls. A smaller proportion (13%) use only external control (performance control).None of the laboratories reported the absence of any control.

3.1.25 Frequency of quality control application in vitamin D assay

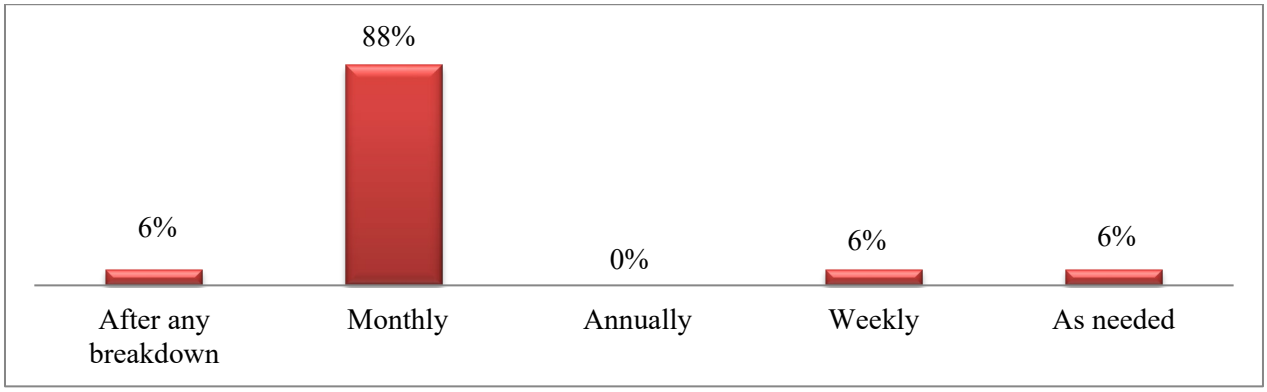

Figure 30 : Distribution of laboratories according to quality control frequency.

The results show that the majority of laboratories (88%) performed quality controls on a monthly basis, while a minority of them performed after breakdown (6%), weekly (6%), or on an as-needed basis (6%).

None of the laboratories reported performing quality controls annually.

3.1.26 Criteria for assessing the performance of vitamin D assay methods

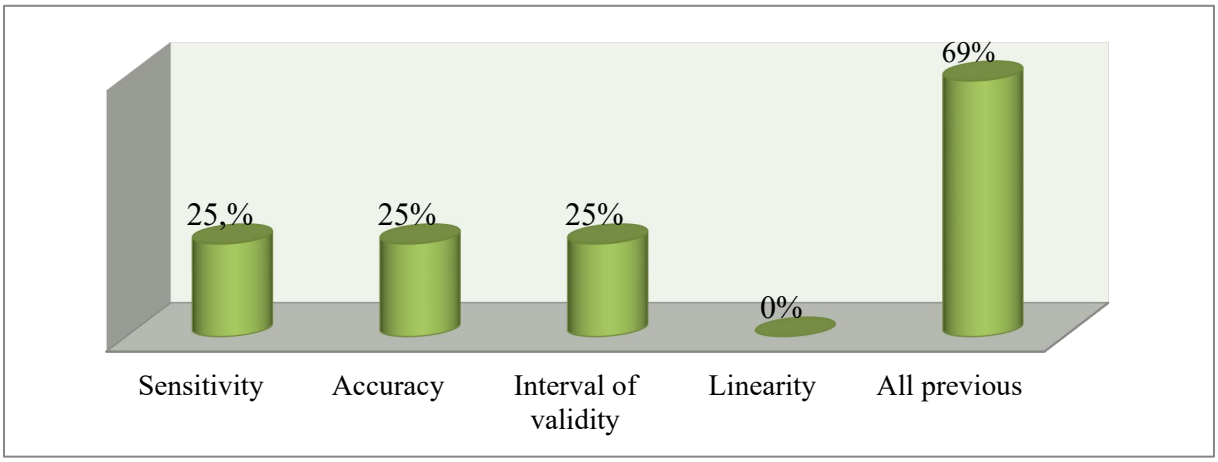

Figure 31: Distribution of criteria used for evaluating the performance of vitamin D assay methods**.**

The majority of laboratories (69%) reported using all key performance criteria including sensitivity, accuracy, linearity and interval of validity to assess the performance of their assay methods. Individually, 25% of laboratories considered sensitivity, accuracy (25%) and interval of validity as the main criteria. None of the laboratories reported using linearity alone as a sole performance criterion.

3.1.27 Frequency of vitamin D assay method validation

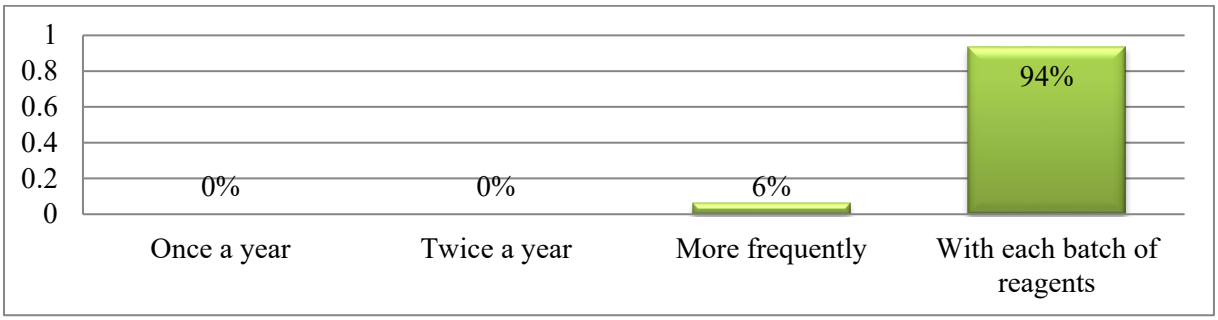

Figure 32 : Frequency of vitamin D assay method validation among surveyed laboratories.

The data show that 94%of the surveyed laboratories validate their vitamin D assay method with each batch of reagents. Only one laboratory reported validating the method more frequently, while none indicated annual or semi-annual validation.

3.1.28 Limit of the detection(LOD)of the analytical method used

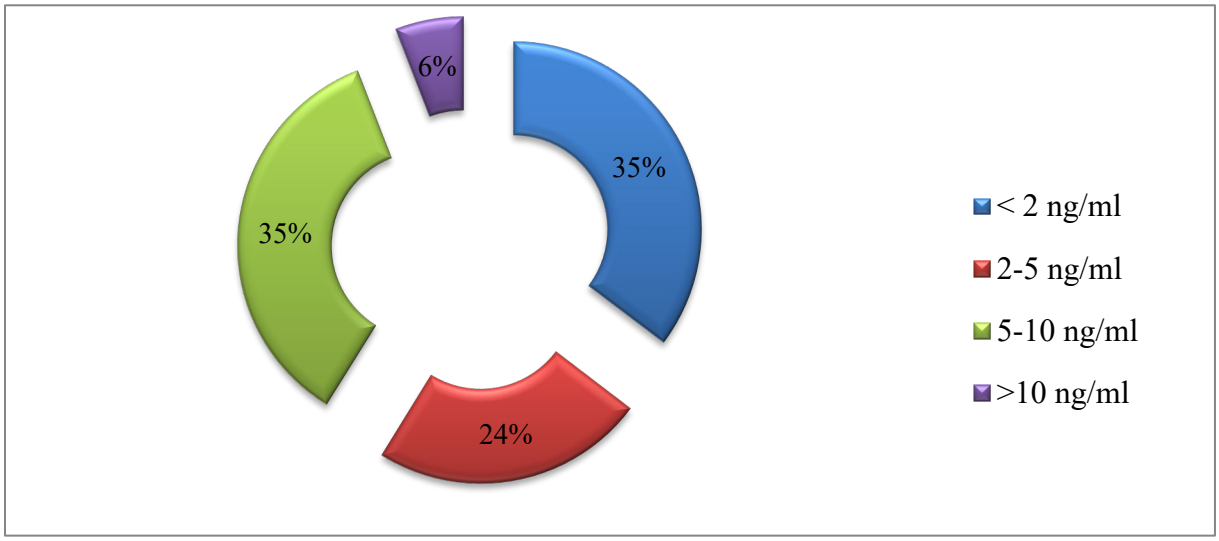

Figure 33 : Distribution of laboratories by limit of detection (LOD) of vitamin D assay method.

According to the graph results, the majority of laboratories reported a limit of detection (LOD) for their vitamin D assay method in the lower range.35% of the laboratories declared an LOD of less than 2 ng/ml, and an equal proportion (35%) reported an LOD between 5 and 10 ng/ml. Additionally, 26% indicated an LOD between 2 and 5 ng/ml, while only 6% reported a detection limit greater than 10 ng/ml.

3.1.29 Distribution of detection limits(LOD)according to the automated systems used by laboratories

Table VIII : Distribution of detection limits (LOD) according to the automated systems used by laboratories

Laboratories	The automated system used	Limit of detection(LOD)
Laboratory 1	Maglumi 800(Snibe),Vidas	2-5 ng/ml, 5-10 ng/ml
Laboratory 2	Mini Vidas ,Maglumi 800(Snibe)	2-5 ng/ml
Laboratory 3	Vidas	<2 ng/ml
Laboratory 4	SIEMENS-Dimension EXL	<2 ng/ml
Laboratory 5/13/16	Vidas	5-10 ng/ml
Laboratory 6	Vidas Kube	5-10 ng/ml
Laboratory 7	Vidas	>10 ng/ml
Laboratory 8	Maglumi 800(Snibe), Vidas	2-5 ng/ml
Laboratory 9	Vidas, Tosoh	2-5 ng/ml
Laboratory 10	ARCHITECT, Manual dosage	<2 ng/ml
Laboratory 11	Vidas PC	<2 ng/ml
Laboratory 12	Maglumi, Vidas	5-10 ng/ml
Laboratory 14	CABUSAM(Roche)	<2 ng/ml
Laboratory 15	CABAS E411	<2 ng/ml

The table enabled the identification of the automated systems used to measure vitamin D, as well as the limits of detection(LOD)associated with these systems.

Automated systems such as ARCHITECT, Siemens EXL, COBAS E411, and CABUSAM (Roche)are exclusively associated with a LOD <2 ng/ml. The various Vidas systems are mostly linked to LOD values between 5-10 ng/ml, and in some cases >10 ng/ml. Maglumi and Tosoh systems fall in the intermediate range, with LODs typically between 2-5 ng/ml.

3.1.30 Units of measurement used for vitamin D assay

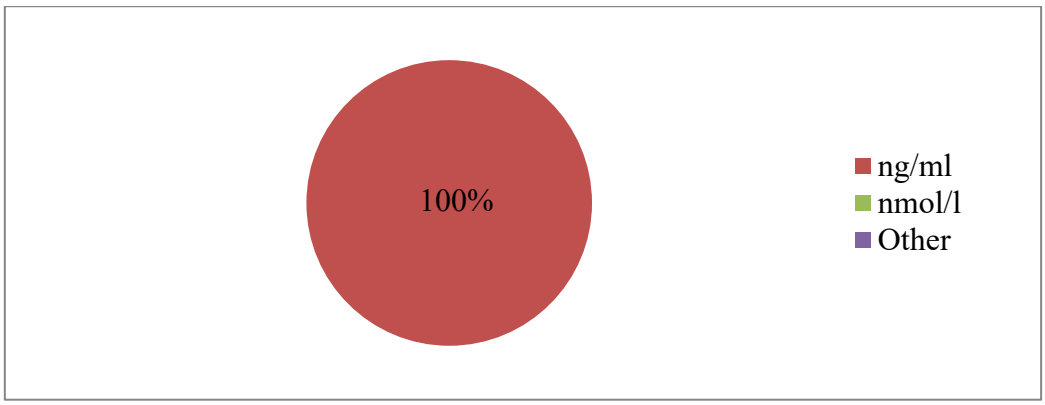

Figure 34 : Units of measurement used for vitamin D assay.

The figure shows a total consistency in the units used: 100% of the laboratories surveyed use ng/ml as the unit for expressing results. No laboratories reported using nmol/ml or any other units.

3.1.31 Source of biological reference intervals for interpreting 25-hydroxyvitamin D results

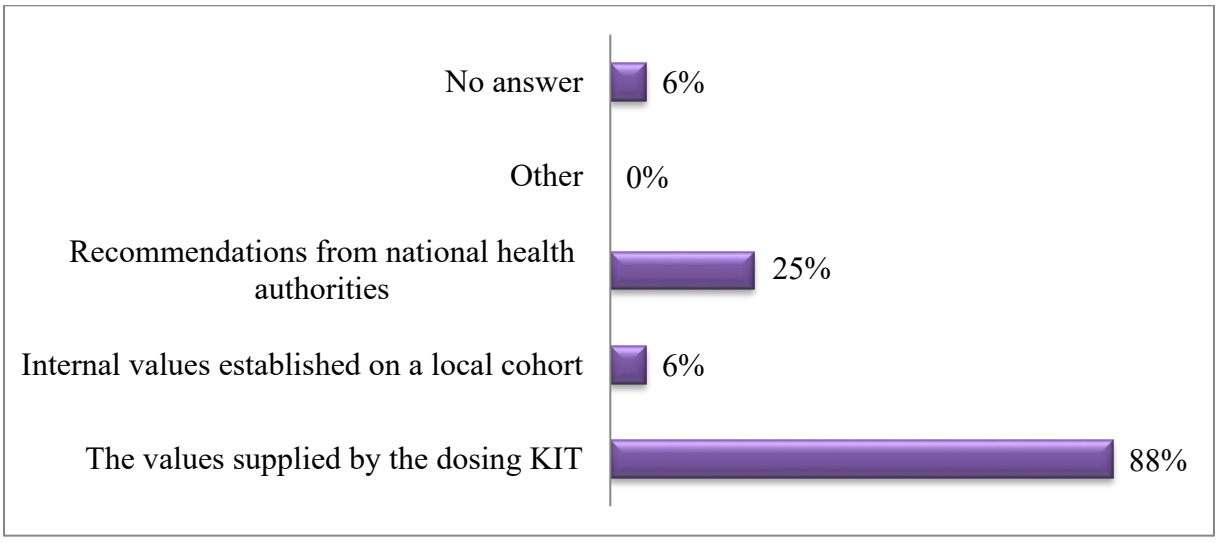

Figure 35 : Distribution of sources of biological references intervals used by laboratories for interpreting 25-hydroxyvitamin D results.

The chart indicates that 88% of laboratories rely on the reference values provided by the assay kits as the basis for interpreting 25-hydroxyvitamin D results. Only one laboratory 6% used internal values based on a local cohort, while 25% laboratories referred to the recommendations of national health authorities. One laboratory did not specify its source.

3.1.32 Average turnaround time for 25-hydroxyvitamin D assay results

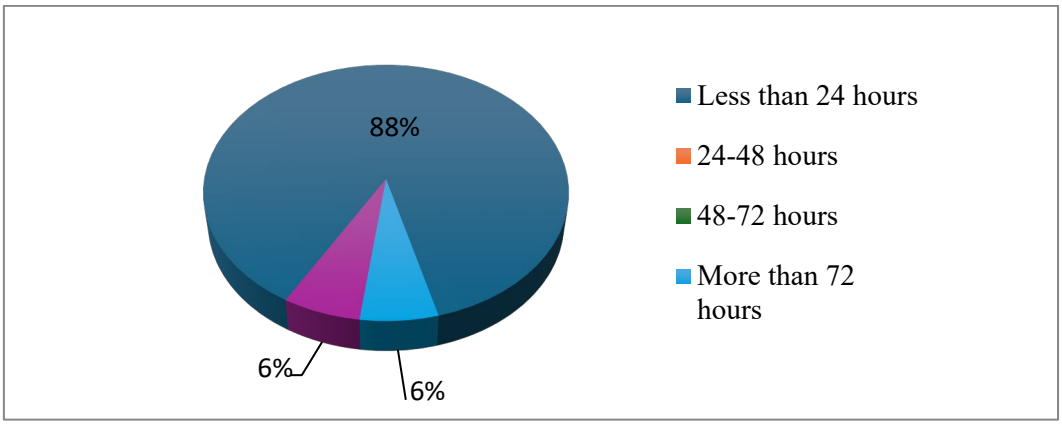

Figure 36: Average turnaround time for 25-hysroxyvitamin D assay results reported by laboratories.

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The majority of laboratories 88% reported that the average time to obtain 25-Hydroxyvitamin D assay results is less than 24 hours. No laboratories indicated a turnaround time between 24-48 hours and between 48-72 hours, while one laboratory 6% reported taking more than 72 hours.

Another laboratory 6% did not answer this question.

3.1.33. Laboratory involvement in studies and analysis of vitamin D assay results

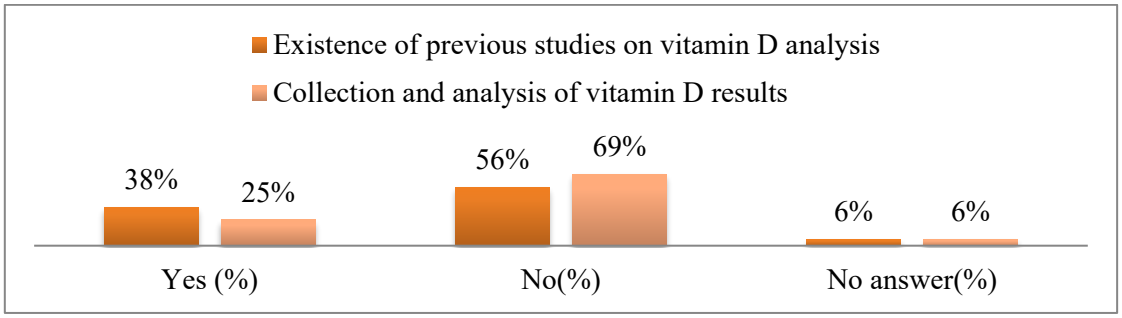

Figure 37 : Distribution of laboratories based in the existence of previous studies and the collect and analysis of vitamin D assay results.

Regarding the existence of previous studies on vitamin D analysis, 38 % of laboratories reported having performed such studies, while 56% had not, and 6% did not respond.

In addition, only 25% of laboratories reported that they collected and analyzed vitamin D results, compared to 69% who did not, and 6% who did not respond.

3.1.34. Tools used for data collection

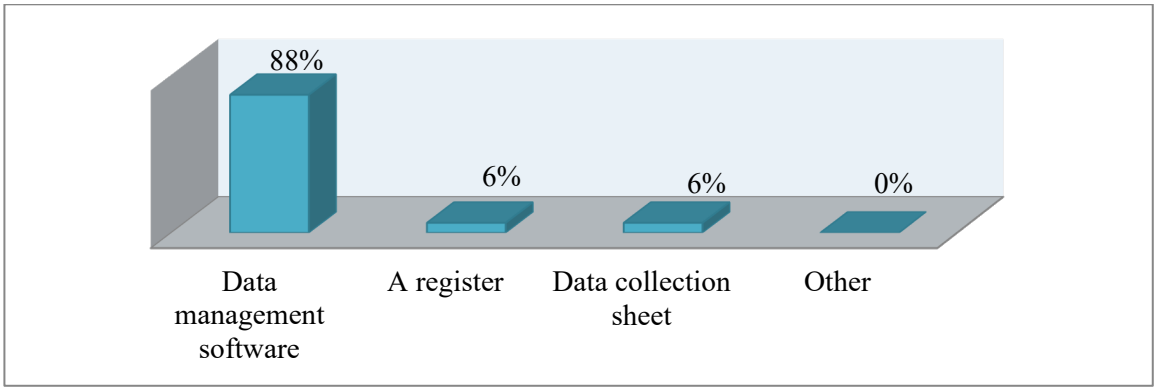

Figure 38 : Tools used for data collection.

The data indicates that 88% of laboratories (14 out of 16) used the data management software to collect information related to vitamin D assays. In comparison, 6 % (1 laboratory) used a manual register, and another 6 % (1 laboratory) relied on a data collection sheet. No laboratories reported using other tools beyond the provided options.

3.2. Part Two: Data collected on the frequency of hypovitaminosis D in children and adolescents

3.2.1. Specialty

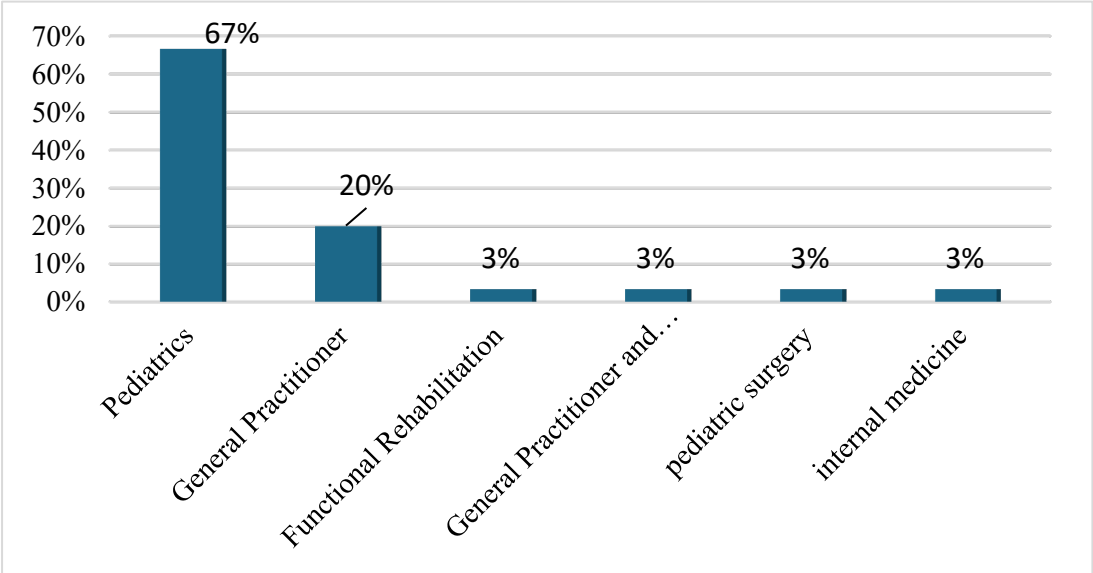

Figure 39 : The various doctors' specialties.

30 of the doctors who took part in this research, 67% were pediatricians, 20% were general practitioners, and 3% were functional rehabilitation, general practitioners and nutritionists, pediatric surgery and internal medicine for each.

3.2.2. Workplace

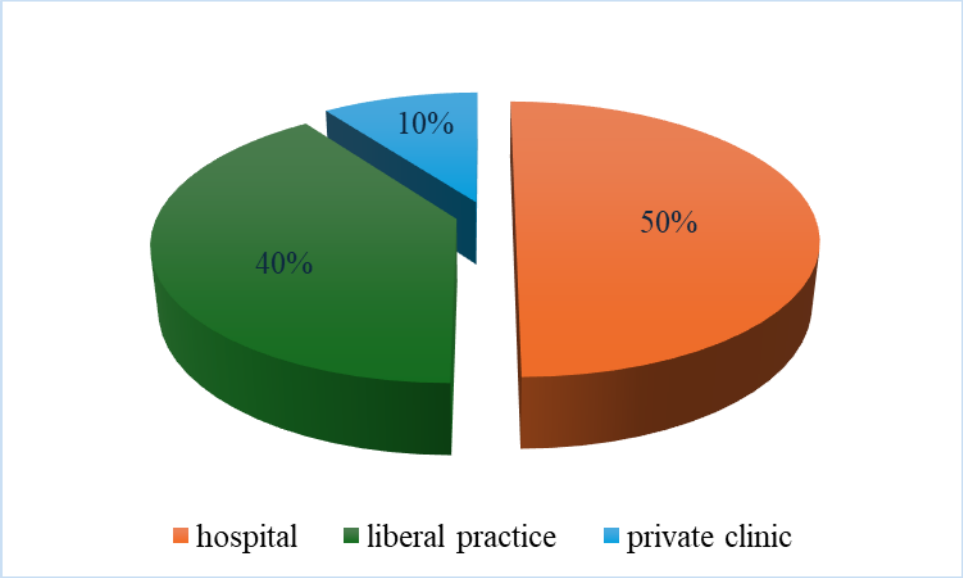

Figure 40 : Different workplace of doctors.

Half of the doctors(50%) taking part in this study work in hospitals, while 40% work in private practices and only 10% in private clinics.

3.2.3. The most frequent clinical manifestations of hypovitaminosis D in children and adolescents

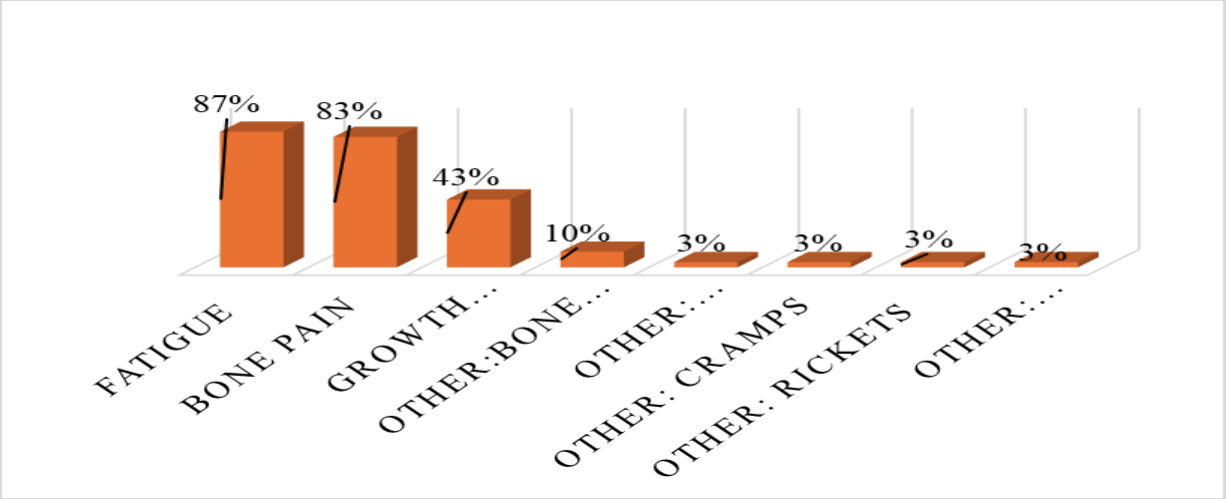

Figure 41 : Clinical manifestations of hypovitaminosis D in children and adolescents.

According to doctors, the most common clinical manifestations of hypovitaminosis D in children and adolescents were fatigue (87%) and bone pain (83%).

43% of them replied that the clinical manifestations were growth problems, and only 10% that they were bone deformities.

Less frequently, with a percentage of 3% for each answer, added that they can be seen in the form of walking delay, cramps, rickets, weight loss, irritability and depression.

3.2.4. Physical signs that may be associated with hypovitaminosis D in children and adolescents

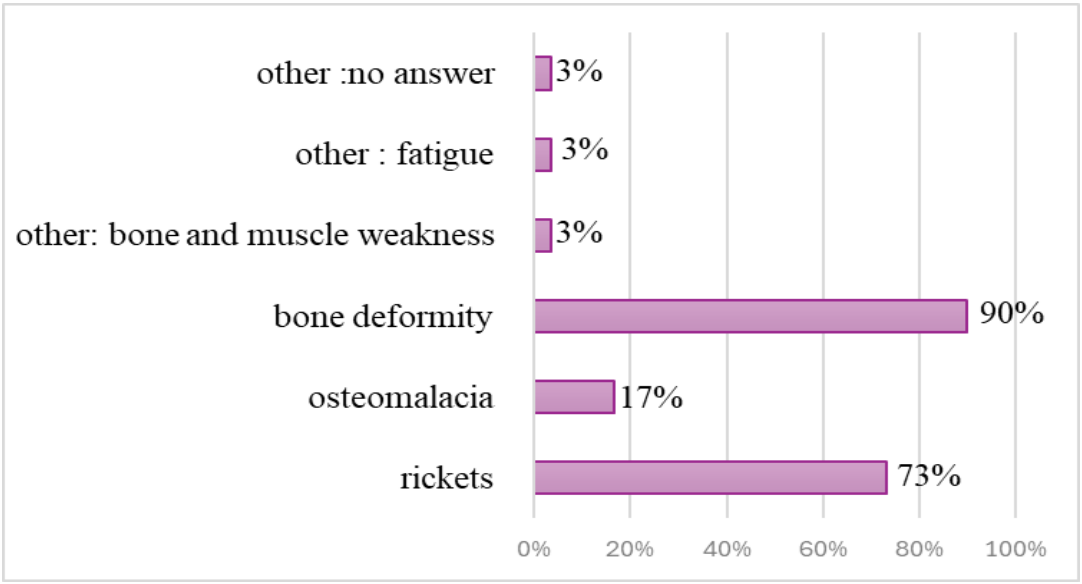

Figure 42 : Physical signs associated with hypovitaminosis D in children and adolescents**.**

In this figure, 90% and 73% of doctors 'responded that the physical signs associated with hypovitaminosis D in children and adolescents were bone deformation and rickets respectively.

Less frequently, osteomalacia (17%), and 3% for other responded : bone and muscle weakness and fatigue. 3% of doctors did not answer this question, seeing that there were no physical signs.

3.2.5. The Main risk factors for vitamin D deficiency in children and adolescents

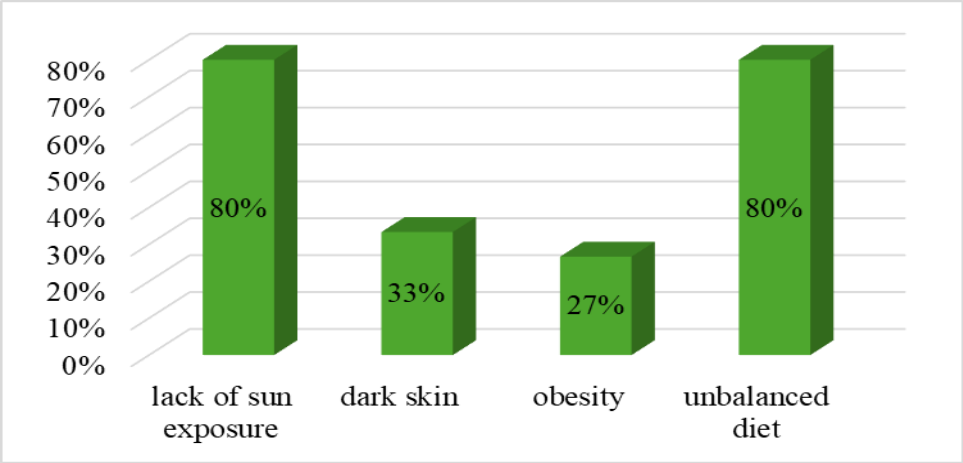

Figure 43 : Risk factors for hypovitaminosis D in children and adolescents.

According to doctors, the main risk factors that can cause hypovitaminosis D in children and adolescents were lack of exposure to sunlight and unbalanced diet(80%). 33% said that dark skin was also an important factor, and only 27% said obesity.

3.2.6 Vitamin D blood tests are sufficient to diagnose hypovitaminosis D

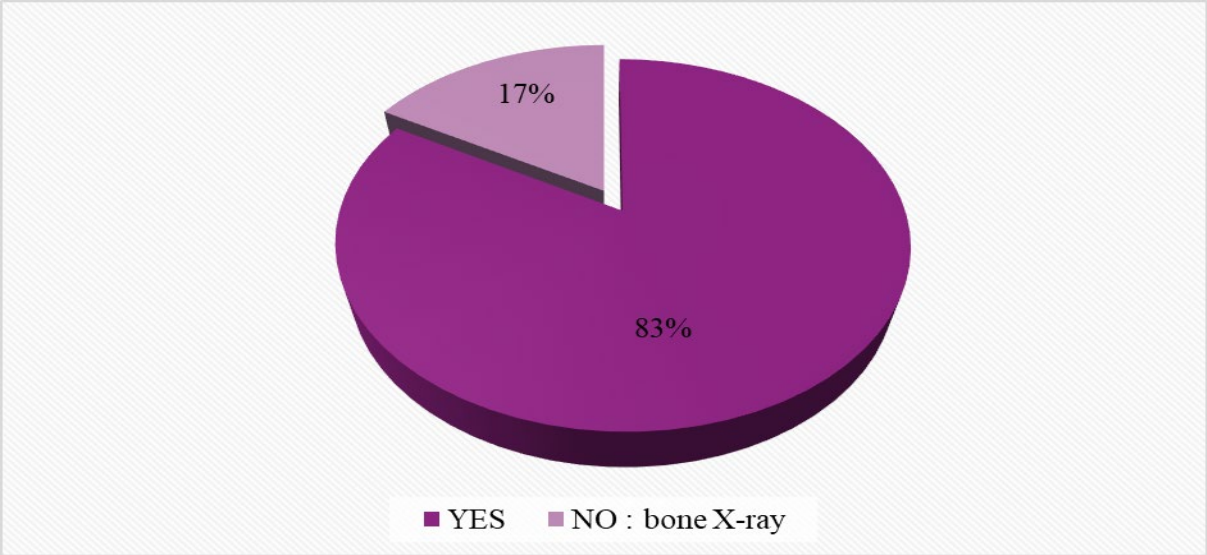

Figure 44 : Reliability of blood vitamin D testing in identifying vitamin D deficiency.

In this graph, 83% of doctors believed that the vitamin D blood test is sufficient to diagnose hypovitaminosis D, but only 17% replied that it is not sufficient, and that a bone X-ray was also necessary to confirm the diagnosis.

3.2.7 Cases where doctors prescribe a vitamin D dosage for children and adolescents

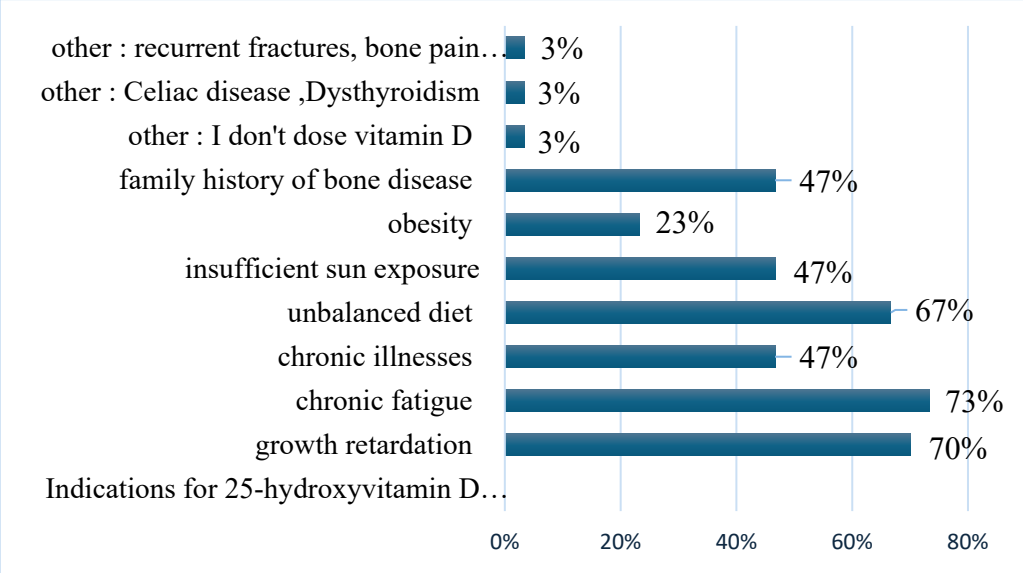

Figure 45 : Indication for vitamin D dosage in children and adolescents.

According to the doctors, the prescription for vitamin D dosage was necessary especially in the following cases: chronic fatigue (73%), growth delay (70%), and in cases of an unbalanced diet (67%). The prescription was also frequent in the case of chronic diseases, insufficient sun exposure, and the presence of family history of bone diseases, with 47% responses for each case, and only 23% doctors prescribed it in cases of obesity. For open responses (other), the doctors specified the following cases: celiac disease, thyroid dysfunction, recurrent fractures, bone pain, and delayed walking (one response for each case). Only one doctor responded that vitamin D dosage in children and adolescents is not done, and the prescription for vitamin D supplementation is made directly if signs of vitamin D deficiency are present.

3.2.8 The Average number of children and adolescent patients (aged 2 to 17 years) at risk seen per week

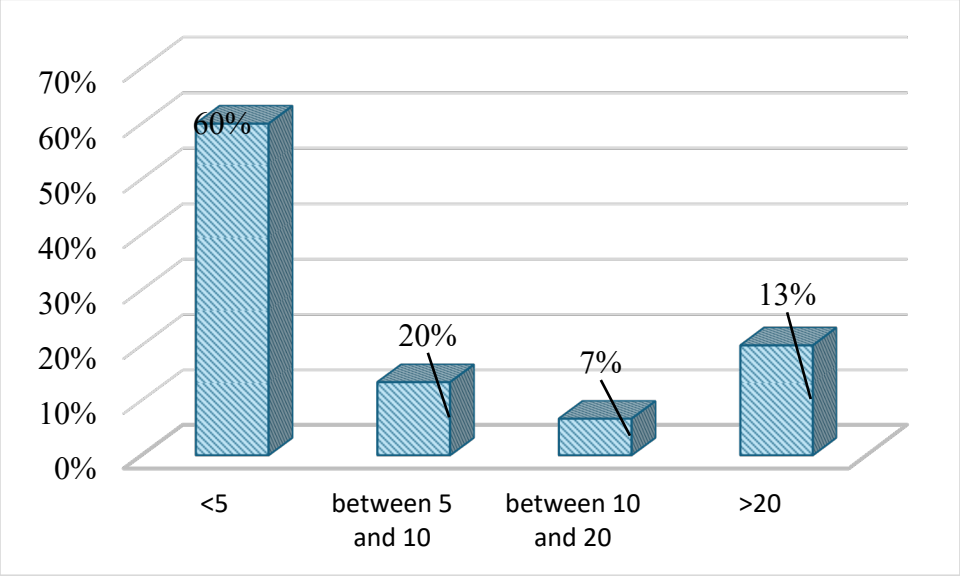

Figure 46 : The frequency of children and adolescents at risk seen per week by doctors.

The average number of at-risk children and adolescents (with one or more risk factors for hypovitaminosis D) seen per week by doctors was mostly less than 5 patient60%, 20%of doctors diagnosed more than 20 at-risk patients, 13% saw between 5 and 10 cases, and only 7% saw between 10 and 20 patients.

3.2.9 The frequency of vitamin D dosage and prescriptions for children and adolescents per week(doctors/laboratories)

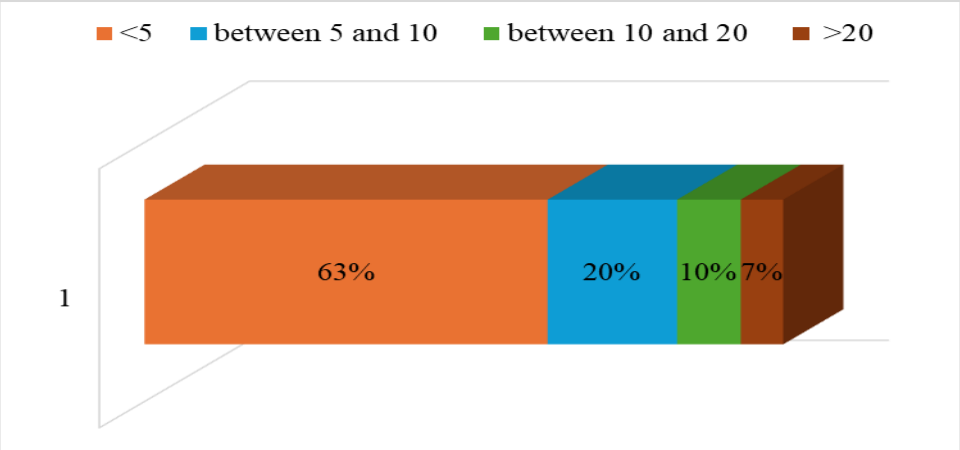

Figure 47 : The number of vitamin D dosage prescriptions from doctors for children and adolescents per week.

Of the 30 doctors questioned, more than half (63%, n=19) replied that vitamin D dosage prescriptions for children and adolescents (2 to 17 years) do not exceed 5 patients per week.

20 %(n=6)said that they prescribed for 5 to 10 patients, 10%(n=3) prescribed for 10 to 20 patients and only 7%(n=2) prescribed vitamin D testing for more than 20 patients per week.

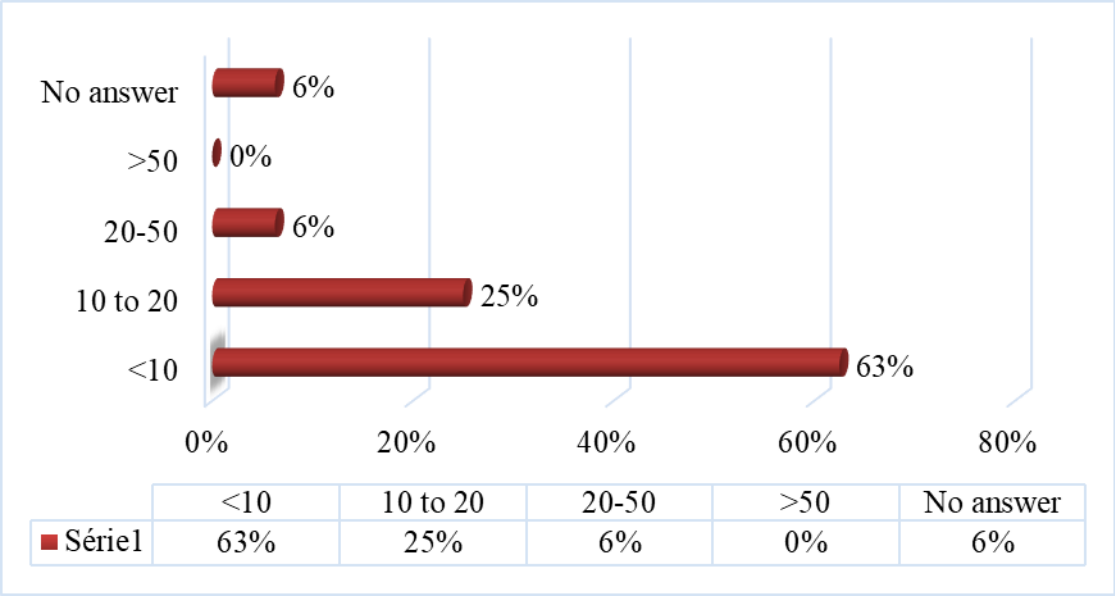

Figure 48 : Number of weekly vitamin D test of children and adolescents in laboratories.

The graph shows the number of vitamin D tests performed in laboratories on children and adolescents aged 2 to 17. With the majority of laboratories (63%) performed fewer than 10 tests per week. A quarter of respondents (25%) performed between 10 and 20 tests per week, while only 6% performed between 20 and 50. No participant reported performing more than 50 tests per week and 6% of respondents gave no answer.

3.2.10 The Frequency of vitamin D prescriptions and dosage in the general population per week(doctors /laboratories) :

Table IX : Frequency of vitamin D dosage prescriptions in general population per week.

Frequency of vitamin D dosage prescriptions in the general population per week	Answers
<5	20%
between 5 and 10	27%
between 10 and 20	20%
>20	30%

The table showed the responses of the 30 doctors on the frequency of prescribing vitamin D dosage in the general population, the most represented category being that of more than 20 prescriptions per week, with30% doctors' responses.

A further 27% of doctors prescribed between 5 and 10 vitamin D tests per week. On the other hand, 20% of doctors replied that they gave between 10 and 20prescriptions, while other doctors with the same percentage gave less than 5 prescriptions per week.

There was only one pediatric doctor who did not answer this question because he did not request vitamin D analysis in the general population.

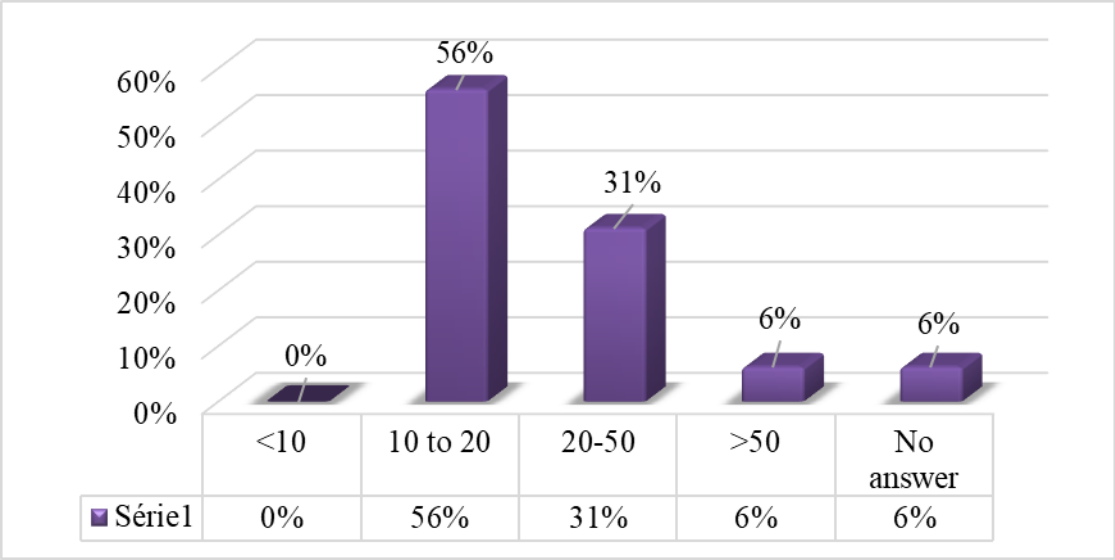

Figure 49 : Number of weekly vitamin D test in laboratories for general population.

The graph showed the weekly number of vitamin D tests in the general population which was moderate to high activity in the majority. Over half (56%) reported performing between 10 and 20 tests per week, while 31% performed between 20 and 50 tests, and a small percentage (6%) exceeded 50 tests. No laboratory performed less than 10 tests per week, and 6% of respondents gave no answer.

3.2.11 The frequency recommended by doctors for checking vitamin D levels in children and adolescents at risk

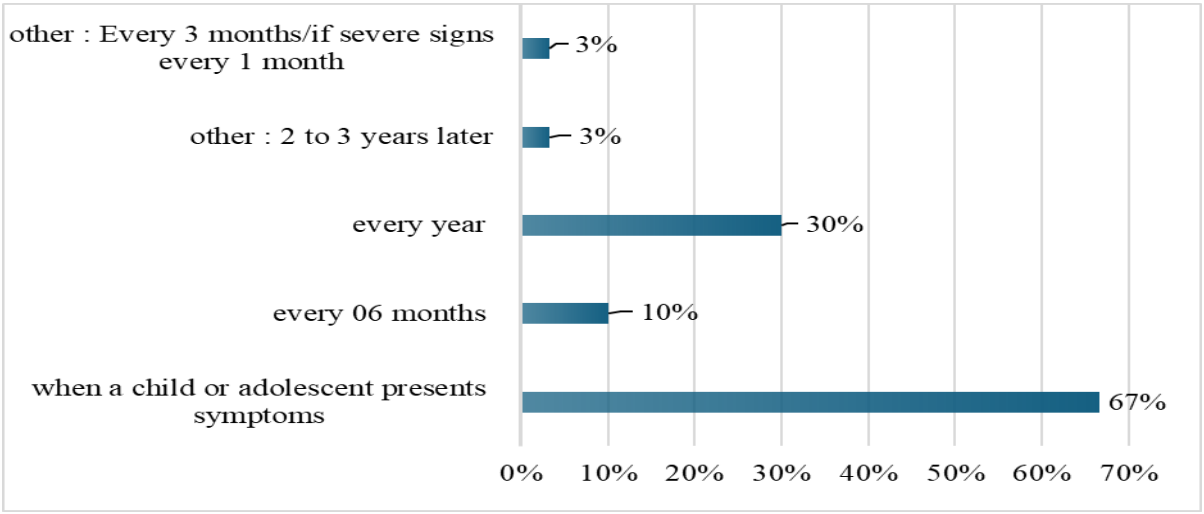

Figure 50 : Frequency of checking 25(OH) D levels in at-risk children and adolescents.

The graph above showed that the majority of doctors, 67% (n=20), recommended checking vitamin D levels in children and adolescents as soon as symptoms appear. A notable proportion, 30% (n=8), recommended annual follow-up, while 10% (n=3) suggested monitoring every six months. A minority responded with other frequencies: 3% (n=1) recommend a longer follow-up, every 2 to 3 years, while 3% (n=1) suggest more frequent monitoring, every three months, or even monthly in cases of very severe signs.

3.2.12 Diagnostic base of vitamin D hypovitaminosis

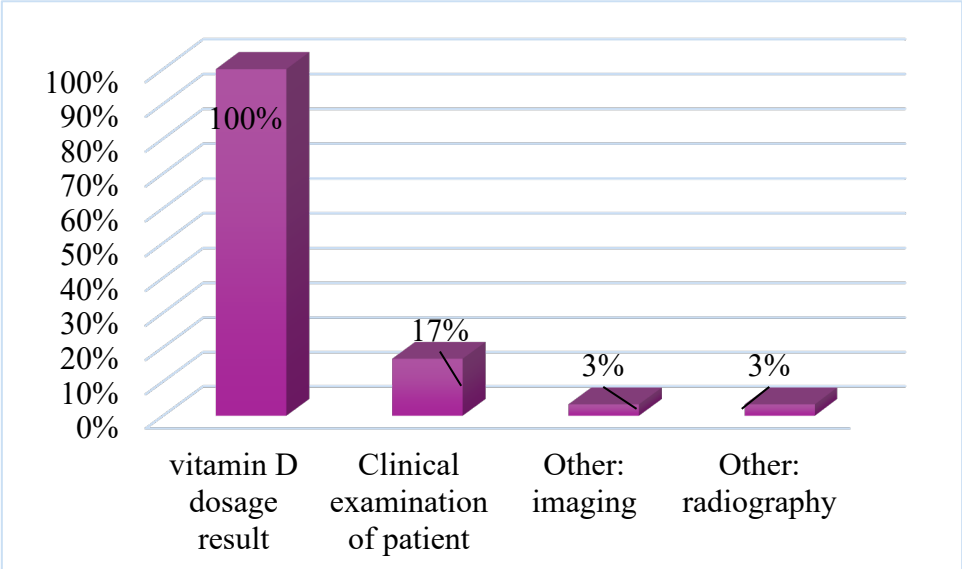

Figure 51 : Diagnostic base of hypovitaminosis D.

The graph shows that the diagnosis of hypovitaminosis D released mainly on the biological assay of vitamin D, which is cited as the diagnostic basis by all doctors (100%). On the other hand, clinical examination of the patient was used much less frequently (17%), and tools such as radiography or imaging were rarely used (3% each).

3.2.13 The evolution of the frequency of hypovitaminosis D in children and adolescents in the year 2024 from the point of view of doctors and laboratories

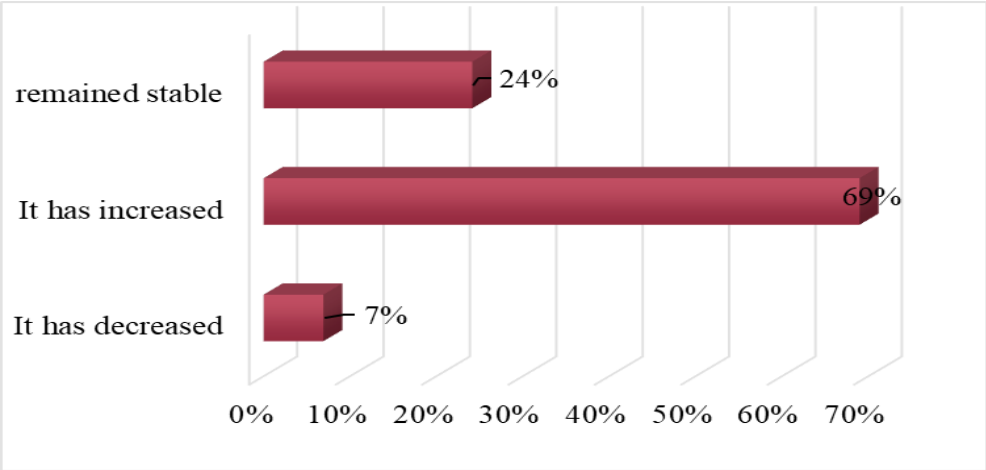

Figure 52: Opinions of doctors on the evolution of the frequency of hypovitaminosis D in children and adolescents over the past few years.

Among doctors, more than half(69%)said that the frequency of hypovitaminosis D in children and adolescents had increased over the past few years**,** 24% showed that it was stable, while only 7% observed that it had decreased.

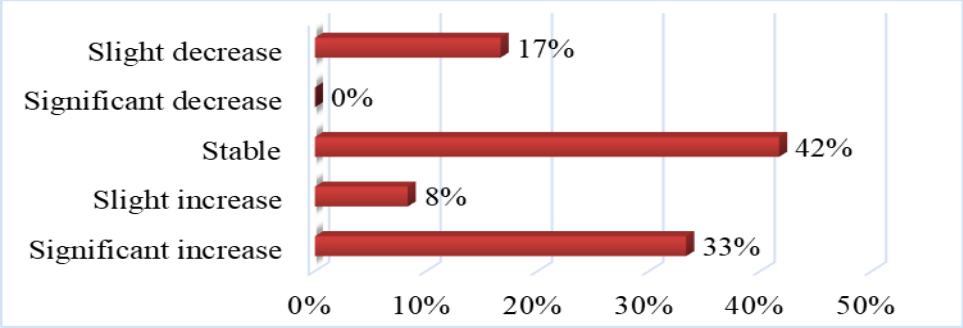

Figure 53 : Opinions of laboratories in the evolution of the frequency of hypovitaminosis D in children and adolescents in 2024

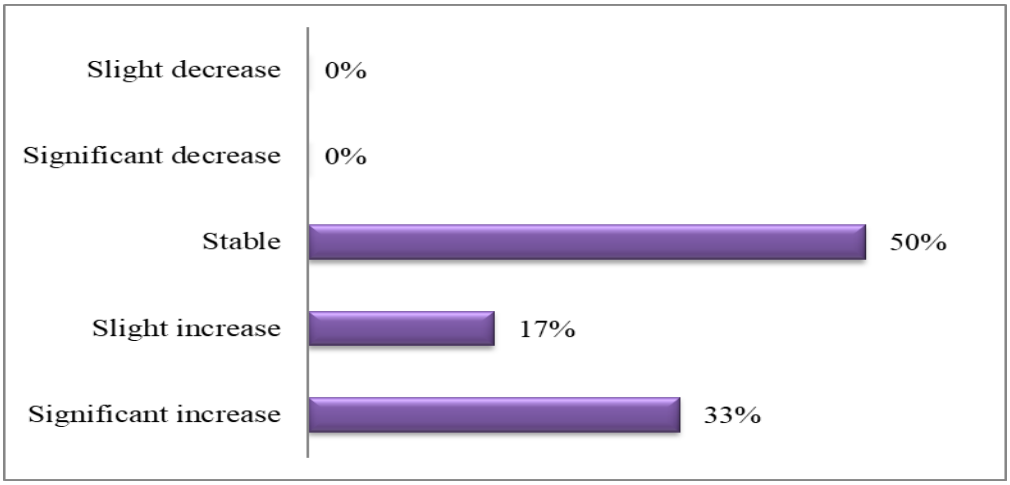

Figure 54 : Opinions of laboratories on the general evolution of the frequency of hypovitaminosis in general population in 2024.

The majority of laboratories answered that the frequency of hypovitaminosis D had remained stable (50% for the general population and 42% for children and adolescents).

Of the others, (33% answers for both groups) reported that the frequency has increased significantly, on the other hand some (17% for the general population and 8% for children and adolescents) observed that it has slightly increased.

No laboratories responded that the frequency is decreasing, except for children and adolescents 17% answered that it is slightly decreasing.

3.2.14 Frequency of vitamin D deficiency findings in the general population and in Children and Adolescents According to Laboratories

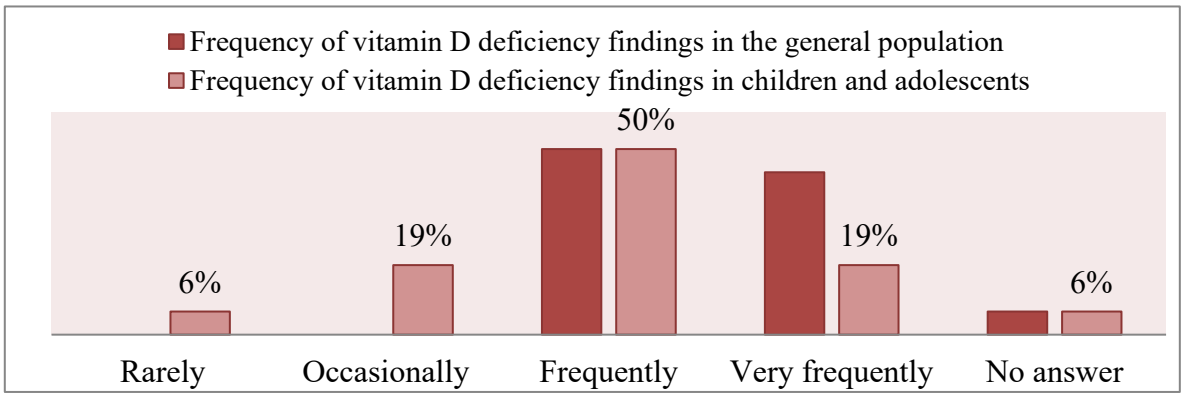

Figure 55 : Frequency of hypovitaminosis D findings in general population and children and adolescents.

The data presented indicate the frequency of cases of hypovitaminosis D observed in two groups: the general population and children/adolescents. For the general population, hypovitaminosis D was reported frequently 50% or very frequently 44% in most responses. For children and adolescents however, the distribution is more evenly spread although the majority of responses indicated frequent frequency 50%, there were also responses indicating very frequent19%, occasional 19% and even rare 6%.

3.2.15 Opinion of laboratories about the importance of vitamin D assay results in clinical decisions for children and adolescents

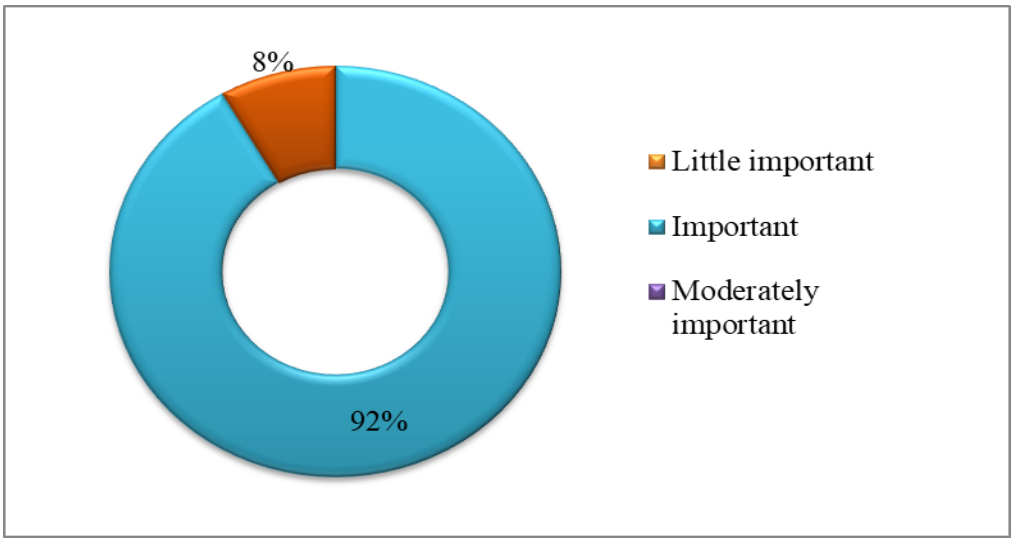

Figure 56 : Importance of results of vitamin D test in clinical decisions for children and adolescents.

The results showed that the vast majority of laboratories (92%) considered vitamin D assays to be important in clinical decision-making for children and adolescents, with a further 8% considered them to be very important.

3.2.16 Doctors' perception of the importance of vitamin D dosage in children and adolescents

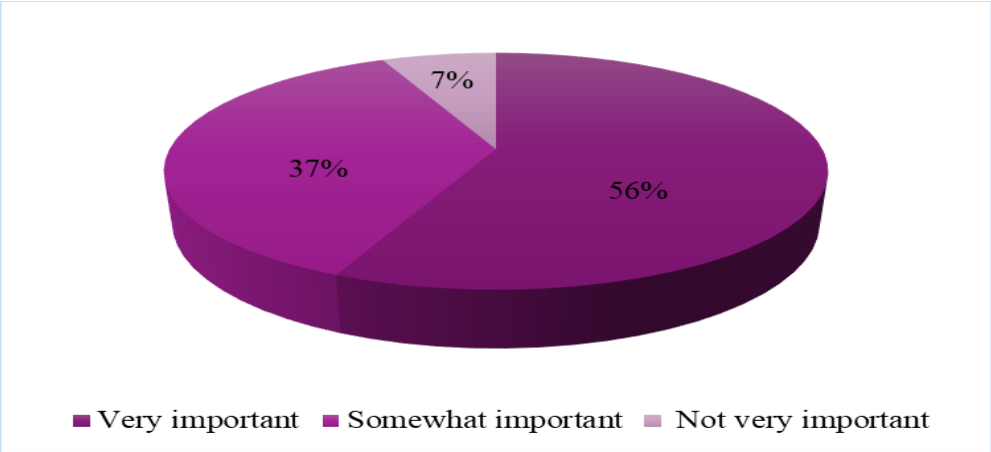

Figure 57 : The perception of doctors about the importance of vitamin D test in children and adolescents.

The data showed that the majority of doctors (56%) considered vitamin D dosage in children and adolescents to be very important. On the other hand, 37% considered it to be fairly important and only 7% of doctors considered it to be of little importance.

3.2.17 Frequency of requests for vitamin D analysis in the general population(2024)

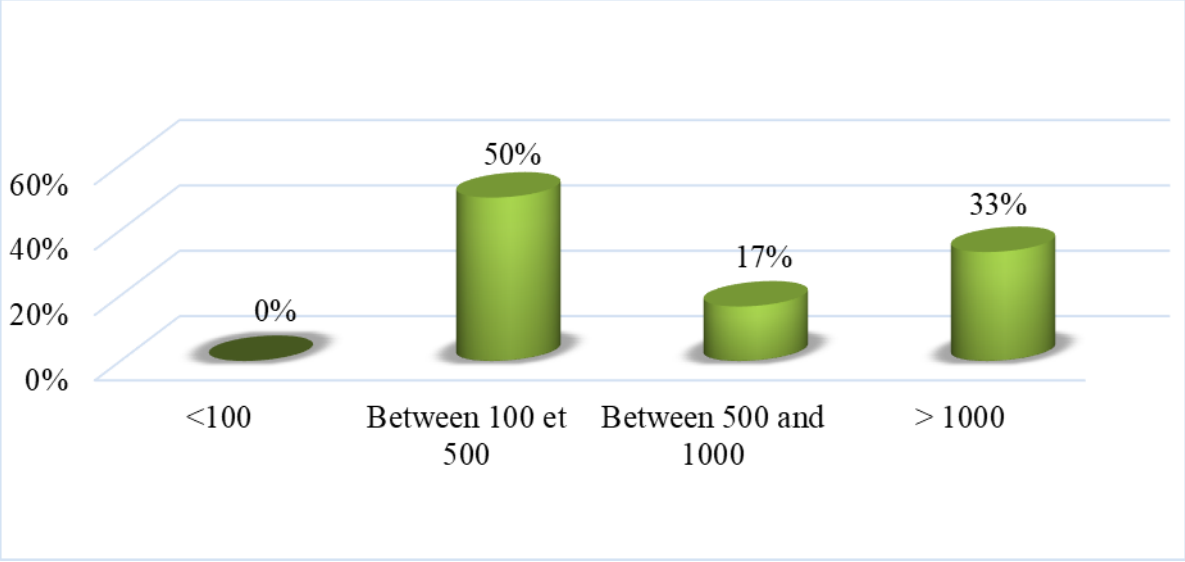

Figure 58 : Frequency of requests of vitamin D test in laboratories for general population (2024)

In 2024, the frequency of requests for vitamin D analyses (from the general population) within laboratories was varied: no laboratory reported fewer than 100 requests. Half of all laboratories (50%) received between 100 and 500 requests, which is the highest proportion. Around 17% of laboratories received between 500 and 1000 requests, while a third (33%) processed more than 1000 tests.

3.2.18 Frequency of requests for vitamin D analysis in laboratories from children and adolescents (2024)

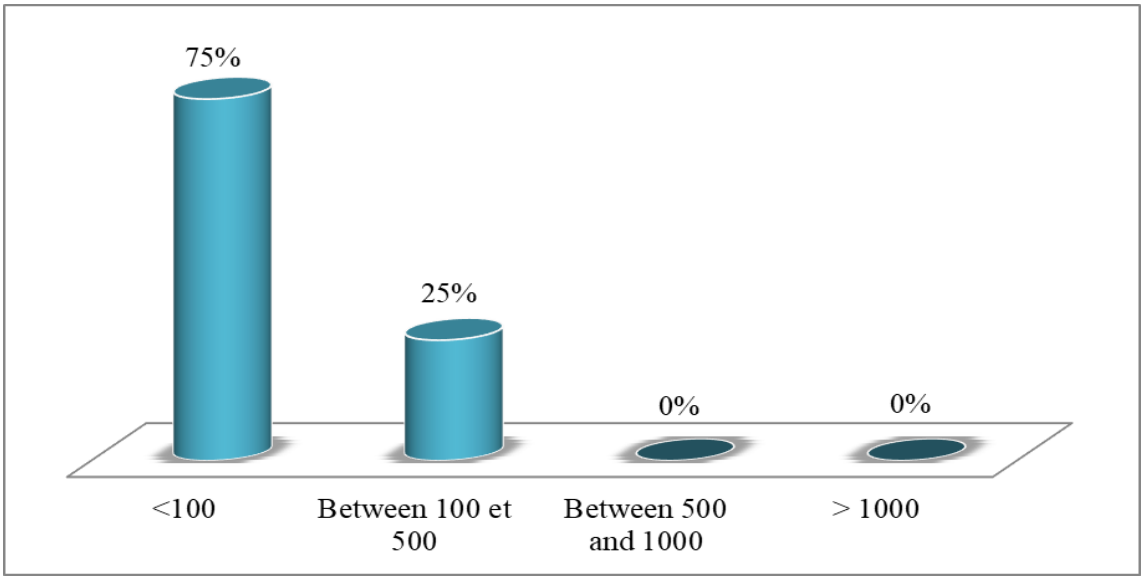

Figure 59 : Frequency of request of vitamin D test in laboratories for children and adolescents (2024).

Most laboratories(75%)declared that they had received fewer than 100 requests of vitamin D test from children and adolescents(2-17 years old), and only 25% between 100 and 500. No laboratory exceeded the threshold of 500 requests.

3.2.19 Global frequency of hypovitaminosis D in the general population in 2024

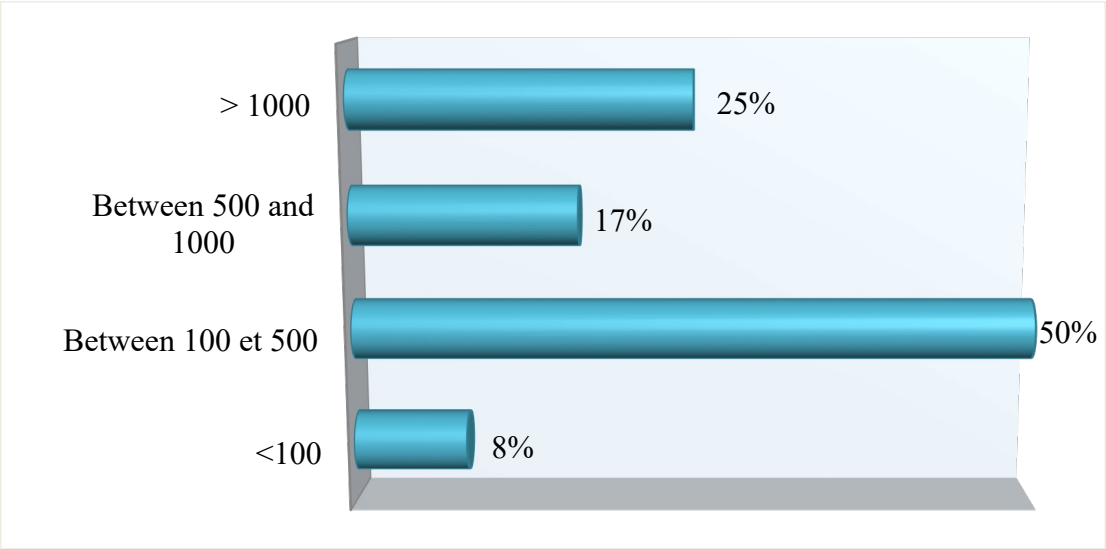

Figure 60 : Frequency of hypovitaminosis in general population according to laboratories in 2024.

In 2024, according to the laboratories participating in the study, the overall frequency of hypovitaminosis D in the general population varied as follows: of the 12 laboratories surveyed, only one(1/12, 8%)reported fewer than 100 cases, while half(6/12, 50%)observed between 100 and 500 cases, making it the most frequent category. Two laboratories (2/12, 17%) recorded between 500 and 1000 cases, and three (3/12, 25%) reported more than 1000 cases.

3.2.20 Global frequency of hypovitaminosis D in children and adolescents in 2024

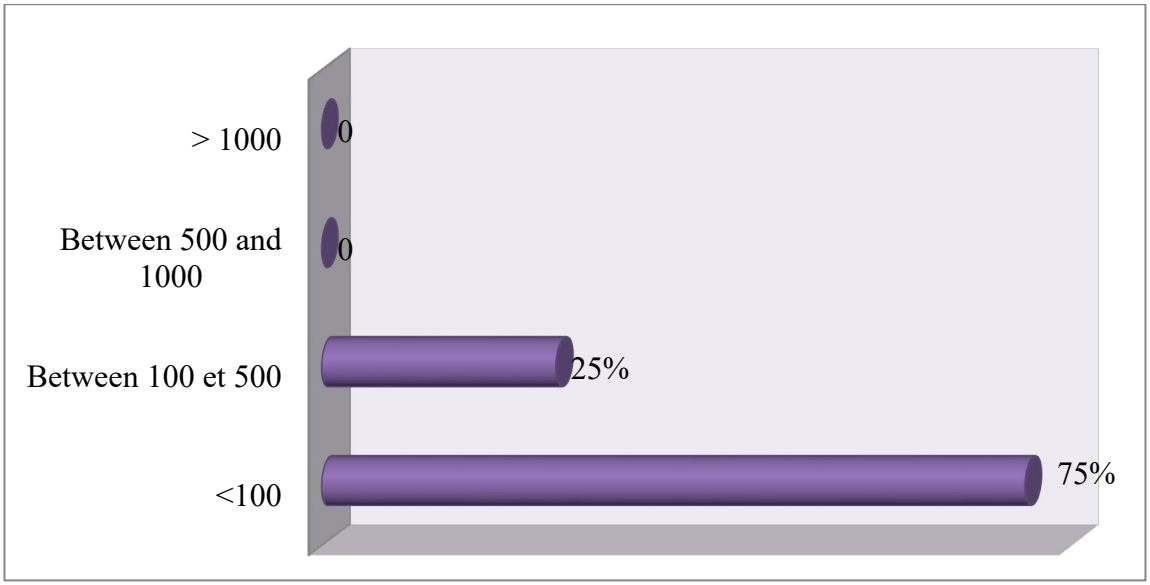

Figure 61 : Frequency of hypovitaminosis D in children and adolescents according to laboratories in 2024.

In 2024, the overall incidence of hypovitaminosis D in children and adolescents remains relatively low, according to data reported by laboratories. Indeed, 9 out of 12 laboratories (75%) reported fewer than 100 cases, while the remaining 3(25%) observed between 100 and 500 cases. No laboratory reported more than 500 cases.

3.2.21 Precise hypovitaminosis D statistics from certain laboratories

Table X : Exact statistics of vitamin D hypovitaminosis in the general population and in children and adolescents in certain laboratories in 2024

Laboratories	Numbers of general population	Frequency of hypovitaminosis D in general population	Frequency of children and adolescents	Frequency of hypovitaminosis D in children and adolescents
Laboratory 1	1864	1073/1864(57,56%)	275	149/275(54,18%)
Laboratory 2	1314	1030/1314 (78,38%)	30	28/30 (93,33%)
Laboratory 3	166	108/166 (65%)	23	15/23 (65,21%)
Laboratory 4	583	366/583 (62,77%)	74	40/74 (54,05%)

The table showed the prevalence of hypovitaminosis D in four laboratories in the Wilaya of Tlemcen for both groups: in the general population and in children and adolescents. In the general population, the rates of vitamin D deficiency exceeded 50% cases, citing the

following percentages: (57.56%), (78.38%), (65%)and(62.77%), in children and adolescents, the prevalence was also high, ranging from 54.05% to 93.33%, noting also (54.18%), (65.21%) cases.

3.2.22 Most frequent type of hypovitaminosis D in children and adolescents (aged 2-17) in 2024

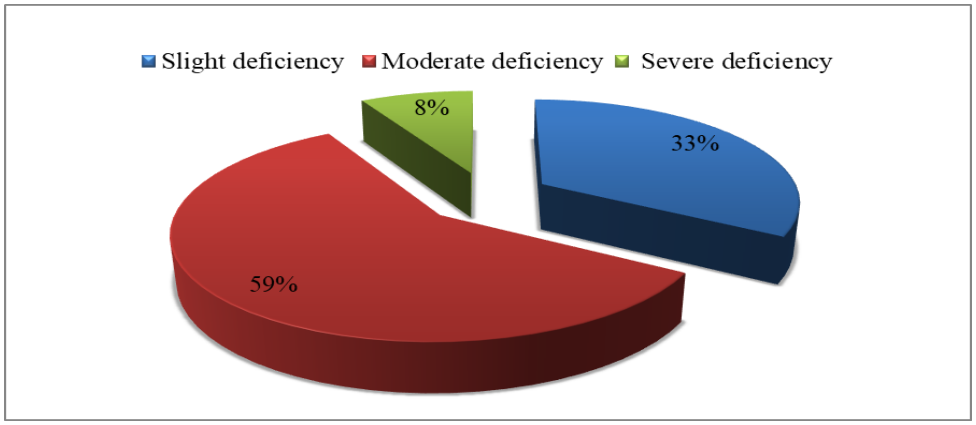

Figure 62 : The most frequent type of hypovitaminosis D in children and adolescents in 2024.

In children and adolescents aged 2 to 17, the most frequent form of hypovitaminosis D reported by laboratories is moderate deficiency, accounting for 59% of cases. This was followed by mild deficiency, affecting 33% of young patients. Severe deficiency remained relatively rare, accounting for only 8% of cases.

3.2.23 The Sex most implicated in vitamin D hypovitaminosis in the general population (laboratories/doctors)

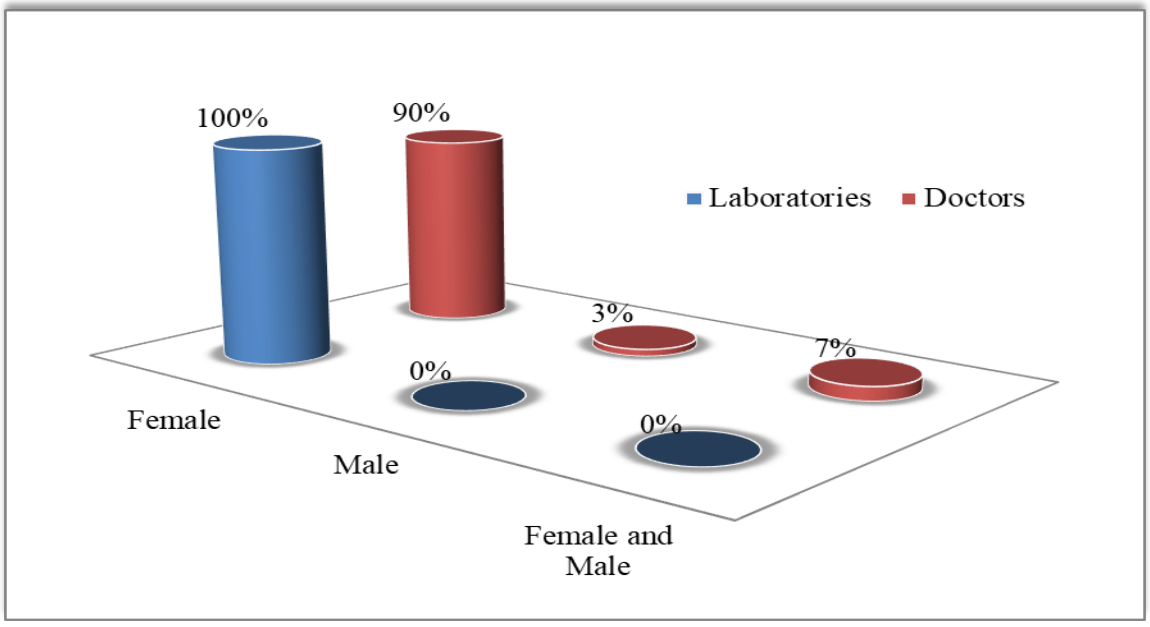

Figure 63 : The most gender incriminated in hypovitaminosis D in the general population (laboratories/ doctors).

The graph showed the perception of healthcare professionals (laboratories and doctors) of the sex most incriminated in cases of hypovitaminosis D in the general population. The data reveal a marked consensus among laboratories, which identified women in 100% of cases as being most affected by this deficiency. On the other hand, 90% of the 30 doctors shared this opinion, while 3% identified men as the group also affected and 7% considered that both sexes are equally affected by hypovitaminosis D.

3.2.24 The gender most incriminated in hypovitaminosis D in children and adolescents 2-17 years old(Laboratories/Doctors)

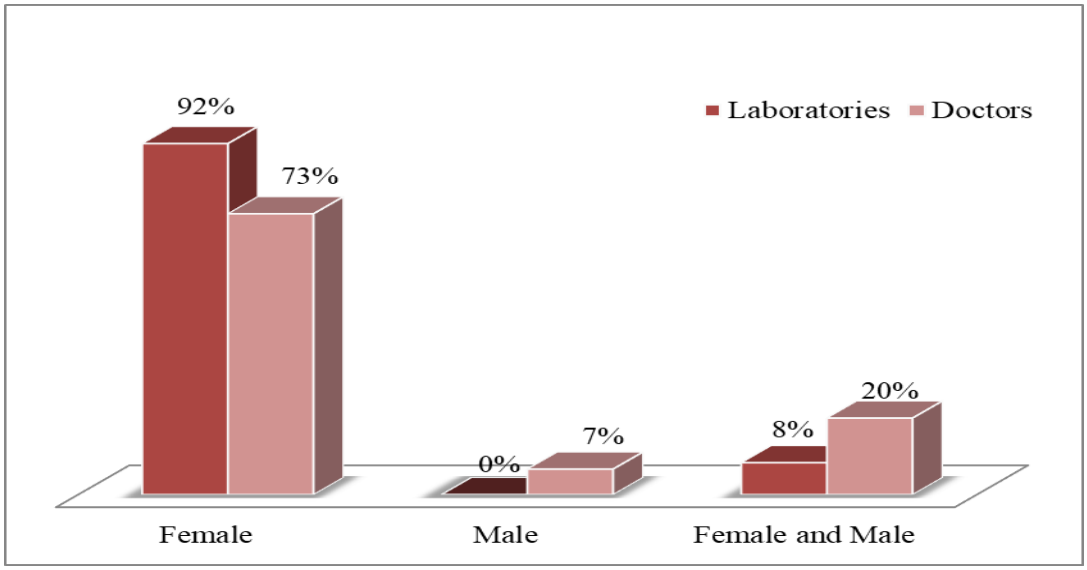

Figure 64 : The most incriminated sex in children and adolescents who resent hypovitaminosis D.

This graph showed the perceptions of laboratories and doctors regarding the gender most implicated in cases of hypovitaminosis D in children and adolescents (aged 2 to 17 years old). Most laboratories identified girls (92%) as being most affected, while a small percentage (8%) considered that both sexes were equally affected, and 0% cases for males. Among doctors, 73% filed that girls were most affected, 7% pointed boys and a considerable percentage 20% showed that both sexes were equally affected.

3.2.25 The season which is associated with a higher risk of hypovitaminosis D (laboratories/doctors)

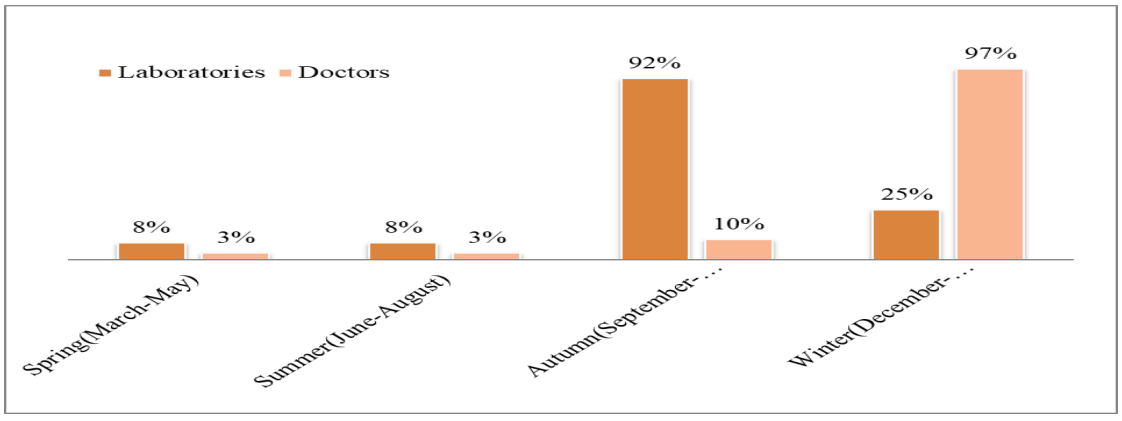

Figure 65 : Seasonal perception of requested for vitamin D dosage and hypovitaminosis D (laboratories/doctors).

This graph showed the perceptions of laboratories and doctors regarding the seasons in which hypovitaminosis D is most frequently observed. The majority of doctors identified winter (97%) as the most critical period for vitamin D deficiency, with a very low proportion in autumn (10%). On the other hand, the majority of laboratories attribute this problem in autumn (92%), with a lower proportion in winter (25%). In the other seasons (spring and summer) were rarely associated with hypovitaminosis D, both by laboratories (8% each) and by doctors (3% each).

3.2.26 The limit concentration of 25(OH)D defining hypovitaminosis D according to doctors' responses

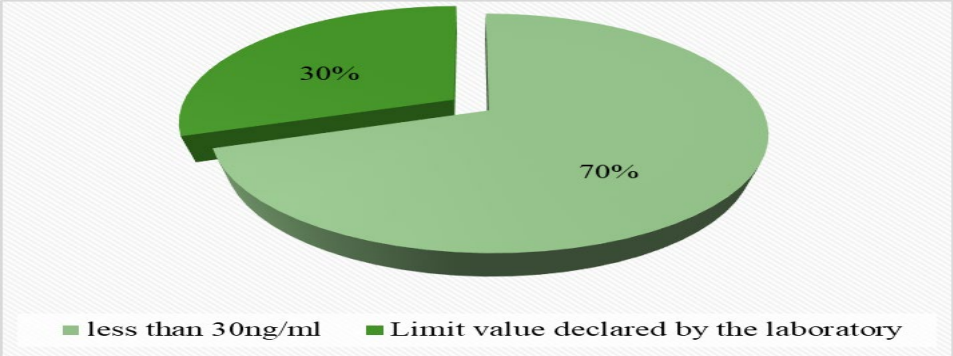

Figure 66 : The limit concentration defining hypovitaminosis D.

The graph showed that most doctors, 21 out of 30 (70%), among the 30 who participated in the study, reported that hypovitaminosis D is defined when the concentration of 25(OH)D is less than 30 ng/ml, while only 30% (9 out of 30)defined it based on the values specified and reported by laboratories as reference values.

3.2.27 Limit concentrations of 25(OH)D which define deficiency and insufficiency(responses of doctors)

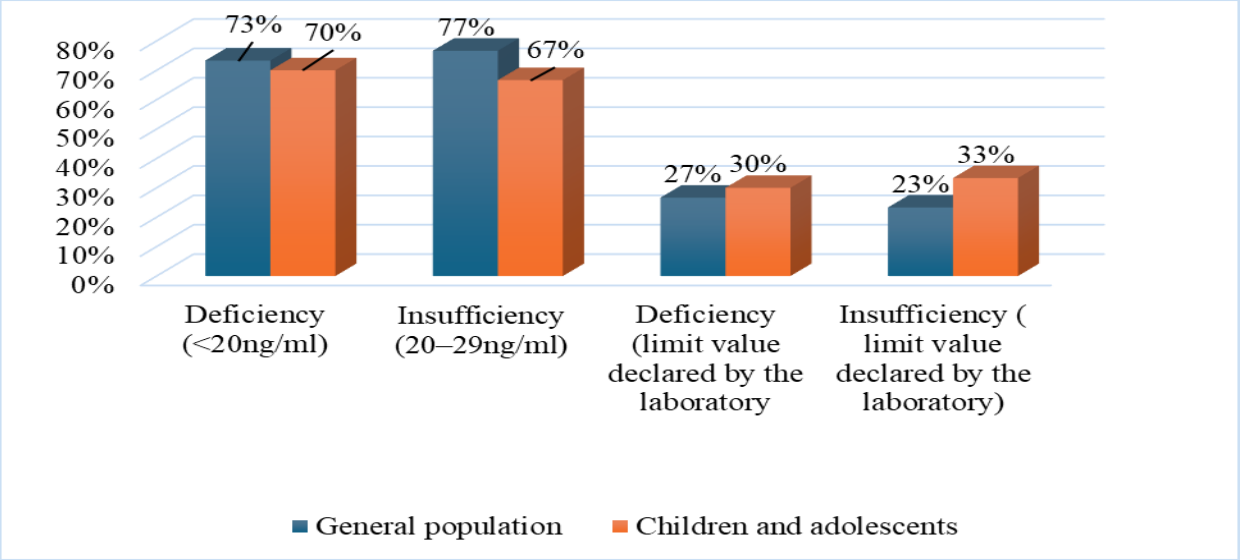

Figure 67 : Limit concentrations which define the difference between deficiency and insufficiency of vitamin D according to the doctor's responses.

The graph above showed the responses from 30 doctors on the reference limit values that define vitamin D deficiency and insufficiency in children and adolescents(aged 2 to 17), and for the general population.

- For vitamin D Deficiency:

Most doctors, with almost equal percentages (73% for the general population and 70% for children and adolescents), replied that deficiency is defined when the concentration of 25(OH) D is less than 20 ng/ml, while the rest (27% and 30% for the general population and children and adolescents respectively) relied on the values defined by medical laboratories.

- About vitamin D insufficiency:

Most doctors defined it as a concentration of 25(OH) D between 20-29 ng/ml (77% for the general population and 67% for children and adolescents), but the others based on the values declared by the laboratories (23% general population and 33% for children and adolescents).

3.2.28 Limit concentrations of 25(OH)D which define deficiency and insufficiency (responses laboratories)

A. General Population

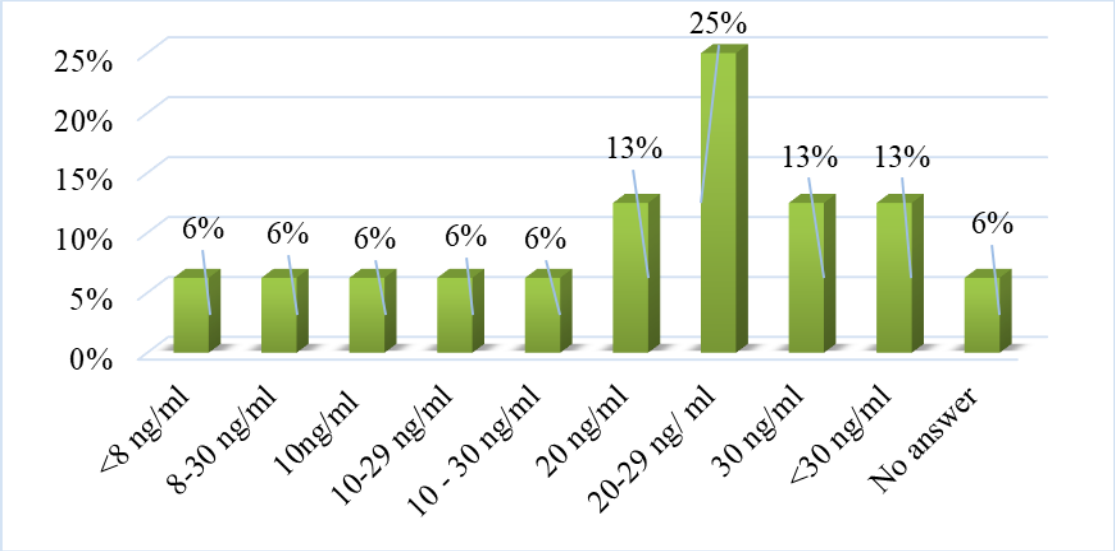

Figure 68 : Limit concentration define insufficiency of vitamin D in general population according to laboratories answers.

This graph presents the different concentration limits of 25(OH) D used to define vitamin D insufficiency in the general population, showed a wide variability in the thresholds chosen: The most frequently cited category is that of 20 to 29 ng/ml (25% of responses), with 13% of responses repeated for each following concentrations: 20 ng/ml, 30 ng/ml, and less than 30 ng/ml. On the other hand, few classes (6%) responded with other limit concentrations for each: less than 8 ng/ml, 8-30 ng/ml, 10 ng/ml, 10-20 ng/ml, 10-30 ng/ml. 6% of laboratories did not respond to this question.

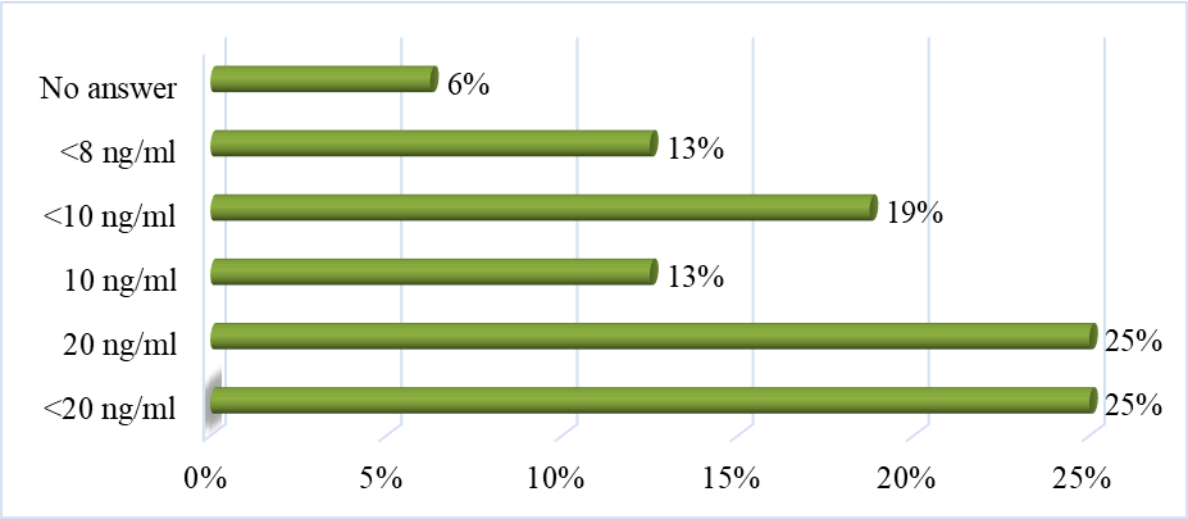

Figure 69 : Limit concentration define vitamin D deficiency according to laboratories answers.

This graph presents the different threshold concentrations of 25(OH) D used to define vitamin D deficiency in the general population. 25% of respondents indicated a lower limit of less than 20 ng/ml, while another quarter (25%) chosen exactly 20 ng/ml. 19% of respondents opted for an even lower limit, below 10 ng/ml, and 13% chosen exactly the value of 10 ng/ml. 13% reported a limit of less than 8 ng/ml, and the remaining 6% did not answer the question.

B. Children and Adolescents

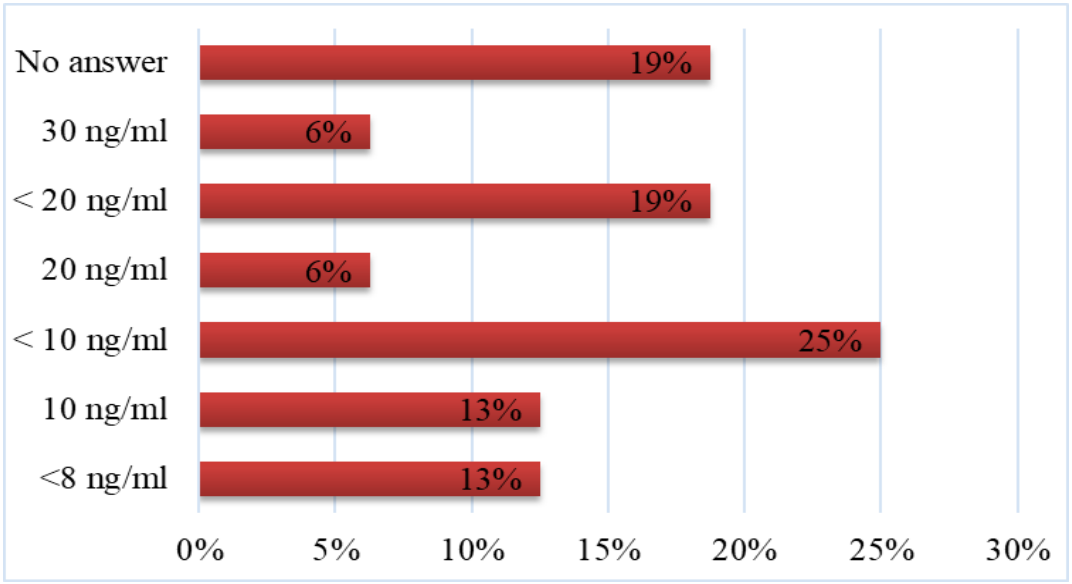

Figure 70 : Limit concentrations define vitamin D deficiency in children and adolescents according to responses of laboratories.

The graph illustrates the variability of the thresholds used to define vitamin D deficiency in children and adolescents. The most frequently mentioned threshold is <10 ng/ml, used by 25% of respondents. Other thresholds are also cited, including <20 ng/ml (19%) and <8 ng/ml (13%) and 10 ng/ml (13%). A small percentage (6%) considered 20 ng/ml or even 30 ng/ml as the limit, and finally, 19% did not answer the question.

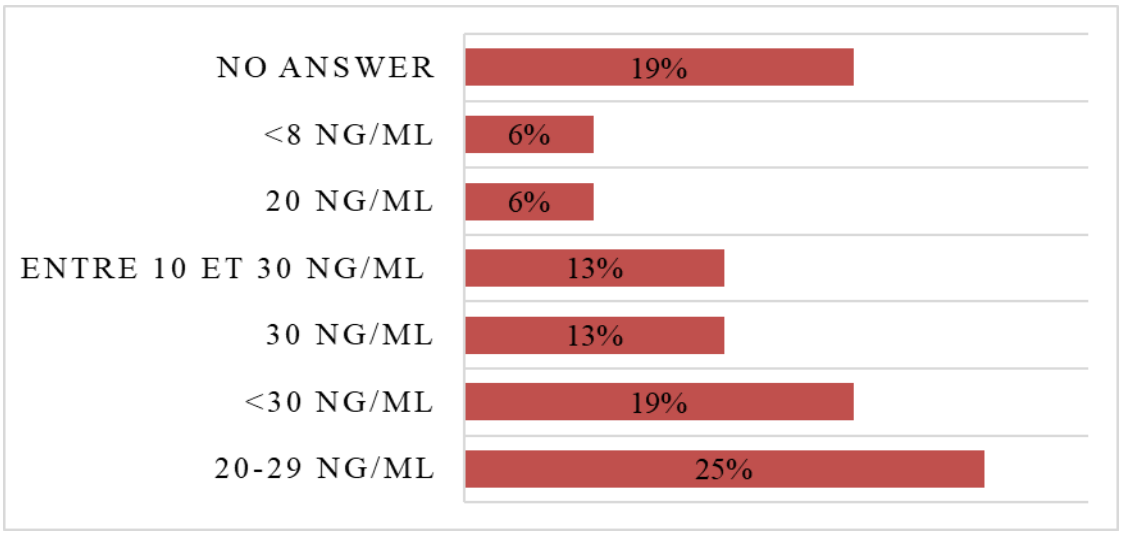

Figure 71 : Limit concentrations define vitamin D insufficiency in children and adolescents according to laboratories answers.

The graph presents the limit concentrations of 25(OH) D used to define vitamin D insufficiency in children and adolescents according to the laboratories. The most commonly cited threshold is the range of 20–29 ng/ml (25% of respondents), closely followed by the threshold <30 ng/ml (18.75%). Furthermore, 12.5% of participants used a fixed value of 30 ng/ml, while another 12.5% defined deficiency within a broader range: from 10 to 30 ng/ml. Some respondents (6.25%) used much lower thresholds, such as 20 ng/ml or even <8 ng/ml, and 18.75% did not provided a response.

3.2.29 Drugs that may interfere with vitamin D absorption or metabolism

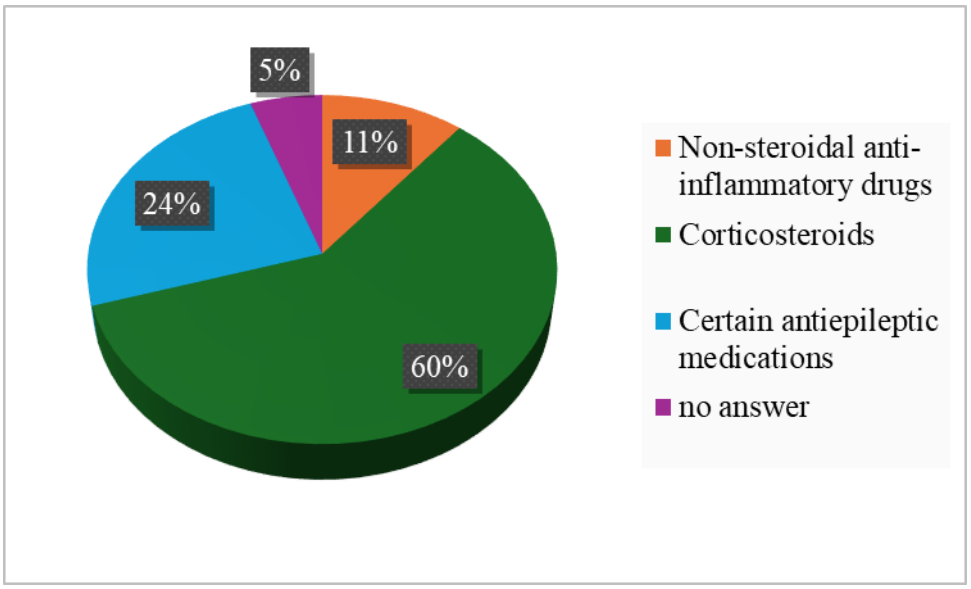

Figure 72 : Medication interfering with absorption or metabolism of vitamin D.

The results revealed that the majority of doctors surveyed recognize that corticoids can interfere with the absorption or metabolism of vitamin D. In comparison, 24% of doctors mentioned anti-epileptic drugs and 11% doctors identified non-steroidal anti-inflammatory drugs (NSAIDs). On the other hand, 5% of doctors did not answer the question.

3.2.30 The frequency of checking 25(OH)D levels in children and adolescents taking supplements

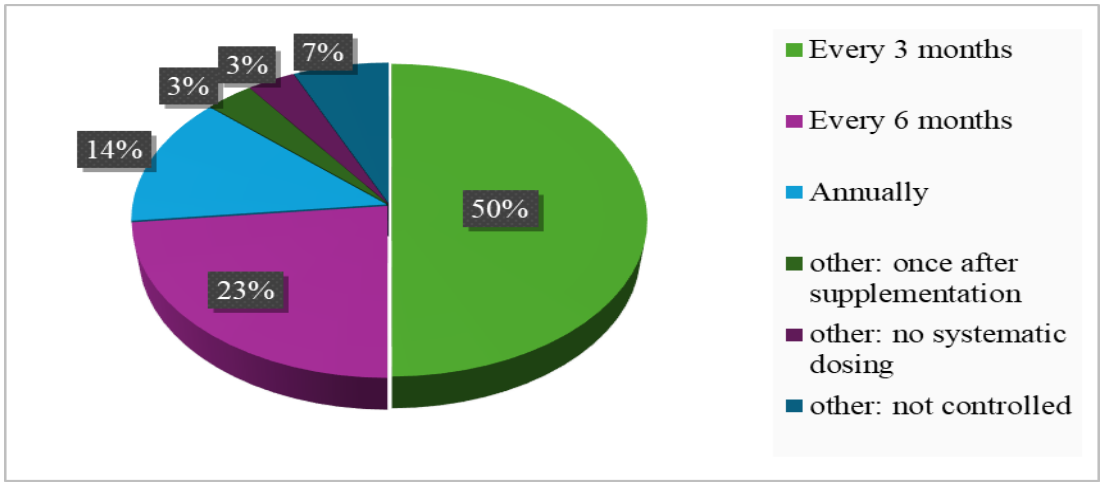

Figure 73 : Frequency of checking vitamin D levels in children and adolescents taking supplements.

The data collected on the frequency of monitoring 25(OH)D levels in children and adolescents on vitamin D supplementation varies:

The majority of doctors (50%) performed a check-up every three months, while others adopted a six-monthly (23%) or annual (14%) rhythm. A few responses indicated non-systematic practices: a single dosage after supplementation (3%), non-systematic check-up (3%) or no check-up at all (7%).

3.2.31 Main challenges in monitoring children and adolescents deficient in vitamin D

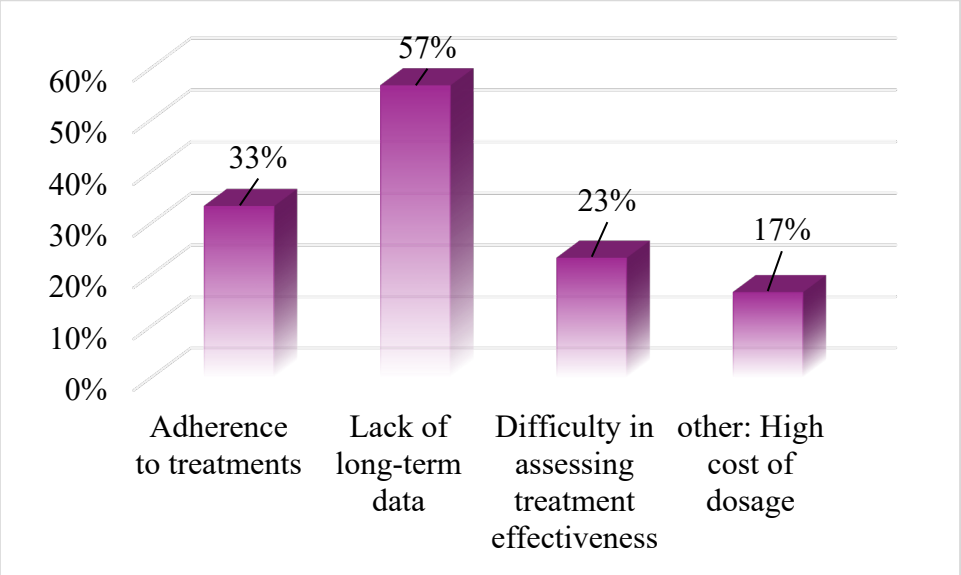

Figure 74 : The challenges faced by doctors in monitoring children and adolescents with hypovitaminosis D.

Doctors face a number of challenges in monitoring children and adolescents with hypovitaminosis D. The main obstacle identified is the lack of long-term data (57%), adherence to treatment also represents a major challenge (33%), evaluation of treatment efficacy remained difficult for some practitioners (23%) and also the high cost of blood tests (17%).

3.2.32 Doctors' opinions on parents' awareness of the importance of vitamin D for their children and adolescents

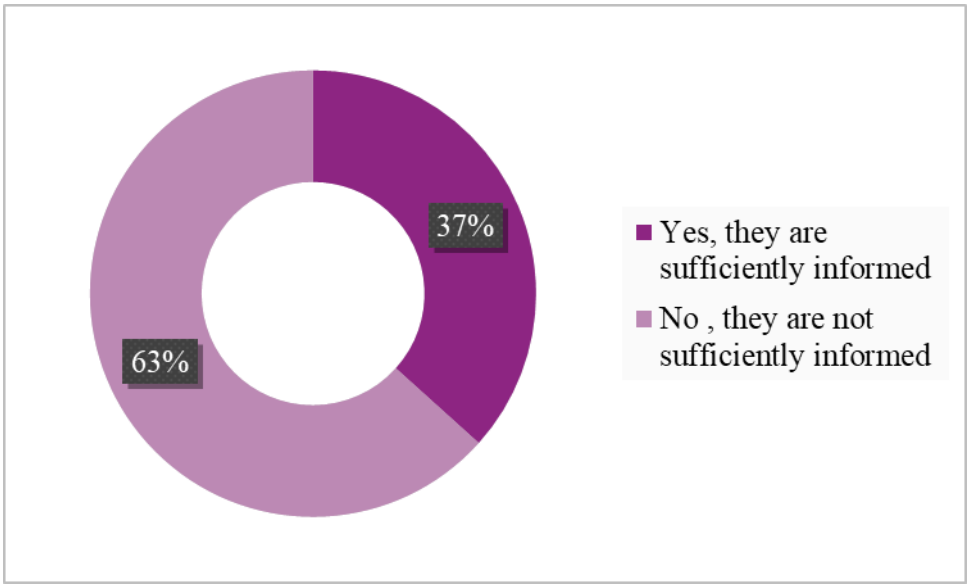

Figure 75 : Parent's awareness of the importance of vitamin D for their children and adolescents.

The results show that the majority of doctors (63%) considered that parents are not sufficiently aware of the importance of vitamin D for the health of their children and adolescents, and only 37% of doctors considered that parents have adequate knowledge on the subject.

4. Discussion

This study primarily aims to identify the analytical methods employed for quantifying vitamin D and to assess hypovitaminosis D in children and adolescents.

This section aims to discuss the results obtained in the course of study by analyzing their significance, exploring possible explanations, and relating them to existing literature.

4.1 Part one : Analytical methods for vitamin D measurement

- Most of the 16 laboratories included in the study (81%) are private, which can be attributed to the limited number of public laboratories equipped to perform vitamin D testing. This high proportion of private sector had a direct influence on the diversity of specialties observed, such as biochemistry (88%), immunology (69%), microbiology (81%), hemobiology (81%) and other specialties less frequently. This disciplinary diversity reflects the multi-sectoral character of private laboratories, which offer a wider range of analytical services to cover the needs of routine medical biology, in particular vitamin D testing. Conversely, public laboratories which often more specialized.

- The majority of laboratories in this study used serum and/or plasma for vitamin D assay, while none using whole blood. As stated by (Janoušek et al.,2022) (39), serum and plasma are the most reliable matrices for quantifying vitamin D metabolites. This choice is in accordance with the indications presented in the technical datasheets for the automated system used, which recommended the use of these types of sample (92–97).

- Heparinzed tubes are employed most frequently (54%), while EDTA tubes are little used (8%). However, both types of tube are recommended in the technical data for the assay kits of all the automated system used (92–97), which indicates that laboratories are adapting to the requirements of the analytical techniques used for vitamin D assays. Despite the high frequency of the use of dry tubes (38%), they are not referenced in the kits documentation, which suggests a possible discordance between the practice of certain laboratories and the recommendations.

- In this study, 58% of laboratories store samples in a refrigerator (2-8°C), this is in line with the recommendations of the kits used with automated analysis systems, and 21% freeze them at -20°C or below, a practice mainly observed in state laboratories. The latter practice consists with the recommendations cited by(Benkirane.2013), who indicates storing samples at -20°C after centrifugation to ensure vitamin D metabolites stability until analysis(57). The results reveal a diversity of storage practices before analysis, often linked to the availability of reagents. This constraint is confirmed by the storage time declared: the majority of laboratories do not exceed 24 hours of storage (70%) (where 44% declared a maximum storage time of 24 hours, 13% declared less than 24 hours, and 13% reported that the samples were not stored), others store up to 48 hours (13%). These practices are mainly observed in private laboratories. Conversely public facilities reported longer storage times, of up to 1 month (6%), or even 3 month at -20°C in some cases (6%), or on the depending the availability of reagents (6%).

The results reveal a wide variability in sample storage practices, related to the type of laboratory and the availability of reagents.

- According to the laboratories, several pre-analytical factors can influence and alter the accuracy of vitamin D assay. The type of tube was most frequently reported (25%), reflecting the attention required for the choice of sampling material. This result is coherent with the recommendations of the kits used on automated systems, which specify the use of particular tubes (EDTA and heparinzed tubes). Hemolysis was also reported as a pertinent pre-analytical factor (23%), which is in accord with the findings of Farrell.al.,(98), who highlights that the presence of free hemoglobin can interfere with vitamin D assays, particularly those performed by immunoassays, thus affecting the accuracy of results. The use of medication (21%) is another recognized factor that can influence circulating vitamin D concentrations; this finding is in accordance with the literature, which indicates that certain medications could change how vitamin D is metabolized, which results in vitamin D deficiency (39). Therefore, it is crucial to consider the medication history during the vitamin D status assessments. Storage temperature (15%), transport time (12%), and exposure of tube to light are also critical factors that can alter certain forms of vitamin D, particularly in the case with prolonged storage in appropriate conditions. Their importance is underlined in the descriptions of the kits used on automated systems of vitamin D dosage (92–95,97).

- Analysis of the professional experience of the laboratories participating in the study reveals that more than half of the laboratories (56%) have between 5 and 10 years experience in vitamin D testing, reflecting a relative stability in this activity and certain establishment of this analysis in their practices. Around a third (31%) had between 1 and 5 years experience, suggesting more recent integration. No laboratory reported less than one year's experience, which is favorable to the reliability of the results.

- Among 16 laboratories included in the study, there was a clear difference between the private and public sectors in terms of the automated equipment used for vitamin D testing.

Private laboratories show a significant predominance of the use of VIDAS system (Classic, PC, Kube, and Mini) presented by 11 out of 13 private laboratories. According to BioMerieux, this system is appreciated for its reliability, availability of reagents and ease of integration into medium-sized laboratories(97), which explains the high proportion of system in laboratories. Other platform include: Maglumi 800(Snibe) used in 4 private laboratories, Tosoh (1/13) and COBAS E411 (1/13).

The state laboratories (3 laboratories) used a wider range of automated equipment: SIEMENS Dimension EXL, ARCHITECT, CABUSAM (Roche), and manual dosage (with Radioimmunoassay).This reflects a lack of standardization in equipment, probably related to equipment inherited from older hospital structures.

- Concerning the period of use of equipment, the data showed that the laboratories keep their analytical systems for long period, with 50% of them having used systems for more than 5 years. This stability may be explained by the high cost of updating automated systems or by the continued reliability of existing systems.

- The study highlights the variety of methods used for vitamin D assay, most of laboratories used automated methods like Enzyme linked fluorescence assay (ELFA) (50%) and chemiluminescence-immune-assay (CLIA) (44%). These methods are commonly used because of their speed and compatibility with current automated systems; these techniques are frequently employed in ordinary practice. This is in line with research by (Janoušek et al.,2022) (39), who reported that CLIA is a fast and simple method**.**

However, methods such as ELISA (25%), fluorescent immunoassay (25%), RIA (6%) and Electrochimiluminescence-assay (6%) are more marginal. No laboratory mentioned the use of HPLC or LC-MS/MS, which are recognized as reference methods by literature (61,74,75),although their high cost, technical complexity and the need for qualified staff (2,75) ,explain why they are not widely used in routine laboratories.
Therefore, there is a clear domination of immunological methods, used by more than 90% of laboratories, and a notable absence of non-immunological methods (HPLC and LC-MS/MS).These predominance is on line with the literature, which indicates that immunological techniques are more frequently used in clinical practice because they allow results to be obtained quickly and are easily automated(49,74).

- Concerning the existence of a reference method for the measurement of vitamin D, the majority of the laboratories surveyed (69%) considered that there was no real reference method for the vitamin D analysis. However, this perception is not in accord with the published literature, which identified LC-MS/MS as the gold standard method for the measurement of 25(OH)D (74,75). On the other hand, a minority of laboratories identified reference methods: 13% cited HPLC, 13% ID-LC-MS/MS (Isotope Dilution-Liquid Chromatography Tandem Mass Spectrometry), and 6% mass spectrometry in general.

- The results of the study reveal that the accuracy and reliability of the results, as well as the cost of the method, are the criteria most frequently cited by laboratories when selecting a vitamin D assay method (43%). These factors reflect a legitimate desire for analytical quality, while taking into account economic constraints. In addition, 28% of laboratories attach particular importance to the rapidity and ease of use of the method. These criteria reflect productivity requirements, particularly in structures with a high volume of analysis. Finally the availability of reagents, cited by 29% of respondents, is also a determining factor, in particular to guarantee continuity of analytical activity without interruption. These selection criteria explain the predominance of immunological methods and are consistent with the advantages reported in the literature(2).

- Among the 16 laboratories surveyed, the majority (10/16) reported measuring total 25-Hydroxyvitamin D, which includes both D2 and D3 forms. This choice consistent with in the

published literature, which consider total 25(OH) D the main indicator if vitamin D status (2), and also respect the recommendations if the manufacturers of the automated systems used, which require the total dosage of the two forms (92–97). However, 6/16 of laboratories focus exclusively on 25-Hydroxyvitamin D3, the most common and biologically active form in humans. On the other hand, no laboratory has reported the exclusive measurement of 25-Hydroxyvitamin D2. This distribution show a relative variability on analytical practices, which may be explained by the technical capacities of the automated systems used or by specific clinical objectives.

- In terms of laboratory satisfaction with the accuracy of their current vitamin D assay method, the majority (10 out of 16) were very satisfied, while 5 laboratories were satisfied. Only one laboratory (1/16) was neutral and none were dissatisfied. This distribution represents a generally positive perception of analytical performance, indicating that the current approach meets the expectations of routine laboratory users.

- Analysis of the laboratories responses reveals that the high cost of reagents is the difficulty most frequently encountered in vitamin D testing, cited by 9/16 of them (57%). This economic difficulty appears to be a limiting factor, particularly in laboratories with limited budgets. Other technical difficulties were reported less frequently: false-positives results by 2 out of 16 of laboratories (13%), while sample instability, false-negative results and lack of standardization were each mentioned by only one laboratory (6%). None of the respondents reported interference with other substances. The lack of these declaration contrasts with the notice of vitamin D assay kits, which mention several interferences (Bilirubin, biotin, cholesterol…)(92–94). It is interesting to note that 4 out of 16 of laboratories (25%) declared that they did not encounter any particular difficulties in the assay of vitamin D. This could indicate a significant level of overall satisfaction with the performance of the current methods, or a satisfactory level of adaptation to technical constraints.
In the final analysis, although analytical difficulties do exist, they are seen as secondary to economic constraints.

- Concerning the errors reported by laboratories during vitamin D analysis and their frequencies, the majority of laboratories (56%) reported no errors; which indicates a perceived

reliability of the methods currently in routine use. This finding is reinforced by the fact that 44% of respondents considered that errors rarely occur.

Among the errors reported, calibration problems topped the list (13%), followed equally by interference due to fibrin, hemolysis and quality control errors, each reported by 6% of respondents. An isolated case was reported concerning systemically high values (in excess of 150 ng/mL) observed when using Maglumi 800 systems in CLIA on heparinzed plasma. In addition, the possible impact of certain drugs on results was also mentioned as a factor of variation.

It is a significant to notice that 2 out of 16 of the laboratories reported encountering errors sometimes or at each analysis, which indicates persistent errors in a limited number of laboratories. In addition 5/16 did not provide an answer to this question of frequency, which could reflect a lack of follow-up or reluctance to report them.

In general, the errors reported remained infrequent, which seems to indicate a satisfactory level of technical control in the majority of these facilities.

- There are several sources of error that can affect the reliability of the vitamin D assay.

Among those identified by the laboratories surveyed, sample storage time was the most frequently mentioned factor, by 44% of participants. This observation underlines the importance of respecting the recommended time between sampling and analysis. Temperature conditions, particularly during transport and storage, came in second place (31%), followed by the sampling method, cited by 19% of laboratories. A small number of laboratories (6%) also mentioned other unspecified factors, while 25% did not answer this question, which may reflect a lack of data monitoring or difficulty in clearly identifying sources of error.

- Analysis of the error management strategies reported by the laboratories reveals significant trends in the way in which the laboratories ensure the reliability of their vitamin D assays. The majority of laboratories adopt a strategy of repeating the analysis (61%). This practice reflects a desire to ensure the validity of the initial results. Automation, used only by 11% remains little used despite its benefits in terms of reliability and speed. The absence of manual correction is a positive point, reflecting compliance with good practices. Corrective actions targeting the sample itself, such as re-sampling or re-centrifugation, were mentioned by 6% of respondents, reflecting attention to the pre-analytical phase. On the other hand, 6%

stated that they did not encounter any errors, which may raise doubts about their detection system, while 10% did not respond.
These data demonstrate variability in practices and underline the importance of standardized protocols for the managements of errors in the laboratory.

- The results of the study show that automatic calibration carried out as needed is the method most frequently mentioned, representing 56% of responses. Although, this approach seems to be adapted to the workload or technical requirements, it steel contradicts the guidelines issued by the manufacturers of automated systems, who require calibration after each series of analysis in order to ensure the reliability and traceability of the results(92–97). Internal calibration is also used, but in a more variable for each laboratory, with some performing it for each series, other weekly, monthly, or as needed. No laboratory reported using external calibration.
These results show certain heterogeneity in practices, which highlights the need of better harmonization of practices and greater adherence to technical recommendations in order to optimize the quality of results.

- Among the control strategies implemented by the laboratories, internal control emerged as the most widely used method, with 62% of participants. This type of control, which integrated into the analytical process, enables continuous monitoring of performance, while 25% of laboratories stated that they use both internal and external control, which combines daily monitoring with comparative evaluation on inter-laboratory scale. Finally, none of the laboratories indicated that no controls had been carried out.
The data describe the diversity of approaches used to ensure quality in vitamin D analysis.
Concerning the frequency with which quality control are performed, the majority (88%) carry out controls on a monthly basis, which is in line with the recommendations of the kit manufacturers, who indicate a frequency of control every 28 to 30 days (92–97). A minority of laboratories (6%) stated that they carry out controls only after a breakdown, on a weekly basis or as needed.
There is some diversity in monitoring practices, but a clear trend emerges in preference of regular monthly controls.

- Rigorous evaluation of the performance of analytical methods is essential to guarantee the reliability and accuracy of vitamin D assays in the laboratory. The results of the study reveal that the majority of laboratories (69%) evaluate their analytical methods based on a full set of performance criteria, including sensitivity, accuracy, linearity, and interval of validity. This practice reflects a complete approach to ensuring the reliability of results.

On the other hand, a proportion of laboratories focus on a single criterion, with 25% highlighting sensitivity, accuracy, and interval of validity as the main evaluation factor. A notable point is that linearity was not chosen by any laboratory as the sole evaluation criterion, the laboratories appear to be well aware that this criterion alone is not sufficient to judge the performance of a method.

Moreover, the frequencies with which methods are validated in another important indicator of analytical rigour. The results also show a good level of rigour, 15 out of 16 (94%) of laboratories perform a validation for each new batch of reagents, which demonstrates a high level of vigilance and limits the risks associated with inter-batch variations. Only one laboratory goes further, with ever more frequent validation, and none is required to perform an annual or biannual validation, practices which are considered insufficient to guarantee the reliability of results on daily basis.

In conclusion, these results show that the essential method validation criteria are taken into account in a satisfactory manner. However, they also highlight the need to harmonize practices through clear recommendations and continuing training in order to improve the quality of analysis.

- In this study, the results indicate a significant variability in the limits of detection (LOD) reported by laboratories for vitamin D dosage. Approximately 35% of laboratories had a very low LOD of less than 2 ng/mL, which enabled them accurately measure even small quantities. At the other extreme, an equivalent proportion reported a higher LOD between 5-10 ng/mL, while others (24%) fall into an intermediate range (2-5 ng/mL). Very few laboratories (6%) used detection limits above 10 ng /mL .

These variations may be explained by the differences in performance between the automated systems used. Some systems such as ARCHITECT, Siemens EXL, COBAS E411 or CABUSAM (Roche) are systemically associated with LOD of less than 2 ng/mL. The results

confirm that the values declared were in accordance with the specifications set out in the kits of the automated systems(92,93,96).

On the other hand, Vidas systems perform more variably, with LODs frequently between 5-10 ng/mL, which is in line with the data published by BioMerieux, and even higher values in some cases. Maglumi and Tosoh systems fall into an intermediate range, with LOD generally between 2-5 ng/mL, which match the kits requirements(94,95). These observations clearly show that the limit of detection LOD is not an isolated parameter: it is extremely dependent on the equipment used. This notice highlights the importance for each laboratory of having full knowledge of the real capabilities of its equipment, particularly in the context of screening for deficiencies where it is essential to be able to measure very low concentrations.

- One of the points that stood out in the study was the complete uniformity of the units used for the measurement of vitamin D. 100% of the laboratories surveyed stated that they used ng/mL. None of the laboratories reported the use of alternative units such as nmol/L or any other form. This homogeneity is a major advantage, particularly for the comparability of analysis between different laboratories, but also reflects a standardization of practices in accordance with current international recommendations (5).

- Concerning the reference values used to interpret 25-hydroxyvitamin D results, 14 out of 16 (88%) of laboratories used the values provided by the assay kits. This majority reflects a standardized practice, easy to implement, but these values do not always take into account the particularities of the local population, which may influence the clinical pertinence of the results. One laboratory (6%) used internal values established on the basis of a local cohort; this choice shows the interest of adapting the references to the local demographic and epidemiological specificities, thus improving the accuracy of the diagnosis, while 4 laboratories (25%) refereed to the recommendations of the national health authorities, illustrating a desire to align with public health standards. One laboratory did not specify the source of its reference values.

In conclusion, these results underline the importance of harmonizing the reference used, in order to ensure reliable interpretation of results, improved comparability between laboratories, and optimal patient monitoring.

- Concerning the average time to obtain the results of 25-hydrixyvitamin D assay, 88% of laboratories reported a turnaround time if less than 24 hours. None of the laboratories reported a period between 24-72 hours. This majority rapidity in the delivery of results indicates a notable efficiency on the processing of analysis within the majority of laboratories, which is essential for effective clinical treatment. However, one laboratory (state laboratory) (6%) indicated a turnaround time of more than 72 hours, which is linked to the availability of reagent.

- The results of the study reveal that only 38% of laboratories have already carried out specific studies on vitamin D assays, while more than half (56%) had not, and 6% did not provide an answer. These statistics reflect the limited involvement of laboratories in a process of reflection or in depth analysis of their practices concerning this analysis.

In addition, the study indicates that only 25% of laboratories collected and used vitamin D dosage results over time, compared with 69% who did not carry any structured monitoring. Once again, this trend points to a lack of use of the available data for quality control or continuous development purpose.

- Concerning the management of data relating to vitamin D testing, the results show that the vast majority of laboratories (88%) used data management's software to collect and organize information's. This high proportion reflects the generalized computerization of practices, favoring traceability, organization of results and rapid access to the data required for interpretation.

On the other hand, a minority of laboratories (12%) continued to use manual methods, such as a paper register or a collection sheet. Although these methods may be adapted to small-scale laboratories or those with limited resources, they have major limitations: risk of transcription errors, difficulty of long term storage.

These results therefore underline the importance of increasing the computerization of process in all types of laboratories.

4.2 Part two: Frequency of hypovitaminosis D

- The study includes 30 participating doctors, most of them are pediatricians (67%) because the study focuses more on the category of children and adolescents, the others are general practitioners (20%), internal medicine doctors, or even functional rehabilitation specialists to assess the prevalence of vitamin D deficiency in the general population. 50% of doctors work in hospitals, the other work in private clinics and liberal practice to obtain different information.

- The most frequent clinical manifestations of hypovitaminosis D in children and adolescents are fatigue (87%) and bone pain (83%), indicating generalized damage, particularly to the skeleton due to vitamin D deficiency, followed by growth disorders (43%), bone deformities (10%) and walking delays (3%), corresponding to more advanced or prolonged forms of hypovitaminosis D. Cramps (3%), rickets (3%), weight loss (3%), irritability, as well as mood disorders such as depression (3%) confirm the importance and wider role of vitamin D.

- The physical signs most frequently observed in children and adolescents suffering from hypovitaminosis D are bone deformity (90%), rickets (73%) and osteomalacia (17%). To a lesser extent, there is also bone and muscle weakness (3%) All these signs occur when there is poor mineralization of the growing skeleton, with the bones becoming soft and weak, leading to typical deformations.

- The main risk factor for vitamin D deficiency in children and adolescents is a lack of sun exposure, affecting about 80% of cases. Indeed, the synthesis of vitamin D primarily occurs in the skin under the effect of UVB rays, and insufficient exposure, often due to a sedentary indoor lifestyle or excessive use of sunscreen and automobiles, reduces this natural production. Additionally, an unbalanced diet, also present in 80% of children and adolescents, contributes to an insufficient intake of foods rich in vitamin D (such as fatty fish, fortified dairy products, or eggs...). Dark-skinned children, representing 33% of cases, are also at risk because melanin acts as a natural filter for UV rays, thereby reducing the skin's ability to produce vitamin D. Finally, obesity (27%) is an aggravating factor, as vitamin D, being fat-soluble, gets trapped in adipose tissue, which reduces its availability in the body, and its levels are inversely proportional to body mass index (BMI).

- Vitamin D dosage is indicated especially if children and adolescents present chronic fatigue (22/30 responses), because it is the most frequent clinical sign followed by growth retardation (21/30 responses), unbalanced diet 20/30 and low sun exposure 14/30 are also widely mentioned.

Other indications in the case of more targeted medical factors:

Chronic diseases 14/30, family history of bone disease14/30, and obesity7/30. Celiac disease, dysthyroidism, recurrent fractures, bone pain and delayed walking were mentioned only once.

Vitamin D dosage is generally indicated if risk factors are present, as mentioned in the following article (5).

- All doctors (100%) replied that vitamin D blood tests are the basis for diagnosing hypovitaminosis D, 83% of whom consider them to be sufficient with the patient's clinical examination, while 17% added bone X-rays, especially in the case of rickets, which has been diagnosed on the basis of radio graphically visible features of the wrists or knees, according to this study (53).

- With regard to assessing the prevalence of hypovitaminosis D in children and adolescents, according to the doctors, 60% estimate that they consult a number no greater than 5 cases at risk per week, and consequently the frequency of prescribing vitamin D dosage in this population group is also less than 5 prescriptions per week (63%).

Others report a higher number: between 5 and 10 cases (20%), and between 10 and 20 cases (10%) per week.

These data are confirmed by the responses of the laboratories that took part in the study, 63% of which stated that the number of vitamin D tests carried out in children and adolescents in their laboratories was less than 10 tests per week.

- In addition, data from laboratories in 2024 show that 75% of them received fewer than 100 patients (children and adolescents) for vitamin D analysis, and results showing hypovitaminosis D are also limited to this number.

On the other hand, precise data from vitamin D dosage results in children and adolescents in certain laboratories have shown that the frequency of hypovitaminosis D exceeds half of

patients and varies between 54.05% and 93.33%, with a predominance of moderate deficiency (vitamin D levels between 10-20 ng/mL).

These results are similar to those of a study carried out on children aged between one month and eighteen years in Kabul, over a one-year period between 2020 and 2021, in which hypovitaminosis D was found to be widespread in 62.5% of the population, but with a predominance of severe deficiency(99).

- The sex most incriminated in hypovitaminosis D in children and adolescents is the female sex according to the responses of doctors (73%) and laboratories (92%), this result is also confirmed in the previous article where there is a predominance of the female sex.

20% of doctors and 8% of laboratories see that both sexes are incriminated and there is no association between sex and vitamin D status, as indicated in a study in Belgium on 14,887 samples between 2014 and 2021 (100)**.**

- Concerning the evolution of the prevalence of hypovitaminosis D, 69% of doctors and 33% of medical laboratories see that it is increasing in recent years, is explained by a set of factors related to modern lifestyle, environment and eating habits, children and adolescents spend more time indoors in schools, in front of screens, therefore there is not sufficient sun exposure, moreover the use of clothing covering and less access to quality food rich in vitamin D remains a frequent cause.

On the other hand, 24% of doctors and 42% of laboratories observed that it was stable, with very few responses saying that there had been a very slight decrease. In fact, during the COVID-19 pandemic, the use of vitamin D supplements increased to prevent acute respiratory tract infections, as well as helping to control the anti-inflammatory process and being used for their role as an immune modulator, in line with the information mentioned in the following article(25)which increases awareness of the importance of vitamin D.

As a result, the frequency of this hypovitaminosis varies according to the degree of sensitization and as a function of daily activities and nutrition; it is mostly frequent, but can also be occasional.

- When it comes to checking vitamin D levels in children and adolescents with risk factors, 67% of doctors replied that it’s only necessary if there is an onset of symptoms of

hypovitaminosis D, and 30% said that it is necessary every year to avoid the complications of hypovitaminosis D.

- 50% of doctors see that the ideal frequency for checking vitamin D levels in children and adolescents on supplementation is recommended every 3 months, suggesting a proactive and rigorous approach to assess the effectiveness of supplementation and adjust doses if necessary to avoid toxicity. A biannual follow-up is practiced by 23% of professionals, especially if the hypovitaminosis is not severe and also because the vitamin D analysis is costly, while 14% recommend annual monitoring.

Concerning the evolution of the prevalence of hypovitaminosis D in the general population, the number of prescriptions for vitamin D dosage per week by doctors in the general population is as follows:

30% more than 20 cases, 27% between 5 and 10 cases, 20% between 10 and 20 cases and 20% less than 5 cases.

Compare these results with the number of vitamin D tests performed in medical laboratories in the general population, which is as follows:

38% more than 20 cases, 56% between 10 and 20 cases and no laboratory reports less than 10 cases per week. This shows that in the general population there is a great demand for vitamin D tests without a doctor's consultation.

- During the year 2024, the prevalence of requests for vitamin D analysis in the general population, there are no reports of less than 100 requests and it is as follows:

50% between 100 and 500 cases, 17% between 500 and 1000, 33% more than 1000 cases.

The prevalence of hypovitaminosis D between 100 and 500 cases is 50% (6/12), and between 500 and 1000 cases is 17% (2/12), means that all the tests that did show a hypovitaminosis D, moreover among the 33% (3/12) of more than 1000 previous requests, 25% present a hypovitaminosis.

Some laboratories declare accurate figures that showed that the prevalence in 2024 varies between 57.56% and 78.38%.

- The sex most incriminated in hypovitaminosis D in the general population according to 90% of doctors and 100% of medical analysis laboratories is women.

- The frequency of hypovitaminosis D in the general population is very high, reflecting a lifestyle that is mainly in the workplace, accompanied by a lack of physical activity and insufficient exposure to the sun, or at home, especially in the elderly, who show a reduction in cutaneous synthesis of vitamin D. In addition, obese people with a BMI over 30 or with dark skin also had low levels of vitamin D (5)**.**

- Women are the sex most incriminate, this is due to the use of scarves in some category among them and daily sun creams with a high sun protection factor (SPF) that can reduce the absorption of UVB rays.

In addition pregnant women are also more widely affected by this hypovitaminosis according to studies made on a total of 578 Saudi women during their first trimester of pregnancy were recruited between January 2014 and December 2015 in three antenatal clinics tertiary care in Riyadh, Saudi Arabia, show that (81%) are deficient (101).

- According to the data from the medical analysis laboratories, hypovitaminosis D in the general population is very frequent (43.75%), while others (50%) see it as frequent, which is shown by the previous results.

Concerning prevalence, half the laboratories observe that it is stable, which indicates that there has been no change in lifestyle, activity or the nature of the diet. On the other hand, 33% said that there had been a significant increase, mainly due to a lack of awareness among the majority of the population.

- The season most responsible for hypovitaminosis D according to 96.67% of doctors is winter, when the temperature falls, the intensity of UVB rays is insufficient, more time is spent indoors and the skin is more covered (warm clothing).

On the other hand, 91.67% of medical laboratories record the highest frequency of hypovitaminosis D in autumn, and this is due to the gradual reduction in sunshine, with less exposed skin because people start to wear covering clothing, and the return to work or school in September, which means more time spent indoors and fewer outdoor activities than in summer, so fewer opportunities for solar synthesis.

- 70% of doctors defined hypovitaminosis D as a limit concentration of less than 30 ng/mL, and this corresponds to what is stated in the following article(35).The other 30% based their interpretation on the values specified by the laboratory.

- When asked to differentiate between vitamin D deficiency and insufficiency, the majority of doctors replied that:

Insufficiency is defined as a blood level of vitamin D between 20 and 29 ng/mL, whereas deficiency is defined as a vitamin D level of less than 20 ng/mL in the general population, including children and adolescents. These definitions are similar to the information cited in the following article(35).

- Regarding the responses from medical analysis laboratories, there is a large diversity of values defining vitamin D deficiency and insufficiency, for the general population, the majority declared the same previous values mentioned by doctors.

For children and adolescents, the largest percentage defined deficiency as a vitamin D blood level of less than 10 ng/mL and insufficiency as a vitamin D level of less than 30 ng/mL without a precise range, 12.5% defined it as a level between 10 and 30 ng/mL.

This diversity is due to the difference in the interpretation values of the automatic testers used in the laboratories, for example:

in the Cobas and Vidas kit: a vitamin D level of less than 20 ng/mL defines a deficiency, and between 21 and 29 ng/mL defines an insufficiency(93,97).

In the Maglumi and Architect kit, deficiency is defined as less than 10 ng/mL, and insufficiency as between 10 and 30 ng/mL (92,94).

The majority of laboratories (92%), and doctors (56%), confirm that vitamin D dosage in children and adolescents is important, as it plays a role in bone and cartilage mineralization(25), as well as being an antimicrobial and immunological agent. Vitamin D deficiency can be associated with acute and chronic systemic diseases, causing rickets, muscular pain and osteomalacia in children and adolescents, so vitamin D dosage is necessary, especially to prevent complications of deficiency and promote healthy growth (1).

- The drugs that can interfere with vitamin D metabolism are mostly (60%) corticosteroids according to doctors, followed by certain anti-epileptics (24%).

Corticosteroids can reduce calcium absorption and affect vitamin D metabolism (5), while some anti-epileptics are CYP enzyme inducers and therefore increase vitamin D catabolism(39).

- The main challenge faced by doctors in monitoring children and adolescents suffering from hypovitaminosis D is the lack of long-term data (17/30), especially in hospitals where doctors change groups. In addition, the majority of patients do not respect the time limit for monitoring after supplementation, partly because vitamin D analyses are expensive and partly because the majority of symptoms disappear or diminish after taking the vitamin D supplement.

Adherence to treatment is also an important point (10/30 responses), as vitamin D is ideally taken with a high-fat meal, consistency is important on a monthly or bi-monthly basis, and this can be neglected by children and adolescents.

- The majority of doctors (63%) say that parents are not sufficiently aware of the importance of vitamin D in their children and adolescents, especially in terms of lifestyle and diet, and as a result the incidence of hypovitaminosis D is still high.

The limitations of this study

Despite the interest of this research, a certain number of limitations were encountered during the realization:

- Limited number of laboratories surveyed: one if the major obstacles was the difficulty of contacting the managers of certain laboratories, particularly when they were absent or not available, which reduced the total number of laboratories surveyed to16, below the initial target of 30.

- Several laboratories responded incompletely, omitting key information such as statistical data on hypovitaminosis D.

- A great deal of variability was observed between laboratories, both in terms of the techniques used and the deficiency thresholds considered, which complicated the comparison.

- The response to the questionnaires was sometimes very slow, taking up to a month for some laboratories. This delay slowed down data collection.

- The difficulty of finding similar studies for comparison limited the possibilities of linking and cross-interpreting the results.

CONCLUSION

Conclusion

Vitamin D plays a central role in bone health and development in children and adolescents. Vitamin D deficiency is a major health issue, requiring accurate and reliable diagnosis.

In this context, the aim of this study was twofold: on one hand, to review current analytical practices in laboratories for the measurement of vitamin D; on the other hand, to assess the frequence and practices of doctors in the face of hypovitaminosis D in children and adolescents. To achieve that, two separate questionnaire were drawn up: one for laboratories, covering the methods used, their frequency, choice and quality control strategies; the other for doctors, exploring their prescribing habits, diagnostic criteria and therapeutic strategies. Analysis of results revealed a wide range of practices; there was great diversity on the techniques used, with a predominance of automated immunological methods (particularly Enzyme linked fluorescent assay (ELFA) and chemiluminescent immune assay (CLIA), and an absence of reference method such as LC-MS/MS (non-immunological).The study also highlighted a differences between public (state) and private laboratories concerning the equipment used to measure vitamin D. It is important to reaffirm that the vitamin D deficiency thresholds used vary from one laboratory to another.

In addition, the survey of medicals practices revealed a growing awareness of vitamin D levels, as well differences in diagnostic and therapeutic strategies.

This work therefore highlights several areas for improvement. It is essential to reinforce standardization and validation of dosage methods in laboratories, in particular to guarantee the comparability of results between different structures. It'sseems crucial to promote harmonization of practices and draw up a clearrecommendationsin order to optimize the prevention and treatment of hypovitaminosis D on children and adolescents.

In conclusion, vitamin D dosage is a key procedure for detecting and preventing deficiency in young people. It is part of a multidisciplinary approach that involves both analytical rigour and clinical responsibility to ensure optimal management of this problem, which is still too often underestimated.

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ANNEXES

Questionnaire of laboratories

FACULTE DE MEDCINE
DEPARTEMENT DE PHARMACIE

Questionnaire sur : « LES METHODES DE DOSAGE DE LA VITAMINE D POUR LE DIAGNOSTIQUE DE L'HYPOVITAMINOSE D CHEZ LES ENFANTS ET LES ADOLESCENTS »

- ✓ Ce questionnaire est à remplir de façon anonyme.
- ✓ Mettez (ou cochez) la réponse appropriée .

Partie I :Les méthodes de dosage de la vitamine D

I.Informations générales sur le laboratoire

1) Type de laboratoire

☐ Laboratoire étatique

☐ Laboratoire privée

2) Quelles sont les spécialités assurées par votre laboratoire ?

☐ Biochimie ☐ Hémobiologie

☐ Microbiologie ☐ Toxicologie

☐ Immunologie ☐ Autre(Précisez) :…….

II. Pré analytique

1) Quels échantillons utilisez-vous pour le dosage de la vitamine D ?

☐ Sang total Sérum ☐ ☐ Plasma

☐ Autres (Précisez) : …….

2) Quel type de tube utilisez-vous pour le dosage de la vitamine D ?

☐ Tube sec ☐ Tube hépariné ☐ Tube EDTA

☐ tre(Précisez) :…….

3) Quelles sont les conditions de conservation des échantillonsavant analyse ?

☐ pérature ambianteCongélateur (-20°C ou inférieur) ☐

☐ igérateur (2-8°C) ☐ re (Précisez) :…….

4) Quelle est la durée maximale de stockage des échantillons avant analyse ?

☐ 24 heures ☐ 48 heures ☐ 1 mois

☐ Autre (Précisez) :…….

5) Quels sont les principaux facteurs pré analytique que vous considérez comme pouvant influencer significativement les résultats du dosage de la vitamine D ?

☐ Le type de tube de prélèvement ☐ l'hémolyse

☐ La durée de transport de l'échantillon ☐ l'exposition à la lumière

☐ La température de stockage avant l'analyse ☐ la prise de médicament

III. Méthodes d'analyse

1) Depuis combien d'années votre laboratoire effectue-t-il des dosages de la vitamine D ?

☐ Moins de 1 an ☐ 5 à 10 ans

☐ 1 à 5 ans ☐ Plus de 10 ans

2) Quel est l'automate utilisé pour le dosage de la vitamine D dans votre laboratoire ?

Précisez :…….

3) Depuis quand vous utilisez cet équipement ?

☐ Moins de 1 an ☐ 1 à 5 ans ☐ Plus

4) Quelle est la méthode utilisée pour le dosage de la vitamine D dans votre laboratoire ? (

☐ HPLC (chromatographie liquide à haut performance)

☐ ELISA (Enzyme –LinkedImmunosorbentAssay)

☐ LC-MS/MS (chromatographie liquide couplée à la spectrométrie de masse)

☐ RIA (radioimmunoassay)

☐ CLIA (chimiluminescence immunoassay)

☐ Immun essai par fluorescence

☐ Autres(Précisez) : ……..

5) D'après vous, existe-t-il une autre méthode que vous considérer comme le Gold standard dans le dosage de la vitamine D ?

☐ Oui ☐ Non

Si oui précisez : ……..
Pourquoi ?:…….

6) Quel type de vitamine D mesurez-vous ?

☐ -hydroxyvitamine D totale

☐ -hydroxyvitamine D2

☐ -hydroxyvitamine D3

☐ …tres(Précisez) :……..

7) Quelles sont les raisons principales de votre choix de méthode de dosage de la vitamine D dans votre laboratoire ?

☐ cision et fiabilité des résultats Coût

☐ pidité de l'analyse Facilité d'utilisation

☐ Disponibilité des réactifs

☐ Autres (Précisez) : …….

8) Dans quelle mesure êtes-vous satisfaits de la précision des résultats obtenus avec votre méthode actuelle de dosage ?

☐ Insatisfait ☐ Neutre

☐ Satisfait ☐ Très satisfait

9) Quels sont les principaux défis que vous rencontrez dans le dosage de la vitamine D ?

☐ Interférences avec d'autres substances ☐ Cout élevé des réactifs

☐ Instabilité des échantillons ☐ manque de standardisation

☐ ésultats faux positifsRésultats faux négatifs ☐

☐ tre(précisez) : …….

10) Quelles sont les erreurs signalées lors du dosage de la vitamine D ?

Précisez :……
……..

11) A quelle fréquence ?

☐ Chaque fois ☐ Rarement

☐ Des fois

12) Quelles sont les principales sources d'erreurs dans le dosage de la vitamine D ?

☐ température de l'échantillon ☐ thode de prélèvement

☐ La durée de stockage de l'échantillon ☐ Autre

13) Comment gérez-vous les erreurs de dosage ?

☐ Répétition des analyses ☐ Correction manuelle

☐ Automatisation ☐ Autre (précisez)……

IV. Qualité et performance de la méthode

1) Quelle méthode de calibration utilisez-vous ?

☐ Etalonnage interne ☐ Etalonnage externe

☐ Calibration automatique ☐ Autre

2) Quelle est la fréquence de calibration ?

☐ Chaque série ☐ Toutes les semaines

☐ Tous les jours ☐ Selon les besoin

☐ Tous les mois

3) Quel type de contrôle effectuez-vous sur votre méthode de dosage de la vitamine D ?

☐ Contrôle interne (contrôle de qualité)

☐ Contrôle externe (évaluation de performance)

☐ Aucun

4) À quelle fréquence réalisez-vous des contrôles de qualité?

☐ Après toute panne

☐ ensuellement

☐ Annuellement

5) Quels critères utilisez-vous pour évaluer la performance de vos méthodes de dosages ?

☐ Sensibilité ☐ Intervalle de validité

☐ Précision ☐ Linéarité

☐ Tous les précédents

6) Quelle est la fréquence à laquelle vous validez votre méthode de dosage ?

☐ Une fois par an ☐ Deux fois par an

☐ Plus fréquemment ☐ A chaque lot de réactifs

7) Quelle est la limite de détection (LOD) de votre méthode ?

☐ ng/ml ☐ ng/ml

☐ 5-10 ng/ml ☐ >10 ng/ml

V. Résultats et interprétation

1) Unités de mesure:

☐ mL ☐ mol/L

☐ re(Précisez) :…….

2) Quelles sont les intervalles de références biologiquesque vous utilisez pour interpréter les résultats de la 25-hydroxyvitamine D chez les enfants et les adolescents ?

☐ Les valeurs fournies par le KIT de dosage

☐ Les valeurs internes établies sur une cohorte locale

☐ Les recommandations des autorités sanitaires nationales

☐ Autre (précisez) :……..

3) Quel est le délai moyen pour obtenir les résultats de dosage ?

☐ ins de 24 heures48-72 heures ☐

☐ 48 heures Plus de 72 heures ☐

4) Quelle est la concentration limite de 25 OHD définissant une carence en vitamine D ?
…….

5) Quelle est la concentration limite de 25 OHD définissant une insuffisance en vitamine D ?
……

6) Quelle est la concentration limite de 25 OHD définissant une carence en vitamine D chez les enfants et les adolescents ?

……

7) Quelle est la concentration limite de 25 OHD définissant une insuffisance en vitamine D chez les enfants et les adolescents ?

……

VI. Collecte des données

1) Quel est le nombre de dosages de la vitamine D effectués par semaine dans votre laboratoire chez la population générale ?

☐ <10 ☐ 10-20

☐ 20-50 ☐ >50

2) Quel est le nombre de dosages de la vitamine D effectués par semaine dans votre laboratoire chez les enfants et les adolescents (2 à 17 ans) ?

☐ <10 ☐ 10-20

☐ 20-50 ☐ >50

3) Existe-t-il des études antérieurs à la notre sur l'analyse de la vitamine D ?

☐ Oui ☐ Non

4) Vos résultats d'analyse sur la vitamine D sont-ils collectés et analysés ?

☐ Oui ☐ Non

5) Si oui , vous visez le lien entre la concentration de 25 OH D et :

☐ Age ☐ Sexe

☐ Pathologies médicales ☐ Autre

6) Quels sont les outils utilisés pour la collecte des données ?

☐ Logiciels de gestion des données ☐ Un registre

☐ Fiche de collecte de données ☐ Autre

7) À quelle fréquence observez-vous des résultats de carence de la vitamine D chez la population générale ?

☐ Rarement ☐ Occasionnellement

☐ FréquemmentTrès fréquemment ☐

8) À quelle fréquence observez-vous des résultats de carence de la vitamine D chez les enfants et les adolescents ?

☐ Rarement ☐ Occasionnellement

☐ FréquemmentTrès fréquemment ☐

Partie Ⅱ :Evaluation de l'hypovitaminose D chez les enfants et les adolescents.

Cette partie concerne une étude sur la prévalence de l'hypovitaminose D chez les enfants et les adolescents effectuant leurs analyses au sein de votre laboratoire durant l'année 2024.

1) Quelles est l'évolution de la prévalence de l'hypovitaminose D chez la population générale au cours de 2024 ?

☐ Augmentation significative ☐ Diminution légère

☐ Augmentation légère ☐ Diminution significative

☐ Stable

2) Quelles est l'évolution de la prévalence de l'hypovitaminose D chez les enfants et les adolescents au cours de 2024 ?

☐ Augmentation significative ☐ Diminution légère

☐ Augmentation légère ☐ Diminution significative

☐ Stable

3) Quelle est la fréquence de la réception de la demande d'analyse de la vitamine D chez la population générale ?(2024)

☐ 00 ☐ ntre 100 et 500

☐ Entre 500 et 1000 ☐ > 1000

4) Quelle est la fréquence de la réception de la demande d'analyse de la vitamine D chez les enfants et les adolescents (2 à 17 ans) ?(2024)

☐ 00 ☐ re 100 et 500

☐ Entre 500 et 1000 ☐ > 1000

5) Quand les demandes de dosage de la vitamine D augmente-elles généralement ?

☐ tomne(septembre-novembre ☐ Printemps (mars-mai)

☐ Hiver (décembre-février) ☐ Eté (juin-aout)

6) Quelle est la fréquence globale de l'hypovitaminose D chez la population générale ?

☐ 00 ☐ ntre 100 et 500

☐ Entre 500 et 1000 ☐ > 1000

7) Quelle est la fréquence globale de l'hypovitaminose D chez les enfants et les adolescents (2 à 17 ans) ?(2024)

☐ 00 ☐ ntre 100 et 500

☐ Entre 500 et 1000 ☐ > 1000

8) Quel type de carence de vitamine D le plus fréquemment chez les enfants et les adolescents (2-17 ans) ?(2024)

☐ Carence légère ☐ Carence modérée

☐ Carence sévère

9) Quel est le sexe le plus incriminé dans l'hypovitaminose D chez la population générale ?

☐ Féminin ☐ Masculin

10) Quel est le sexe le plus incriminé dans l'hypovitaminose D chez les enfants et les adolescents ?

☐ Féminin ☐ Masculin

11) D'après vous,quelle est l'importance des résultats de dosage de la vitamine D dans les décisions cliniques pour les enfants et les adolescents ?

☐ Peu importante ☐ Importante

☐ dérément importante ☐ s importante

MERCI POUR VOTRE PARTICIPATION !

The request

Université Abou BekrBelkaid Tlemcen

FACULTE DE MEDECINE

DEPARTEMENT DE PHARMACIE

TLEMCEN le : 23 /01/2025.

M^lle AmmourImene

Interne en 6 année pharmacie

E-mail :imeneammour1@gmail.com

N° Tel :0674727157

A monsieur le Directeur du Laboratoire d'Analyse Médicales

«…… »

Objet : Demande d'autorisation pour une enquête.

Monsieur le directeur,

Je vous écris afin de solliciter votre autorisation pour mener une enquête et distribuer un questionnaire au sein de votre laboratoire sur le dosage de la vitamine D.

En effet, cette collecte de donnée s'inscrit dans le cadre de notre mémoire de fin d'études en pharmacie intitulé **''Analytical Methods for Quantification of Vitamin D and implications for diagnosis of vitamin D deficiencyamongchildren and adolescents'',** encadré par **Dr.Ghenimi F** et **Dr. Boubris.** Votre collaboration serait déterminante pour garantir la pertinence et la qualité de cette étude.

Cette démarche vise à recueillir des données essentielles sur :

- Les équipements et les méthodes de dosage de la vitamine D utilisées dans votre laboratoire.
- Les difficultés rencontrées dans la réalisation de ces dosages.
- Collecte des données sur la fréquence de l'hypovitaminose D chez les enfants et les adolescents testés dans votre laboratoire.

Nous tenons à vous informer que notre engagement à respecter l'anonymat du laboratoire et le caractère confidentiel des données recueillies.

En vous remerciant par avance pour l'attention portée à notre demande, nous vousprions d'agréer, Madame/Monsieur, l'expression de nos salutations distinguées.

Université Abou BekrBelkaid Tlemcen

FACULTE DE MEDECINE

DEPARTEMENT DE PHARMACIE

TLEMCEN le : 23 /01/2025.

M^{lle} Belhadj Ilham

Interne en 6 année pharmacie

E-mail : belhadjilham2019@gmail.com

N° Tel :0663499811

A monsieur le Directeur du Laboratoire d'Analyse Médicales

«…… »

Objet : Demande d'autorisation pour une enquête.

Monsieur le directeur,

Je vous écris afin de solliciter votre autorisation pour mener une enquête et distribuer un questionnaire au sein de votre laboratoire sur le dosage de la vitamine D.

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Questionnaire of doctors

FACULTE DE MEDCINE

DEPARTEMENT DE PHARMACIE

Dans le cadre de notre mémoire de fin d'étude en pharmacie intitulé : « Analytical Methods for Quantification of Vitamin D and implications for diagnosis of vitamin D deficiencyamongchildren and adolescents », nous sollicitons votre collaboration dans cette étude qui vise à recueillir des informations sur l'hypovitaminose D chez les enfants et les adolescents.

Votre expertise précieuse serait déterminante pour garantir la pertinence et la qualité de cette étude.

- ✓ Ce questionnaire est à remplir de façon anonyme.
- ✓ Mettez (ou cochez) la(les) réponse(s) appropriée(s) .

1. Informations générales :

1.1. Spécialité :

☐ Pédiatrie ☐ Endocrinologie

☐ decine généraliste ☐ Autre(précisez) :……

1.2. Exercez-vous dans :

☐ Hôpital ☐ Cabinet libérale

☐ Clinique privée ☐ Autre (précisez) : ……

2. Hypovitaminose D chez l'enfant et l'adolescent :

2.1. D'après vous, quelles sont les manifestations cliniques les plus fréquentes de l'hypovitaminose D chez l'enfant et l'adolescent ?

☐ atigue ☐ Les douleurs osseuses

☐ troubles de la croissance ☐ Autre (précisez) : ……

2.2.D'après vous , quels signes physiques pouvantelle associés à l'hypovitaminose D chez les enfants et les adolescents ?

☐ Rachitisme ☐ Ostéomalacie

☐ Déformation osseuse ☐ Autre (précisez) :…….

2.3. D'après vous les principaux facteurs de risque de l'hypovitaminose D chez les enfants et les adolescents sont :

☐ manque d'exposition au soleil ☐ peau foncée

☐ L'obésité ☐ Alimentation déséquilibrée

☐ utre (précisez) :……..

2.4.D'aprèsvous ,un dosage sanguine de la vitamine D est-il suffisant pour diagnostiquer l'hypovitaminose D ?

☐ Oui ☐ Non

Si non , quels sont les autres examens complémentaire ?

☐ La biopsie osseuseLa radiographie des os ☐

☐ Autre (précisez) :……..

2.5.Dans quels cas prescrivez-vous systématiquement un dosage de la 25-hydroxyvitamine D chez les enfants et les adolescents ?

☐ Retard de croissance ☐ Fatigue chronique

☐ Maladies chroniques ☐ Alimentation déséquilibrée

☐ Exposition solaire insuffisante ☐ Obésité

☐ Antécédents familiaux de maladies osseuses

☐ Autre(précisez) : ……

2.6.Quel est le nombre moyen des patients enfants et adolescents (2 à 17 ans) à risque vus par semaine ?

☐ <5 ☐ 5-10

☐ 10-20 ☐ >20

2.7.Quel est la fréquence de prescription de dosage de la vitamine D chezlesenfantset adolescents (2 à 17 ans) par semaine ?

☐ <5 ☐ 5-10

☐ 10-20 ☐ >20

2.8.Quel est la fréquence de prescription de dosage de la vitamine D chez la population générale par semaine ?

☐ <5 ☐ 5-10

☐ 10-20 ☐ >20

2.9.D'aprèsvous , quelle est votre perception de l'importance du dosage de la vitamine D chez les enfants et les adolescents ?

☐ Très important ☐ Assez important

☐ Peu important

2.10. A quelle fréquence recommandez-vous de vérifier les niveaux de la vitamine D chez les enfants et les adolescents à risque ?

☐ Lorsqu'un enfant ou adolescents présente des symptômes

☐ Chaque 6 mois

☐ Chaque année

☐ Autre (précisez) :…….

2.11. Quelle est le sexe le plus incriminé dans l'hypovitaminose D chez la population générale ?

☐ Féminin ☐ Masculin

2.12. Quelle est le sexe le plus incriminé dans l'hypovitaminose D chez les enfants et les adolescents ?

☐ Féminin ☐ Masculin

2.13. Quelle saison est associée à un risque plus élevé d'hypovitaminose D ?

☐ printemps ☐ L'été

☐ utomne ☐ L'hiver

2.14.D'après vos constatations ,quelle est l'évolution de la prévalence de l'hypovitaminose D chez les enfants et les adolescents au cours des dernières décennies ?

☐ Elle a diminuéElle a augmenté ☐

☐ Elle est restée stable

3. <u>Interprétation et traitement :</u>

3.1.D'après vous, quelle est la concentration limite de 25 OHD définissant une hypovitaminose D ?

☐ Inferieur à 30ng/mlValeur limite déclarer ☐e laboratoire

3.2.Quelle est la concentration limite de 25 OHD définissant une carence en vitamine D ?

☐ Moins de 20 ng/MlValeur limite déclarer p☐ laboratoire

3.3.Quelle est la concentration limite de 25 OHD définissant une insuffisance en vitamine D ?

☐ Entre 20 et 29 ng/mLValeur limite dé☐er par le laboratoire

3.4.Quelle est la concentration limite de 25 OHD définissant une carence en vitamine D chez les enfants et les adolescents ?

☐ Moins de 20 ng/Ml Valeur limite☐larer par le laboratoire

3.5.Quelle est la concentration limite de 25 OHD définissant une insuffisance en vitamine D chez les enfants et les adolescents ?

☐e 20 et 29 ng/mL Valeur limite déclar☐ar le laboratoire

3.6.Vous déclarer une hypovitaminose D à partir :

☐ De résultat de dosage de vit D ☐ Examen clinique de patient

☐ Autres (précisez) :……

3.9.Quel traitement médicamenteux peut interférer avec l'absorption ou le métabolisme de la vitamine D ?

☐anti-inflammatoires non stéroïdiensLes corticoïdes ☐

☐certains médicaments antiépileptiques ☐Autre (précisez) :……

3.10.À quelle fréquence doit-on contrôler le taux de 25-hydroxyvitamine D chez un enfant et adolescent sous supplémentation ?

☐ Tous les 3 mois ☐ Tous les 6 mois

☐ Annuellement ☐ Autre (précisez) :……..

3.11. Quels sont les principaux défis que vous rencontrez dans le suivi des enfants et des adolescents présentant une carence en vitamine D ?

☐ Adhérence aux traitements ☐ Manque de données à long terme

☐ Difficulté à évaluer l'efficacité du traitement

☐ Autre (précisez) :……

3.12. Considérez-vous que les parents sont suffisamment informés sur l'importance de la vitamine D pour leurs enfants et adolescents ?

☐ i ☐ Non

MERCI POUR VOTRE PARTICIPATION !

Abstract

Accurate measurement of vitamin D is essential for diagnosing and managing vitamin D deficiency in both children, adolescents, and the general population. This study evaluates analytical methods used by medical laboratories and estimates hypovitaminosis D prevalence in these groups. Two surveys were conducted: one with 16 laboratories assessing assay methods, quality controls, and hypovitaminosis frequency, the other with 30 doctors providing clinical insight. Results show varied practices, mostly using immunoassays, with differences in quality control. Hypovitaminosis D was frequent in both populations. The study emphasizes the need to harmonize assay methods and improve collaboration between laboratories and clinicians for better diagnosis and optimal care.

Keywords : Vitamin D, hypovitaminosis, children and adolescents, Analytical method.

Résumé : La mesure précise de la vitamine D est essentielle pour diagnostiquer et gérer les carences en vitamine D chez les enfants, les adolescents et la population générale. Cette étude évalue les méthodes analytiques utilisées par les laboratoires médicaux et estime la prévalence de l'hypovitaminose D dans ces groupes. Deux enquêtes ont été menées : l'une auprès de 16 laboratoires pour évaluer les méthodes d'analyse, les contrôles de qualité et la fréquence de l'hypovitaminose, l'autre auprès de 30 médecins pour obtenir des informations cliniques. Les résultats montrent des pratiques variées, utilisant principalement des dosages immunologiques, avec des différences dans le contrôle de la qualité. L'hypovitaminose D était fréquente dans les deux populations. L'étude souligne la nécessité d'harmoniser les méthodes de dosage et d'améliorer la collaboration entre les laboratoires et les cliniciens pour un meilleur diagnostic et des soins optimaux.

Mots-clés : Vitamine D, hypovitaminose, enfants et adolescents, Méthode analytique.

ملخص

القياس الدقيق لفيتامين (د) ضروري لتشخيص نقص فيتامين (د) ومعالجته لدى كل من الأطفال والمراهقين وعامة السكان. تقيّم هذه الدراسة الطرق التحليلية التي تستخدمها المختبرات الطبية وتقدر معدل انتشار نقص فيتامين د في هذه المجموعات. أُجري استبيانان: أحدهما مع 16 مختبرًا لتقييم طرق الفحص وضوابط الجودة وتواتر نقص الفيتامين د، والآخر مع 30 طبيبًا يقدمون رؤية سريرية. أظهرت النتائج ممارسات متنوعة، معظمها باستخدام المقايسات المناعية، مع وجود اختلافات في مراقبة الجودة. كان نقص فيتامين د متكررًا في كلتا المجموعتين. تؤكد الدراسة على الحاجة إلى مواءمة طرق الفحص وتحسين التعاون بين المختبرات والأطباء من أجل تشخيص أفضل ورعاية مثلى .

الكلمات المفتاحية: فيتامين د، نقص الفيتامين، الأطفال والمراهقين، طريقة التحليل.