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Title

The impact of Vitamin D deficiency on Anxiety and Depression in region of Saïda and Sidi Bel Abbes

Presented by :

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The university is not only the place where students must enrich their knowledge and perfect it, nor that of teachers to manage their careers, but it is the universe which allows to irradiate and diffuse a light spectrum worthy of charisma and the motivation of each person. Dr Nasr-eddine KEBIR

Dedication

The sublime beauty lies in presenting the fruits of our dedication and labor, emanating from the profound depths of our hearts, to express gratitude and appreciation to those we cherish and acknowledge during our existence. It is with humility that I offer this humble endeavor :

First and foremost, I thank Allah (SWT) for guiding me and putting me in the right path to accomplish this thesis through.

In the luminance of my days, I dedicate this endeavor to the individual who nurtured me with affection and optimism, to the wellspring of irreplaceable love, to you, my cherished mother, and no expression will suffice to convey my deepest gratitude. Thank you, dear mother, for all that you have bestowed upon me, your boundless love, unwavering support, indomitable strength, and enduring encouragement throughout my educational journey.

To the pillar of my life, my perpetual exemplar, my moral compass, and the wellspring of my elation and contentment, who selflessly strived to witness my success. For providing me with unyielding emotional and material support to this day, for your love, to you my dearest father.

May the Divine protect you, granting you vitality, eloquence, longevity, and perennial felicity.

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To all my dear ones who have accompanied me on this journey.

IBTISSEM.

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In conclusion, I extend my appreciation to the academic staff, and all my professors that helped and participated in my academic journey.

Abbreviations

ADHD : attention deficit hyperactivity disorder

MHQ : mental health quotient

GAD : generalised anxiety disorder

PTSD : post-traumatic stress disorder

CBT : Cognitive Behavioral Therapy

ACT : acceptance and commitment therapy

PDD : Persistent depressive disorder

DMDD : Disruptive mood dysregulation disorder

PMDD : Premenstrual dysphoric disorder

PMS : premenstrual syndrome

ECT : electroconvulsive therapy

TMS : transcranial magnetic stimulation

VNS : vagus nerve stimulation

D2 : ergocalciferol

D3 : cholecalciferol

PTH : parathyroid hormone

VDR : vitamin D receptor

DBP: vitamin D-binding globulin

VDBP : vitamin d binding protein

NADPH : nicotinamide adenine dinucleotide phosphate hydrogen

FGF : fibroblast growth factor

VDRE : vitamin D response element

TRPV5/6 : transient receptor potential vanilloid 5/6 (calcium channel) plays a major role in the maintenance of ca2+ in blood

NPFRR2 : neuropeptide FF receptor2, associated with human diseases

UVB : ultraviolet B

BMI : body mass index

VDD : vitamin D deficiency

RDA : recommended dietary allowance

BMD : bone mineral density

DVD : developmental vitamin D

RXR : retinoid X receptor

DA : dopamine antagonist

LRP : low density lipoprotein receptor-related protein

GDNF : glial derived neurotrophic factor

NURR1 : nuclear receptor ;essential for the induction of dopaminergic phenotype

TPH1 : enzyme tryptophan hydroxylase 1

TPH2 : enzyme tryptophan hydroxylase 2

Ang2 : angioprotein ; endothelial growth factor that ligands to tyrosine kinase receptor

GM : gut microbiota

MDD : major depressive disorder

SCFAs : short-chain fatty acids

LPS : lipopolysaccharide

BDNF : brain-derived neurotrophic factor

TMAO : trimethylamine N-oxide ;dietary component that belong to the class of amine oxides

CUMS : chronic unpredictable mild stress

MWM : Morris Water Maze

NRC: nuclear hormone receptor coregulatormodulator

VGLUT2 : glutamate transporter 2

GRIK3 : kainate receptor 3

CREB : camp responsive element binding protein 3

NR4A2 : nuclear receptor subfamily4 groupA member2;downstream mediator of TNF-α

CaMKIIδ : ca2+/calmodulin-dependant protein kinase II delta

CYP : genes which encode enzymes involved in vitamin D metabolism

BBB : blood brain barrier

GABA : γ-aminobutyric acid

p57kip2 : cyclin dependent kinase inhibitor

VMAT2 : vesicular monoamine transporter 2

DAT : dopamine antagonist transporter

SRC : steroid receptor coactivator

DRIP : vitamin D receptor interacting protein

NLS : nuclear localization signal

HAT : histone acetyl transferase

IP3 : inositol triphosphate

PIP2 : phosphoinositol bisphosphate

GDP : guanosine diphosphate

GTP : guanosine triphosphate

RANKL : receptor activator of NF-κB ligand

CRH : corticoliberin-releasing hormone

HPA : hypothalamic-pituitary-adrenal

ACTH : adrenocorticotropic hormone

CRF : corticotropin releasing factor

CNS : central nervous system

MS : multiple sclerosis

MGP : matrix Gla protein

OC : osteocalcin

Tables list

Figures list

Abstract

Depression and anxiety are psychiatric and mood disorders that affect health and therefore quality of life and increase the global burden of disease. Vitamin D, a fat-soluble vitamin, is well-known for its role in bone health, is involved in several brain processes, including neuroimmune regulation, neurotrophic factor regulation, neuroprotection, neuroplasticity, and brain development and recently emerged it's impact on mental health where the brain regions involved in the pathophysiology of depression and anxiety and vitamin D metabolism intersect. Vitamin D deficiency is widespread worldwide impacts brain structure and function and it has been linked to an increased risk of depression and anxiety. One of the possible mechanisms in the pathophysiology of these psychiatric disorders has been reported as oxidative stress and inflammation. In light of this information, our goal is to determine the importance of vitamin D on psychiatric disorders such as depression and anxiety.

We conducted two evaluations of vitamin D levels. The initial assessment involved 525 individuals from the general population to gauge their vitamin D status. Among them, 412 individuals, constituting 78% of the total, had insufficient vitamin D levels (<30 ng/mL), with 86% being women and 14% men. Acceptable vitamin D levels (30 to 50 ng/mL) were observed in 83 individuals, comprising 15% of the total population, with 87% being women and 13% men. Approximately twenty individuals, accounting for 3% of the total, had optimal vitamin D levels (50-70 ng/mL), with 95% being women and 5% men. Therapeutic levels (≥70 ng/mL) were found in 8 individuals, representing 1.5% of the total, with 62% being women and 37% men. The prevalence of vitamin D levels exceeding 100 ng/mL was extremely low, with only 2 individuals, or 0.3% of the total, and they were exclusively female.

The second evaluation on a population of 100 subjects with psychiatric disorders which included three groups of different ages and sexes: 1 to 15 years, 15 to 50 years, and 50 years and above. Among those aged 1 to 15 years, 1% were girls, and in the 15 to 50 years group, 52% were women and 29% men, while in the 50 years and above group, 14% were women and 3% men. Within these groups, individuals suffered from various mental disorders such as schizophrenia, Alzheimer's, bipolar disorder, anxiety, depression, OCD (obsessive-compulsive disorder), GAD (generalized anxiety disorder), and social phobia. Among them, 80 individuals had vitamin D deficiency. The prevalence of vitamin D insufficiency (<30 ng/mL) associated with mental disorders primarily affected women, with 66% of women and 33% of men suffering from anxiety and depression experiencing this deficiency. Of the 100 subjects evaluated, almost the majority (80%) suffered from vitamin D deficiency.

Clearly spending time in the sunshine and/ or exercising outdoors, eating food that is rich of vitamin D, taking dietary supplements to improve vitamin D deficiency may improve mental well-being in patients with depression. Vitamin D screening should be performed in the prevention and treatment planning of these mood disorders.

Keywords: vitamin D, depression, anxiety, immune system, inflammation, neuroinflammation psychiatry.

خلاصة

يعد الاكتئاب والقلق من الاضطرابات النفسية والمزاجية التي تؤثر على الصحة وبالتالي نوعية الحياة وتزيد العبء العالمي للمرض. فيتامين د، وهو فيتامين قابل للذوبان في الدهون، معروف بدوره في صحة العظام، ويشارك في العديد من عمليات الدماغ، بما في ذلك تنظيم المناعة العصبية، وتنظيم عامل التغذية العصبية، والحماية العصبية، والمرونة العصبية، ونمو الدماغ، وظهر مؤخرًا تأثيره على الصحة العقلية. حيث تتقاطع مناطق الدماغ المشاركة في الفسيولوجيا المرضية للاكتئاب والقلق واستقلاب فيتامين د. يؤثر نقص فيتامين د على نطاق واسع في جميع أنحاء العالم على بنية الدماغ ووظيفته، وقد تم ربطه بزيادة خطر الإصابة بالاكتئاب والقلق. تم الإبلاغ عن إحدى الآليات المحتملة في الفيزيولوجيا المرضية لهذه الاضطرابات النفسية على أنها الإجهاد التأكسدي والالتهاب. وفي ضوء هذه المعلومات فإن هدفنا هو تحديد أهمية فيتامين د على الاضطرابات النفسية مثل الاكتئاب والقلق.

أجرينا تقييمين لمستويات فيتامين د. شمل التقييم الأولي 525 فردًا من عامة السكان لقياس حالة فيتامين د لديهم. من بينهم، كان 412 فردًا، يشكلون 78% من المجموع، لديهم مستويات غير كافية من فيتامين د (أقل من 30 نانوجرام/مل)، وكان 86% منهم من النساء و14% من الرجال. وقد لوحظت مستويات مقبولة من فيتامين د (30 إلى 50 نانوغرام/مل) في 83 فردًا، يشكلون 15% من إجمالي السكان، 87% منهم نساء و13% رجال. ما يقرب من عشرين شخصًا، وهو ما يمثل 3٪ من المجموع، لديهم مستويات مثالية من فيتامين د (50-70 نانوغرام / مل)، مع 95٪ من النساء و 5٪ من الرجال. تم العثور على المستويات العلاجية (≤70 نانوغرام/مل) في 8 أفراد، وهو ما يمثل 1.5% من المجموع، مع 62% من النساء و37% من الرجال. كان معدل انتشار مستويات فيتامين د التي تتجاوز 100 نانوغرام/مل منخفضًا للغاية، مع وجود فردين فقط، أو 0.3% من المجموع، وكانوا من الإناث حصريًا.

تم إجراء التقييم الثاني على مجموعة مكونة من 100 شخص يعانون من اضطرابات نفسية، وشمل ثلاث مجموعات من مختلف الأعمار والجنسين: من سنة إلى 15 سنة، ومن 15 إلى 50 سنة، و50 سنة فما فوق. ومن بين الذين تتراوح أعمارهم بين 1 إلى 15 سنة، كانت نسبة الفتيات 1%، وفي المجموعة من 15 إلى 50 سنة، 52% نساء و29% رجال، بينما في المجموعة 50 سنة فما فوق، 14% نساء و3% رجال. ضمن هذه المجموعات، عانى الأفراد من اضطرابات عقلية مختلفة مثل الفصام، ومرض الزهايمر، والاضطراب ثنائي القطب، والقلق، والاكتئاب، والوسواس القهري ، والرهاب الاجتماعي. وكان من بينهم 80 فرداً يعانون (GAD)(اضطراب الوسواس القهري)، واضطراب القلق العام من نقص فيتامين د. يؤثر انتشار نقص فيتامين د (<30 نانوغرام/مل) المرتبط بالاضطرابات العقلية في المقام الأول على النساء، حيث يعاني 66% من النساء و33% من الرجال من القلق والاكتئاب الذين يعانون من هذا النقص. من بين 100 شخص تم تقييمهم، عانت الأغلبية تقريبًا (80٪) من نقص فيتامين د.

من الواضح أن قضاء الوقت تحت أشعة الشمس و/أو ممارسة الرياضة في الهواء الطلق، وتناول الأطعمة الغنية بفيتامين د، وتناول المكملات الغذائية لتحسين نقص فيتامين د قد يحسن الصحة العقلية لدى المرضى الذين يعانون من الاكتئاب. يجب إجراء فحص فيتامين د في الوقاية والتخطيط العلاجي لهذه الاضطرابات المزاجية.

الكلمات المفتاحية: فيتامين د، الاكتئاب، القلق، جهاز المناعة، الالتهاب، التهاب الأعصاب، الطب النفسي

Résumé

La dépression et l'anxiété sont des troubles psychiatriques et de l'humeur qui affectent la santé et donc la qualité de vie et augmentent la charge mondiale de morbidité. La vitamine D, une vitamine liposoluble, est bien connue pour son rôle dans la santé des os, est impliquée dans plusieurs processus cérébraux, notamment la régulation neuro-immune, la régulation des facteurs neurotrophiques, la neuroprotection, la neuroplasticité et le développement cérébral, et son impact sur la santé mentale a récemment été révélé. où se croisent les régions cérébrales impliquées dans la physiopathologie de la dépression et de l'anxiété et le métabolisme de la vitamine D. La carence en vitamine D est répandue dans le monde entier et a un impact sur la structure et le fonctionnement du cerveau et a été associée à un risque accru de dépression et d'anxiété. L'un des mécanismes possibles dans la physiopathologie de ces troubles psychiatriques a été signalé comme étant le stress oxydatif et l'inflammation. À la lumière de ces informations, notre objectif est de déterminer l'importance de la vitamine D sur les troubles psychiatriques tels que la dépression et l'anxiété. Nous avons effectué deux évaluations des niveaux de vitamine D. L'évaluation initiale a porté sur 525 individus de la population générale pour évaluer leur statut en vitamine D. Parmi eux, 412 personnes, soit 78 % du total, présentaient des taux de vitamine D insuffisants (<30 ng/mL), dont 86 % étaient des femmes et 14 % des hommes. Des niveaux acceptables de vitamine D (30 à 50 ng/mL) ont été observés chez 83 individus, représentant 15 % de la population totale, dont 87 % étaient des femmes et 13 % des hommes. Environ vingt individus, représentant 3 % du total, présentaient des niveaux optimaux de vitamine D (50 à 70 ng/mL), dont 95 % étaient des femmes et 5 % des hommes. Des niveaux thérapeutiques (≥70 ng/mL) ont été trouvés chez 8 individus, représentant 1,5 % du total, dont 62 % étaient des femmes et 37 % des hommes. La prévalence des taux de vitamine D supérieurs à 100 ng/mL était extrêmement faible, avec seulement 2 individus, soit 0,3 % du total, et ils étaient exclusivement des femmes. La deuxième évaluation a porté sur une population de 100 sujets souffrant de troubles psychiatriques qui comprenait trois groupes d'âges et de sexes différents : 1 à 15 ans, 15 à 50 ans et 50 ans et plus. Parmi les personnes âgées de 1 à 15 ans, 1 % étaient des filles, et dans le groupe des 15 à 50 ans, 52 % étaient des femmes et 29 % des hommes, tandis que dans le groupe des 50 ans et plus, 14 % étaient des femmes et 3 % des hommes. Au sein de ces groupes, les individus souffraient de divers troubles mentaux tels que la schizophrénie, la maladie d'Alzheimer, le trouble bipolaire, l'anxiété, la dépression, le TOC (trouble obsessionnel-compulsif), le TAG (trouble d'anxiété généralisée) et la phobie sociale. La prévalence de l'insuffisance en vitamine D (<30 ng/mL) associée aux troubles mentaux touchait principalement les femmes, avec 66 % des femmes et 33 % des hommes souffrant d'anxiété et de dépression connaissant cette carence. Sur les 100 sujets évalués, la quasi-majorité (80 %) souffrait d'une carence en vitamine D. Il est clair que passer du temps au soleil et/ou faire de l'exercice à l'extérieur, manger des aliments riches en vitamine D, prendre des compléments alimentaires pour améliorer la carence en vitamine D peuvent améliorer le bien-être mental des patients souffrant de dépression. Un dépistage de la vitamine D doit être effectué dans le cadre de la prévention et de la planification du traitement de ces troubles de l'humeur.

Mots clés : vitamine D, dépression, anxiété, système immunitaire, inflammation, psychiatrie de neuroinflammation.

Summary

Chapter I : Mental health

1. Introduction

Since the creation of human history, the environment and its lightfull elements have been associated with happiness and positive feelings, On the other hand, dark, urban environments, covered by heavily polluted skies are often associated with misery and fear, Nevertheless, since rickets had been a widespread, observably crippling disorder, its disappearance as a public health problem became a stunning accomplishment of modern medicine, inspiring increased sun exposure in the first half of the 20th century, in parallel ricket disease highlighted on an important element that can effect the human wellbeing and its major role in maintaining a healthy life. (Mats B. Humble.2010)

Vitamin D insufficiency affects almost the total half of the population worldwide. An estimated 1 billion people worldwide, across all ethnicities and age groups, have a vitamin D deficiency (VDD). Vitamin D deficiency or hypovitaminosis D can mainly be attributed to lifestyle (for example, reduced outdoor activities) and environmental (for example, air pollution) factors that reduce exposure to sunlight, which is required for ultraviolet-B (UVB) induced vitamin D production in the lower layers of the epidermis. (Nair R, Maseeh A.2012)

Studies demonstrated that, vitamin D inadequacy is a average health question in two together developed and underdeveloped countries. It is postulated that one billion people worldwide have vitamin D deficiency or insufficiency due to reduced sun exposure or inadequate intake for various reasons. Its status is very various with various nations, which is on account of the distinctness in many factors containing light part of every 24 hours exposure. (Ali SM, Salih LMA, Saeed E.2019)

The high prevalence of vitamin D insufficiency is a particularly important public health issue because hypovitaminosis D is an independent risk factor for total mortality in the general population, leads to both medical and psychosocial questions. It has rearose as an epidemic that influences the youth populations, Emerging research supports the likely duty of vitamin D against tumor, myocardial infarction, fractures and falls, autoimmune diseases, disease that is widespread, type-2 diabetes and mental illnesses (Nair R, Maseeh A.2012)

Vitamin D is involved in numerous brain processes, regulation of neurotrophic factors, neuroprotection, neuroplasticity and brain development. it is important not only for skeletal health but for proper brain development and psychological functioning. Low vitamin D levels are linked to mental health issues such as depression, schizophrenia, and seasonal affective disorder in adults. However, there are disagreements about the connection between vitamin D deficiency and psychological issues. (Mohammad Reza T and al.2014)

2. Mental health

Mental health is understood to be a state of mental well-being that enables individuals to manage life's stresses, recognize their talents, learn effectively, work effectively, and contribute to their community, Mental health is more than the absence of mental disorders, It exists on a complex continuum, which is experienced differently from one person to the next, with varying degrees of difficulty and distress and potentially very different social and clinical outcomes. (World Health Organisation [WHO], 2018).
Mental health conditions cover mental disorders and psychosocial disabilities, as well as other mental states that are linked to significant distress, impairment in functioning, or risk of self-harm. Although this is often the case, it is not always or necessarily so. (Galderisi S, Heinz A and al.,2015).
mental health include subjective well-being, perceived self-efficacy, autonomy, competence, intergenerational dependence and recognition of the ability to realize one's intellectual and emotional potential. (Srivastava K.2011)

2. 1).Mental health around the world

Global mental health refers to mental health needs of all countries, focusing on communities at greatest risk for mental health disparities , it consists on four foundational pillars ; First, mental health is a global public good. Second, mental health problems exist along a continuum. Third, the mental health of an individual is a unique product of one's social and environmental influences along with their genetic and biological predisposition. Fourth, mental health is a fundamental human right and requires a rights-based approach.

Most illnesses,mental and physical are influenced by a combination of biological,psychological and social factors. (Moitra M, Owens S, Hailemariam M and al.,2023)

Figure (01) the interaction of biological, psychological and social factors in the development of mental disorders (Kitanovska and al.,2023)

In 2019, more than 970 million people globally were living with a mental disorder, with anxiety and depression the most common. (Dhungana RR, Pandey AR, Joshi S and al.,2019)

Globally, mental disorders account for 1 in 6 years lived with disability, People with severe mental health conditions die 10 to 20 years earlier than the general population. And having a mental health condition increases the risk of suicide and experiencing human rights violations.

Hundreds of millions suffer from them yearly, and many more do over their lifetimes. It's estimated that 1 in 5 women and 1 in 8 men will experience major depression in their lives. Other conditions, such as schizophrenia and bipolar disorder, are less common but still have a large impact on people's lives.(World Health Organisation [WHO], 2022).

According to recent estimates, more than 20% of adolescents (1 in 7;from 10 to 18 years old) globally have a mental disorder, with common mental disorders such as anxiety and depressive , the rest of ages variates between adults from 18 to 50 years old and above. (Wu Y, Wang L, Tao M and al.,2019)

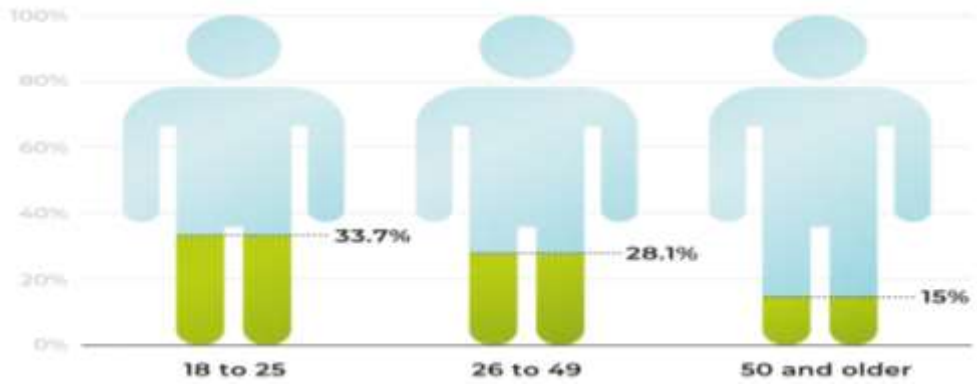

Figure (02) prevalence of mental illness in adults by age group (Mulvik and al., 2021)

Mental disorder prevalence continues to show consistent variation by gender with depression and anxiety being more common among females and attention deficit hyperactivity disorder (ADHD) and conduct disorder being more common among males . The prevalence of substance use disorders also continues to vary by gender with the prevalence in males being twice as high as that of females. (Solberg BS and al.,2018)

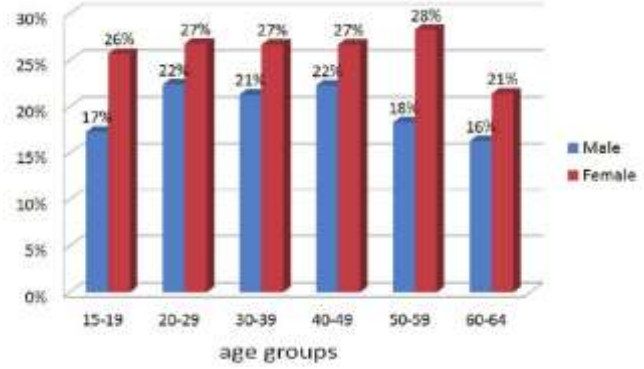

Figure (03) Twelve month prevalence of any psychiatric disorder by sex and age groups (Sharifi,Vandad and al.,2015)

The prevalent cases of mental disorders around the world in 2022. Anxiety and depressive disorders were the most prevalent, followed by other mental disorders

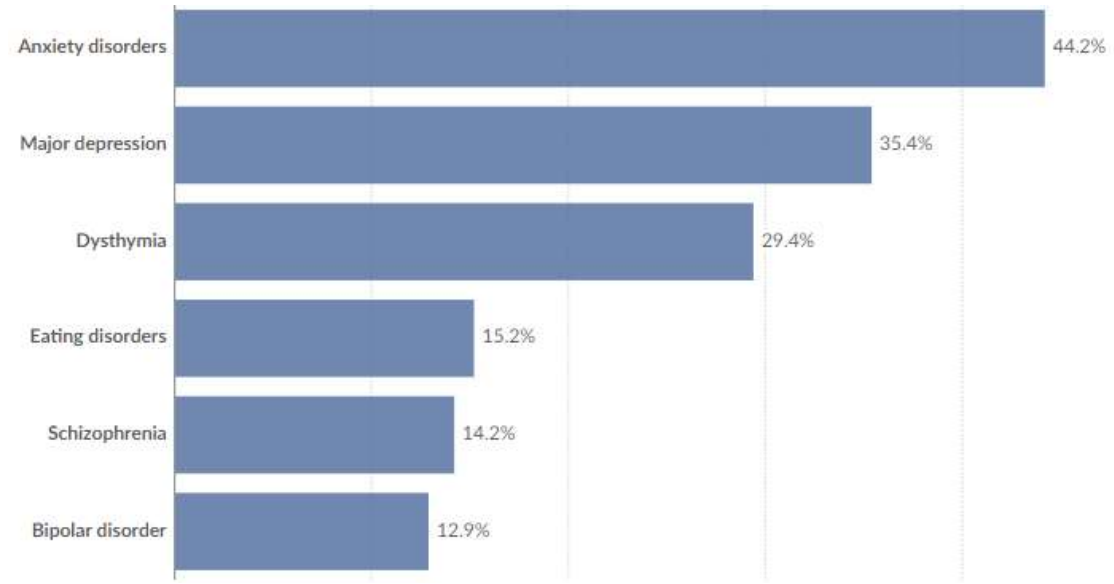

Figure (04) burden disease from each mental illness (Baxter and al.,2013)

Each disorder is a consequence of many factors that are biological, psychological and social, it variates also from a region to another, sex, age and the maintained life style.

MHQ (mental health quotient), a questionnaire that was developed as a standardized instrument to evaluate our mental wellbeing, by using the psychometric principles for psychological assessments , the result goes from -100 to 200 to see in which spectrum of mental wellbeing we are dealing. (Newson JJ, Thiagarajan TC,2020)

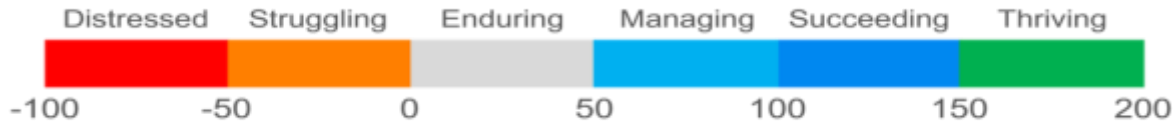

Figure (05) the MHQ score range (sapienlabs.org.,2016)

The questionnaire consists of 37 items that can be grouped into six main categories:

1. cognition (ability to perform basic cognitive functions, make sense of complex sets of events and situations and display a longer-term perspective in your thoughts and behavior)
2. Adaptability & Resilience (ability to shift your behaviour and outlook in response to changing circumstances and cope with the challenges and setbacks that you encounter)
3. Social Self (How you interact with, relate to and see yourself with respect to others)
4. Mood and Outlook (ability to manage and regulate your emotions effectively and to have a constructive or optimistic outlook for the future)
5. Drive and Motivation(Your ability to work towards achieving your desired goals and to initiate, persevere and complete activities in your daily life)
6. Mind-Body Connection (The regulation of the balance between your mind and body) (sapienlabs.org.,2016)

Figure (06) Scores across 6 dimensions of mental health (sapienlabs.org.,2016)

The MHQ will automatically provide the individual with a composite mental wellbeing score when all questions are answered .

The mental state of the world in 2022 relative to previous years

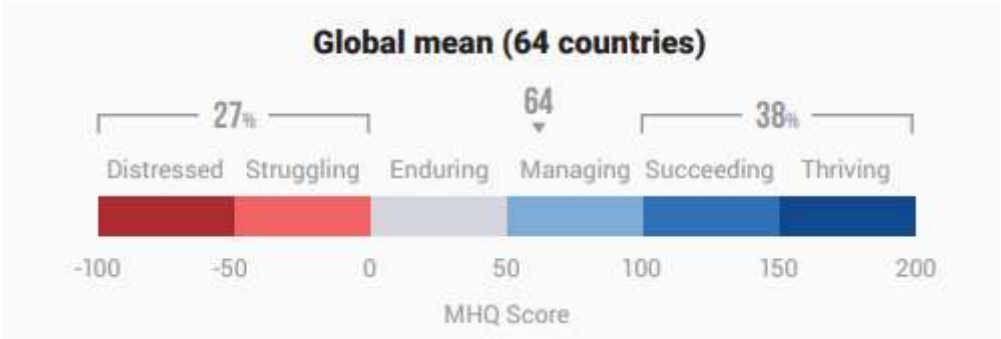

Figure (07) the average mental health quotient score measured in 2022 (sapienlabs.org.,2016)

The average MHQ score across the 64 countries measured in 2022 was 64 on the 300-point MHQ scale as shown above. Across the spectrum of mental wellbeing 27% of respondents were Distressed or Struggling (MHQ scores of below 0, typically indicating 5 or more clinical symptoms), while 38% were Succeeding or Thriving (MHQ scores above 100). (Newson, Jennifer and al.,2020)

3. Anxiety

Anxiety is a feeling of unease, such as worry or fear, that can be mild or severe, Anxiety disorders are characterised by excessive fear and worry and related behavioural disturbances.

Symptoms are severe enough to result in significant distress or significant impairment in functioning; There are several different kinds of anxiety disorders, such as:

1. generalised anxiety disorder GAD (characterized by excessive worry)
2. panic disorder (characterized by panic attacks),
3. social anxiety disorder (characterized by excessive fear and worry in social situations)
4. separation anxiety disorder (characterized by excessive fear or anxiety about separation from those individuals to whom the person has a deep emotional bond), and others
5. phobias, such as agoraphobia or claustrophobia
6. post-traumatic stress disorder (PTSD)
7. Effective psychological treatment exists, and depending on the age and severity, medication may be considered. (Bandelow B, Michaelis S.2015)

3)1.The signs and symptoms of anxiety

3).1.1.Generalized anxiety disorder

Generalized anxiety disorder (GAD) usually involves a persistent feeling of anxiety or dread, which can interfere with daily life. It is not the same as occasionally worrying about things or experiencing anxiety due to stressful life events. People living with GAD experience frequent anxiety for months, if not years. (National Institutes of Health [NIMH], 2022).

Symptoms of GAD include:

1. Feeling restless, wound-up, or on-edge
2. Being easily fatigued
3. Having difficulty concentrating
4. Being irritable
5. Having headaches, muscle aches, stomachaches, or unexplained pains
6. Difficulty controlling feelings of worry
7. Having sleep problems, such as difficulty falling or staying asleep

3).1.2.Panic disorder

People with panic disorder have frequent and unexpected panic attacks. that are sudden periods of intense fear, discomfort, or sense of losing control even when there is no clear trigger, During a panic attack, a person may experience:

1. Pounding or racing heart
2. Sweating
3. Trembling or tingling
4. Chest pain
5. Feelings of impending doom
6. Feelings of being out of control (National Institutes of Health [NIMH], 2022).

People with panic disorder often worry about when the next attack will happen and actively try to prevent future attacks by avoiding places, situations, or behaviors they associate with panic attacks. (Taylor, C. B.2006)

3).1.3.Social anxiety disorder

Social anxiety disorder is an intense, persistent fear of being watched and judged by others. For people with social anxiety disorder, the fear of social situations may feel so intense that it seems beyond their control. For some people, this fear may interrupt them from doing their basic daily activities. (National Institutes of Health [NIMH], 2022).

People with social anxiety disorder may experience:

1. Blushing, sweating, or trembling
2. Pounding or racing heart
3. Stomachaches
4. Rigid body posture or speaking with an overly soft voice
5. Difficulty making eye contact or being around people they don't know
6. Feelings of self-consciousness or fear that people will judge them negatively. (Minesh Khatri, MD.2021)

3).1.4.Phobia-related disorders

A phobia is an intense fear to specific objects or situations. Although it can be realistic to be anxious in some circumstances, the fear people with phobias feel is out of proportion to the actual danger caused by the situation or object.

People with a phobia may :

1. Have an irrational or excessive worry about encountering the feared object or situation
2. Take active steps to avoid the feared object or situation
3. Experience immediate intense anxiety upon encountering the feared object or situation
4. Endure unavoidable objects and situations with intense anxiety. (O'Connor K, Audet JS.2019)

There are several types of phobias and phobia-related disorders:

Specific Phobias (sometimes called simple phobias): As the name suggests, people who have a specific phobia have an intense fear of, or feel intense anxiety about specific types of objects or situations. Some examples of specific phobias include the fear of :

1. Flying
2. Heights
3. Specific animals, such as spiders, dogs, or snakes
4. Receiving injections
5. Blood

3).1.5.Social anxiety disorder (previously called social phobia): People with social anxiety disorder have a general intense fear toward social or performance situations. They worry that actions or behaviors associated with their anxiety will be negatively evaluated by others, leading them to feel embarrassed. This worry lead people with social anxiety to avoid social situations. Social anxiety disorder can manifest in a range of situations, such as within the workplace or the school environment. (Rector et al., 2006)

3).1.6.Agoraphobia: People with agoraphobia have an intense fear of two or more of the following situations:

1. Using public transportation
2. Being in open spaces
3. Being in enclosed spaces
4. Standing in line or being in a crowd
5. Being outside of the home alone

People with agoraphobia often avoid these situations, in part, because they think being able to leave might be difficult or impossible in the event they have panic-like reactions or other embarrassing symptoms. where the most severe form of agoraphobia, an individual can become housebound. (Balaram K, Marwaha R.2024)

3).1.7.Separation anxiety disorder :

Separation anxiety is often thought of as something that only children deal with. However, adults can also be diagnosed with separation anxiety disorder. People with separation anxiety disorder fear being away from the people they are close to. They often worry that something bad might happen to their loved ones while they are not together. This fear makes them avoid being alone or away from their loved ones. (Feriante J and al.,2024)

3).1.8.Post-traumatic stress disorder (PTSD) :

PTSD is a real disorder that develops when a person has experienced or witnessed a scary, shocking, terrifying, or dangerous event that marked him. These stressful or traumatic events usually involve a situation where someone's life has been threatened. (Jabeen Begum, MD,2024)

3).2.Anxiety treatment

Anxiety disorders are generally treated with psychotherapy, medication, or both

Psychotherapy

Psychotherapy or “talk therapy” can help people with anxiety disorders. To be effective, psychotherapy must be directed at a specific anxieties and tailored to certain needs. (Nita V.2024)

Cognitive behavioral therapy

Cognitive Behavioral Therapy (CBT) is an example of one type of psychotherapy that can help people with anxiety disorders. It teaches people different ways of thinking, behaving, and reacting to situations to help you feel less anxious and fearful. (Kaczkurkin, A.2015)

Exposure therapy is a CBT method that is used to treat anxiety disorders. Exposure therapy focuses on confronting the fears underlying an anxiety disorder to help people engage in activities they have been avoiding. Exposure therapy is sometimes used along with relaxation exercises. (Jabeen Begum, MD.2024)

Acceptance and commitment therapy

Another treatment option for some anxiety disorders is acceptance and commitment therapy (ACT). ACT takes a different approach than CBT to negative thoughts. It uses strategies such as mindfulness and goal setting to reduce discomfort and anxiety. Compared to CBT, ACT is a newer form of psychotherapy treatment, so less data are available on its effectiveness. (huanghu F, Dongyan D.2023)

Medication

Medication does not cure anxiety disorders but can help relieve symptoms. Health care providers, such as a psychiatrist or primary care provider, can prescribe medication for anxiety. Some states also allow psychologists who have received specialized training to prescribe psychiatric medications. The most common classes of medications used to combat anxiety disorders are antidepressants, anti-anxiety medications (such as benzodiazepines), and beta-blockers. (Anthony Cull, MD.2023)

Antidepressants

Antidepressants are used to treat depression, but they can also be helpful for treating anxiety disorders, Antidepressants can take several weeks to start working so it's important to give the medication a chance before reaching a conclusion about its effectiveness. The patient should not stop taking antidepressants without the help of care provider, the provider can help slowly and safely decrease the doses. Stopping them abruptly can cause withdrawal symptoms. （Institute for Quality and Efficiency in Health Care (IQWiG); 2006)

In some cases, children, teenagers, and adults younger than 25 may experience increased suicidal thoughts or behavior when taking antidepressant medications, especially in the first few weeks after starting or when the dose is changed. (Andrade C, Rao NS.2010)

Anti-anxiety medications

Anti-anxiety medications can help reduce the symptoms of anxiety, panic attacks, or extreme fear and worry. The most common anti-anxiety medications are called benzodiazepines. Although benzodiazepines are sometimes used as first-line treatments for generalized anxiety disorder, they have both benefits and drawbacks. (Centre for Addiction and Mental Health [CAMH].2012)

Benzodiazepines are effective in relieving anxiety and take effect more quickly than antidepressant medications. However, some people build up a tolerance to these medications and need higher and higher doses to get the same effect. Some people even become dependent on them. (National Institutes of Health [NIMH], 2024).

To avoid these problems, health care providers usually prescribe benzodiazepines for short periods of time, If people suddenly stop taking benzodiazepines, they may have withdrawal symptoms, or their anxiety may return. Therefore, benzodiazepines should be tapered off slowly,the provider can help slowly and safely by decreasing the doses. (Evans-Lacko S, Aguilar-Gaxiola S, Al-Hamzawi A, and al.2018)

Beta-blockers

Although beta-blockers are most often used to treat high blood pressure, they can help relieve the physical symptoms of anxiety, These medications can help people keep physical symptoms under control when taken for short periods. They can also be used "as needed" to reduce acute anxiety, including to prevent some predictable forms of performance anxieties. (Alisha D.2023)

4).Depression

Depression is a mood disorder that causes a persistent feeling of sadness and loss of interest in things and activities you once enjoyed. It can also cause difficulty with cognition, eating and sleeping, depression is different from grief in that it persists practically every day for at least two weeks and involves other symptoms than sadness alone. (World Health Organisation [WHO], 2023).

4)1.Types of depression

1. **Clinical depression (major depressive disorder):** A diagnosis of major depressive disorder means you've felt sad, low or worthless most days for at least two weeks while also having other symptoms such as sleep problems, loss of interest in activities or change in appetite.This is the most severe form of depression and one of the most common forms.
2. **Persistent depressive disorder (PDD):** Persistent depressive disorder is mild or moderate depression that lasts for at least two years. The symptoms are less severe than major depressive disorder. Healthcare providers used to call PDD dysthymia.
3. **Disruptive mood dysregulation disorder (DMDD):** DMDD causes chronic, intense irritability and frequent anger outbursts in children. Symptoms usually begin by the age of 10.
4. **Premenstrual dysphoric disorder (PMDD):** With PMDD, the individual can have premenstrual syndrome (PMS) symptoms along with mood symptoms, such as extreme irritability, anxiety or depression
5. **Depressive disorder** due to another medical condition: Many medical conditions can create changes in your body that cause depression; include hypothyroidism, heart disease, Parkinson's disease and cancer. (Smitha Bhandari, MD.2023)

There are also specific forms of major depressive disorder, including:

Seasonal affective disorder (seasonal depression): This is a form of major depressive disorder that typically arises during the fall and winter and goes away during the spring and summer.

Prenatal depression and postpartum depression: Prenatal depression is depression that happens during pregnancy. Postpartum depression is depression that develops within four weeks of delivering a baby.

Atypical depression: Symptoms of this condition, also known as major depressive disorder with atypical features, vary slightly from "typical" depression. The main difference is a temporary mood improvement in response to positive events (mood reactivity). (Bains N, Abdijadid S. 2023)

4).2.Symptoms of depression

The symptoms of depression can vary slightly depending on the type and can range from mild to severe. In general, symptoms include:

1. Feeling very sad, hopeless or worried. Children and adolescents with depression may be irritable rather than sad.
2. Not enjoying things that used to bring joy.
3. Being easily irritated or frustrated.
4. Eating too much or too little, which may result in weight gain or weight loss.
5. Trouble sleeping (insomnia) or sleeping too much (hypersomnia).
6. Having low energy or fatigue.
7. Having a difficult time concentrating, making decisions or remembering things.
8. Having thoughts of self-harm or suicide. (Nicole W.2023)

4).3.Causes of depression

Brain chemistry: An imbalance of neurotransmitters, including serotonin and dopamine, contributes to the development of depression.

Genetics: first degree relative (biological parent or sibling) with depression, are more likely to develop the condition than the general population.

Stressful life events: Difficult experiences, such as the death of a loved one, trauma, divorce, isolation and lack of support, can trigger depression.

Medical conditions: Chronic pain and chronic conditions like diabetes can lead to depression.

Medication: Some medications can cause depression as a side effect. Substance use, including alcohol, can also cause depression or make it worse. (Shadrina M, Bondarenko EA.2018)

4).4.Treatment options include:

Psychotherapy: Psychotherapy (talk therapy) involves talking with a mental health professional. a therapist helps to identify and change unhealthy emotions, thoughts and behaviors. There are many types of psychotherapy cognitive behavioral therapy (CBT) is the most common. (National Institutes of Health [NIMH], 2023).

Medication: Prescription medicine called antidepressants can help change the brain chemistry that causes depression. Some antidepressants have side effects, which often improve with time.

Complementary medicine: This involves treatments that a patient may receive along with traditional Western medicine. People with mild depression or ongoing symptoms can improve their well-being with therapies such as acupuncture, massage, hypnosis and biofeedback.

Brain stimulation therapy: Brain stimulation therapy can help people who have severe depression or depression with psychosis. Types of brain stimulation therapy include electroconvulsive therapy (ECT), transcranial magnetic stimulation (TMS) and vagus nerve stimulation (VNS). (Hasler G.2010)

Chapter II : Vitamin D

1).historical background

The history of vitamin D is a rich and storied subject and is now over 350 years old. It started in the early 16's with the first descriptions of the human deficiency disease: rickets in children and osteomalacia in adults. There were no precise medical details that distinguished it from other bone diseases, but treaties and lithographs describing the symptoms that time showing bone deformities resembling rickets leave little doubt that it was vitamin D deficiency. It took another 250 years to define the cause of vitamin D deficiency in the 1900–1920 period when physicians and biochemists elucidated the role of sunlight and identified the chemical structure of the two main forms of the vitamin D molecule, vitamin D2 and vitamin D3. (Jones G.2022)

The four phases of vitamin D history

1) 1650–1890: history of vitamin D deficiency (rickets)
2) 1890–1930: history of the discovery of vitamin D and its structural elucidation
3) 1930–1975: history of the discovery of vitamin D metabolites including 1,25-(OH)2D3
4) 1975–present: history of the discovery of the vitamin D cellular machinery, functions and vitamin D-related human diseases.

1650–1890: history of vitamin D deficiency (rickets)

Rickets and osteomalacia were first clearly described by Daniel Whistler in the Netherlands (1645) as a condition in which the skeleton was poorly mineralized and deformed. Francis Glisson (1650) provided the first documented records with his book entitled De Rachitide. It features a lithograph of children with bowing of the legs and skeletal deformities which are the major marks of vitamin D deficiency

Rickets is characterized by a deformed and misshaped skeleton, particularly bending and bowing of the long bones and enlargement of the epiphyses of the joints of the rib cage, arms, legs and neck. Accompanied with painful movements of the rib cage and difficulty breathing. (Light J,Retrouvey M,Wellman LL, Conran RM.2022)

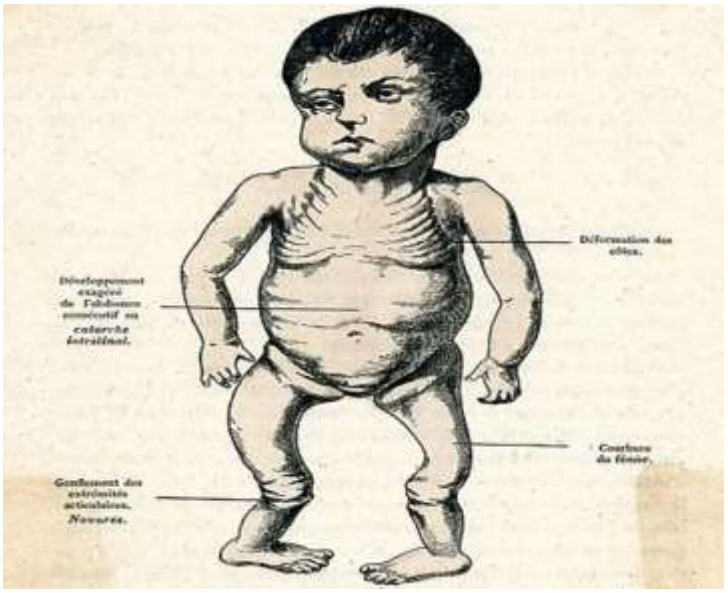

Figure (08) bone deformities in a child with rickets (from "La nature et l'homme" of Rengade 1881 Private collection)

However, rarely is rickets life threatening, it certainly lowers the quality of life for the afflicted individual and leads to secondary problems. One of these secondary effects of rickets occurs in young women who had vitamin D deficiency in childhood causing deformities of the pelvis, which result in difficulties in childbirth. Shorter speculates that rickets in early life must have resulted in numerous deaths of women during their first delivery. (Uday S, Högler W.2020)

The advent of the Industrial Revolution in Western Europe heralded in massive air pollution in the form of smoke from mills and burning of fossil fuels were dramatically reducing the amount of UV light reaching the ground. Since the workers needed for these new industrial jobs were required to move from their rural locations into dingy, poorly lit cities, their exposure to UV light diminished and skin synthesis of vitamin D was reduced. Rickets resulted and was associated with lack of exposure to sufficient sunlight. Thus, the 18th and 19th centuries saw a higher increase in rickets in the industrialized cities of northern Europe, Despite the fact that rickets seemed to be associated with lack of exposure to sunlight, by the late 1700s, some, including Percival in the UK, were advocating the use of cod liver oil for the treatment of rickets suggesting a nutritional aspect to vitamin D. (Deluca HF.2014)

1890–1930: history of the discovery of vitamin D and its structural elucidation

By the 1890s, some researchers such as Owen and Palm, who clearly supported the environmental theory, produced evidence that there were big geographical differences in the incidence of rickets in different parts of the UK and northern and southern China. Palm, a medical missionary, went on to suggest that exposure of children to sunlight would cure rickets. Subsequently, researchers in Europe and the United States namely Buchholtz (1904), Raczynski (1913), Huldshinsky (1919), and later Chick (1922) and Hess & Weinstock (1924) performed experiments in which laboratory animals and children with rickets could be cured with sunlight or light from mercury arc lamps. This clearly demonstrated that lack of exposure to UV light was one cause of rickets. (Deluca HF.2014) But the proponents of the theory that a dietary factor could also be involved continued with their experiments too. The early 20th century was a momentous period in nutritional research in which nutritionists showed that a diet of highly purified carbohydrates, protein, fat and salt is unable to fully support growth and life of experimental animals. By adding various ‘trace factors’, researchers were able to restore growth and a full range of physiological actions. The first of these trace factors was thiamin discovered by Funk which cured neuritis in what Funk termed the ‘vital amine or vitamin theory.’ Thiamin was later renamed vitamin B1, but it was one of a number of vitamin substances that are defined as ‘trace compounds which are derived from the diet and are required in small amounts per day and perform an essential role critical to life.’ Vitamin D was identified as one of these substances playing a critical role in skeletal growth and calcium and phosphate homeostasis. (Spedding S.2013)

The discovery of the nutritional factor, later termed vitamin D by McCollum, came largely as the result of the work of a number of researchers: Mellanby, McCollum, Steenbock and Hart working independently. Sir Edward Mellanby in the UK reasoned that rickets might be due to a dietary deficiency and managed to produce beagle dogs with severe rickets by feeding them oatmeal and then cured their rickets with cod liver oil. (Hector F.Deluca.2011) Since cod liver oil is a mixture of lipids and a rich source of vitamin A, it was not clear what the active ingredient might be. McCollum , working first at the U Wisconsin and then Johns-Hopkins,

heated and bubbled oxygen through the cod liver oil to destroy the vitamin A and found that the product still cured rickets. Building on the new vitamin nomenclature, he termed the new substance vitamin D. But how was the field to reconcile the apparently unconnected findings that UV light and a nutritional substance termed vitamin D could both cure rickets,Harry Steenbock also working at the U Wisconsin-Madison performed the definitive experiment. Steenbock & Black experimented with the diets of goats and found that sunlight or UV irradiation of the animals or their diets resulted in rickets being cured in the goats . Steenbock traced the bioactive substance in irradiated food to the non-saponifiable fraction of lipids in the diet and showed that it cured rickets . Dietary vitamin D was born. (Jones G.2022)

In the late 1920s, Windaus and colleagues isolated the key anti-rachitic substance from a mixture of irradiated plant sterols and named it vitamin D1, although they did not identify its structure. Later, vitamin D1 was shown to be a mixture of vitamin D2 and tachysterol. A British group headed by Askew successfully identified and determined the structure of the anti-rachitic, plant-derived sterol as vitamin D2 or ergocalciferol. Windaus's group confirmed the structure of vitamin D2 and also isolated and identified the animal-derived, anti-rachitic vitamin D3 or cholecalciferol and its skin precursor, 7-dehydrocholesterol . (Glenville J.2022)

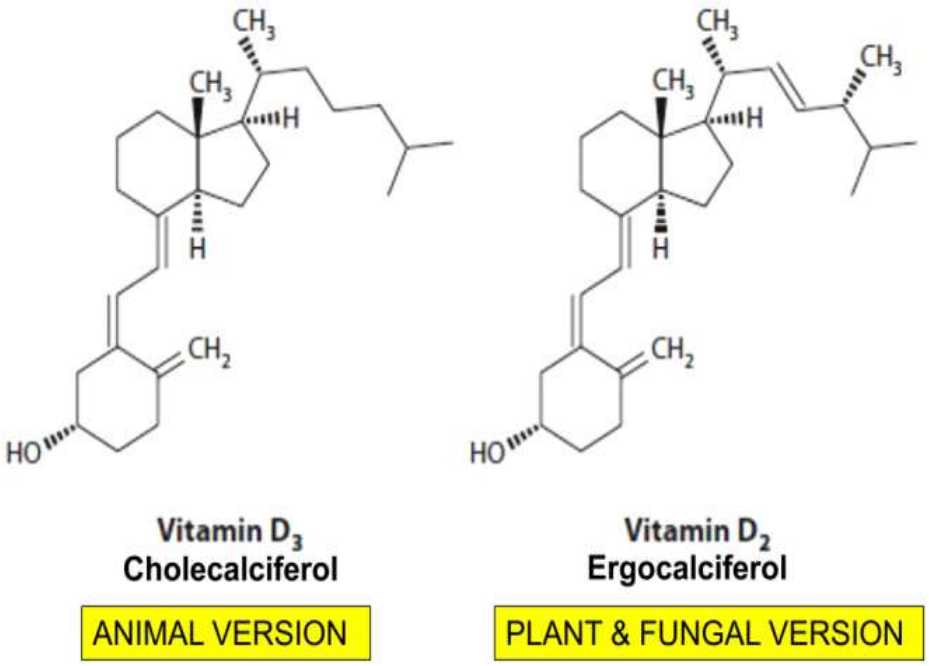

Figure (09) chemical structure of vitamin D2 and D3 (Jones, Glenville.2022)

1930–1975: history of the discovery of vitamin D metabolites including 1,25-(OH)2D3

Chemically synthesized vitamin D2 and vitamin D3 have been available since the 1930s and made the way for the study of their biological functions and metabolism. The physiological roles of vitamin D are primarily its roles in calcium and phosphate homeostasis and include:

1. stimulation of intestinal calcium and phosphate absorption
2. mobilization of calcium from bone
3. renal reabsorption of calcium.

All three of these functions serve to raise blood calcium and phosphate and ensure that these ions are available to ensure health and prevent rickets. Elucidating the details of these physiological functions became the main foci during the 1930–1960 time period, and research revealed that vitamin D was intimately connected to the roles of other calcium and phosphate-related hormones including parathyroid hormone (PTH) and calcitonin. (Anawalt B, Blackman MR, et al.,2000)

In the 1960s, there was considerable debate over whether the functions of vitamin D were carried out by vitamin D itself or its possible metabolites. Consequently, intense effort was put into studying the metabolism of vitamin D by using chemically synthesized radioactive versions of vitamin D2 and vitamin D3. The pioneer in this area was Egon Kodicek at the Dunn Nutritional Laboratories, U Cambridge UK. After 10 years of work, Kodicek concluded that vitamin D was active without being metabolized. In retrospect, the radioactive vitamin D that his group were using was insufficiently labeled to detect its metabolites. However, Hector DeLuca, again at the U Wisconsin-Madison, and the final graduate student of Harry Steenbock, synthesized radioactive vitamin D3 with much higher specific activity and was able to demonstrate metabolism to more polar metabolites, the principal one being 25-hydroxyvitamin D3 (25-OH-D3)made in the liver and the first identified natural vitamin D metabolite, 25-OH-D3 proved to be more potent biologically than vitamin D3 and was present in the bloodstream at a higher concentration. (Bikle DD.2014)

1975–present: history of the discovery of the vitamin D cellular machinery, functions and vitamin D-related human diseases

The discovery of the active forms of vitamin D heralded in a search for :

a. the signal transduction mechanisms to explain how 1,25-(OH)2D3 was able to produce its various biological effects;
b. identification of the enzymes responsible for the synthesis and catabolism of 1,25-(OH)2D3
c. a clear understanding of the regulation of the vitamin D endocrine system

These studies started almost as soon as metabolism was recognized in the late 1960s when Mark Haussler, in AW Norman's laboratory, demonstrated that vitamin D metabolites were associated with the chromatin. Clear evidence of the protein that is now termed the vitamin D receptor (VDR) was produced by Haussler's lab . The VDR protein from various species was later purified and its gene was cloned by Haussler's group . Study of the pure protein has led to a determination of its crystal structure . Over the past 30 years, Mark Haussler, Wes Pike and colleagues have demonstrated that 1,25-(OH)2D3 works through a VDR-mediated mechanism that involves many coactivators and repressors to directly interact with and regulate hundreds of genes around the body. Other researchers, most notably Anthony Norman , have proposed that some of the actions of vitamin D occur through rapid non-genomic signaling pathways, possibly involving a plasma membrane VDR but this protein has never been fully characterized at the molecular level. Nevertheless, there remains some uncertainty that all vitamin D ligands and analogs produce their effects through a genomic VDR mechanism (Jones G.2022)

These are vitamin D-binding globulin and the cytochromes P450-containing enzymes that metabolize vitamin D into its many metabolites . (Pop TL, Sîrbe C, Benţa G, Mititelu A, Grama A.2022)

Being a fat-soluble vitamin, vitamin D requires a protein to transport it around the body and the vitamin D-binding globulin performs this function. (Jones G, Prosser DE, Kaufmann M.2014) The cytochrome P450-containing enzymes (CYPs) responsible for vitamin D metabolism were first studied in the early 1970s in tissue extracts of liver and kidney and then in tissue culture and given names based upon their hydroxylation activity: 25-hydroxylase, 1α-hydroxylase and 24-hydroxylase. In the early 1990–2005 period, all three enzymes were purified, cloned and expressed in cell culture systems, principally by Canadian group of St-Arnaud as well as the Japanese groups of Kato S, Okuda and Sakaki as well as Russell's group at the U Texas . The three enzymes are now known as CYP2R1, CYP27B1 and CYP24A1. (Holick, Michael F. 2023)

History of the main protein components of the specific* vitamin D signal transduction machinery.

Protein	Abbreviation	source	Biological function	Discovery	Gene cloning
Vitamin D-binding globulin	DBP	Liver	Transport of vitamin D and its metabolites	Daiger *et al.* 1975 (64)	Cooke *et al.* 1991 (79)
Vitamin D receptor	VDR	Most tissues except liver	Regulation of vitamin D-dependent genes	Haussler 1969 (80) Brumbaugh *et al.* 1975 (55)	McDonnell *et al.* 1987 (56)
25-Hydroxylase	CYP2R1	Liver	25-hydroxylation of vitamins D_2 and D_3	Cheng *et al.* 2003 (81)	Cheng *et al.* 2004 (75)
1α-Hydroxylase	CYP27B1	Kidney (major) Extra-renal sites	1α-hydroxylation of 25-OH-D_2 & 25-OH-D_3	Fraser *et al.* 1970 (42)	St-Arnaud *et al.* 1997 (70) Takeyama *et al.* 1997 (71)
24-Hydroxylase	CYP24A1	Kidney (major) Extra-renal sites	24-hydroxylation of (& 23- & 26-hydroxylation) 25-OH-D_2 & 25-OH-D_3 Complete catabolism of vitamin D	Knutson *et al.* 1972 (66)	Ohyama & Okuda 1991 (72)

Table (01) history of the main protein components of the specific vitamin D signal transduction machinery (Jones G.2022)

2).Vitamin D definition

Vitamin D is a steroidal substance required by all vertebrates including humans to maintain blood calcium and phosphate to support a healthy skeleton, muscle contraction, immune function and optimal cellular functions in many locations around the body ,The name vitamin D is a term coined by nutritionists, and is not a chemical term, which is defined as 'a substance with anti-rachitic properties that will cure rickets'.In human biology,vitamin D usually refers to two substances:vitamin D3 (usually known as cholecalciferol) of animal origin and vitamin D2 (referred to as ergocalciferol) of plant or fungal origin.These two forms have roughly equal potencies,similar metabolic patterns.(Jones G.2022)

Although vitamin D2 and D3 were considered equally active for many years, current knowledge indicates that Vitamin D3 is three times more potent than Vitamin D2. The potential responsible factors are different metabolic pathways and different affinity of the active metabolites of vitamins D2 and D3 toward vitamin D receptor (VDR). (Janoušek, J.and al.2022)

Vitamin D3 is the main form of vitamin D in humans, and indeed, it is estimated that about 80-90% of the vitamin D requirements are covered by the endogenous synthesis in the skin. The extent of the skin vitamin D synthesis is dependent on the length of sun exposure, the season of the year, and latitude . A 20-min long whole body exposure to the summer sun is able to produce up to 250 μg of vitamin D3, which yields the recommended serum level (>30 ng/mL) of its metabolite and systemic indicator, 25-hydroxyvitamin D [25(OH)D], which is also known as calcifediol or calcidiol . (Mostafa WZ, Hegazy RA, 2015)

3).Vitamin D sources

Vitamin D is unique because it can be made in the skin from exposure to sunlight only, this vitamin exists in two forms, vitamin D2 and vitamin D3, which differ in the structure of their side chains that are known as ergocalciferol and cholecalciferol, respectively. Vitamin D2 is obtained from the UV irradiation of the yeast sterol ergosterol and is found naturally in sun-exposed mushrooms. UVB light from the sun strikes the skin, and humans synthesize vitamin D3, so it is the most "natural" form. (Feingold KR and al.,2000)

Human beings do not make vitamin D2, and most oil-rich fish such as salmon, mackerel, and herring contain vitamin D3. Vitamin D (D represents D2, or D3, or both) that is ingested is incorporated into chylomicrons, which are absorbed into the lymphatic system and enter the venous blood. (Lu Z, Chen TC, Zhang A and al.,2007)

During the exposure to sunlight, UV-B photochemical reaction where photons penetrate the epidermis and the absorbed energy causes the photolysis of 7-dehydrocholesterol, present in the plasma membrane of keratinocytes, into the previtamin D3. The formed previtamin D3 is thermodynamically unstable and rapidly isomerizes to vitamin D3. At 37 °C, 80% of previtamin D3 is isomerized to vitamin D3 within 8 h. Generated vitamin D3 is then released from the plasma membrane into the extracellular space, wherefrom it moves into the capillary bed and binds to plasma proteins. (Holick MF.1987)

The main food sources of vitamin D vary largely according to eating habits and age,An overview of the most important sources of vitamin D shows that vitamin D3 dominates over vitamin D2 in natural dietary intake. Although fatty fish and fish oil are the richest sources of vitamin D3, they do not account for a large proportion of vitamin D intake in many countries (cod liver oil as a traditional preparation for the treatment of rickets was described in the eighteenth century). Liver oil from wild cod fish contains up to 1,250 μg of vitamin D3 per 100 g. Other fish liver oil, especially various tuna species, has even higher vitamin D content than that from the cod. Interestingly, in comparison with other fish (e.g. mackerel, salmon, herring), cod and tuna flesh usually contain lower amounts of vitamin D3. Of the freshwater fish, rainbow trout and tilapia have a significant content of vitamin D3. (Jäpelt RB, Jakobsen J.2013)

Another important source of vitamin D3 is eggs, specifically the egg yolk. There are no significant differences in vitamin D3 contents between the individual animal species whose eggs are commonly consumed (e.g. chicken, duck, goose, quail), with values ranging from 2 to 5 µg/100 g of yolk. In poultry, vitamin D3 supplementation is of huge importance, as a significant increase in egg yolk vitamin D content can be achieved (Janoušek J and al.,2022)

Dairy products are also an important source of vitamin D, especially in younger individuals. That some fatty dairy products like butter, whipping cream, and cheese, contain significant amounts and usually also contain some vitamin D2.

Nine food groups to measure the contribution of particular food categories to vitamin D intake:

1. fish/fish products (e.g. fish, canned fish, seafood)
2. eggs
3. fats/oils (e.g. butter, lard, margarine, oil)
4. bread/bakery products (e.g. bread, toast, cake, biscuits)
5. milk/dairy products (e.g. milk, cheese, yoghurt, curd, cream)
6. potatoes/fruits/ vegetables and related products
7. nutriments (e.g. pasta, rice, cereals, corn flakes)
8. meat/meat products (e.g. meat, innards, sausages, cold cuts, ham)
9. And the category others inclusive of, for example, sauces, sweets, snacks, and beverages. (Janoušek, J and al.,2022)

4).Biochemistry of vitamin D (Vitamin D Metabolism and Homeostasis)

Vitamin D wether is synthetized or ingested requires its first hydroxylation in the liver by the enzyme D-25-hydroxylase (25-OHase) to 25(OH)D. then ,25(OH)D requires a further hydroxylation in the kidneys by the 25(OH)D-1-OHase (CYP27B1) to form the biologically active form of vitamin D 1,25(OH)2D.1,25(OH)2D stimulates intestinal calcium absorption. (Jiang Q, Zhang M, Mujumdar AS.2020).

Moreover, both vitamins D2 and D3 are metabolized by their conversion to the 25-hydroxy form and then to the 1,25-dihydroxy metabolite in the kidney, which is the bioactive form of this vitamin.(Ross AC, Taylor CL, Yaktine AL and al.,2011)

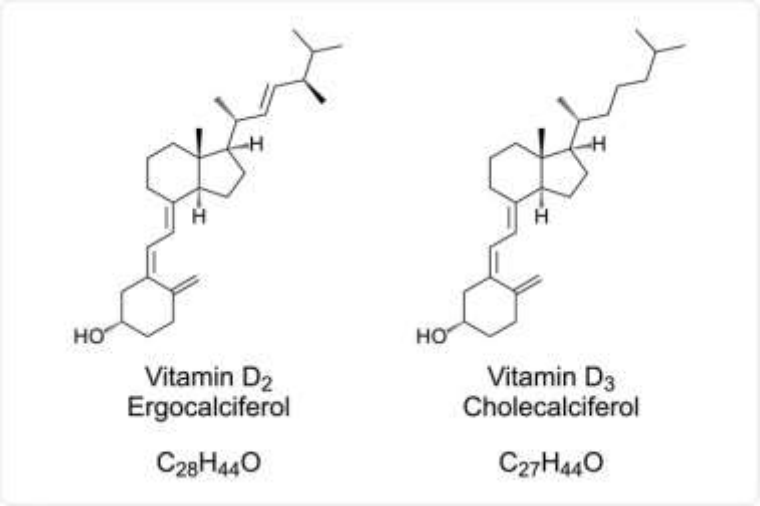

Figure (10) chemical structure of vitamin D2 and D3 (Singh, Vinita and al.,2022)

5).Vitamin D metabolism and homeostasis

Vitamin D3 produced in the epidermis must be further metabolized to be active. The first step, 25-hydroxylation, takes place primarily in the liver, 25OHD is the major circulating form of vitamin D. However, in order for vitamin D metabolites to achieve maximum biologic activity they must be further hydroxylated in the 1α position by the enzyme CYP27B1; 1,25(OH)2D is the most potent metabolite of vitamin D and accounts for most of its biologic actions. The 1α hydroxylation occurs primarily in the kidney, although as for the 25-hydroxylase, other tissues have this enzyme. (Martin K.2003)

Vitamin D and its metabolites, 25OHD and 1,25(OH)2D, can also be hydroxylated in the 24 position. This may serve to activate the metabolite or analog as 1,25(OH)2D and 1,24(OH)2D have similar biologic potency, However, 24-hydroxylation of metabolites with an existing 25OH group leads to further catabolism. (Christakos S, Ajibade DV, Dhawan P, Fechner AJ, Mady LJ.2010)

The three main steps in vitamin D metabolism, 25-hydroxylation, 1α-hydroxylation, and 24-hydroxylation are all performed by cytochrome P450 mixed-function oxidases (CYPs). These enzymes are located either in the endoplasmic reticulum (ER) (e.g., CYP2R1) or in the mitochondria (e.g., CYP27A1, CYP27B1, and CYP24A1). (Anderson PH, May BK, Morris HA.2003)

Biosynthesis of 25OHD3 (25-hydroxylase) in liver

Vitamin D3 synthesized in the skin is released into the systemic circulation and all forms are transported by binding to VDBP in serum. A portion of vitamin D, a fat-soluble vitamin, is stored in adipose tissue for use when necessary. The ability of vitamin D to be stored in adipose tissue extends its total half-life in the body up to approximately 2 months. When vitamin D3 is transported to the liver, it is first converted into 25OHD3 by the cytochrome P450 25-hydroxylase enzyme (Ramasamy I.2020). 25OHD3 is the main circulating form of vitamin D, Various enzymes that show 25-hydroxylase properties have been described in the body. Among these, the first one is CYP27A1 located in mitochondria, and the second is microsomally found CYP2R1 . CYP27A1 also exerts 27-hydroxylase effect and is involved in bile acid synthesis. Although CYP27A1 is expressed in different tissues of the body, the tissues where it is most commonly found are liver and skeletal muscle tissues . In experimental studies, it was reported that the serum 25OHD3 levels were increased in mice which possess an inactivated CYP27A1 gene, and that rickets did not occur in these mice . Interestingly, in this study, it was shown that CYP2R1 expression increased after CYP27A1 gene inactivation, and consequently 25-hydroxylation activity increased . (Sezer A.2020)

Studies have suggested that CYP2R1 is the major enzyme responsible for 25-hydroxylation in the human body. The 25-hydroxylase encoded by the CYP2R1 gene was first described by Cheng et al. It was first reported by Chen et al. that homozygous inactivating mutations of this gene lead to clinically observed rickets (vitamin D-dependent rickets type IB) in Nigerian families. It has been reported that these cases gave suboptimal response to standard vitamin D (inactive vitamin D2 or D3 forms) treatment . (Zhu JG and al.,2013)

It has been shown that in CYP2R1-null mice, the level of 25OHD3 decreases by 50%, when both CYP2R1 and CYP27A1 are inactivated, and that serum 25OHD3 levels decrease by 70%, and serum 25OHD3 level remains at a measurable level in both cases. This supports the view that serum vitamin D level is compensated by other enzymes with recruitable 25-hydroxylase enzyme activity. (Cheng JB, Levine MA, Bell NH, Mangelsdorf DJ, Russell DW.2004)

Formation of active vitamin D [1,25 (OH) 2D3] by 1-alpha hydroxylase (CYP27B1) in the kidney

The kidney is the major source of circulating levels of 1,25(OH)2D. Unlike 25-hydroxylation, there is only one enzyme recognized to have 25OHD 1α-hydroxylase activity, and that is CYP27B1 (Bikle D.2014)

The principal regulators of CYP27B1 activity in the kidney are parathyroid hormone (PTH), FGF23, calcium, phosphate, and 1,25(OH)2D. Extrarenal production tends to be stimulated by cytokines such as IFN-gamma and TNF-α more effectively than PTH and may be less inhibited by calcium, phosphate, and 1,25(OH)2D depending on the tissue. Administration of PTH in vivo or in vitro stimulates renal production of 1,25(OH)2D. (Bacchetta J and al.2013)

The final step of active vitamin D formation takes place in the proximal tubules of the kidney, led by the enzyme 1-alpha hydroxylase. 25OHD3, which is bound to VDBP, is taken into tubule cells and metabolized through megalin and cubilin, which are transmembrane proteins located in renal tubules and act as surface receptors for VDBP in tubules. 25OHD3, which then undergoes 1-alpha hydroxylation. resulting in the formation of 1,25 (OH) 2D3 (active form of Vitamin D). (Ramasamy I.2020)

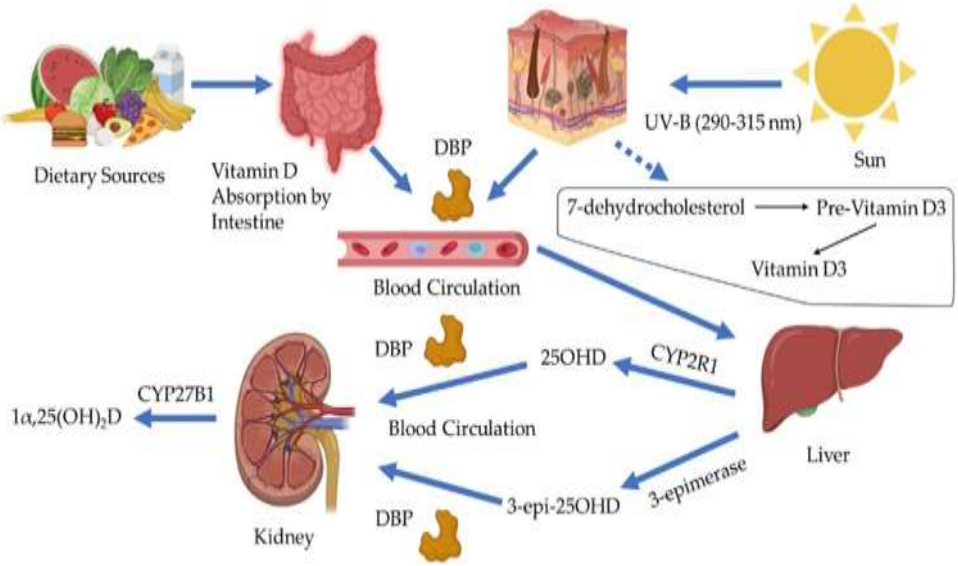

Figure (11) Unraveling the complex interplay between obesity and vitamin D metabolism (Alzohily, Band al.,2024)

Inactivation of vitamin D by 24-hydroxylase (CYP24A1)

The 24-hydroxylase enzyme is located in the mitochondrial inner membrane of the cells found in the proximal kidney. The CYP24A1 enzyme, encoded in 20q13 chromosomal region and having 24-hydroxylase enzyme activity, initiates catabolic processes that lead to the inactivation of vitamin D. This enzyme can use both 25OHD3 and 1.25 (OH) 2D3 as substrates, but has a higher affinity for 1.25 (OH) 2D3. As a result of a series of enzymatic reactions, calcitroic acid is formed, which becomes biologically inactive that in the other hand it will be exerted to the bile (Cappellani D, Brancatella A, Kaufmann M and al.,2019)

Transport in blood

The vitamin D metabolites are transported in blood bound primarily to vitamin D binding protein (DBP) (85-88%) and albumin (12-15%) . DBP concentrations are normally 4-8μM, well above the concentrations of the vitamin D metabolites, such that DBP is only about 2% saturated. DBP has high affinity for the vitamin D metabolites (Ka=5x108M-1 for 25OHD and 24,25(OH)2D, 4x107M-1 for 1,25(OH)2D and vitamin D), such that under normal circumstances only approximately 0.03% 25OHD and 24,25(OH)2D and 0.4% 1,25(OH)2D are free. Conditions such as liver disease and nephrotic syndrome resulting in reduced DBP and albumin levels will lead to a reduction in total 25OHD and 1,25(OH)2D levels without necessarily affecting the free concentrations . Similarly, DBP levels are reduced during acute illness, potentially obscuring the interpretation of total 25OHD levels. Bouillon R, Schuit F, Antonio L, Rastinejad F.2020)

The vitamin D metabolites bound to DBP are in general not available to most cells. Thus, the free or unbound concentration is that which is critical for cellular uptake as postulated by the free hormone hypothesis. Support for the concept that the role of DBP is to provide a reservoir for the vitamin D metabolites but that it is the free concentration that enters cells and exerts biologic function comes from studies in mice in which DBP has been deleted and in humans in which the gene is mutated. In DBP knockout mice the vitamin D metabolites are presumably all free and/or bioavailable. These mice do not show evidence of vitamin D deficiency unless placed on a vitamin D deficient diet despite having very low levels of serum 25OHD and 1,25(OH)2D (Xue Y, Fleet JC.2009). Tissue levels of 1,25(OH)2D were found to be normal in the DBP knockout mice as were markers of vitamin D action such as expression of intestinal TRPV6, calbindin 9k, PMCA1b, and renal TRPV5 . Recently a family in which a large deletion of the coding portion of the DBP gene (and adjacent NPFFR2 gene) has been reported . The proband had normal calcium, phosphate and PTH levels with vitamin D supplementation despite very low levels of 25OHD, 24,25(OH)2D, and 1,25(OH)2D that were not responsive to massive doses of vitamin D (oral or parenteral). The free 25OHD was nearly normal. The carrier sibling had vitamin D metabolite levels between those of the proband and the normal sibling. Thus, both the studies in DBP null mice and humans support the free hormone hypothesis while also supporting the role of DBP as a circulating reservoir for the vitamin D metabolites. Therefore, there is currently a debate as to whether the free concentration of 25OHD, for example, is a better indicator of vitamin D nutritional status than total 25OHD, given that DBP levels, and hence total 25OHD levels, can be influenced by liver disease, nephrotic syndrome, pregnancy, and inflammatory states .(Feingold KR, Anawalt B, Blackman MR and al.,2000)

5).Physiological actions of vitamin D

Vitamin D is a fat-soluble vitamin that acts as a steroid hormone. In humans, the primary source of vitamin D is UVB-induced conversion of 7-dehydrocholesterol to vitamin D in the skin , Vitamin D influences the bones, intestines, immune and cardiovascular systems, pancreas, muscles, brain, and the control of cell cycles (Nair R and al.,2012)

Vitamin D and its metabolites are steroid hormones and hormone precursors. About 80% derive from ultraviolet B (UVB) induced photoconversion in the skin of 7-dehydrocholesterol to previtamin D3, and the remainder from the diet and from food supplements. Whether derived from skin, food, or supplements, previtamins D2 and D3 are biologically inactive and, in the liver and in the kidney, undergo two stages of hydroxylation to the biologically active form of vitamin D, 1,25(OH)2D . Vitamin D and its metabolites are transported in the circulation by vitamin D binding protein (VDBP), and having reached their target cells, they dissociate from the VDBP and enter the cells (Nair R, Maseeh A.2012),Vitamin D undergoes two hydroxylations in the body for activation. Calcitriol (1,25-dihydroxyvitamin D3), the active form of vitamin D, has a half-life of about 15 h, while calcidiol (25-hydroxyvitamin D3) has a half-life of about 15 days.Vitamin D binds to receptors located throughout the body. 25(OH)D is transformed by renal or extrarenal 1α-hydroxylase into 1,25-dihydroxyvitamin D (1,25[OH]2D), which circulates at much lower serum concentrations than 25(OH)D, but has a much higher affinity to the VDR (Martin K.2003) Studies have, however, shown that many other cell types, including those of the vascular wall, express 1α-hydroxylase with subsequent intracellular conversion of 25(OH)D to 1,25(OH)2D, which exerts its effects at the level of the individual cell or tissue before being catabolized to biologically inactive calcitroic acid. Factors such as fibroblast growth factor 23 and Klotho, which suppress 1α-hydroxylase expression, have also been shown to regulate the renal conversion of 25(OH) D to 1,25(OH)2D. (Brandi ML.2010)

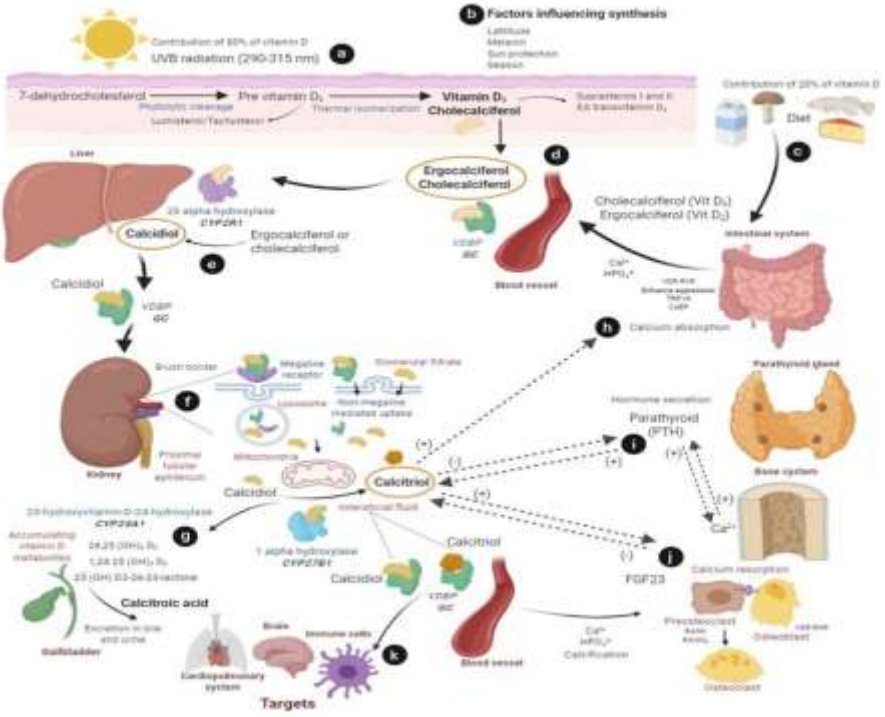

Figure (12) Functional effects of vitamin D: From nutrient to immunomodulator (Meza-Meza, M. R and al., 2022)

6).The mechanism of Vitamin D actions

Vitamin D provides its biological effect in two different ways. The first is by directly affecting gene transcription (genomic effect) as other steroid hormones. This effect is relatively slow and usually occurs within hours or days. The second is the non-genomic pathway whose biological effect is relatively faster (within minutes). Vitamin D exerts its non-genomic effect by directly altering the trans-membrane passage of some ions (Ca, Cl) or by affecting intracellular signaling pathway activities (cAMP, PKA, PLC, PI-3 kinase and MAP kinase). (Christakos S, Dhawan P and al.,2016)

Genomic effect of Vitamin D

The active form of vitamin D displays this effect through the vitamin D receptor (VDR). Knowing that VDR is a member of the nuclear hormone receptor superfamily, which includes steroid, thyroid hormone, and retinoic acid receptors. The VDR gene located on chromosome 12 consists of 427 amino acids encoded by. The structure of the VDR consists of a relatively short N-terminal domain compared to other nuclear receptors, two zinc-fingers that allow the receptor to bind to DNA, and a highly variable C-terminal region, and the hinge region connecting binding these domains . The DNA-binding region of the receptor is rich in cysteine, and the sequence of this region is largely conserved between species (Zenata O, Vrzal R.2007). The zinc-finger structure close to the C-terminal part of VDR determines the specificity for the VDRE (vitamin D response element), which is the binding site on the DNA. The other zinc-finger structure is involved in the heterodimerization of VDR with RXR (retinoid X receptor). The ligand-binding part of the receptor consists of 12 α-helix structures (H1-12; the H12 part is also called AF2) and 3 β-sheet structures (S1-3). The AF-2 region located at the end of the C-terminal is the binding site of co-activator complex structures such as SRC (steroid receptor coactivator) and DRIP (vitamin D receptor interacting protein). (Rochel N.2022)

Transcription is initiated by binding co-activators to this region . Apart from these functional domains, there are NLS (nuclear localization signal) regions within the DNA binding region of VDR, which are necessary for maintaining transcriptional activity. In addition, there is a hinge region between the ligand-binding and DNA-binding domains of the VDR that ensures molecule stabilization. (Pike JW, Christakos S.2017)

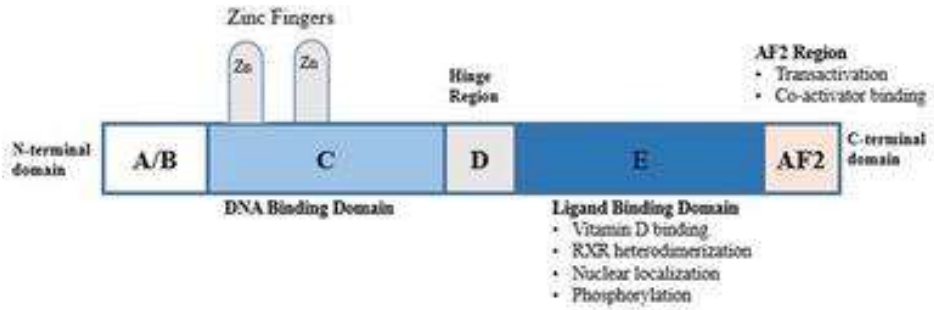

Figure (13) : The structure of the Vitamin D receptor (VDR). (Öner Ö.2021)

After active vitamin D crosses the target cell membrane, it interacts with the ligand-binding domain of its own receptor (VDR) in the cytoplasm of the cell. Vitamin D is embedded in the ligand-binding domain, and subsequently, in the H12 alpha-helix H12 (AF-2) region, which is located at the end of the ligand binding part . This critical conformational change of AF-2 facilitates the binding of co-activators in later stages. In the next step, vitamin D-bound VDR binds to RXRα to form a VDR/RXR heterodimer structure that binds to cognate VDR elements (VDRE) in the promoter region in the target genes with a high affinity to initiate gene activation or inhibition (Sylvia Christakos,Puneet D and al.,2015). There are many gene-specific VDREs associated with bone metabolism, xenobiotic detoxification, drug resistance, cell growth and differentiation, angiogenesis, mammalian hair growth cycle, lipid synthesis regulation, apoptosis, and immune functions, suggesting that vitamin D has numerous regulatory roles in various organs or tissues in the body. (Starska-Kowarska K.2023)

After active vitamin D-VDR-RXR-VDRE interaction, the progression of transcription is controlled by co-activator and co-repressors. The best known co-activators are the p160 co-activator family (eg CBP/p300 and p/CAF) and SRC 1,2,3. Both bind to the AF-2 part and have histone acetyl transferase (HAT) activity, which enables the opening of the histone structure and thus facilitates gene expression. The SRC complex has three NR regions that facilitate binding and contain LxxLL (L, leucine; x, any amino acid) motifs. Likewise, the DRIP complex (Mediator) also has NR regions with LxxLL motifs consisting of 15 or more amino acids . Unlike SRC, DRIP complex does not have HAT activity. This suggests the fact that both protein complexes play a complementary role in the initiation of transcription. The mediator multi-protein complex DRIP205/MED1 (also known as MED1) accumulates around RNA polymerase 2of the initiation complex. This complex then interacts with the TATA region in the promoter region and enables transcription to be initiated . Co-repressors (eg SMRT and NCoR) have histone de-acetylase activity and inhibit transcription by preventing unfolding of the histone core. (Takeyama K, Masuhiro Y, Fuse H and al.,1999)

Non-genomic effects of vitamin D

Some of the hormones that act on the nuclear hormone receptor can also exert their biological effects on the membrane receptor without the need for additional gene regulation. The non genomic effect occurs through messenger mediated pathways. Estrogen, progesterone, testosterone, corticosteroids and thyroid hormones have been reported to exert their effects by using both genomic and non-genomic pathways (Ren B, Zhu Y.2022) Vitamin D has been shown to directly regulate the activation or distribution of various ion-transport channel proteins (for calcium and chloride) and of enzymes (protein kinase C and phospholipase C) through the membrane receptor in osteoblast, liver, muscle, and intestinal cells. In order to demonstrate the non-genomic effect of vitamin D, many studies have been conducted on intestinal calcium absorption. Rapid vesicular calcium absorption (also called transcaltachia) has been shown in the chick intestinal tract. Further experimental studies have shown that intestinal calcium transport cannot be blocked by the administration of actinomycin D (which inhibits the genomic effect), whereas calcium absorption can be blocked by inhibition of voltage-gated L-type calcium channel proteins or by protein kinase C. (Nemere I, Norman AW.1990)

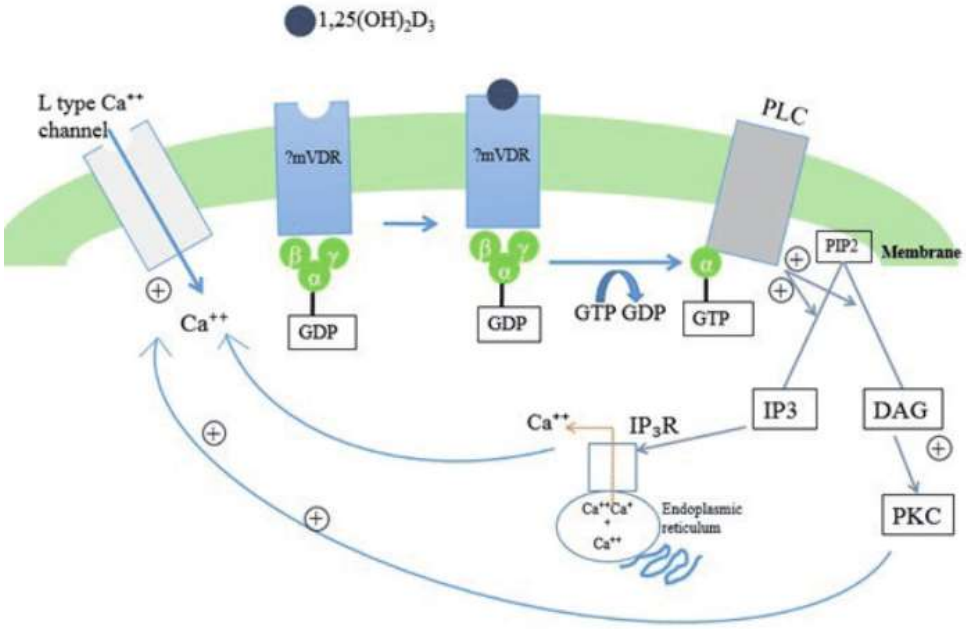

Figure (14) Representation of the signal transduction pathways where Vitamin D has its non-genomic effect (Acar, Sezer and al.,2021)

After vitamin D binds to the membrane receptor, GDP in the G protein α-subunit turns into GTP and activation occurs. The α-subunit of the G protein is separated from other subunits and binds to phospholipase C (PLC). The PLC is then activated to convert phosphoinositol bisphosphate (PIP2) to inositol triphosphate (IP3) and diacylglycerol (DAG). Calcium release from the endoplasmic reticulum via the IP3 receptor (IP3R); DAG activates PKC. PKC, on the other hand, provides calcium entry into the cell via the L-type calcium channel in the membrane. (Donati S, Palmini G, Romagnoli C and al.2021)

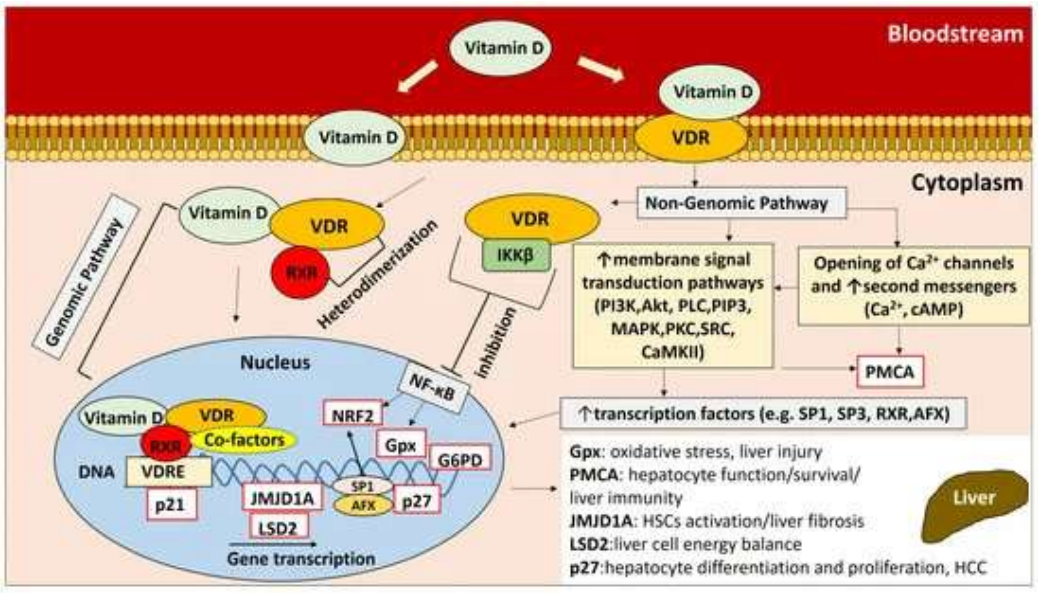

Figure (15) Vitamin D-VDR pathways and regulation of downstream gene expression (Tourkochristou, Evanthia and al.,2023)

7).Effects of Vitamin D on calcium and phosphorus

Intestinal calcium absorption

One of the most important functions of vitamin D is to increase calcium absorption from the intestines. Calcium absorption from the intestinal tract occurs trans-cellular and para-cellular processes mediated through genomic and non-genomic effects. Among these, the trans-cellular pathway largely utilized by the intestinal system, which is regulated by vitamin D. The absorption effect of vitamin D with non-genomic effect of calcium occurs directly on the membrane (transcaltachia). The channel-mediated calcium absorption effect of vitamin D occurs more slowly. (Christakos S and al.,2011)

Calcium enters the epithelian cell by the effect of an electrical and chemical gradient via calcium channel protein TRPV6 (which has significant sequence homology to TRPV5 in the kidney), the transmembrane protein at the lumenal brush border edge of the intestinal epithelial cell. The expression of TRPV6 is activated by vitamin D. Reduced intestinal calcium transport is observed in TRPV6 null mice. Calcium entering the cell binds to calmodulin (CaM), which is bound with myosin 1A (also known as brush border myosin I). This formed complex allows calcium to be transported across the microvilli. Subsequently, the transport of calcium up to the basolateral membrane occurs inside the vesicle via calbindin-D9k (CaBP). The affinity of calcium for calbindinin is greater than for calmodulin, and better facilitates calcium transport inside the cell. The calcium reaching the basolateral membrane is pumped out of the cell to systemic circulation via the Ca-ATPase (PMCA1b) pump located on the membrane. In addition, although it is less important, NCX (sodium/calcium exchanger), located in the basolateral region, also plays a role in excretion of calcium . (Christakos S, Dhawan P and al.2011)

Intestinal calcium absorption, serum calcium level and bone mineral content in Kalbindin D9k null mice (regardless of dietary calcium level) have been shown to be similar to normal mice. Intestinal calcium absorption was found to be normal in calbindin D9k and TRPV6 null mice when a diet containing the daily requirement for calcium was given. These findings indicate there is a mechanism other than the genomic effect through which vitamin D exerts its action (a non-genomic effect) in calcium absorption in the intestines when the amount of calcium in the diet is sufficient. (Benn B and al,2008)

Phosphate, another important molecule for bone mineralization, is actively absorbed mostly in the jejunum, with absorption influenced by vitamin D . This absorption is provided by sodium-phosphate co-transporter IIb (NaPi IIb). In experimental studies, it has been shown that phosphate absorption is blocked when cycloheximide, which inhibits protein synthesis, is given This situation supports that phosphate absorption occurs by genomic effect. Vitamin D increases NaPi-IIb expression and thus phosphate absorption. (Foster BL, Tompkins KA, Rutherford RB and al.,2008)

The effect of vitamin D on the kidneys

Most of the calcium that reaches the kidney tubules is absorbed from the proximal and distal tubules and approximately. Approximately 65% of calcium absorption in the kidney is passively absorbed para-cellularly from the proximal tubules with the sodium gradient and independent of vitamin D direct action. The rest of the calcium is absorbed from the ascending limb of the loop of Henle (20%), the distal tubules (15–20%), and the collecting ducts (5%) . Vitamin D plays an important role in calcium absorption in the distal tubules and provides active calcium absorption via the trans-cellular pathway with the help of an electrochemical gradient. Calcium is taken into the cell by TRPV5 channel on the surface of the tubular cell and is transported inside the cell by calbindin-D9k and D28k. Transported to the basolateral part of the cell, calcium is released into the systemic circulation by NCX1 (sodium/calcium exchanger) and PMCA1b. This mechanism is similar to that in the intestinal tract. Vitamin D increases the expression of TRPV5, calbindin, NCX and PMCA1b. (Hanna R and al.,2022)

Phosphate is reabsorbed by sodium-dependent phosphate carrier proteins (NaPi-IIa and NaPi-IIc) in proximal tubular cells under vitamin D control. In addition, for phosphate reabsorption, a Na/K-ATPase channel located in the basolateral membrane is also needed. The impact of vitamin D on transport channels is not clearly known. While PTH increases the lysosomal degradation of phosphate transport channels, FGF23 causes a decrease in the expression of these channels. (Hanna RM, Ahdoot RS and al.,2022)

The effect of vitamin D on bone tissue

Calcium, phosphorus and vitamin D are important molecules for bone metabolism and health. Calcium is one of the most abundant minerals in the body and is obtained entirely from dietary sources. In addition to its various biological effects in the body, it is also essential for bone metabolism. More than 99% of the total body calcium is found in the bone tissue as a calcium-phosphate mineral complex, while the remaining <1% is distributed between the intracellular and extracellular compartments. While 40% of calcium outside bone tissue is bound to protein, 9% forms ionic complexes, and the remaining 51% is found as free ions. Ionized calcium balances the calcium pool in the intracellular-extracellular area and plays an important role in bone metabolism (Abseyi SN, Şıklar.2023) This balance is provided by the cooperation of various hormones (PTH, vitamin D) and the organs they affect (kidney, bone and intestinal system). Where there is vitamin D deficiency (nutritional or genetic) or VDR-inactivating mutations, serum levels of calcium and phosphate, which play an important role in bone development and growth, are reduced and thus rickets/osteomalacia emerge. Rickets is a disease characterized by excessive osteoid tissue accumulation and defective mineralization of the epiphyseal plate, which occurs as a result of insufficient mineralization in the epiphyseal plates of growing bones. Osteomalacia is a disease characterized by a deterioration in the mineralization of the newly formed osteoid and a decrease in bone turnover. (Ross AC, Taylor CL, Yaktine AL and al,2011)

There is a continuous remodeling cycle consisting bone tissue resorption and mineralization. When calcium, phosphorus, and vitamin D are sufficient, this cycle continues in a balanced manner. In the case of negative calcium balance caused by insufficient calcium intake with diet or increased renal calcium loss, vitamin D increases bone resorption in osteoblasts through VDR signaling, resulting in calcium passage from bone to blood, which leads to impaired bone mineralization. (Wacker M, Holick MF.2013)

8).Vitamin D toxicity

Vitamin D displays a wide therapeutic window, with toxicity being observed only at extremely high doses (150-200ng/ml). As such, the number of cases of vitamin D intoxication reported is low but affects all age groups, hypercalcemia appears when serum 25(OH)D3 concentration is higher than 150 ng/mL (375 nmol/L). As such, a concentration of 100 ng/mL is accepted by the Endocrine Society as posing no risk of developing hypercalcemia (Parvaiz A,Sheikh Hilal A, Feroze A and al.,2011), Vitamin D toxicity is usually iatrogenic or due to accidental overdose. Supplements containing vitamin D are now easily obtainable over the counter in pharmacies, grocery and food markets, and other retail stores. Some of these supplements exists as unregulated or unlicensed formulations. at times many cases of vitamin D intoxication result from doses of vitamin D prescribed far above the permissible limits. These factors together with the lack of public education for safe dosing has likely contributed to increasing reported cases of vitamin D toxicity.(Lim, K., & Thadhani, R.2020).

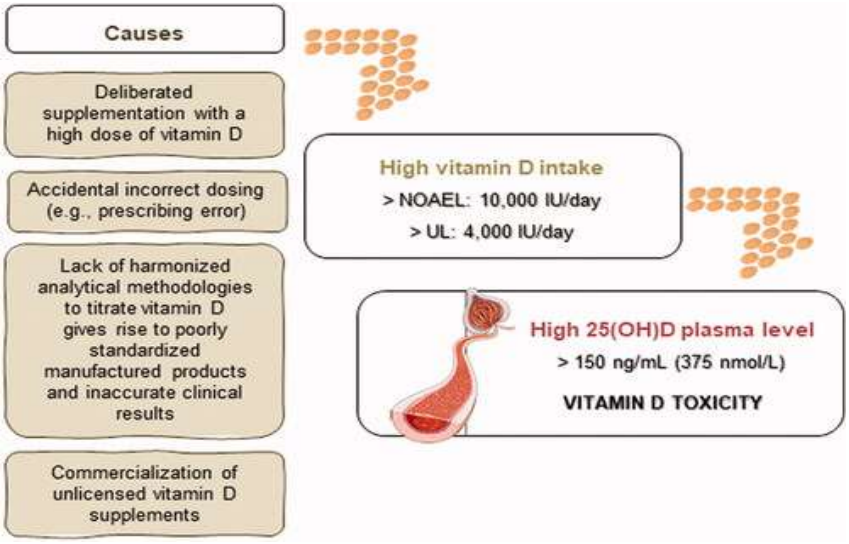

Figure (16) Vitamin D overdose (Jiří J and al.,2022)

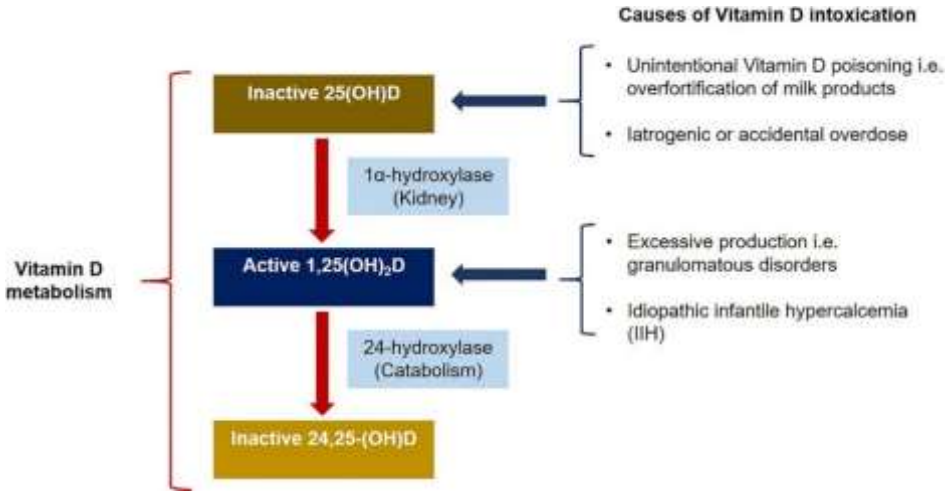

Figure (17) Causes and metabolism of vitamin D intoxication (Lim K, Thadhani R.2020)

Certain diseases make patients more prone to vitamin D toxicity. Individuals suffering from idiopathic infantile hypercalcemia, lymphoma, and granulomatous disorders such as sarcoidosis, tuberculosis, leprosy, fungal diseases, infantile subcutaneous fat necrosis, giant cell polymyositis,and berylliosis are hypersensitive to vitamin D increases both from exogenous sources or endogenous synthesis. In granulomatous diseases, hypervitaminosis D and hypercalcemia are the results of abnormal local synthesis of calcitriol in macrophages. The rise in the active form of vitamin D in idiopathic infantile hypercalcemia patients is related to the malfunction of deactivating enzyme CYP24A1, while in patients with lymphoma (Marcinowska-Suchowierska, Ewa and al.,2018)

8).1.Clinical manifestations

Clinical manifestations of vitamin D toxicity are varied, but largely related to hypercalcemia and include neuropsychiatric (such as confusion, psychosis, stupor, or coma), gastrointestinal (abdominal pain, vomiting, polydipsia, anorexia, constipation, pancreatitis), cardiovascular (hypertension, shortened QT interval, ST segment elevation, bradyarrhythmias, first degree heart block), and renal (hypercalciuria, acute kidney injury (AKI), dehydration and nephrocalcinosis) complications. Additional complications of hypercalcemia include band keratopathy, hearing loss, and painful periarticular calcinosis(Lim, K., & Thadhani, R.2020). An excessive high intake of vitamin D2/3 is correlated to increased 25(OH)D in the blood, which may lead to augmented calcium absorption by the gut and bone resorption. For these reasons, the main conditions associated with hypervitaminosis D are hypercalcemia and hypercalciuria (Tebben, Peter J and al.,2016)

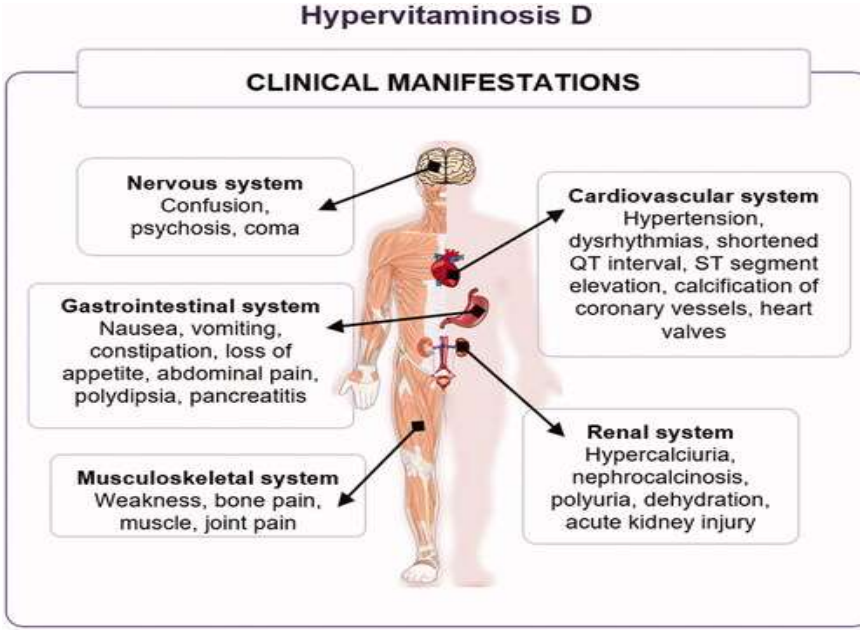

Figure (18) Clinical manifestations of hypervitaminosis D (Jiří J and al.,2022)

8).2.Mechanisms of toxicity

There are three major theories to explain the mechanisms underlying vitamin D toxicity. All of these theories are related to high plasma concentrations of vitamin D metabolites and the activation of nuclear VDR in the target cells, stimulating transcriptional machinery. (Anderson, P H and al.,2003)

The first mechanism proposed for explaining vitamin D toxicity involves a plasma increase in calcitriol [1,25(OH)2D]. This active form of vitamin D has low affinity to the vitamin D binding protein (vDBP) and high affinity to the vitamin D receptor (VDR), leading to a critical increase in calcitriol in the target cells and subsequent overstimulation of the gene expression machinery. A second theory proposes an increase in plasma vitamin D metabolites to concentrations that saturate vDBP, allowing high free levels of these metabolites to enter the target cells, in particular 25-hydroxyvitamin D [25(OH)D] that has a greater affinity to VDR. The last mechanism is related to the release of calcitriol from vDBP because it has the lowest affinity for this plasma protein compared to other vitamin D metabolites. (Tebben, Peter J and al.,2016)

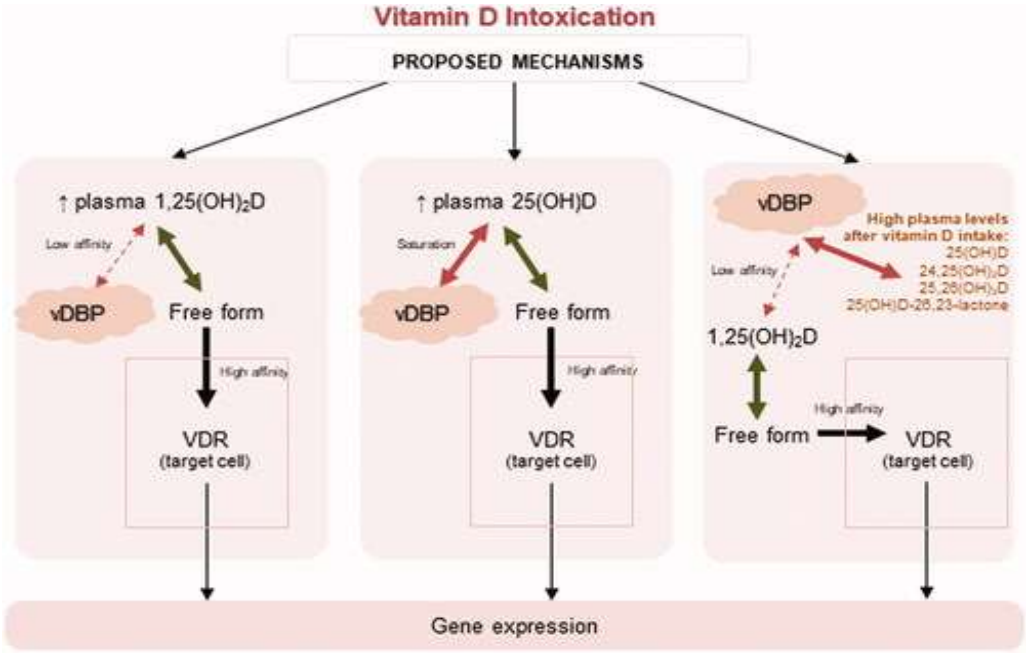

Figure (19) Theories proposed by Jones et al. (Jiří J and al.,2022)

The first theory involves an increase in plasma calcitriol concentration with a subsequent increase in the target cells, for instance, due to the inability to suppress the 1-hydroxylase in response to high 25(OH)D levels. Calcitriol has low affinity to the transport protein, vDBP, and high affinity to VDR, leading to critical overstimulation of the gene expression machinery. This hypothesis appears to be the most probable for explaining vitamin D toxicity in patients with already elevated plasma calcitriol levels (e.g. certain granulomatous disorders with unregulated 1-hydroxylase). Of note, disturbances in the calcitriol catabolic system (e.g. genetic defects in 24-hydroxylase) also make certain individuals particularly susceptible to vitamin D toxicity.

The free calcitriol was responsible for toxicity, despite no elevated plasma concentration of the active metabolite being observed in their cases of vitamin D toxicity, in line with other human and animal data. (de Brito Galvao, Joao F and al.,2013)

The second theory postulates the increased plasma levels of vitamin D metabolites following vitamin D intoxication, especially 25(OH)D, to concentrations that saturate vDBP, allowing high levels of free 25(OH)D to enter the target cells. Compared to others, this metabolite has a greater affinity to VDR, stimulating gene expression in a concentration-dependent manner

The last hypothesis is related to the presence of vitamin D and metabolites at levels so high that vDBP is saturated. Of note, in such a case, calcitriol is released from vDBP due to its lower affinity for this protein compared to other vitamin D metabolites, including 25(OH)D, 24,25(OH)2D, 25,26(OH)2D, and 25(OH)D-26,23-lactone, or even vitamin D itself, which will be found at higher concentrations after vitamin D intake. (Ramasamy, Indra.2020) The active metabolite is then free to enter cells and bind to VDR. Nevertheless, Deluca et al.reported that both wild-type controls and 1-hydroxylase knockout mice (i.e. unable to produce calcitriol) suffered from vitamin D toxicity after a high dose of vitamin D, suggesting that other vitamin D metabolites may also contribute to hypercalcemia. In fact, VDDR type 1 patients respond therapeutically to extreme doses of 25(OH)D (Bikle DD.2021)

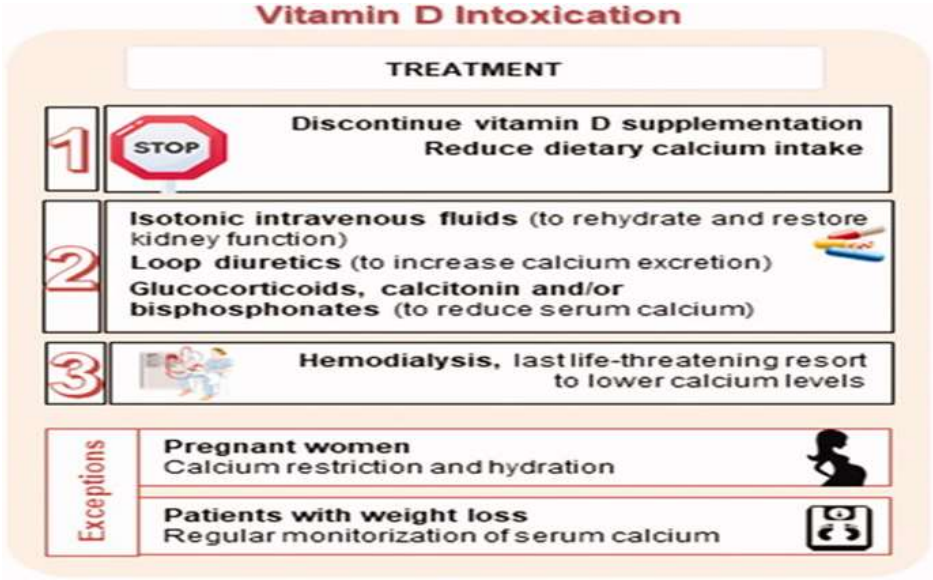

Figure (20) Treatment approaches for hypervitaminosis D. (Jiří J and al.,2022)

8).3.Treatment of intoxication

The main goal of treatment during vitamin D toxicity is emergent resuscitation in an unstable patient and correction of hypercalcemia. Hypercalcemia due to vitamin D overdose theoretically can last up to 18 months following discontinuation of administration. This is due to the slow release of stored vitamin D from fat deposits. Therefore, sustaining normocalcemia is just as pivotal as acute treatment of hypercalcemia.(Lim, K., & Thadhani, R.2020). Additionally, vitamin D2 or D3 has a high lipid solubility in liver, muscles, and fat tissues and

a long half-life in the body. However, 25(OH)D and 1,25(OH)2D have shorter half-lives at 15 days and 15 hours, respectively. Therefore, overdose of 25(OH)D can persist for weeks. (Jones, K S and al.2014)

As a first-line approach, the patient should stop taking vitamin D and reduce calcium intake from the diet. For granulomatous disorders, lymphoma, and idiopathic intracranial hypertension patients, sunlight and other UV-B light exposures are not recommended. (Gianella, Fabiola and al.,2020)

To treat hypercalcemia, isotonic intravenous fluids to correct dehydration and restore kidney function should be considered. Furthermore, loop diuretics (e.g. furosemide) can be added to increase calcium excretion; however, this approach has its limitations due to potential adverse reactions.(Khoury, N., & Carmichael, K. A.2011) Therapy with glucocorticoids (e.g. prednisone) can also be successfully applied to reduce serum calcium levels. These steroid hormones prevent active reabsorption of calcium in the kidneys and also alter vitamin D metabolism, favoring the synthesis of inactive metabolites, which lowers plasma calcitriol concentration and consequently reduces intestinal calcium absorption. Nevertheless, it should be noted that chronic glucocorticoid treatments are also associated with adverse effects, such as secondary osteoporosis, osteonecrosis, and muscle weakness, among others. Calcitonin and bisphosphonate therapies (e.g. pamidronate and alendronate) can be useful in severe cases to reduce calcium serum levels by inhibiting bone resorption. In some reports, bisphosphonates are described as the most effective treatment of vitamin D toxicity in children. As a last resort, when no other treatment has been successful, hemodialysis can be used to rapidly lower calcium levels. (Homik, J and al.2000)

9).Correlation between Zinc, Magnesium and Vitamin K Supplementation in Vitamin D Deficiency:

Zinc, magnesium, and vitamin K are important nutrients for humans. There are various factors that contribute to the development of their deficiency, which might result in or exacerbate various diseases. These nutrients can also interact with vitamin D metabolism and activity. (Bleizgys A.2024)

It is well known that the metabolism and the levels of different VitD metabolites, in particular the calcitriol levels (calcitriol = active or hormonic form of VitD) are influenced by numerous substances parathormone (PTH), fibroblast growth factor-23, serum calcium (Ca) levels, magnesium (Mg), etc. Interestingly, zinc (Zn) and vitamin K (VitK) can also have some "crosstalk" with Vitamin D. (de Brito Galvao JF, Nagode LA, Schenck PA, Chew DJ.2013)

Nonetheless, it could be speculated that the undervalued (unsuspected, undiagnosed, and, consequently, untreated) insufficiency of nutrients like Mg, Zn, and, to some degree, VitK could weaken the action of calcitriol in various organs and tissues. This may also explain why many clinical trials did not show the beneficial outcomes that had been initially presumed, and, in some cases, why adverse reactions (e.g., calcification of soft tissues) developed after supplementation with VitD. Of note, the deficiency of various nutrients (including Zn, VitK and Mg), also called "the hidden hunger" is widespread in many countries and might lead to serious illnesses. (Bleizgys A.2024)

Vitamin D and Magnesium

An adult body contains approximately 25 g magnesium, with 50% to 60% present in the bones and the rest in soft tissues. Less than 1% of total magnesium is available in the blood serum (Nasr-Eddine K, Touria Z.2021) ,Mg functions as a structural or catalytic component of enzymes, as well as of substrates, in hundreds of enzymatic processes. It is involved in numerous processes ion channel activity and signal transduction, stabilization of membranes, aerobic and anaerobic metabolism, proliferation, differentiation and apoptosis of cells, and angiogenesis. The potentially beneficial impact of Mg on glucose and lipid metabolism, innate and adaptive immunity, and the nervous system deserves particular attention (Li X, Yang Y, Zhang B and al.,2022). A deficiency of Mg is associated with headaches, hyperemotionality, generalized anxiety, insomnia, asthenia, depressive states, muscle weakness, numbness and cramps, exacerbations of bronchial asthma, increased risk of stroke, progression of diabetes mellitus and congestive heart failure, worse control of arterial hypertension, higher risk of cardiac arrythmias, alterations in blood lipids and progression of atherosclerosis, severe forms of infectious diseases, long-term COVID-19 syndrome, increased risk of cancers, and many other illnesses. (Matias, Patricia and al.,2023)

All enzymes in the metabolism of Vitamin D appear to be dependent on magnesium, which acts as a co-factor in enzymatic reactions occurring in the liver and kidneys (Nasr-Eddine K, Yahia B, Wassila C.2024) such as VitD binding to VitD-binding protein, 25OH-D synthesis, calcitriol synthesis, activity of 24-hydroxylase (i.e., the enzyme, deactivating 25OH-D and calcitriol), and VitD receptor (VDR) expression for cellular effects. Low Mg levels enhance the release of PTH, but very low Mg concentrations suppress the secretion of PTH. Thus, indirectly, severe Mg deficiency could also contribute to reduced renal synthesis of calcitriol, since PTH is known to stimulate renal 1α-hydroxylase. Mg deficiency notably influences the VitD levels in people with a high risk of low VitD, such as women, non-Hispanic African Americans, obese people, or persons with the highest levels of PTH in the blood. (Glenville J and al., 1998)

On the other hand, VitD could itself impact Mg metabolism. The majority of Mg is absorbed regardless of VDR or VitD; nevertheless, calcitriol can increase Mg absorption in the gut by upregulating intestinal VDR, and this contributes to maintaining Mg homeostasis. Interestingly, VitD can increase renal Mg excretion and therefore decrease Mg retention; possibly, this is realized via PTH suppression (by calcitriol), since PTH contributes to renal Mg reabsorption. (Matias P, Ávila G and al.,2023), The beneficial effects of ingestion or magnesium status on a multitude of metabolic disorders can be explained by several mechanisms, including improvement of glucose and insulin homeostasis, oxidative stress, lipid metabolism, vascular or myocardial contractility, endothelium-dependent vasodilatation, anti-arrhythmic effects, coagulant or antiplatelet and anti inflammatory effects (Nasr Eddine K, Touria Z, Meghit Boumediene K and Mustapha D.2021)

Vitamin D and Zinc

After iron, Zn is the second most common trace mineral in the body. The body has 2–4 g of Zn, of which approximately 90% is located in the bones and muscles. Only 0.1% of the total Zn is present in plasma, where it is primarily bound to albumin, and in a very small quantity, bound to metallothionein and transferrin (Bleizgys A.2024)

Zn is crucial for body growth, development, and functioning. Zn is a part of numerous proteins, over 2500 transcription factors, and over 600 enzymes. The roles of Zn in the human body can be grouped into three general functional classes: structural (component of proteins), catalytic, and regulatory. As a catalytic factor, Zn acts in enzymes from six main classes: oxidoreductases (dehydrogenases), transferases, hydrolases, lyases, isomerases, and ligases; Zn is also an important signaling mediator in the endocrine, paracrine, and autocrine systems. Zn is essential in lipid, carbohydrate, and protein metabolism, in particular for the regular synthesis, storage, and secretion of insulin within pancreatic beta cells, as well as for hepatic insulin clearance and insulin sensitivity. (Maares M, Haase H.2020)

It appears that Zinc homeostasis and Vitamin D functioning are linked. Zinc can regulate the expression of vitamin D-dependent genes via contributing to VDR conformational changes and intensifying the activity of specific vitamin D-dependent promoters; therefore, Zinc is considered an essential cofactor for Vitamin D activity. On the other hand, VitaminD can directly augment the expression of Zn transporters, such as ZnT10; an upregulation of the ZnT10 protein allows Zinc to migrate out of the cytosol, and increased concentrations can be available for extracellular use. However, it was also hypothesized that, by improving the intracellular Zn concentrations, Vitamin D can mitigate oxidative damage; and increased intracellular oxidative stress is known to contribute to the development of many pathologies, such as cardiovascular disease, neurological disorders, cancer, diabetes mellitus or ischemia. (Bleizgys A.2024)

Vitamin D and Vitamin K

VitK, a fat-soluble vitamin, is actually a group of molecules that share a common methylated naphthoquinone ring but with different side chains. Naturally, VitK exists in two main forms: K1 (=phylloquinone) and K2 (=menaquinones). Vitamin K2 (VK2) comprises a collection of different compounds known as menaquinone-n (MK-n), with variation in the length of the unsaturated isoprene side chain in the molecule (n means the number of isoprene units), ranging from MK-2 to MK-15; MK-4, MK-7, and MK-9 are the most studied menaquinones. Vitamin K3 (menadione) is a synthetic water-soluble form of the vitamin and is converted into VK2 by the liver. Vitamin K3 is primarily used for industrial and research purposes. Other VitK forms K4 and K5 also exist, but they are only available in a synthetic form. (Marta Z,Beata S,Krystyna P.2021)

Vitamin D appears to have some synergistic effects with Vitamin K. Calcitriol upregulates MGP and OC expression, and Vitamin K is required for the proper γ-carboxylation of these proteins. Another area of Vitamin D and Vitamin K cooperation is inflammation, since both vitamins are known to decrease the production of certain proinflammatory cytokines. (Shea MK and al.,2008). In addition, Vitamin D and Vitamin K can cooperate in preventing arterial stiffness and vascular calcification. (van Ballegooijen AJ and al.2017), Moreover,Vitamin K supplementation may offer a defense against any unfavorable consequences of Ca supplementation. In summary, Vitamin D and Vitamin K cooperation is useful in combating the “calcium paradox”, the resultant increase in arterial calcification and decrease in the Ca content of bone when Ca metabolism is impaired. Of note, if was suggested that excessive amounts of Vitamin D can augment Vitamin K requirements, since direct stimulation of the synthesis of VKDPs can lead to a relative Vitamin K deficiency, Interestingly, Vitamin K and Vitamin D can have some metabolic overlap at the cellular level. SXR, which can be activated

by VK2, is able to crosstalk with VDR, and this SXR–VDR interaction can suppress the activity of the VDR-mediated CYP24 promoter; this results in decreased CYP24-mediated hydroxylation, i.e., reduced catabolism of calcitriol (and also reduced deactivation of 25OH-D). On the other hand, calcitriol can enhance the reductive recycling of VK2. Therefore, Vitamin K and Vitamin D can mutually intensify each other's metabolism. (Ziemińska M, Sieklucka B, Pawlak K.2021)

A plethora of risk factors were shown to contribute to the development of various deficiencies. the main risk factors regarding Mg, Zn and VitK deficiency. It appears that these deficiencies share many common risk factors with low VitD status, e.g., intestinal malabsorption, unhealthy diet, aging, or increased requirements for certain nutrients during some periods of life (Ghada E, Maivel E and al.,2024), However, in clinical practice, some nutrient deficiencies, particularly in their mild forms, may remain undiagnosed and this may lead to undesirable results, such as worsening or progression of certain chronic diseases. (Bleizgys, Andrius.2024)

Overall, from the clinical point of view, Vitamin D appears to have a beneficial synergism with the studied nutrients. Therefore, once low Vitamin D is diagnosed, searching for possible deficiencies of other nutrients might be useful. In case the assessment of the Mg, Zn or VitK status is not currently available but the deficiency of one or several nutrients is highly possible, for some patients it might be reasonable to recommend additional supplementation with prophylactic doses of the nutrients.(Bleizgys A.2024), For adults, it might be 300–400 mg of Mg, 15–20 mg of Zn, and/or 90–120 μg of VitK. These doses would be considered "safe" for many patients. The rationality behind similar recommendations is obvious: since a patient is highly suspected to have a deficiency of one or several nutrients, the current nutrient status is expected to improve with the help of appropriate supplementation. This amelioration of the nutrient status likely results in the improvement of the patient's health, e.g., in better control of chronic disease(s), and in a lower burden for healthcare systems. (Kiani AK, Dhuli K, Donato K and al.,2022)

A higher increase in the serum 25OH-D levels might also appear, but this should not be the main target when supplementation with Mg, Zn or VitK is started. One of the main goals of this supplementation, in particular when it is combined with VitD supplementation, should be to achieve higher intracellular levels of calcitriol and to increase the activity of calcitriol in various cells, expecting the improvement of many physiological processes; the latter does not necessary correlate with better serum 25OH-D levels. (Máčová L, Bičíková M.2021)

10).Vitamin D blockers

Drug interactions

Vitamin D supplements may interact with several types of medications, Corticosteroids can reduce calcium absorption which results in impaired vitamin D metabolism, Since vitamin D is fat soluble, Orlistat and Cholestyramine can reduce its absorption and should be taken several hours apart from it. Phenobarbital and phenytoin increase the hepatic metabolism of vitamin D to inactive compounds and decrease calcium absorption, which also impairs vitamin D metabolism. (Robien K, Oppeneer SJ and al.,2013)

Drugs that Deplete vitamin D

Inhalant, Systemic, and Topical Corticosteroids

1. Beclomethasone
2. Budesonide
3. Dexamethasone
4. Fluticasone
5. Hydrocortisone
6. Methylprednisolone
7. Mometasone Furoate
8. Prednisone
9. Triamcinolone

Anti-inflammatory Medications

- Inhalant, Systemic, and Topical Corticosteroids

Antibiotic Medications

- Antituberculosis Agents

Anticonvulsant Medications

- Barbiturates
- Hydantoin Derivatives

Cholesterol-Lowering Medications

- Bile Acid Sequestrants

Laxatives

- Lubricant Laxatives

Ulcer Medications

- Histamine H2 Antagonists

Antacids, Taking certain antacids for long periods of time may alter the levels, metabolism, and availability of vitamin D.

Antiseizure medications. These medications include:

1. Phenobarbital
2. Phenytoin (Dilantin)
3. Primidone (Mysoline)
4. Valproic acid (Depakote)

Bile acid sequestrants. Used to lower cholesterol. These medications include:

1. Cholestyramine (Questran, Prevalite)
2. Cholestipol (Colestid)

Rifampin. Used to treat tuberculosis.

Mineral oil. Mineral oil also interferes with absorption of vitamin D.

Orlistat (Alli). A medication used for weight loss that prevents your body from absorbing fat. Because of how it works, orlistat may also prevent the absorption of fat-soluble vitamins, such as vitamin D. (Efird JT, Anderson EJ, Jindal C and al.2021)

11).Clinical benefits of vitamin D

Cancer

Vitamin D decreases cell proliferation and increases cell differentiation, stops the growth of new blood vessels, and has significant anti-inflammatory effects. Many studies have suggested a link between low vitamin D levels and an increased risk of cancer, with the strongest evidence for colorectal cancer. In the Health Professionals Follow-up Study (HPFS), subjects with high vitamin D concentrations were half as likely to be diagnosed with colon cancer as those with low concentrations. A definitive conclusion cannot yet be made about the association between vitamin D concentration and cancer risk, but results from many studies are promising. ernández-Barral A, Bustamante-Madrid P and al.,2021)

Heart disease

Several studies are providing evidence that the protective effect of vitamin D on the heart could be via the renin–angiotensin hormone system, through the suppression of inflammation, or directly on the cells of the heart and blood-vessel walls.In the Framingham Heart Study, patients with low vitamin D concentrations (30 ng/mL). (Mozos I, Marginean O.2015)

Hypertension

The third National Health and Nutrition Examination Survey (NHANES-III),which is representative of the noninstitutionalized US civilian population, showed that systolic blood pressure and pulse pressure were inversely and significantly correlated with 25(OH)D levels among 12,644 participants. Age-associated increase in systolic blood pressure was significantly lower in individuals with vitamin D sufficiency.The prevalence of arterial hypertension was also associated with reduced serum 25(OH)D levels in 4030 participants of the German National Interview and Examination Survey,in 6810 participants of the 1958 British Birth Cohort,and in other study populations. (Robert S, MaryFran S, Colin B,2007)

Obesity

Low concentrations of circulating vitamin D are common with obesity and may represent a potential mechanism explaining the elevated risk of certain cancers and cardiovascular outcomes. Levels of 25(OH)D are inversely associated with BMI, waist circumference, and body fat but are positively associated with age, lean body mass, and vitamin D intake. The prevalence of VDD is higher in black versus white children regardless of season predictors of VDD in children include black race, female sex, pre-pubertal status, and winter/ spring season.Weight loss is associated with an increase in 25(OH)D levels among postmenopausal overweight or obese women. (Mason C, Xiao L, Imayama I and al.2011)

Type 2 diabetes

A trial of nondiabetic patients aged 65 years and older found that those who received 700 IU of vitamin D (plus calcium) had a smaller rise in fasting plasma glucose over 3 years versus those who received placebo. A correlation between vitamin D and the risk diabetes can be ruled in from the results. (Pittas AG, Harris SS, Stark PC, Dawson-Hughes B.2007)

Depression

A Norwegian trial of overweight subjects showed that those receiving a high dose of vitamin D (20,000 or 40,000 IU weekly) had a significant improvement in depressive symptom scale scores after 1 year versus those receiving placebo. The result determines a correlation between vitamin D and the risk of depression. (Menon V, Kar SK, Suthar N, Nebhinani N.2020)

Cognitive impairment

In the Invecchiare in Chianti (InCHIANTI) Italian population based study, low levels of vitamin D were associated with substantial cognitive decline in the elderly population studied during a 6-year period. Low levels of 25(OH)D may be especially harmful to executive functions, whereas memory and other cognitive domains may be relatively preserved. (lewellyn DJ, Lang IA, Langa KM and al.2010)

Parkinson's disease

Parkinson's disease is a major cause of disability in the elderly population. Unfortunately, risk factors for this disease are relatively unknown. Recently, it has been suggested that chronically inadequate vitamin D intake may play a significant role in the pathogenesis of Parkinson's disease. A cohort study based on the Mini-Finland Health Survey demonstrated that low vitamin D levels may predict the development of Parkinson's disease. (Agim ZS, Cannon JR.2015)

Fractures and falls

Vitamin D is known to help the body absorb calcium, and it plays a role in bone health. In addition, VDRs are located on the fast-twitch muscle fibers, which are the first to respond in a fall. It is theorized that vitamin D may increase muscle strength, thereby preventing falls.Many studies have shown an association between low vitamin D concentrations and an increased risk of fractures and falls in older adults (Bischoff-Ferrari HA, Shao A, Dawson-Hughes B, Hathcock J, Giovannucci E, Willett WC.2010). A combined analysis of 12 fracture-prevention trials found that supplementation with about 800 IU of vitamin D per day reduced hip and nonspinal fractures by about 20%, and that supplementation with about 400 IU per day showed no benefit.Researchers at the Jean Mayer USDA Human Nutrition Research Center on Aging at Tufts University have examined the best trials of vitamin D versus placebo for falls. Their conclusion is that "fall risk reduction begins at 700 IU and increases progressively with higher doses. (Nair R, Maseeh A.2012)

Autoimmune diseases

VDD can contribute to autoimmune diseases such as multiple sclerosis (MS), type 1 diabetes, rheumatoid arthritis, and autoimmune thyroid disease.A prospective study of white subjects found that those with the highest vitamin D concentrations had a 62% lower risk of developing MS versus those with the lowest concentrations. A Finnish study that followed children from birth noted that those given vitamin D supplements during infancy had a nearly 90% lower risk of developing type 1 diabetes compared with children who did not receive supplements. (Fletcher J, Bishop EL, Harrison SR and al.2022)

Chapter III : Vitamin D deficiency

1).Definition of Vitamin D Deficiency

A significant debate in the field of vitamin D revolves around the precise definition of hypovitaminosis. The specific blood level of 25(OH)D that constitutes a deficiency is still a subject of controversy. This ongoing debate is evident in the varying recommendations provided by different authorities, including European bodies, the Institute of Medicine (IOM), and the Endocrine Society. The definitions of vitamin D deficiency set by the Scientific Advisory Committee on Nutrition (SACN), IOM, European Food Safety Authority (EFSA), Endocrine Practice Guidelines, and the Australian Working Group are discussed in **Table (02)**

Table (02) : Recommended serum levels for 25(OH)D. (Ramasamy I.2020)

	ESPG	SACN	IOM	EFSA	Australian Working Group
Vitamin D deficiency	<50 nmol/L (<20 ng/mL)	<25 nmol/L (<10 ng/mL)	Persons are at risk of deficiency relative to bone health at serum 25OHD levels <30 nmol/L (<12 ng/mL)		Severe <12.5 nmol/L (<5 ng/mL); Moderate 12.5–29 nmol/L (5–11.6 ng/mL)
Vitamin D insufficiency	52.5–72.5 nmol/L (21–29 ng/mL)		Some, but not all, persons are potentially at risk of inadequacy at serum 25OHD levels 30–50 nmol/L (12–20 ng/mL)		30–49 nmol/L (12–19.6 ng/mL) Mild deficiency
Sufficient	75–250 nmol/L (30–100 ng/mL)		50 nmol/L (20 ng/mL) (covers the requirements of 97.5% of population)*	≥50 nmol/L (≥20 ng/mL)	≥50 nmol/L (≥20 ng/mL) (at the end of winter)*†

Groups at risk of vitamin-D inadequacy

Obtaining sufficient vitamin D from natural food sources alone is difficult. Consumption of vitamin D-fortified foods and exposure to some sunlight are essential for maintaining a healthy vitamin D status. Dietary supplements might be required to meet the daily need for vitamin D in some group of people. (Poonam S.2024)

Breastfed infants

Vitamin D requirements cannot ordinarily be met by human milk alone, which provides <25 IU/L to 78 IU/L. Vitamin D content of human milk is related to the mother's vitamin D status; therefore mothers who supplement with high doses of vitamin D may have high levels of vitamin D in their milk. International associations of Paediatricians recommended that exclusively and partially breastfed infants must be supplemented with 400 IU of vitamin D per day, the recommended daily allowance for this nutrient during infancy. (Dawodu A, Salameh KM, Al-Janahi NS, Bener A, Elkum N.2019)

Older adults

Older adults are at high risk of developing vitamin D insufficiency because of aging. Their skin cannot synthesize vitamin D as efficiently, they are likely to spend more time indoors, and they may have inadequate intakes of the vitamin. (Boucher BJ.2012)

People with limited sun exposure

Homebound individuals, women who wear long robes and head coverings for religious reasons, and people with occupations that limit sun exposure are unlikely to obtain adequate vitamin D from sunlight. The significance of the role that sunscreen may play in reducing vitamin D synthesis is still unclear. Intake of RDA (recommended dietary allowance) levels of vitamin D from foods and/or supplements will provide adequate amounts of this nutrient to these individuals. (Nair R, Maseeh A.2012)

People with dark skin

Larger amounts of the pigment melanin in the epidermal layer result in darker skin and reduce the skin's ability to produce vitamin D from sunlight. It is not sure that lower levels of 25(OH)D for persons with dark skin have significant health consequences. Intake of RDA levels of vitamin D from foods and/or supplements will provide adequate amounts of this nutrient to these individuals. (Brenner M, Hearing VJ.2008)

People with fat malabsorption

Vitamin D is fat soluble, therefore it requires some dietary fat in the gut for absorption. Individuals with reduced ability to absorb dietary fat might require vitamin D supplements. Fat malabsorption is associated with a variety of medical conditions including some forms of liver disease, cystic fibrosis, and Crohn's disease. (Nair R, Maseeh A.2012)

People who are obese or who have undergone gastric bypass surgery

A BMI value of ≥30 is associated with lower serum 25(OH)D levels compared with nonobese individuals. Obese people may need larger than usual intakes of vitamin D to achieve 25(OH)D levels comparable to those of normal weight. Greater amounts of subcutaneous fat sequester (captivate) more of the vitamin and alter its release into the circulation. Individuals who have undergone gastric bypass surgery may become vitamin D deficient over time without a sufficient intake of vitamin D from food or supplements; moreover part of the upper small intestine where vitamin D is absorbed is bypassed. (Dos Santos MTA, Suano-Souza FI and al.2018)

2).Vitamin D deficiency Consequences

VDD results in abnormalities in calcium, phosphorus, and bone metabolism. VDD causes a decrease in the absorption of dietary calcium and phosphorus, resulting in an increase in PTH levels. The PTH-mediated increase in osteoclastic activity creates local foci of bone weakness and causes a generalized decrease in bone mineral density (BMD), resulting in osteopenia and osteoporosis. An inadequate calcium–phosphorus product causes a mineralization defect in the skeleton. In young children who have little mineral in their skeleton, this defect results in a variety of skeletal deformities classically known as rickets. VDD also causes muscle weakness; affected children have difficulty in standing and walking, whereas the elderly have increasing sway and more frequent falls, thereby increasing their risk of fracture. (Nair R, Maseeh A.2012)

3).Vitamin D hypothalamic-pituitary-adrenal (HPA) axis and depression

The hypothalamic-pituitary-adrenal (HPA) axis plays an important role in the body's adaptation to stressful situations. HPA axis malfunction occurs in many mental diseases, including depression and schizophrenia. A relationship has been shown between disorders caused by stressful stimuli, especially long-term ones, and depression. (Murphy F, Nasa A, Cullinane D and al.2022)

The HPA axis plays a key role in maintaining body homeostasis and the body's response to stress. Stress results in the release of corticoliberin-releasing hormone (CRH) from the hypothalamus. This information is then transmitted to the anterior lobe of the pituitary gland, where the secretion of adrenocorticotropic hormone (ACTH) takes place. This leads to stimulation of cortisol release into the blood from the adrenal cortex . Increased cortisol level plays a negative feedback that leads to inhibition of CRH and ACTH secretion. (Mikulska J, Juszczyk G, Gawrońska-Grzywacz M, Herbet M.2021)

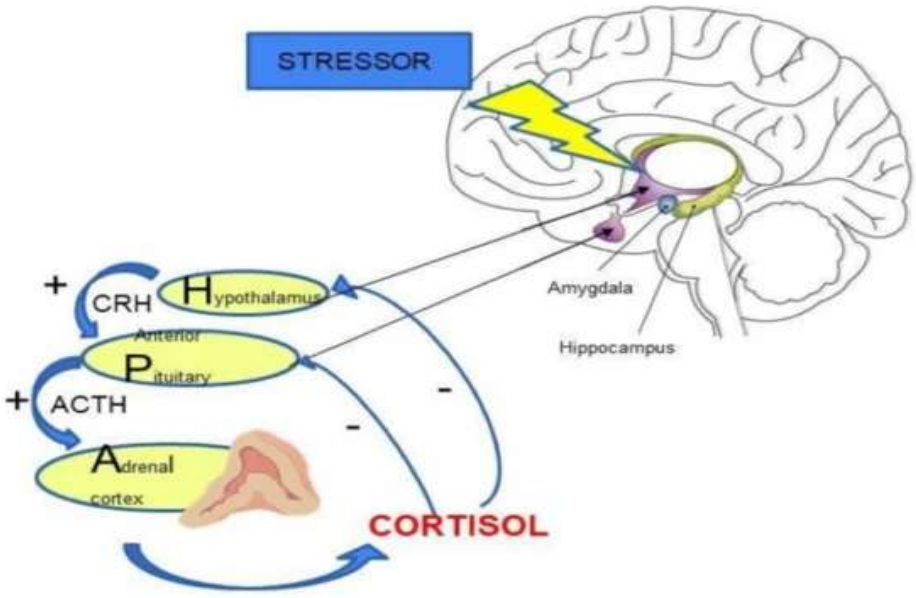

Figure (21) Regulation of hypothalamic-pituitary-adrenal (HPA) axis activity: stress as a factor activating the HPA axis. (Mikulska, Joanna and al.,2021)

The importance of the HPA axis is mainly based on the action of cortisol . Cortisol is secreted in stressful situations as a defense response of the body. It reduces the inflammatory response, stimulates gluconeogenesis and is responsible for protecting the body from an excessive immune response. Activation of the HPA axis also occurs in non-stress-related situations. This is related to the regulation of circadian rhythms, e.g., the highest cortisol levels are observed in the morning (Herman JP, McKlveen JM, Ghosal S and al.,2016)
Further more ,vitamin D modulates the hypothalamic-pituitary-adrenal axis, which regulates the production of the monoamine neurotransmitters epinephrine, norepinephrine, and dopamine in the adrenal cortex and also protects against the depletion of dopamine and serotonin. (Stephens MA, Wand G.2012)

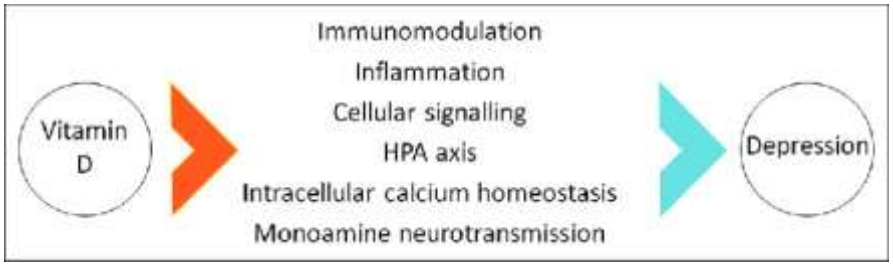

Figure (22) Postulated biological links between vitamin D and depression-HPA (Menon, Vikas and al.,2020)

Vitamin D receptors and vitamin D metabolizing enzymes are present in the central nervous system. Calcitriol (the active vitamin D hormone) affects numerous neurotransmitters and neurotrophic factors, relevant for mental disorders. (Humble MB.2010)
The active form of vitamin D, calcitriol (1,25(OH)2-vitamin D), is a seco-steroid (steroid molecule where one of the connected rings is cut open) with potent endocrine, paracrine and autocrine effects, induced by binding to its specific ligand, the vitamin D receptor (VDR). Like other hormones with nuclear receptors, it affects the gene expression of a multitude of target genes.Concerning neurotransmitters, calcitriol activates the gene expression of the enzyme tyrosine hydroxylase (which is considered the rate-limiting step in the synthesis of the catecholamines), thereby increasing the availability of dopamine, noradrenaline and adrenaline. Also, calcitriol may enhance cholinergic function, both by increasing the activity of choline acetyltransferase (the key enzyme for acetylcholine synthesis) and by decreasing the activity of acetylcholine esterase (the enzyme that limits acetylcholine synapse transmission). Dopamine, noradrenaline and acetylcholine are well-known actors in the pathophysiology of e.g. mood disorders, attention deficit/hyperactivity disorders and Alzheimer's disease.(Norman AW, Nemere I, Zhou LX.1992)

The role for vitamin D within the CNS concerns its influence on several neurotrophic factors. Thus,calcitriol is a potent enhancer of nerve growth factor (NGF) and glial derived neurotrophic factor (GDNF). Also, it increases neurotrophin3(NT-3) and decreases neurotrophin 4 (NT-4) activity (Blais M, Lévesque P, Bellenfant S, Berthod F.2013). NGF is important for the developing brain prenatally, but is also believed to counteract degeneration of the cholinergic system in Alzheimer's disease . In psychiatry, much research has focussed on brain derived neurotrophic factor (BDNF, not regulated by vitamin D), but recent research has shown that NGF, NT-3 and GDNF may also be involved in both depression and schizophrenia. In addition,

GDNF, strongly linked to dopaminergic functions, has a postulated therapeutic potential in Parkinson's disease as well as dependence disorders. (Neto FL, Borges G and al.,2011)

Vitamin D and VDR are widely distributed in the hypothalamus,pituitary and adrenal.The hypothalamus-pituitary-adrenal (HPA) axis is the center of balance, stress response, energy metabolism and neuropsychiatric function in the body . Hormones released by hypothalamus, pituitary gland and adrenal gland are corticotropin releasing factor (CRF), adrenocorticotropin (ACTH) and cortisol, respectively. The adrenal gland is the terminal effector organ of HPA axis, and the glucocorticoid secreted by adrenal is the key regulatory factors of the metabolism of sugar, fat and protein. It has been proved that HPA axis dysfunction played an important role in the occurrence and development of metabolic diseases. Additionally, the hyperfunction of the HPA axis is one of the common neurobiological manifestations of depression, which is a prominent feature of depression.therefore vitamin D is one of the antioxidant that can decrease the CRH levels consequently ACTH and cortisol levels that cause depression. (Cui X, Eyles DW.2022)

3).1.Vitamin D and dopamine

Vitamin D has been identified as a key factor in dopaminergic neurogenesis and differentiation, Consequently, developmental vitamin D (DVD) deficiency has been linked to disorders of abnormal dopamine signalling with a neurodevelopmental basis such as schizophrenia , vitamin D plays a major role as a mediator of dopaminergic development that it increases neurite outgrowth, neurite branching, presynaptic protein re-distribution, dopamine production and functional release in various in vitro models of developing dopaminergic cells, Mainly vitamin D increases the capacity of developing neurons to release dopamine. (Pertile R, Brigden R, Raman V, Cui X, Du Z, Eyles D.2023)

Vitamin D is a nuclear steroid. Its signaling is via a single nuclear receptor called the VDR which is expressed widely throughout the human and rat brain . The VDR shares structural characteristics with other nuclear steroid receptors. After ligand binding the VDR forms a heterodimer with the retinoid X receptor (RXR). This complex binds to vitamin D response elements (VDRE) in the promoters of a number of genes; to regulate their transcription . Expression of the VDR begins early in development and increasing levels of VDR coincide with increasing levels of apoptosis and decreasing levels of mitosis (Jimenez-Lara AM, Aranda A.1999). However, it is the coincident expression of the VDR within developing DA neurons and projections in the brain that suggest an important role for vitamin D in the developing DA system.

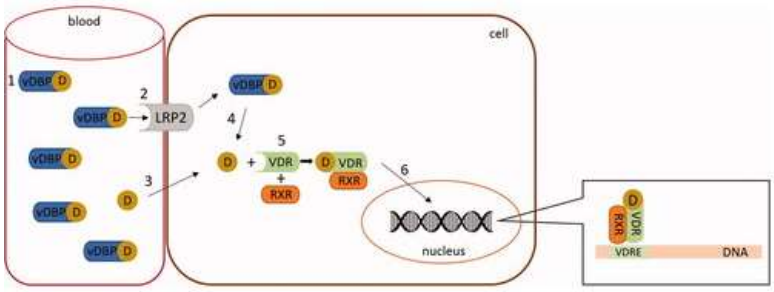

Figure (23) mechanism of vitamin D (calcitriol) action (Janoušek, Jiří and al.,2022)

vitamin D should be understood as its active form - calcitriol. The majority of circulating vitamin D is bound to vitamin D binding protein (vDBP) (1). This complex may only enter cells with the megalin/cubulin system (LRP2) (2). Free vitamin D can enter any cell through passive diffusion (3). vDBP-bound vitamin D is released inside the cells (4). In the cytoplasm, vitamin D interacts with its receptor (VDR) and creates a heterodimer with retinoid X receptor (RXR) (5). The active VDR complex enters the nucleus (6) and binds to the responsive elements (VDRE) of regulated genes. (Janoušek, Jiří, Pilarova and al.,2022)

Effects of Vitamin D on Dopamine Differentiation and Innervation

Expression of the VDR can be found as early as E12 in the neuroepithelium coinciding with the peak differentiation of monoamine cells in the substantia nigra; the primary source of midbrain dopaminergic projections to the basal ganglia. As dividing mesencephalic DA progenitor cells stop proliferating, they immediately begin to express specification factors [initially Nurr1 with p57Kip2 expressed soon after that help to establish the neurotransmitter phenotype of these cells. DVD-deficient E12 embryos show decreased gene expression of Nurr1, p57Kip2 and TH suggesting altered vitamin D signaling affects early monoamine cell development, perhaps even prior to E12. Not surprisingly, all three of these factors are linked and it would appear that Nurr1 is the upstream effector that results in altered p57Kip2 and TH levels. For example, Nurr1 has been shown to activate the expression of p57kip2 which then cooperates with Nurr1 in the maintenance of DA neurons . Moreover, Nurr1 has been shown to regulate important proteins in DA synthesis and function including TH, vesicular monoamine transporter 2 (VMAT2) and DAT . Thus a decrease in Nurr1 expression would be expected to result in decreased p57Kip2 and TH as found in the DVD-deficient embryo.(Maria TM ,Alves D, Marten P.2011)

Monoaminergic striatal innervation occurs from E14-17 with functional release observed at E18 . Consistent with the premise that vitamin D plays a role in dopaminergic cell development, VDR expression in the differentiating field of the midbrain and basal ganglia can be observed by E15. Furthermore, DVD-deficient embryos show decreased expression of Nurr1 at E15 (Gáll Z, Székely O.2015) Thus, the appearance of the nuclear expression of the VDR in the mesencephalon at the peak period of DA neuron differentiation raises the possibility that the absence of vitamin D at this point may lead to changes consistent with the absence of this ligand. Namely, increased rates of DA neuron proliferation and delayed differentiation. This is consistent with the reduction in the expression of post-mitotic specification factors such as Nurr1. Interestingly, although Nurr1 gene expression in the mesencephalon peaks from E13 to E15 the levels of Nurr1 in the developing rat cortex show a different temporal window of expression with peak protein levels occurring later . Whether the levels of cortical Nurr1 are decreased or delayed as observed in the mesencephalon of DVD-deficient rats is currently unknown. (Kesby JP, Cui X, Burne TH, Eyles DW.2013)

How the absence (or presence) of vitamin D could alter Nurr1 levels remains unknown. However, retinoid function and specifically the interactions of retinoid receptors and Nurr1 have led researchers to suggest that retinoid signaling may be one link between the genetic and environmental susceptibility to schizophrenia (García-Yagüe ÁJ, Cuadrado A.2023) . Both Nurr1 and the VDR form heterodimers with the RXR. Indeed, signaling through the RXR-Nurr1 heterodimer is involved in the neuroprotective actions of Nurr1 in DA neurons. However, in rat neural precursor cells the RXR-Nurr1 heterodimer has been shown to reduce Nurr1

activity in DA neuron generation and reduce TH promoter activity. It is important to note that levels of the VDR are unaltered in DVD-deficient pups allowing for ligand-independent actions. The interactions between, and functions of, the VDR and RXR appear to be extremely dependent on the presence of vitamin D. For example, the VDR-RXR heterodimer with no ligand acts as a weak transcriptional repressor. Furthermore, when no ligand is present the RXR increases the nuclear accumulation of VDR by slowing nuclear export whereas, when bound to vitamin D, the VDR regulates the import of the RXR into the nucleus . Thus the non-ligand bound VDR may lead to decreased levels of cytosolic VDR and reduced competition for RXR compared with Nurr1. This may lead to an increase in the inhibitory functions of the RXR-Nurr1 heterodimer on DA neuron generation . Decreased DA neuron generation would subsequently lead to reduced levels of Nurr1 and TH as found in DVD-deficient embryos. The presence and interaction of the VDR (minus ligand) on the availability or function of the RXR-Nurr1 heterodimer is unknown but this remains an intriguing target. (Haussler MR, Livingston S and al.,2020),In addition, Nurr1 expression induces the expression of the glial derived neurotrophic factor (GDNF) receptor, Ret, in adult nigral DA neurons. The decreased Nurr1 levels may therefore decrease GDNF signaling in DVD-deficient embryos. Moreover, vitamin D positively regulates GDNF levels in the developing mesencephalon. GDNF is an important factor involved in DA neuron development, survival and function. Thus, the combined local expression these three factors (VDR, RXR, and Nurr1) in addition to their cooperative signaling capabilities may be causal for the downstream effects of DVD-deficiency on DA neuron development. (Decressac M, Kadkhodaei B and al.,2012)

3).2.Vitamin D and Serotonin

vitamin D plays an important role in serotonin and melatonin regulation, which further indicates the relevance of vitamin D in mental health, especially the regulation of mood and sleep, vitamin D can influence serotonin regulation in the brain and in peripheral (non-brain) tissues. In peripheral tissues outside the blood–brain barrier, sufficient serum 25(OH)D levels, binding to vitamin D response elements (VDREs) on TPH genes, are proposed to inhibit the expression of the enzyme tryptophan hydroxylase 1 (TPH1) so that less serotonin is produced. In addition, having sufficient 25(OH)D levels in the brain increased expression of the enzyme tryptophan hydroxylase2 (TPH2), which is necessary for sufficient serotonin production. Sufficient serotonin in the brain, in turn, could positively affect, amongst others, mood, cognition, impulse control, and social behavior. (Laura M. Huiberts, Karin C.H.J. Smolders.2021)

one potential important implication of the inhibiting role of vitamin D on serotonin production outside the blood–brain barrier is the regulation of melatonin levels . In fact, the pineal gland converts serotonin produced via TPH1 expression into melatonin during the evening and nighttime (Laura M. Huiberts, Karin C.H.J. Smolder.2020) . The pineal gland, although located in the brain, belongs to these peripheral tissues as this is a circumventricular organ (i.e., laying outside of the blood–brain barrier) . During the evening and nighttime, it is suggested that TPH1 expression in the pineal gland is increased when serum 25(OH)D levels (as well as 1,25(OH)2D levels) decrease, promoting serotonin production which can then be converted into melatonin. This process of increased peripheral TPH1 expression during the evening and night, stimulating melatonin production, and increased TPH2 expression during daytime enabling sufficient serotonin production in the brain, is optimal when serum 25(OH)D and 1,25(OH)2D levels follow a 24h pattern with higher levels during the day and lower levels in the evening and night (Yao L, Chen M, Zhang N and al.2023) As with daily variation in natural light exposure,

the variation from relatively high serum 25(OH)D during daytime to relatively lower levels during nighttime may be necessary for optimal TPH1 expression in the pineal gland for melatonin regulation as well as optimal TPH2 expression for serotonin regulation in the brain. During natural day–night rhythms, serum vitamin D3 (cholecalciferol) increases rapidly as a result of UVB exposure, which occurs mostly between 11:00 and 15:00 h at higher latitudes since UVB is largely absent before and after these times due to the large solar zenith angle . Also after supplement intake, serum cholecalciferol starts rising in a similar (rapid) fashion as after UVB exposure. It is not unlikely that increases in cholecalciferol levels during the time window in which UVB exposure naturally occurs is most optimal for subsequent processing of vitamin D metabolites in the liver and kidneys. Especially because organ metabolism (i.e., nutrient uptake and processing in the liver and kidneys) is also regulated by circadian clocks. For example, liver metabolism is regulated in such a way that most nutrient uptake and processing activity occurs during daytime under natural light dark cycles. Optimizing this circadian process of vitamin D metabolism may subsequently play a role in promoting sufficient and well-timed serotonin and melatonin synthesis. (Prono F, Bernardi K, Ferri R, Bruni O.2022)

Overall, the serotonergic pathway of vitamin D not only has important implications for the potential role of vitamin D in the development of mental disorders, but also for mental wellbeing in healthy people (Gáll Z, Székely O.2021). That is, sufficient serum 25(OH)D is likely needed to ensure adequate local 1,25(OH)2D production which promotes TPH2 activation in the brain that is needed to produce serotonin which has a wide range of benefits for mood and cognitive functioning during the waking episode. Moreover, TPH1 activation in the evening due to decreasing serum 25(OH)D and local 1,25(OH)2D production may promote serotonin production in the pineal gland which is then transformed into melatonin to facilitate sleep . Good sleep may, in turn, also benefit mood during the waking episode,Vitamin D also upregulates serotonin synthesis by activating the transcription of the serotonin-synthesizing enzyme tryptophan hydroxylase 2 in the brain . (Akpınar Ş, Karadağ MG.2022)

3).3.Vitamin D and norepinephrine

Norepinephrine, also called noradrenaline, is a neurotransmitter that belongs to a class of compounds known as catecholamines. Catecholamines are released into the blood in response to both physical and emotional stress. Norepinephrine is synthesized from dopamine and released from the adrenal medulla into the brain. It works as a neurotransmitter in the central nervous system and sympathetic nervous system, where it’s released from the noradrenergic neurons. (Paravati S, Rosani A, Warrington SJ.2022)

The 1,25(OH)2D3 dosing enhances pressor responses to norepinephrine and Ang II independent of the elevation of serum calcium level. In addition, the administration of 24,25(OH)2D3, an inactive vitamin D3 analogue,24 had no effect on pressor responses to norepinephrine and Ang II, suggesting that pressor enhancement by 1,25(OH)2D3 is not due to its nonspecific action. Because of the augmentation of responses to norepinephrine by 1,25(OH)2D3, consequently to that 1,25(OH)2D3 can modulate sympathetic tone directly, because norepinephrine release at presynaptic nerve endings depends on its intracellular calcium concentration.25 However, Baksi and Hughes26 reported that vitamin D supplementation did not affect norepinephrine release or metabolism (Veldurthy V, Wei R, Campbell M, Lupicki K, Dhawan P, Christakos S.2015). In fact, in the present study, the changes in heart rate with infusion of norepinephrine or Ang II corresponded to those in MAP,

although that increased pressor response to norepinephrine by 1,25(OH)2D3 may be the result of enhanced vascular smooth muscle cell contractility through nonspecific agonist-mediated receptor reaction. 1,25(OH)2D3 could augment not only the pressor response to norepinephrine but also that to Ang II. Norepinephrine- or Ang II-induced contraction of vascular smooth muscle cell is dependent on an increased concentration of intracellular free ionized calcium. (Zhou C, Lu F, Cao K, Xu D, Goltzman D, Miao D.2008)

Recent studies on vasoactive substances and endothelium lead us to conclude that endothelial function is one of the important factors in regulating vascular tone. It is known that there are 1,25(OH)2D3 receptors on endothelial cells. Although little is known about the effect of 1,25(OH)2D3 on endothelial function, hypervitaminosis D in rats caused endothelial degeneration. In contrast, a recent study by Xue et al3 ' indicated that 1,25(OH)2D3 preserved endothelial function in culture conditions. In in vitro conditions, vascular relaxation with acetylcholine is well preserved when active vitamin D3 is added in media. Our study conditions are different from the conditions in the report of Xue et al,31 but releasing ability of endothelium-derived relaxing factor might not be the cause of the vasoconstriction observed. The apparently discrepant results may be due to different experimental conditions between our study and Xue's. The noncalcemic effect of 1,25(OH)2D3 on endothelial function still remains to be determined. Finally, because of the active vitamin D3-induced enhancement of pressor and vasoconstrictor responses to vasoactive substances, we should consider the possible role of active vitamin D3 on vascular hypertrophy. Evidence includes the existence of 1,25(OH)2D3 receptors on vascular smooth muscle cells and the 1,25(OH)2D3 modulation of the growth of vascular smooth muscle cells in vitro and in vivo. (Sandoo A, van Zanten JJ and al.,2010)

Noradrenaline can suppress a programmed cell death induced by 1,25(OH)2D3, could potentially have both physiological and pharmacological implications , thus it has been proposed that the capacity of activated microglial cells to produce 1,25(OH)2D3 could be part of the mechanisms limiting the extension of some glioma in vivo Results suggest that if a such mechanism exists it might be inoperative in the close vicinity of noradrenergic neurons. In addition, in view of the possible role of 1,25 OH D analogs in the treatment of proliferative diseases, it could be important to determine whether the cross-talk observed here in glioma between noradrenaline and the 1,25 OH D -induced programmed cell death is also observed in other cancer cells, such as breast,colonic, and prostate carcinoma cells, for which an antiproliferative action of 1,25 OH D has been demonstrated. (Canova C, Baudet C and al.,1997)

Chapter IV : Human Gut Microbiota and Mental Health

1).The relationship between Human Gut Microbiota and Mental Health

Gut microbes play prime role in human health and have shown to exert their influence on various physiological responses including neurological functions. Growing evidences in recent years have indicated a key role of gut microbiota in contributing to mental health. The connection between gut and brain is modulated by microbes via neural,neuroendocrinal and metabolic pathways that are mediated through various neurotransmitters and their precursors, hormones,cytokines and bioactive metabolites(Suganya K,Koo BS.2020).Impaired functioning of this connection can lead to manifestation of mental disorders. Around 1 billion of the world population is reported to suffer from emotional, psychological and neurological imbalances, substance use disorders and cognitive,psychosocial and intellectual disabilities.Thus,it becomes imperative to understand the role of gut microbes in mental disorders. Since variations occur in the conditions associated with different mental disorders and some of them have overlapping symptoms, it becomes important to have a holistic understanding of gut dysbiosis in these disorders. In this review, we consolidate the recent data on alterations in the gut microbes and its consequences in various neurological, psychological and neurodegenerative disorders. Further, considering these evidences, several studies have been undertaken to specifically target the gut microbiota through different therapeutic interventions including administration of live microbes (psychobiotics) to treat mental health disorders and/or their symptoms. The human gut microbiome is involved in a bi-directional communication pathway with the central nervous system (CNS), termed the microbiota gut brain axis. The microbiota gut brain axis is believed to mediate or modulate various central processes through the vagus nerve. The microbiota gut brain axis is involved with the production of microbial metabolites and immune mediators which trigger changes in neurotransmission, neuroinflammation, and behavior. (Verma H, Phian S.2020)

2).Vitamin D and microbiome -Vitamin D may serve as a key regulator in the gut-brain axis to modulate gut microbiota and alleviate symptoms of depression and anxiety-

The gut microbiota (GM) is emerging as a key regulator of the central nervous system homeostasis by exerting fundamental immune and metabolic functions. In turn,the brain influences the intestinal microbial composition through neuroendocrine signals, within the so called gut microbiota brain axis.The balance of this bidirectional crosstalk is important to ensure neurogenesis, preserve the integrity of the blood-brain barrier and avoid neuroinflammation. Conversely, dysbiosis and gut permeability negatively affect brain development, behavior, and cognition. Furthermore, changes in the GM composition in depressed patients are reported to influence the pharmacokinetics of common antidepressants by affecting their absorption, metabolism, and activity. (Varesi A, Campagnoli LIM and al.2023) Consequently,strategies aimed at re-establishing the correct homeostatic gut balance (i.e., prebiotics, probiotics, fecal microbiota transplantation, and dietary interventions) represent an innovative approach to improve the pharmacotherapy of depression. (Jian S, Wen Q, Li Kar S and al.,2024)

The term gut microbiota (GM) refers to a complex ecosystem composed of bacteria, fungi, protozoa, and viruses that populate our intestines. Under physiological conditions, a balanced intestinal flora ensures the maintenance of homeostasis by strengthening the host immune system, regulating hormonal signaling, and secreting a plethora of metabolites. (Thursby E, Juge N.2017)

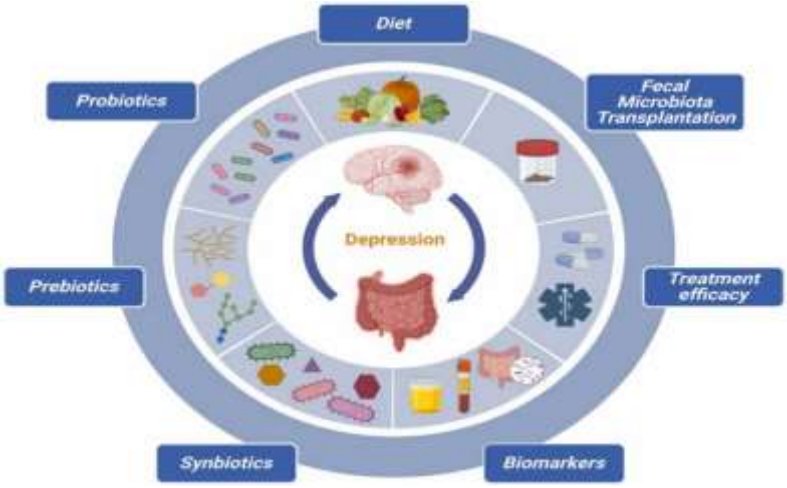

Figure (24) common factors known to impinge on gut microbiota brain axis activity (Angelica, Lucrezia and al.,2023)

Changes in the relative abundance of the different bacterial species lead to an imbalanced GM community, a condition known as dysbiosis. Several external factors have been linked to intestinal dysbiosis, including dietary habits, drug intake, environmental pollutants, and psychological distress. (Intili G, Paladino L, Rappa F and al.,2023)

Due to the existence of a complex bidirectional and dynamic crosstalk between the gut and the brain (the so-called gut microbiota-brain axis),an increased understanding of the close relationship has emerged in these last years between GM composition and various neuropsychiatric and mood disorders, For example, it has been established that recurrent or early exposure to antibiotics increases the risk of developing psychopathological disorders, including anxiety and depression (Marazziti D, Buccianelli B, Palermo S and al,.2022). To date, although some inconsistencies remain, several studies have shown changes in the abundance of various microbial taxa in depressed people compared to healthy controls. Generally, the gut of depressed patients is often characterized by an outgrowth of "pro-inflammatory" bacteria at the expense of the beneficial "anti-inflammatory" species. (Carabotti M, Scirocco A and al.,2015)

An increased relative abundance of Bacteroidetes and Proteobacteria is reported in major depressive disorder (MDD) patients, with a concurrent reduction in the Firmicutes phylum also described. It should be noted that although the Firmicutes/Bacteroidetes ratio has been established as a relevant gut health biomarker, some controversies still exist in using this parameter as a depression-related biomarker. (Liu L, Wang H and al.,2023)

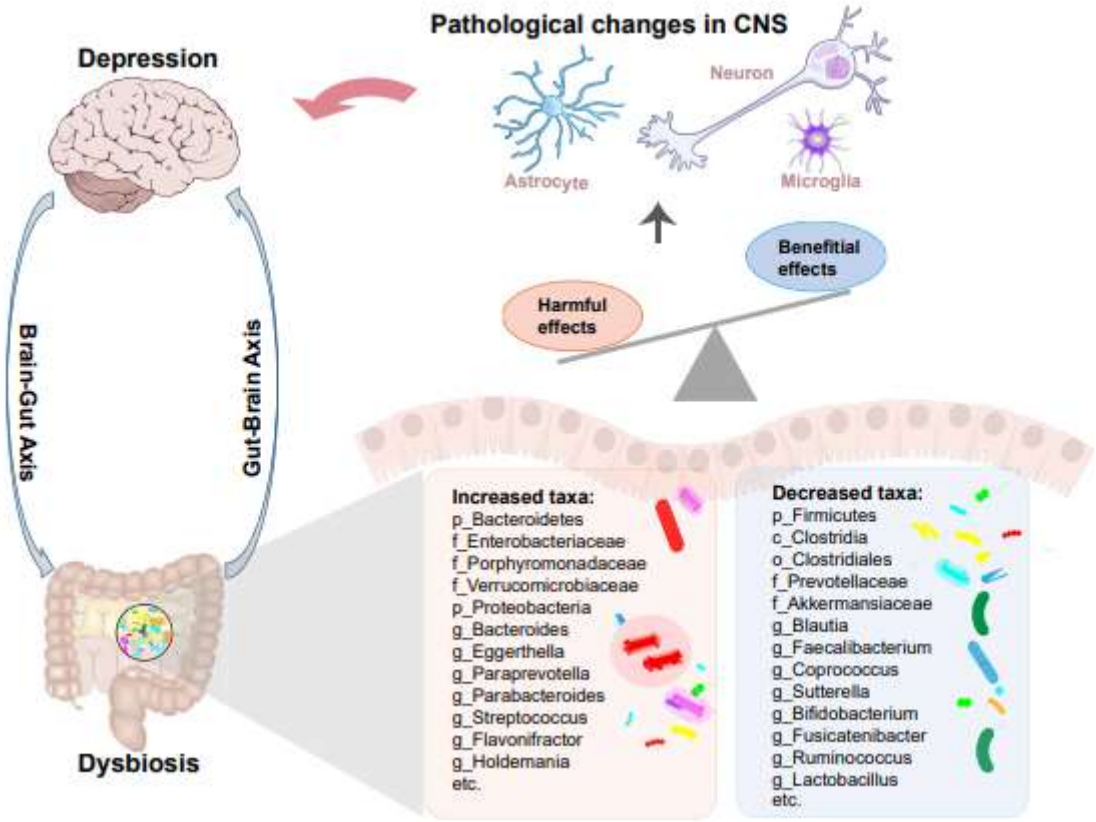

Figure (25) the association between dysbiosis and central pathological changes during the devlopment of depression (Lanxiang, Haiyang and al.,2023)

At the family level, decreased Ruminococcaceae, Prevotellaceae, and Akkermansiaceae are detected in favor of enhanced Enterobacteriaceae, Lachnospiraceae, Verrucomicrobiaceae, and Porphyromonadaceae. More extensively, different microbial genera, including Coprococcus, Eggerthella, Subdoligranulum, Hungatella, Sellimonas, Sutterella, and Eubacterium have been associated with depression across all the studies. Among these, Sutterella and Eggerthella appear to be consistently linked with disease manifestations, with the growth of the first being disfavored over the second in MDD patients. (Angelica V, Lucrezia I and al.,2023)

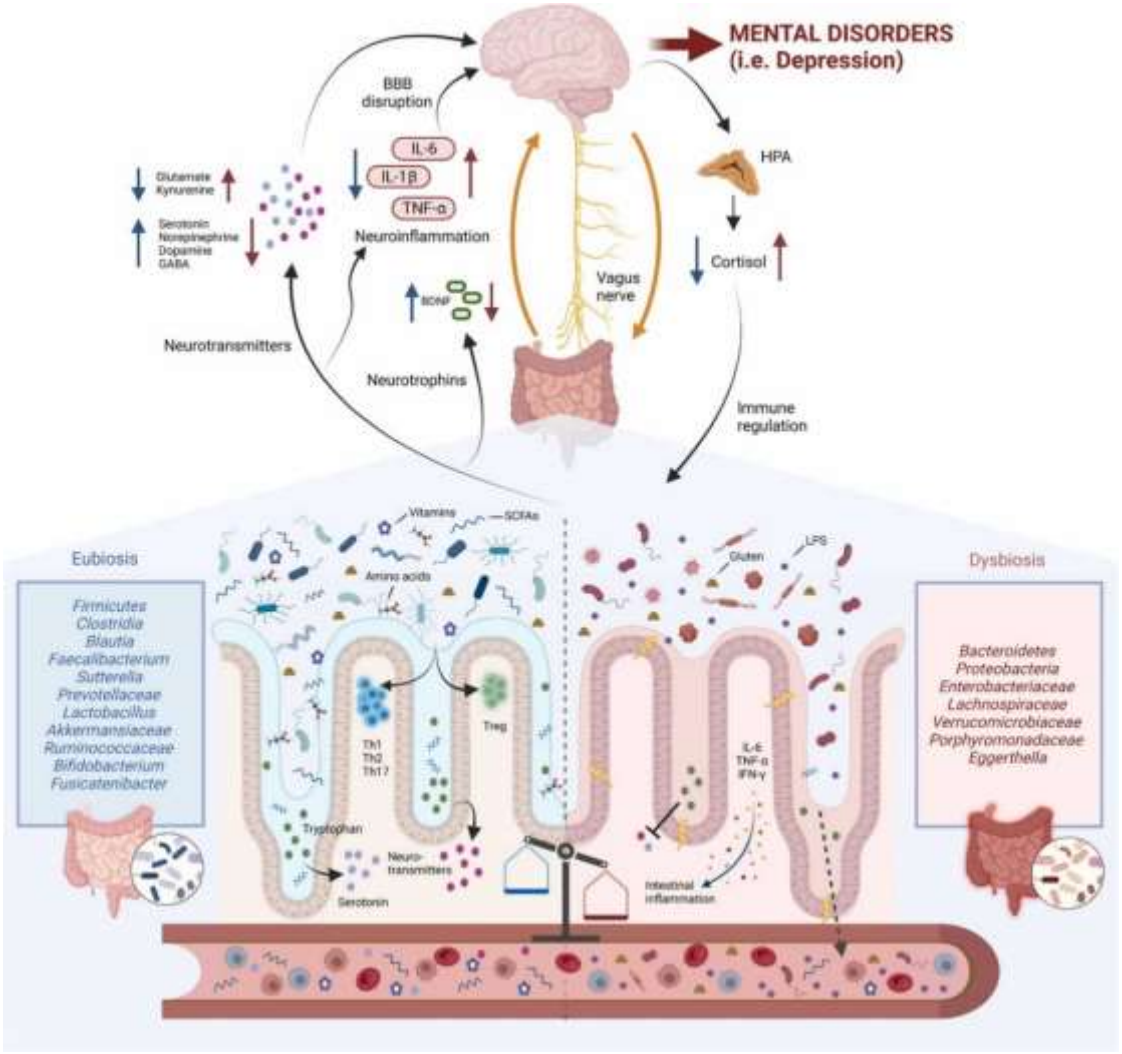

Figure (26) gut microbiota brain axis in healthy subjects and depressed patients (Angelica, Lucrezia and al.,2023)

In healthy conditions (left), a great variety of anti-inflammatory microbes colonize the gut mucosa and maintain the integrity of the intestinal epithelial barrier by producing several key metabolic products, including SCFAs, vitamins and amino acids. In these conditions, intestinal immunity is reinforced, and adaptive T helper and T regulatory cells are primed for development. SCFAs, vitamins, amino acids, together with serotonin obtained by the conversion of tryptophan at the level of the intestinal epithelium enter the circulation and preserve brain health. In depressed patients, the overgrowth of harmful and pathogenic microbes create a dysbiosis condition characterized by gut inflammation and intestinal permeability. Intestinal immunity is not ensured, and the tryptophan metabolic pathway is blocked. Commensal bacteria, their components (i.e., LPS), and other pro-inflammatory molecules (i.e., IL-6, TNF-α, IFN-γ, and gluten) can enter the circulation and disrupt brain homeostasis by reducing the abundance of neurotrophins (such as BDNF), inducing neuroinflammation, and altering the levels of neurotransmitters. In turn, altered hormonal signals from the brain modify the cortisol levels and impair the immune system functioning, which is instead reinforced during gut microbiota brain axis homeostasis. (Angelica V, Lucrezia I and al.,2023)

Often, gut dysbiosis and intestinal inflammation are linked to increased gut permeability (leaky gut).Notably,this term refers to a condition in which a loss of the epithelial barrier integrity allows commensal bacteria and their pro-inflammatory components (LPS) to enter the circulation (metabolic endotoxemia) and trigger Accordingly, higher serum levels of anti-LPS IgA and IgM deriving from Gram-negative enterobacteria have been reported in MDD patients showing a disrupted intestinal mucosa. Other leaky gut biomarkers, such as interleukin (IL) 6, C reactive protein (CRP), zonulin (a tight junction modulator), LPS binding protein, and intestinal fatty acid binding protein (which is released upon gut mucosal damage) have also been associated with depression in independent studies. These events, together with the notion that an increased enterobacterial translocation triggers the activation of oxidative and nitrosative pathways, lead to the establishment of an immune-inflammatory status typically found in depression. Indeed, it should be kept in mind that the interplay between bacterial species and the gut mucosal immune cells is crucial to maintain the correct balance within the mucosal immune system,systemic inflammation. (Brandl K, Schnabl B.2015)

GM also exerts fundamental metabolic functions. These include the production of short chain fatty acids such as propionate, butyrate, and acetate, the synthesis of neurotransmitters (i.e., serotonin, glutamate, GABA), the transformation of bile acids by bacterial enzymes as well as the production of vitamins, amino acids, and amino acids-related metabolites, which in turn contribute to the healthy activity of many organs, including myocardial function, metabolism, and brain homeostasis (Praveenraj SS, Sonali S, Anand N and al,.2022). Interestingly, some of these microbial-derived metabolites [e.g., folate, butyrate, choline, and trimethylamine-N-oxide (TMAO, a product of choline metabolism linked to brain damage and neurodegeneration) can also act by modulating histone modifications, DNA methylation, and noncoding RNA associated gene silencing, thus acting as epigenetic factors. It has been reported that SCFAs and secondary bile acids (glycolithocholic acid, taurolithocholic acid, and lithocholic acid 3-sulfate) are decreased in MDD patients, while the serum levels of TMAO correlate with the symptomatology of depression. Of note, the supplementation with SCFAs in animal models of brain damage and vascular depression improves hippocampal neurogenesis, preserves blood-brain-barrier integrity, and ameliorates depression-like behavior, possibly by favoring the tryptophan conversion into serotonin over kynurenine (whose production is instead favored in the context of depression). (Chen Y, Xu J, Chen Y.2012)

2).1.Gut microbiota-based interventions: probiotics

Probiotics are living microorganisms that when consumed in adequate amounts are beneficial for the host. Although nowadays the use of probiotics is widely prevalent, their actual preventative and therapeutic role against a wide range of diseases remains to be clarified (Aponte M, Murru N, Shoukat M.2020) Concerning psychological disorders, several preclinical studies have shown that probiotics supplementation attenuates depression by reshaping the GM composition and regulating the abundance of SCFAs (short-chain fatty acids). In particular, amelioration of depressive behavior, increased social interaction, and reduced anxiety have been reported upon probiotics administration to mice and rats subjected to CUMS or social defeat stress. The probiotics administered include Lactococcus lactis, Lactobacillus paracasei (L. paracasei) and L. rhamnosus, Akkermansia muciniphila (A. muciniphila), L. paragasseri , L. kefiranofaciens , L. plantarum , Bifidobacterium breve, and multi-strain probiotic formulations containing different species of Bacillus, Lactobacillus,and Bifidobacterium, the behavioral benefits were accompanied by increased hippocampal levels of norepinephrine, serotonin,and

brain derived neurotrophic factor (BDNF), decreased serum corticosterone and adrenocorticotropic hormone, as well as improved neurite outgrowth in the dentate gyrus. At the intestinal level, reduced gut dysbiosis and constipation, increased α-diversity, re-established plasma levels of tryptophan and tryptophan metabolites, and enriched fecal abundance of SCFAs (particularly acetate and butyrate) were reported in depressed animals receiving probiotics compared to their untreated counterparts. (Plaza-Diaz J, Ruiz-Ojeda FJ and al.,2020)

Very similar results have been reported also in other animal models of depression, including E. coli-induced depression, alcohol-mediated depression, and corticosterone-induced chronic stress. When taken together, all these studies indicate that probiotics mediate intestinal and brain benefits through the modulation of neuroinflammation and oxidative stress. According to this concept, probiotics-induced reshaping of the GM re-establishes eubiosis and intestinal barrier integrity, thus preventing LPS from entering the bloodstream and triggering systemic inflammation. This results in reduced levels of brain IL-1β and tumor necrosis factor-alpha (TNF-α), an increased hippocampal abundance of BDNF+ /NeuN+ (a marker of postmitotic neurons), less NF-kB+ /Iba1 + (a microglial marker) and IL-1R+ cells, and re-established levels of serotonin, which accounts for anti-depressive effects. (Angelica V, Lucrezia I and al.,2023)

2).1.Gut microbiota-based interventions: prebiotics and symbiotics

The term “prebiotics” refers to molecules deriving from the diet that can be digested by the gastrointestinal microbiota and mediate beneficial effects on individual health by favoring the growth of anti-inflammatory bacterial species over their harmful counterpart (Wu H, Chiou J.2021) Although the present scientific data cannot be considered definitive as the number of studies is still limited, evidence from the literature highlights controversial results for the use of prebiotics alone as a treatment for depression. Several studies show that individual prebiotics do not have either a preventive capacity against the onset of depressive disorders or therapeutic effects in patients affected by MDD. Accordingly, a recent randomized clinical trial pointed out that the intake of the prebiotic lactosucrose does not alleviate symptoms of depression. Similarly, the sole administration of the prebiotic inulin was not related to significant improvements in depressive disorder. Furthermore, a study on the student population tested before the execution of an academic exam showed that prebiotics deriving from the diet do not modify the occurrence of symptoms of depression. Despite all these negative results regarding the use of a single prebiotic, the co-administration of two different types of prebiotics seems to be a more promising strategy against depressive disorders. In this respect, a combination of fructo-oligosaccharides and galacto-oligosaccharides resulted in reduced depressive like symptoms, higher levels of hippocampal BDNF,and increased cortical serotonin. Mechanistically, there is evidence that fructo-oligosaccharides and galacto-oligosaccharides work together in preventing changes in the proportion of Actinobacteria and Proteobacteria, typical of MDD patients. Furthermore, fish oil was demonstrated to restore gut flora eubiosis and to alleviate depressive symptoms in rats. (Yang Y, Zhou B and al.,2023)

Besides prebiotics co-administration, promising data are emerging on the combinations of prebiotics with probiotics (symbiotics) or postbiotics, which consist of inactivated microorganisms or parts of them leading to health improvements. To date, symbiotic mixtures have been observed to alleviate depressive symptoms not only in murine models of depression but also in a cohort of patients suffering from coronary artery diseases. (Leo et al., 2021)

Gut microbiota-based interventions: diet

The influence of inadequate nutrition on the pathogenesis and progression of several diseases, including neurodegenerative, cardiovascular, and liver disorders, metabolic syndrome, diabetes, as well as mental illness, such as depression, has been well clarified. Regarding depression, some cross-sectional and longitudinal studies underscored the association of an unhealthy diet rich in calories, sugars, and saturated fats and poor in fruits, vegetables, fibre, and antioxidants, with an increased risk of disease developing, as well as a rise in symptom severity and chronicity. Furthermore, the relevant role of diet on the gut-microbiota-brain axis, which is shown to be involved in the pathogenesis of psychiatric disorders, including depression. the potential role of a healthy diet in reducing depression risk and recurrent depressive symptoms, that a combination of healthful lifestyle habits and adherence to specific diets, such as the Mediterranean diet (MD) or the ketogenic diet (KD), may protect against the onset of depression. (Mrozek W, Socha J, Sidorowicz K and al.2023)

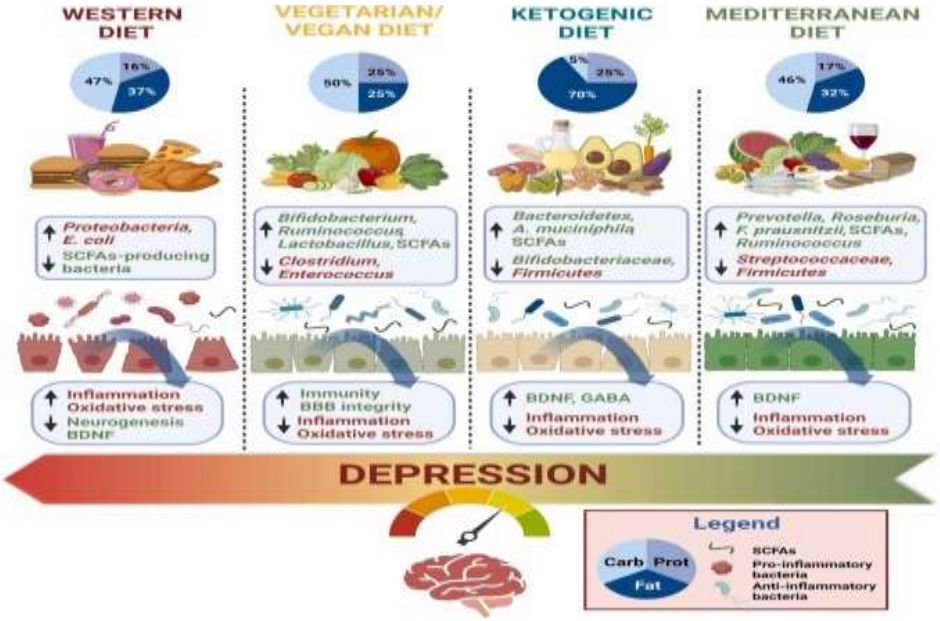

Figure (27) Dietary habits shape the risk of depression (Angelica, Lucrezia and al.,2023)

Diets based on the high intake of calories, saturated fatty acids, and sugars (i.e., Western Diet) are associated with gut dysbiosis by favoring the overgrowth of pro-inflammatory bacteria (listed in red and depicted in red) at the expense of SCFAs-producing species (listed in green and depicted in blue).This triggers a pro-inflammatory state associated with intestinal permeability and sustained systemic oxidative stress (listed in red), with a negative impact on neurogenesis and brain health (listed in green). On the contrary, Vegetarian or Vegan diet, Ketogenic diet, and Mediterranean diet are inversely correlated to the risk of depression by promoting eubiosis, increasing the levels of BDNF and GABA as well as reducing inflammation and oxidative stress. Beneficial microbes and positive effects are listed in green; harmful microbes and negative effects are listed in red. (Canale MP, Noce A, Di Lauro M and al.,2021)

Chapter V : Vitamin D and cognitive decline

1).Vitamin D prevents cognitive decline

Vitamin D, a secosteroid hormone known for its role in bone and calcium homeostasis, is now well recognized for its many diverse functions and actions on a variety of tissues and cell types, including the brain. Inadequate vitamin D status also correlates with a greater risk for cognitive decline in the elderly, that optimal levels may promote healthy brain aging . Because the brain expresses vitamin D receptors (VDRs) and can synthesize the active form of the hormone, the possible cognitive enhancing effects of vitamin D may reflect a primary action in the brain rather than a result of secondary systemic effects . Indeed, as well as the biologically active form of the hormone, 1,25-dihydroxyvitamin D, has direct neuroprotective actions and can reduce some biomarkers of brain aging. (Latimer CS, Brewer LD, Searcy JL and al.,2014)

To test the hypothesis that higher vitamin D levels improve cognitive function in aging animals, middle-aged male F344 rats were placed on diets containing low, medium, or high levels of VitD3 (or cholecalciferol) for 5–6 months. The middle-age period was chosen because it increasingly appears to be an important window of time at which to initiate interventions designed to preserve cognitive function into the geriatric period. At midlife, subtle cognitive impairments begin to appear, along with structural and genomic changes associated with brain aging . the results show that higher than normal dietary VitD3 may improve the chances of successful brain aging and that changes in neuronal synaptic function in the hippocampus may underlie its protective effects against age-related cognitive decline. (Latimer CS, Brewer LD and al.,2014)

cognitive task

Morris Water Maze.

After 5–6 months of dietary VitD3 manipulation, performance in the Morris water maze (MWM) was evaluated to determine effects on spatial learning and memory. All groups learned the task equally well, as demonstrated by similar path lengths and latencies to the hidden platform during the 3-d training period . Twenty-four hours later, memory was assessed during the probe trial (platform removed). There was no statistical difference between groups on either latency ($P = 0.14$) or path length ($P = 0.22$) , although animals on the low VitD3 diet showed trends toward higher latencies and path lengths.

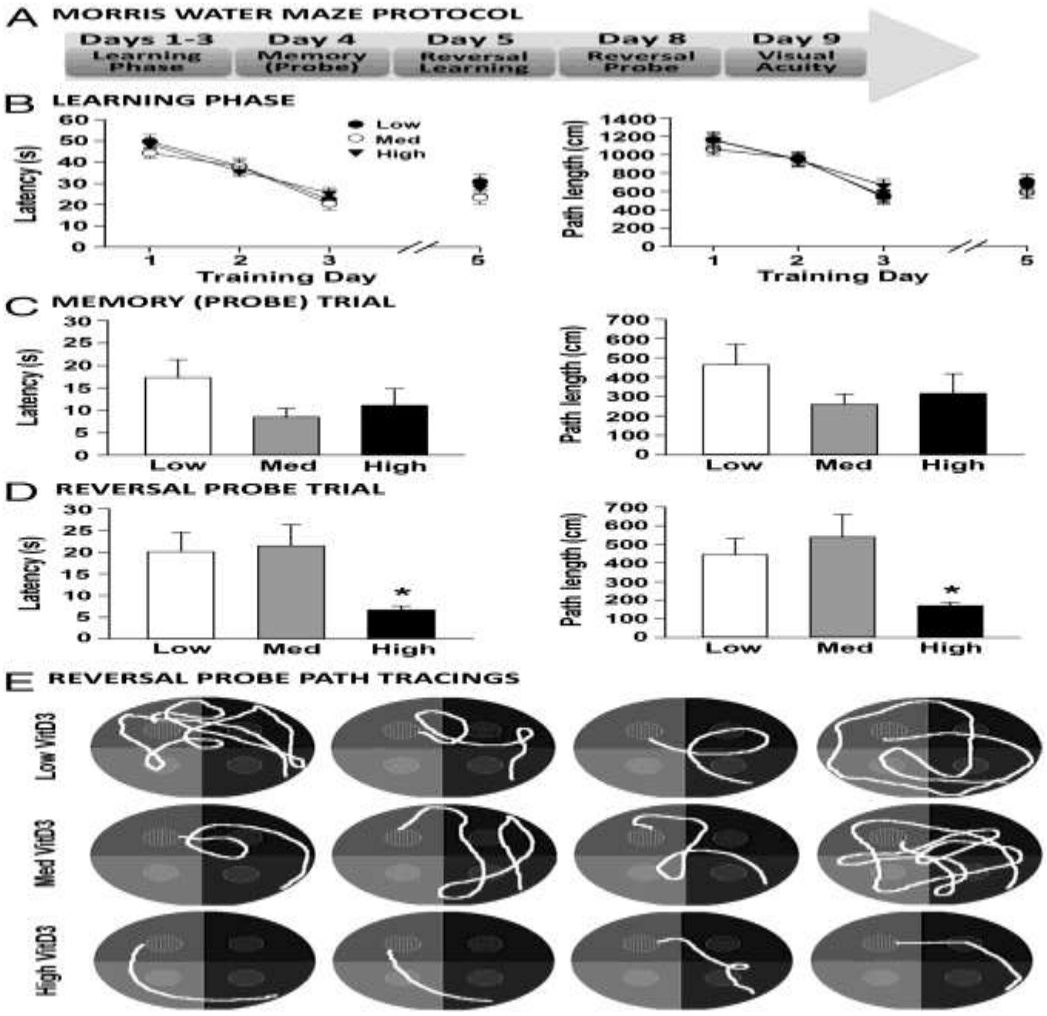

Figure (28) Morris Mater Maze protocol (Latimer CS, Brewer LD, Searcy JL and al.,2014)

High dietary VitD3 improves performance on a cognitive task. (A) MWM protocol. (B) Latencies and path lengths during training days 1–3. All groups showed a similar performance and improvement in reaching the platform. On day 5, all groups learned the new platform location during spatial reversal training (results are the average of the second and third trials). (C) Latency and path length to the goal during the probe trial (day 4) did not differ significantly between groups [latency: $F(2,52) = 2.02$, $P = 0.14$; path length: $F(2,52) = 1.57$, $P = 0.22$]. (D) On day 8 (reversal probe trial), the high-VitD3 group significantly outperformed the other groups on latency [$F(2,52) = 3.8$, $P = 0.03$] and path length [$F(2,52) = 4.0$, $P = 0.02$; $n = 16–20$ per group]. (E) Representative reversal probe path tracings. (Latimer CS, Brewer LD and al.,2014)

Despite growing concerns that vitamin D deficiency is a risk factor associated with unhealthy cognitive aging, consequently there must a relationship between chronic vitamin D (VitD3) supplementation and cognitive decline with aging . that the effects of long-term dietary manipulation of serum vitamin D (25OHD) and tested the hypothesis that cognitive decline with aging can be slowed or prevented by higher vitamin D levels. Middle-aged male F344 rats were fed diets containing low, medium (NRC-required), or high VitD3 for 5–6 mo, followed by assessments of cognitive function, hippocampal electrophysiology, and gene expression. The results provide evidence of a potential cause-and-effect relationship because raising 25OHD levels prevented age-related cognitive decline. In addition, these identify effects on

synaptic function as a mechanism by which vitamin D may promote healthy brain aging. (Latimer CS, Brewer LD and al.,2014)

There are relatively few risks associated with increased vitamin D intake, especially when taken in the inactive form (VitD3 or cholecalciferol). The most concerning potential side effect is hypercalcemia; however, hypercalcemia is rarely seen except at serum 25OHD levels far exceeding those levels recommended for optimum health. The animals on the high-VitD3 diet had 25OHD levels of ~30 ng/mL with no changes in serum calcium. (Amrein K, Papinutti A, Mathew E, Vila G, Parekh D.2018)

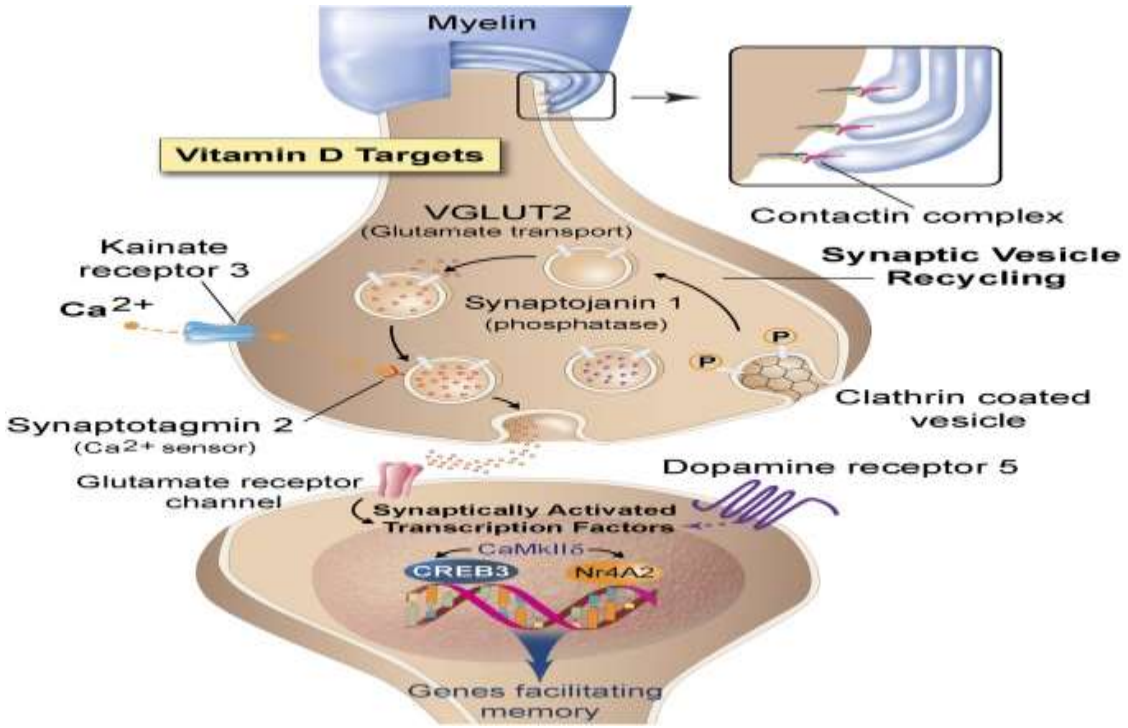

Figure (29) Proposed model of synaptic changes with Vitamin D3 (Latimer CS, Brewer LD, Searcy JL and al.,2014)

Optimal levels of VitD3 stabilize myelin structure and enhance synaptic vesicle recycling and transcription factors facilitating cognitive processes.

Together, these changes preserve memory in aging. (Inset) Anchoring of the myelin to the axon facilitated by contactin. (Presynaptic targets) Empty clathrin-coated vesicles undergo endocytosis or recycling driven, in part, by the phosphatase synaptojanin 1. Vesicle refilling of glutamate (red molecules) occurs via vesicular glutamate transporter 2 (VGLUT2). Ca2+ enters the synapse via kainate receptor 3 (Grik3), and docking of the filled vesicle is facilitated by Ca2+ binding to synaptotagmin 2. The synapse also contains a dopamine-filled synaptic vesicle (purple molecules), which can colocalize with kainate receptor 3 terminals. (Postsynaptic targets) Stimulation of glutamate (or dopamine 5) receptors initiates a CaMKIIδ signaling cascade resulting in activation of transcription factors (CREB3 and NR4A2) and subsequent expression of genes promoting memory-related processes. (Latimer CS, Brewer LD, Searcy JL and al.,2014

Higher Vitamin D Levels Reduce Cognitive Deficits at Middle Age

The MWM task is used to test spatial reference memory and is widely thought to have relevance for human hippocampal-dependent memory. Although not as commonly used, reversal learning in the MWM is more difficult than the standard task, and therefore can be used to detect subtle memory deficits. Further, because of its more complex nature, reversal learning may be viewed as a task of executive function. The middle-age period is often characterized by the onset of such subtle changes in cognitive performance, most notably in executive function and processing speed. It was assessed spatial reference memory, where rats were trained to locate a hidden platform in a specific location, and found no significant differences among groups of middle-aged animals treated with varying levels of vitamin D. During spatial reversal, however, the animals were required to learn a new platform location in a single day and then to remember the new location after several days (Bizon JL, Foster TC, Alexander GE, Glisky EL.2022). This more difficult task required differentiating an old, now irrelevant, memory from a new one, and it may involve both the hippocampus and higher cortical regions. The low- and medium-VitD3 groups demonstrated deficits and struggled to recall the new platform location, appearing lost or confused during the reversal probe. The high-VitD3 group, on the other hand, performed this task extremely well, reaching the goal in half of the time and distance compared with the other two groups. Therefore, these results suggest that higher serum levels of 25OHD may be protective against some of the early, subtle changes in cognitive function. (Blazer DG, Yaffe K, Liverman CT.2015)

Several aspects of brain aging in this animal model mirror brain aging in humans. First, the initial signs of cognitive aging, characterized by subtle deficits, occur at approximately the same time in the lifespan of humans and rats, during middle age. Second, the behavioral task used , assessing hippocampal-dependent spatial memory, also has relevance for human memory because patients with hippocampal lesions perform poorly in a virtual maze test. Finally, the vitamin D levels achieved here model clinically relevant levels found in humans, ranging from deficient to sufficient, and animals with the highest 25OHD levels, considered “optimal” by some recommendations, outperformed animals with lower levels on a challenging cognitive task . (Latimer CS, Brewer LD, Searcy JL and al.,2014)

2).Vitamin D and Psychiatric Illnesses

2).1.Psychiatric Illnesses

A mental disorder is characterized by a clinically significant disturbance in an individual’s cognition, emotional regulation, or behaviour. It is usually associated with distress or impairment in important areas of functioning. Mental disorders may also be referred to as mental health conditions. The latter is a broader term covering mental disorders, psychosocial disabilities and other mental states associated with significant distress, impairment in functioning, or risk of self-harm. (Nigussie K, Tesfaye D and al.,2023)

In 2019, 1 in every 8 people, or 970 million people around the world were living with a mental disorder, with anxiety and depressive disorders the most common . In 2020, the number of people living with anxiety and depressive disorders rose significantly because of the COVID-19 pandemic. Initial estimates show a 26% and 28% increase respectively for anxiety and major depressive disorders in just one year. (Kumbet JS, Oseni T and al.,2023), Mental disorders (or mental illnesses) are conditions that affect thinking, feeling, mood, and behavior. They may be occasional or long lasting (chronic). They can affect the ability to relate to others and function each day. (Villas-Boas S, Kaplan S, White JS, Hsia RY.2023)

2).2.Types of mental disorders

There are many different types of mental disorders. Some common ones include:

1. Anxiety Disorders, Including Panic Disorder, Obsessive-Compulsive Disorder, And Phobias
2. Depression, Bipolar Disorder, And Other Mood Disorders
3. Eating Disorders
4. Personality Disorders
5. Post-Traumatic Stress Disorder
6. Psychotic Disorders, Including Schizophrenia.(World Health Organisation [WHO], 2022).

2).3.Causes of mental disorders

1. Genes And Family History
2. Life Experiences, Such As Stress Or A History Of Abuse, Especially If They Happen In Childhood
3. Biological Factors Such As Chemical Imbalances In The Brain
4. A Traumatic Brain Injury
5. A Mother's Exposure To Viruses Or Toxic Chemicals While Pregnant
6. Use Of Alcohol Or Recreational Drugs
7. Having A Serious Medical Condition Like Cancer. (Smitha B.2023)

2).4.Relationship between Vitamin D and Psychiatric Illnesses

Vitamin D is known not only for its essential role in calcium homeostasis and bone health but also for maintaining a healthy mind. A number of recent studies, in fact, have demonstrated a correlation between vitamin D deficiency and psychiatric illness. In addition to all its other functions, vitamin D acts as a potent neurosteroid hormone, critical to brain development and normal brain function; it is known for its anti-inflammatory properties, which are able to affect many aspects of human health. The vitamin D receptor, which mediates many of its biological actions, has been found throughout the body, including in the central nervous system. Vitamin D deficiency is common in patients with serious mental illnesses, such as depression, schizophrenia and neurocognitive disorders. (Zhang S, Miller DD, Li W.2021) Several risk factors, such as genetic and environmental factors, season of birth, latitude and migration, have been linked to vitamin D deficiency and can explain, at least in part, the association between hypovitaminosis D and mental illness. The causal link between hypovitaminosis D and mental illness is probably bi-directional; mental illness increases the risk of hypovitaminosis D, and hypovitaminosis D increases the risk of developing mental illness. The biological mechanism at the base of the relationship between hypovitaminosis D and mental illness is most likely related to vitamin D action on the regulation of inflammatory and immunological processes, which in turn can act as mediators or modulators for the development of clinical symptoms and/or treatment response. (Ghaseminejad-Raeini A, Ghaderi A, Sharafi A and al.2023)

Since its discovery in 1921, vitamin D has been known for its role in calcium homeostasis and bone health. Low levels of vitamin D have been associated with bone disorders such as rickets, osteomalacia and osteoporosis. However, these disorders can be considered as simply “the tip

of the iceberg" in vitamin D deficiency. Recent studies have shown that most tissues and cells of the human body, including the brain, have vitamin D receptors, thus providing new information about its function . Vitamin D plays an important role in the pathophysiology of psychiatric diseases, as has been shown by various studies on the presence of this vitamin, its receptors (Vitamin D Receptors, or VDRs) and its associated enzymes (CYP24A1 and CYP27B1) in many parts of the brain (Alessandro C.2019). The expression of vitamin D receptors (VDRs) in the prefrontal cortex, cingulate gyrus, thalamus, hypothalamus, amygdala, hippocampus and substantia nigra suggests a possible key role of vitamin D in the pathophysiology of psychiatric illnesses such as depression and psychosis . It has been proved that vitamin D plays an important role in neurodevelopment, neuroprotection, neuroplasticity and neuromodulation, not only by exercising its biological action, but also by influencing gene expression at the cellular level. In addition, there is new evidence regarding the neuroprotective mechanism of vitamin D action on inflammatory processes in the brain , such as the upregulation of pro-inflammatory cytokines associated with depression and with mental illness (Cuomo A, Maina G, Bolognesi S and al,.2019). The discovery of vitamin D receptors in extraskeletal systems has caused increased interest in its function in these systems. Further studies have shown a relationship between vitamin D deficiency and cancer, chronic conditions such as diabetes, and metabolic, autoimmune, infective and cardiovascular diseases. It has further been shown that patients with mental disorders are at a higher risk for vitamin D deficiency than the general population. In particular, patients with schizophrenia have a greater risk of lacking vitamin D than those affected by other mental illnesses. (Ristic S, Kocic SS, Milovanovic DR and al.,2017)

Vitamin D is crucial for overall health; physical and mental, Vitamin D deficiency has received increasing attention over the last decade, Lower levels of serum vitamin D have been associated with cardiovascular disease, hypertension, neurodegenerative diseases, diabetes, metabolic syndrome, and cancers.(Kim S, Seok H, Kim D.2016) in the other hand vitamin D insufficiency has been a major problem that trigger off mental disorders such as anxiety and depression, consequently in their turn they will effect on the individual's life on many aspects (social , personal and professional life) and that is why the prevention measures should be taken in consideration such as getting sufficient exposure of sunlight (UVB), rich dietary , supplements, working out to avoid obesity in order to have a healthier life, therefore, governments, policy makers, health workers, supervisors at schools should attach the importance to the high prevalence of vitamin D deficiency as well the awareness of the individuals mental state in society and making their prevention a health priority, as well it is known as an important contributor to psychiatric illnesses so we should not leave this serious issue unresolved. (Tanna NK, Karki M, Webber I, Alaa A, El-Costa A, Blair M.2023)

Chapter VI : Statistical study

Statistical study

I. Introduction

Vitamin D has implications both on bone health and on non-skeletal aspects. Deficiency or insufficiency in vitamin D has significant consequences in various health conditions. Therefore, it is important to understand its prevalence, Is there an interest in screening and in a supplementation of deficiency or insufficiency in vitamin D and in the prevention of many diseases ? A wider assessment of that population of both the prevalence and the benefits in the short and in the long term of supplementation in vitamin D would be necessary to determine the best medical care

II /1.Materials and Methods:

The data for this study lasted from January to May 2024 and was obtained from three parts

The first was the collection of vitamin D analyzes from laboratories in the wilaya of Saïda **table 03**,then we carried out a questionnaire relating to the vitamin D **table 05** ,and the third step was the study of 100 patients suffering from mental disorders from the Wilaya of Sidi Bel Abbes **table 06** and who carried out the vitamin D analysis.

III/1.1. The objective of the study:

The first step : The main objective of this study is to estimate the prevalence of vitamin D deficiency in a group of 525 patients of different age groups in Saida.

Secondary step: the secondary objective is to research the risk and protective factors of insufficiency in this group of 140 people, its effect on their mental state and the quality of their life, therefore the precautions to take as a preventive measure.

Third step of the study was to assess the influence of vitamin D levels on the mental health of 100 people with mental disorders in Wilaya of Sidi Bel Abbes.

III /2. Methods:

Vitamin D analysis is one of the many tests that the VIDAS device can perform.

The VIDAS device uses the Enzyme-Linked Fluorescent Assay (ELFA) principle for analysis. The main difference in this technology is the use of enzyme-linked to antibodies instead of radiation to bind known substances in liquid samples.

Using this technology, the VIDAS device can analyze Vitamin D accurately and efficiently, providing important information about Vitamin D levels in the body. Vitamin D levels are essential for many body functions, including bone health, immune system function, and many other functions.

Another test that comes with high performance and precision; the ARCHITECT 25-OH Vitamin D assay is a chemiluminescent microparticle immunoassay (CMIA) for the quantitative determination of 25- hydroxyvitamin D (25-OH vitamin D) in human serum and plasma

Immunochemical assays analyzed on Abbott Architect i-2000 (Abbott Park, IL, USA). The original Architect (Abbott Diagnostics, Lake Forest, IL, USA) 25-OH Vitamin D assay determine 25(OH) D2 and D3 in human serum and plasma. It is a delayed one-step immunoassay including a sample pre-treatment for the quantitative determination of vitamin D in com petitive chemiluminescent microparticle immuno assay (CMIA) technology with flexible assay protocols

Architect 25-OH D vitamin assay revealed excellent precision with a total coefficient of variance (CV %) <5%.

Procedure

1. Sample and pre-treatment reagents are combined. An aliquot of the pre-treated sample is combined with assay diluent and paramagnetic anti-vitamin D coated microparticles to create a reaction mixture. Vitamin D present in the sample binds to anti-vitamin D coated microparticles forming an antigen-antibody complex
2. After incubation a biotinylated vitamin D anti-Biotin acridiniumlabeled conjugate complex is added to the reaction mixture and binds to unoccupied binding sites of the anti-vitamin D coated microparticles.
3. After washing, pre-trigger and trigger solutions are added to the reaction mixture.
4. The resulting chemiluminescent reaction is measured as relative light units (RLUs).

III/2. 1. Type of study:

A prevalence survey carried out in region of Saïda from different laboratories, this is a descriptive study of the status in vitamin D in a sample of 525 patients aged 1 to plus 50 years

III /2.1.1. Target population and sampling :

III /2.1.1.1. Target population :

The target population of this study are subjects aged 1 to +50 years old, in Saïda, who do not present 100% of major health problems interfering with the metabolism of Vitamin D.

III /2.1.1.1. A. Inclusion criteria :

The inclusion criteria were all anonymous patients from three laboratories, where they were divided male/female and divided into 3 age groups, to which 140 of them responded to a questionnaire on vitamin D.

III /2.1.1.1. B. Non-inclusion criteria :

The exclusion criteria for the study were essentially all chronic pathologies responsible for hypovitaminosis (with Crohn's disease, ulcerative colitis or celiac disease) or the lifestyle/diet of the subject (spending most of the time at indoors or outdoors) interfering with the development of vitamin D insufficiency.

III /2.1.1.2. Sampling method and sample size

Our study is composed of three samples as follows: The first sample is composed of 525 people including 454 women and 71 men spread over 3 age groups, the first group from 1 to 15 years old, the second group from 15 to 50 years old and the third group over 50 years old **Table (03)**

The second sample questionnaire covering a population of 140 people categorized according to sex and age; 61 women, 54 men aged 18 to ≥50 years and a group of adolescents aged 25 years, all boys whose ages ranged between 10 and 18 years. **: Table (05)**

The third sample is made up of 100 people including 64 women and 36 men spread over 3 age groups, the first group from 1 to 15 years old, the second group from 15 to 50 years old and the third group from over 50 years old **Table (06)**

III /2.1.1.3. Statistical processing of data :

Statistical tests were carried out using Microsoft Office Excel 2019 for the analysis of the quantitative data collected through the questionnaire.

Results and analyzes

First evaluation: Vitamin D analysis survey

Table (03) Vitamin D level (ng/ml)

25(OH) D based on sex and group age									
	male	female	1-15 years		15-50 years		≥50 years		Total
			male	female	male	female	male	female	
Vitamin D <30 ng/mL	57	355	8	26	30	215	19	114	412
Vitamin D 30-50 ng/mL	10	73	1	5	2	32	7	36	83
Vitamin D 50 -70 ng/mL	1	19	1			11		8	20
Vitamin D ≥70 ng/mL	3	5			1	3	2	2	8
Vitamin D ≥100 ng/mL		2		1				1	2
Total	71	454	10	32	33	261	28	161	525

Levels of 50 nmol/L (20 ng/mL) or more are sufficient for most people. In contrast, the Endocrine Society stated that, for clinical practice, a serum 25(OH)D concentration of more than 75 nmol/L (30 ng/mL) is necessary to maximize the effect of vitamin D on calcium, bone, and muscle metabolism

First evaluation: the subject group consists of 525 people whose minimum age was 1 year and maximum age was over 50 years, classified according to gender and age groups

There was 412 person that were having vitamin D deficiency <30ng/mL from 525 tested subjects:

Group age 1-15 contained 76% girl and 23% boys

Group age 15-50 contained 87% women and 12% men

Group age +50 contained 85% women and 14% men

Prevalence of vitamin D deficiency (<30ng/ml) is the highest: 412 person from a sum of 525 person (78%), where 86% of them are women and 14% men.

Prevalence of vitamin D sufficiency (30-50 ng/mL) medium: 83 person from a sum of 525 person (15%), where 87% of them are women and 13% men.

Prevalence of vitamin D adequacy (50-70 ng/mL) medium low: 20 person from a sum of 525 person (3%), where 95% of them are women and only 5% of them are men.

Prevalence of vitamin D therapeutic (≥70 ng/mL) low: 8 persons from a sum of 525 person (1.5%), where 62% are women and 37% of them are men.

Prevalence of vitamin D level (≥100 ng/mL) lowest : there's only 2 persons from a sum of 525 person (0.3%) it includes only women

The general prevalence is 525 people; among them, 454 (86%) are women and 71 (13%) are men. Age (1-15) = 42 people; 76% girls and 23% boys, while age (15-50) = 294 people; 88% women and 12% men, also in the age range (≥50 years) = 189 people; 85% women and 15% men.

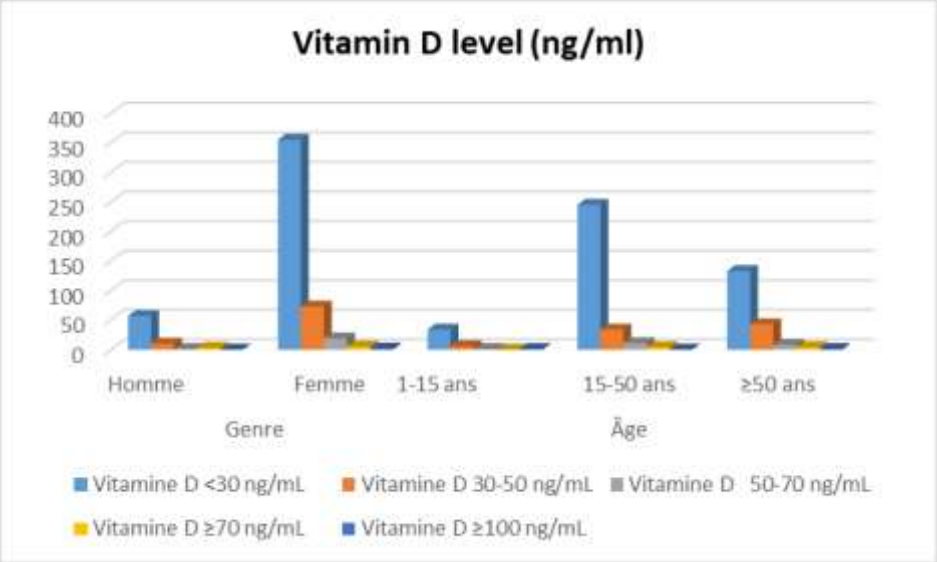

Figure (30) Vitamin D level (ng/ml)

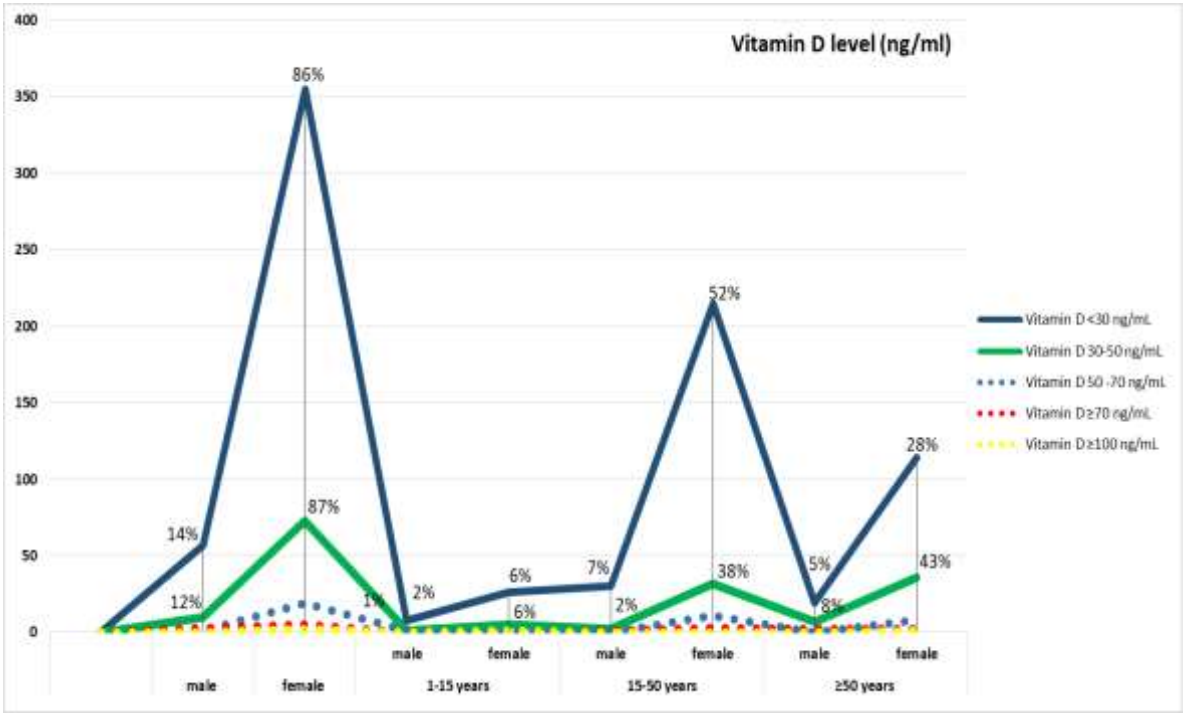

Figure (31) vitamin D level (ng/ml)

Table (04) Vitamin D level (<30ng/mL)

Population	Women	Men	Total
1-15 years	26	8	34
15-50 years	215	30	245
≥50 years	114	19	133
Total	355	57	412

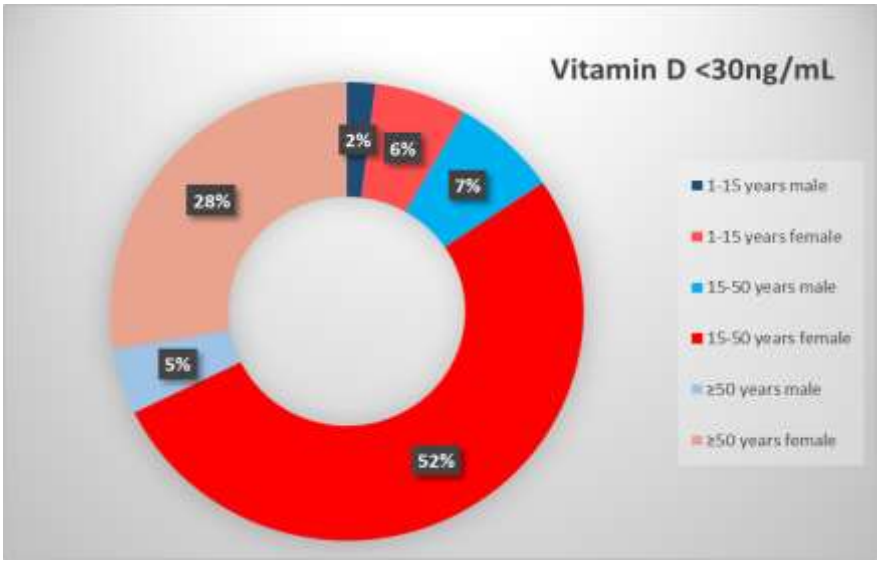

Figure (32) Vitamin D level (<30ng/mL)

Prevalence of vitamin D deficiency (<30ng/ml) demonstrated that from 412 subject, women in all group ages are more prone to be deficient than men, where :

Group age 1-15 contained 6% girl and 2% boys

Group age 15-50 contained 52% women and 7% men

Group age +50 contained 28% women and 5% men

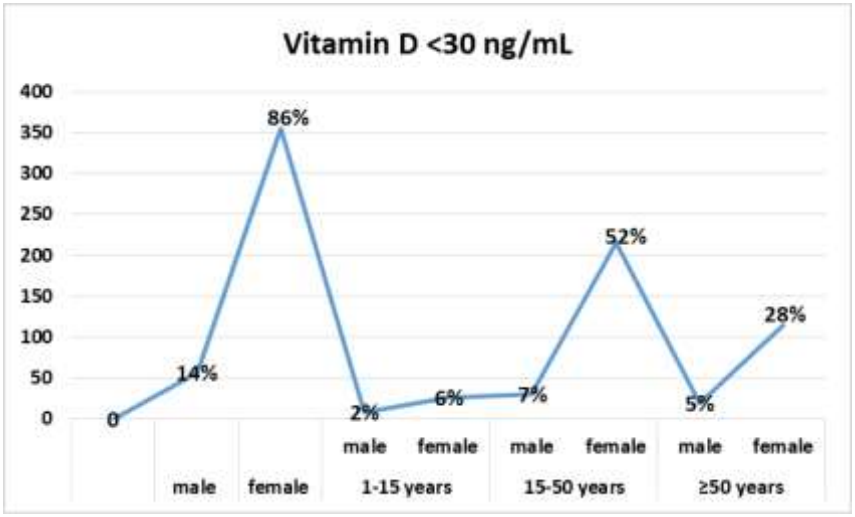

Figure (33) Vitamin D level (<30ng/mL)

Second evaluation based on questionnaire.

Vitamin D based on population's daily life

From a sample questionnaire of a population of 140 person categorized on sex and age; 61women, 54 men between the ages 18 to ≥50 years, and teenage group were 25 all of them boys their age varied between 10 and 18 years.

Table (05) Vitamin D sample questionnaire

	Men		Woman		Child	
	18-+50		18-+50		10-18 years	
	yes/in	no/out	yes/in	no/out	yes/in	no/out
Have you ever heard of vitamin D?	54 yes	0 no	61 yes	0	17 yes	8 no
Do you work mainly indoors or outdoors?	40% In	59% out	65% in	34% out	80% in	20% out
Do you live in an apartment or a house?	74% Ap	25% Home	85% Ap	14% home	100% Ap	0% Home
Do you like being in contact with the sun?	100% yes	0% no	90% yes	9% no	100% yes	0% no
Are you afraid of sun exposure	0% yes	100% no	81% yes	18% no	100% yes	0% no
Did you know that sunlight can provide you with vitamin D?	74% yes	25% no	70% yes	29% no	24% yes	76% no
Did you know that darker skin tones are more prone to vitamin D deficiency?	3% yes	96% no	6% yes	93% no	0% yes	100% no
Do you use sunscreen? SPF factor?	0% yes	100% no	40% yes	59% no	0% yes	100% no
What are the sources of vitamin D?	74% fish/milk	25% Egg/tuna	49% Dairy product	50% vegetable	52% egg	48% Milk
Do you take vitamin D supplements?	5% yes	94% no	16% yes	83% no	0% yes	100% no
Do you consume a lot more products of animal or plant origin?	50% animal	49% vegetal	49% animal	50% vegetal	50% animal	49% vegetal
Do you have the ability to eat well? (Allow red meat, cow's milk, butter for example).	100% yes	0% no	95% yes	4% no	100% yes	0% No
Do you take cow's milk, do you eat butter, meat (approximate quantity)	90% yes	9% no	86% yes	13% no	100% yes	0% no
Have you been diagnosed with Crohn's disease, ulcerative colitis or celiac disease?	35% yes	64% no	24% yes	75% no	8% yes	92% no
Do you suffer from Chronic Intestinal Disorder?	27% yes	72% no	6% yes	93% no	0% yes	100% no

The questions were practically related to their knowledge about vitamin D (source, intake, sun exposure) and the possibility to have any chronic diseases or health problems, consumption, ability to eat well (diverse products from different origins) beside their daily habits and working environment (indoors/outdoors)

Additional evaluation: questionnair**e** table consists of 15 question that were asked to 140 person from different age groups, sex

The answers depended on the individual's knowledge, occupation, place of living, their interaction with sun light and protection, dietary and their health state

The answers varied from a subject to another depends on their life routine, occupation, age and sex, where 100% of both men and women, as well 68% of teenage group were having an idea about vitamin D

The exposure to sunlight was basically depending on their occupation (in/out doors), activities and also the type of habitat they were living in, that men were most likely to be exposed to sunlight unlike women that were less exposed to it due to their occupation where 59% of men and 25% of women were working outdoors, therefore the majority of women 90% were afraid to be exposed to sunlight ,40% of women were using a sunscreen with spf 50, and all of them didn't know that it affects the vitamin D absorption, because the women who didn't know about the fact that sunlight exposure is the main source of vitamin D were frightened to be exposed to sunlight due to preserve their skin from getting darker (aesthetic prevention).

Population's dietary was balanced between products of animal and vegetal sources with the exception of 5% were vegan, in the other hand 69% of this subjects had a good knowledge about vitamin D sources (fish,mushrooms,dairy products, egg yolk), and they were all having the ability to eat well (red meat, dairy product, butter) except the vegetarians 5% and the subjects who were diagnosed with diseases (Crohn's disease, ulcerative colitis or celiac disease) 35% of men and 24% of women and the others who were struggling from chronic intestinal disorders 13% (27% men/ 6% women); these were on a diet to avoid stomach/intestinal inflammation.

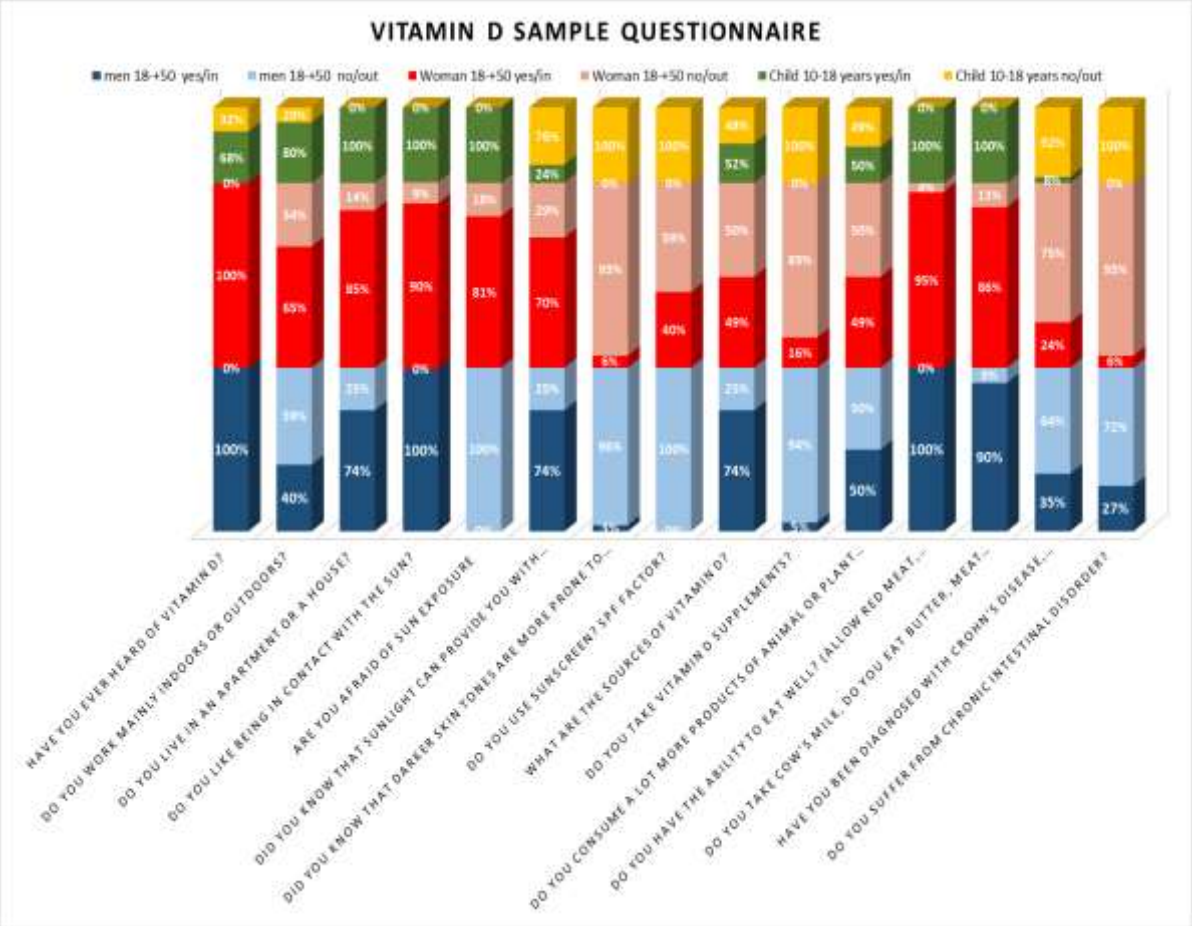

Figure (34) Vitamin D sample questionnaire

Risk factors related to the questionnaire

A large percentage of the population is aware of the existence of vitamin D, but living in apartments rather than houses with terraces where you have the choice and surface area to expose yourself to the sun adds to this fear exposure in the sun and the use of sunscreen represents an obstacle to the synthesis of vitamin D.

1. therefore the majority of women 90% were afraid to be exposed to sunlight
2. 40% of women were using a sunscreen with spf 50, and all of them didn't know that it affects the vitamin D absorption, because the women who didn't know about the fact that sunlight exposure is the main source of vitamin D were frightened to be exposed to sunlight due to preserve their skin from getting darker (aesthetic prevention).

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Third evaluation: Vitamin D deficiency and mental disorders

From a 100 subject that were struggling from mental disorders such as schizophrenia, Alzheimer, Bipolar trouble, anxiety, depression, TOC (obsessional compulsive trouble), TAG (generalized anxiety trouble) and social phobia, it was found that 80 of them were having vitamin D deficiency.

Table (06) Vitamin D level of patients with mental disorders

25(OH) D based on sex and group age									
	male	female	1-15 years		15-50 years		≥50 years		Total
			male	female	male	female	male	female	
Vitamin D <30 ng/mL	27	53	1	1	23	42	2	11	80
Vitamin D 30-50 ng/mL	8	10			6	8	2	2	18
Vitamin D 50 -70 ng/mL		1						1	1
Vitamin D ≥70 ng/mL									
Vitamin D ≥100 ng/mL	1				1				1
Total	36	64	1	1	30	50	4	14	100

Patients that were having mental disorders associated with VDD (80%) from 100 subject, where women represented 66% and men 33% varied on age groups that :

Group age 1-15 contained 1% girl and 1% boys

Group age 15-50 contained 52% women and 29% men

Group age +50 contained 14% women and 3% men

Prevalence of vitamin D insufficiency (<30ng/ml) associated with mental disorders contained 66% women and 33% men that were dealing with anxiety and depression, that from 100 subject almost the majority (80%) were having Vitamin D deficiency.

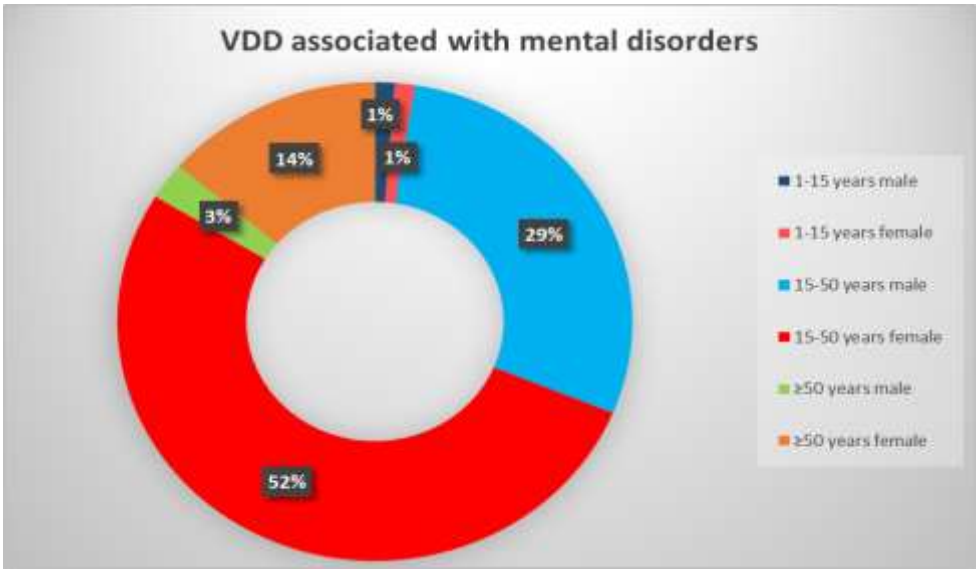

Figure (35) Vitamin D level of patients with mental disorders

Psychiatric disorders were varied between men and women, that :

1. Depression considered as the major disorder since it contained most of patients where 20% were women and 8% were men
2. Panic trouble contained 10% men and 5% women
3. TAG contained 3% men and 8% women
4. Social phobia 5% men and 0% women
5. Addiction trouble 7% men and 0% women
6. Anxiety contained 2% men and 3%
7. After a general observation, the frequency of hypovitaminosis D (serum 25-hydroxyvitamin D < 30ng/mL) (86%) women and (13%) men of the studied population
8. Patients that were having mental disorders associated with VDD (80%) from 100 subject, where women represented 66% and men 33% varied on age groups that :
9. Group age 1-15 contained 1% girl and 1% boys
10. Group age 15-50 contained 52% women and 29% men
11. Group age +50 contained 14% women and 3% men
12. That was also obvious that there was a correlation between Vitamin D deficiency that led to psychiatric disorders development

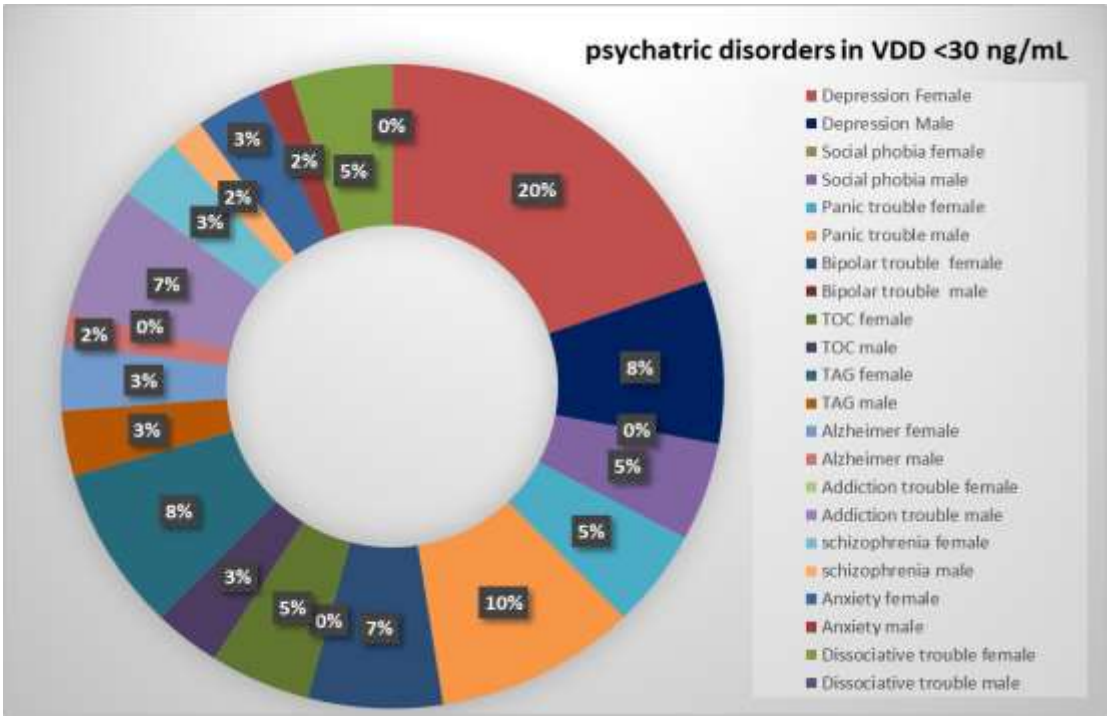

Figure (36) psychiatric disorders in VDD (<30ng/mL)

Discussion

This study was conducted to determine the association between vitamin D deficiency and psychological burden within the population of Saida and that of Sidi Bel Abbes. Further research was conducted to determine the link between mental health and vitamin D deficiency status. We also studied other variables that affect the emergence of psychological burdens and

compared their impact to that of a vitamin D deficiency. The results of the inferential analysis highlight a considerable impact of vitamin D deficiency on the occurrence of depression, anxiety and stress. Vitamin D is a steroid hormone that performs various essential roles in the body, including its psychological roles (Bičíková M, Máčová L.2023), Correlational scientific evidence continually demonstrates an adverse association between low vitamin D levels and mental health issues, including depression and anxiety, across all age groups (Wen Z and al.,2024)

The criteria for interpretation of vitamin D values are divided into four main categories: vitamin D deficiency (less than 20 ng/mL), vitamin D insufficiency (21–29 ng/mL),vitamin D sufficiency (equal to or more than 30 ng/mL), vitamin D intoxication (more than 150 ng/mL) (Khan B and al.,2022)

In the **first survey**, the prevalence of vitamin D deficiency was highest among 525 people, with 412 individuals (78% of the population) having levels below 30 ng/ml. Of these, 86% were women and 14% were men. In this study sample, the female gender emerged as an important factor in the development of psychological burdens, an association supported by many reports. In the **second study**, 80% of the 100 patients with mental disorders associated with vitamin D deficiency were women (66%) and men (33%), with variations observed across different age groups. This was corroborated by Tang and Zhang, Javaid et al (Tang J, Zhang T.2022). (Javaid SF and al.,2023). According to studies conducted, women are more likely than men to suffer from significant levels of depression and anxiety. (Albert PR.2015). Why is depression more prevalent in women?

Moreover, our study revealed a strong correlation between vitamin D deficiency and female gender in our sample, consistent with findings from prior research.(Jhee J and al.,2017). Vitamin D deficiency is significantly associated with depression in patients with chronic kidney disease.(Asdaq Sand al.,2020).

We attribute this correlation to prevalent local cultural norms that restrict skin exposure to sunlight, coupled with a lack of attention to sun exposure.(Raymond-Lezman R,Riskin S.2023). Therefore, with vitamin D deficiency, women are more likely to develop psychological distress. This was confirmed by Moy et al (Moy F and al.,2017).

A systematic review of vitamin D prevalence and predictors in the Middle East and North Africa region revealed that despite abundant sunlight, hypovitaminosis D is widespread, with rates ranging from 30% to 90%. Among adults, risk factors include older age, female gender, multiparity, seasonality, clothing style, socioeconomic status, and urban residency. Therefore, it is crucial to take measures to enhance vitamin D levels, which can significantly improve the mental well-being of the overall population, especially females.

Conclusions

Vitamin D inadequacy or deficiency (VDID) has been reported in a high percentage of otherwise healthy individuals. Factors that may contribute to the high prevalence of VDID in people with mental disorders include diet low in vitamin D, poor sunlight exposure, decrease in cutaneous vitamin D synthesis, intake of certain medications, VDID has been correlated to a host of adverse conditions, including rickets, osteoporosis, osteomalacia, muscle diseases, psychiatric disorders, cognitive dysfunction, and even certain cancers.

The major source of vitamin D for children and adults is exposure to natural sunlight. Very few foods naturally contain or are fortified with vitamin D. Thus, the major cause of vitamin D deficiency is inadequate exposure to sunlight. Wearing a sunscreen with a sun protection factor of 30 reduces vitamin D synthesis in the skin. People with a naturally dark skin tone have natural sun protection and require at least three to five times longer exposure to make the same amount of vitamin D as a person with a white skin tone, therefore a range of people avoid to get exposed to sunlight in order to not develop cancer or skin problems

Vitamin D deficiency has become a major problem not only by affection the physical health of a human being but also in affecting his mental state, that the majority of them struggles from mental disorders as anxiety, depression and schizophrenia

Vitamin D deficiency could affect people from different ages, sex that the previous prevalence showed that women are more prone to have VDD, most likely due to pregnancy and also for not being exposed to UVB and that's often due to passing most of the time indoors, clothing style, using skin cosmetic products that prevents Vitamin D absorption

Dietary also plays a proportional role in maintaining adequate vitamin D levels, that the most known sources of Vitamin D are fish, egg yolk, cod liver oil..etc, with adopting a healthy life style including physical activities

Recommendation

Algeria is a sunny region, ensuring sufficient sunlight exposure. However, people generally avoid outdoor activities, and in the studied region, women, in particular, have a phobia of sun exposure and frequently use sunscreen. These factors must be taken into consideration.

Vitamin D represents an important element for mood and any deficiency leads to mental disorders like depression and anxiety, but what is important is not to focus on the level of vitamin D is so important that we must go further by favoring its sources, whether in terms of exposure to the sun which represents more than 80% of the vitamin D that our body needs, then come food sources based on products of animal and then plant origin.

Vitamin D is an essential nutrient that our bodies need for many vital processes, including building strong bones and maintaining a good humor in our daily life and prevent us from mental illnesses, that's why it is crucial to adopt a measurement plan as a prevention from VDD that includes :

1. Spending time in sun light (taking in consideration skin tone, age, geographical location, season, sunscreen and clothing)
2. Dietary that is rich in Vitamin D sources as fatty fish, seafood, mushrooms, egg yolk, fortified food

3. Taking supplements
4. Vitamin D screening in case where a deficiency is found, better treating it in the first stages before it's getting severe

Better education of the medical community about the risk factors for the deficiency of these nutrients, its consequences for human health, the diagnostic possibilities, as well as measures for improving the nutrients status is also needed. These means are expected to help many patients in improving the control of their diseases as well as their quality of life and attach the importance to the high prevalence of vitamin D deficiency as well the awareness of the individual's mental state in society and making their prevention a health priority

Following our study we see that women are the most vulnerable regarding vitamin D deficiency compared to men and among the most relevant factors remain the reduction or absence of exposure to the sun by default or by fear of ideas received concerning cancer or by choice for aesthetics.

For vitamin D to exert its full power, it needs magnesium, an essential element to be active, just as VDBP and VDR need magnesium to perform their respective tasks.

Zinc and vitamin K2 also have an important role to play in the biochemistry of vitamin D but vitamin K2 has a particular role which helps avoid the toxicity of vitamin D relating to hypercalcemia

Based on our study, sun exposure is the primary recommendation for anyone looking to correct a deficiency or maintain normal vitamin D levels. The optimal time for this is between 10 a.m. and 3 p.m., when UVB availability is highest. However, the use of total sunscreens can inhibit vitamin D synthesis. The situation is further exacerbated for individuals who live indoors or engage in activities sheltered from the sun.

Globally, vitamin D deficiency has become a concern.The high prevalence of vitamin D deficiency is believed to increase the global burden of associated diseases. Thus, governments, policy makers, health professionals and individuals should attach importance to the high prevalence of vitamin D deficiency and make its prevention a public health priority, without forgetting self-medication which remains nevertheless a potential danger.

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Annexe

Vitamin D analysis surveys

Table : Vitamin D level (ng/ml)

Data were taken from three laboratories in Wilaya of Saïda, Vitamin D serum levels of 525 subjects classified based on sex and age groups

25(OH) D based on sex and group age									
	male	female	1-15 years		15-50 years		≥50 years		Total
			male	female	male	female	male	female	
Vitamin D <30 ng/mL	57	355	8	26	30	215	19	114	412
Vitamin D 30-50 ng/mL	10	73	1	5	2	32	7	36	83
Vitamin D 50 -70 ng/mL	1	19	1			11		8	20
Vitamin D ≥70 ng/mL	3	5			1	3	2	2	8
Vitamin D ≥100 ng/mL		2		1				1	2
Total	71	454	10	32	33	261	28	161	525

Vitamin D levels : D<30 ng/ml ; 30-50 ng/ml ; 50-70 ng/ml ; ≥70 ng/ml ; ≥100 ng/ml

Table : Vitamin D level (D<30 ng/ml)

412 subjects having Vitamin D defeciency from the total population of 525 people

Population	Women	Men	Total
1-15 years	26	8	34
15-50 years	215	30	245
≥50 years	114	19	133
Total	355	57	412

Table : Vitamin D levels of patients struggling from psychiatric disorders in Wialaya of Sidi Bel Abbes

Data were classified based on sex and age groups

25(OH) D based on sex and group age									
	male	female	1-15 years		15-50 years		≥50 years		Total
			male	female	male	female	male	female	
Vitamin D <30 ng/mL	27	53	1	1	23	42	2	11	80
Vitamin D 30-50 ng/mL	8	10			6	8	2	2	18
Vitamin D 50 -70 ng/mL		1						1	1
Vitamin D ≥70 ng/mL									
Vitamin D ≥100 ng/mL	1				1				1
Total	36	64	1	1	30	50	4	14	100

Table : Vitamin D sample questionnaire

This questionnaire is formed of 15 questions, that were asked on 140 person classfied based on sex and age groups

	Men		Woman		Child	
	18-+50		18-+50		10-18 years	
	yes/in	no/out	yes/in	no/out	yes/in	no/out
Have you ever heard of vitamin D?	54 yes	0 no	61 yes	0	17 yes	8 no
Do you work mainly indoors or outdoors?	40% In	59% out	65% in	34% out	80% in	20% out
Do you live in an apartment or a house?	74% Ap	25% Home	85% Ap	14% home	100% Ap	0% Home
Do you like being in contact with the sun?	100% yes	0% no	90% yes	9% no	100% yes	0% no
Are you afraid of sun exposure	0% yes	100% no	81% yes	18% no	100% yes	0% no
Did you know that sunlight can provide you with vitamin D?	74% yes	25% no	70% yes	29% no	24% yes	76% no
Did you know that darker skin tones are more prone to vitamin D deficiency?	3% yes	96% no	6% yes	93% no	0% yes	100% no
Do you use sunscreen? SPF factor?	0% yes	100% no	40% yes	59% no	0% yes	100% no
What are the sources of vitamin D?	74% fish/milk	25% Egg/tuna	49% Dairy product	50% vegetable	52% egg	48% Milk
Do you take vitamin D supplements?	5% yes	94% no	16% yes	83% no	0% yes	100% no
Do you consume a lot more products of animal or plant origin?	50% animal	49% vegetal	49% animal	50% vegetal	50% animal	49% vegetal
Do you have the ability to eat well? (Allow red meat, cow's milk, butter for example).	100% yes	0% no	95% yes	4% no	100% yes	0% No
Do you take cow's milk, do you eat butter, meat (approximate quantity)	90% yes	9% no	86% yes	13% no	100% yes	0% no
Have you been diagnosed with Crohn's disease, ulcerative colitis or celiac disease?	35% yes	64% no	24% yes	75% no	8% yes	92% no
Do you suffer from Chronic Intestinal Disorder?	27% yes	72% no	6% yes	93% no	0% yes	100% no