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Evaluation the Level of Vitamin D Receptor, Retinoid X Receptor, and Vitamin D Binding Protein in Patients with Chronic Plaque Psoriasis and Its Relationship with Disease Severity

A Thesis
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جامعة بابل
كلية الطب

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رسالة مقدمة إلى
مجلس كلية الطب – جامعة بابل
كجزء من متطلبات نيل درجة دكتوراه فلسفة في الكيمياء الحياتية السريرية

من قبل

محمد عبد الحسين خليل غياض

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Summary

Psoriasis is a chronic recurrent inflammatory disease that occurs due to the appearance of important changes in the growth and differentiation of epidermal cells. The disease is multi-factorial and there is difficulty in identifying the exact cause for it, but there is a strong relationship with genetic factors in addition to the deficiency of certain substances such as vitamins or over-expression of certain immunologic factors from the activated T cell.

There are different triggering factors for psoriasis such as trauma, infection bacterial, or viral.

Psoriatic lesions are sharply red or pink in color with white to silver scale plaque, plaque either spread or localized in a single place from the skin. Many different psoriatic patients have psoriatic arthritis which is the main complication of psoriasis. Psoriasis has a negative impact on patient life.

The prevalence of psoriasis is 3% over the world. The onset of the disease can occur at any age from early life to the older extreme age.

Epidermal keratinocyte hyperproliferation with the increase in the infiltration of the inflammatory cell to the epidermis in association with the increase in angiogenesis is the main pathological mechanism of psoriasis. In the normal condition the consumed time for normal proliferation, differentiation, and then emigration of the keratinocyte from the basal layer of the skin to reach the upper dermal layer until it becomes senescence usually required 30 days in the normal condition, During the disease, there is an increase in the rate of keratinocyte proliferation subsequently followed by a decrease in the rate of differentiation with rapid keratinocyte emigration thorough the different skin layer reaching

the upper layer, Losing of the granular layer and acanthosis also it can occur during psoriasis.

All patients that were included in the study were collected from the department of dermatology after diagnosing the type of psoriasis as chronic plaque psoriasis by the specialist dermatologist was founded in the department of dermatology in the Marjan teaching hospital in al Hilla city. The study was designed as a case-control and 89 cases were collected in the study which is patient and control groups respectively.

Patients information such as name, gender, age, address, date of the onset and duration of the involvement, height, weight, BMI calculation, presence or absence of the psoriatic co-morbidities, psychological stress, itching, erythema induration, and calculation of the PASI score are collected directly from the involved patients are directly collected from the patients after an interview with those patients and the documented in the questionnaire and finally patients signature.

The mean age for the patient and control group in the study was 36.6905 ± 13.862281and 40.2414 ± 18.33662 respectively with the P value 0.148, the mean BMI for the patients and control group was 27.2663 ± 6.59598 and 28.3227 ± 4.66561 respectively with the P value 0.380, the mean exposure to the sunlight among patients and control group was 137.9885 ± 14.10315 and 125.0460 ± 9.45214 respectively with the P value 0.56

The result of the current study explains there is no significant statistical difference between age, BMI, and exposure to sunlight between the patient's group and the control group.

There is a significant difference between the level of the DBP between the patient and control group where the level of the DBP is

higher in the patient than in the control group. The level of the DBP in the patient and control group was 435.0364 ± 171.36762 and 266.1936 ± 217.30586 respectively with the P value 0.01

The VDR level was more deficient in the patient's group than in the control. The level of the VDR in the patient and control group was 2.4283 ± 0.15848 and 3.2821 ± 1.7089 respectively with the P value 0.001

The level of the alpha RXR was higher in the patient's group than in the control. The level of the alpha RXR among the patient and control group was 22.2989 ± 9.55601 and 8.1467 ± 4.39137 respectively with the P value 0.001

The author of the current study's opinion about the result of the current study was the DBP was elevated in the patient group because of the deficiency status of the vitamin D in the patient's group. The VDR was little in the patient's group may be attributed to the higher level of the vitamin D binding protein. The high level of the alpha RXR is attributed to the deficiency in the level of the VDR.

The current study concluded that the DBP, VDR, and alpha RXR are important in the lower the PASI score in the psoriatic patient, which makes it a high novelty in the end.

Dedication

I want to thank my God to help me in completing my work, my great thanks to all people who help me in this work beginning with the family father, mother, brother, sister, and professor Hussein Al Sultany.

My great thanks to Dr. Saffa alwash, Ali shukur, and abu Fatima.

My great thanks for the my frind Rahad, shahd and zainab with Dhay to help me in my work.

Acknowledgment

In the beginning great thanks to my father and mother who support me to complete my study and then to my supervisor and all the people who help me in completing my work. Very great thank you for me to carry out different difficulties during my study in the end.

Even in the presence of difficulties, God helps me to enface these difficulties, My supervisor Hussein Abbas Al sultany played important roles in supporting me in all the scientific information included in my study and learn me the different things considered helpful for me in my routine life.

All the teachers that taught me in my academic life left a very beautiful impression on them in my heart and I express all my respect and thanks to them.

My father and mother are great humans and provide me with the power to enfacing all the difficulties during the period of work.

I want to express my respect to Dr. Saffa Alwash and Dr. Ali Shukur and abu Fatima who help me with different scientific information and make me a brother to them**.**

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List of abbreviation

Abbreviation	Mean
AF-2	Activated function – 2
Ag	Antigen
AIDS	Aquired immunodeficiency syndrome
Alpha RXR	Alpha retinoid X receptor
BB-UVB	Broad band ultraviolet B
BMI	Body mass index
cAMP	Cyclic adenosine monophosphate
CE	cornified envelope
Dab-2	Disabled protein
DBD	DNA binding domain
DBP	Vitamin D binding protein
DBP-MAF	Macrrrophage activated factor - D binding protein
DHA	docosahexaenoic acid
DM	Diabetes mellitus
DR3	Direct repeate 3
EGFR	epidermal growth factor receptor
EGR-1	early growth response -1
EPA	eicosapentaenoic acid
ER 6	everted repeat 6
GC 2	group-specific component 2
GC1	group-specific component 1
GC1f	Group specific component 1- fast
GC1s	Group specific component 1 – slow

GM-CSF	Granulocyte monocyte – colony stimulating factor
HIF	hypoxia-inducible factors
HPA	Hypothalamous pituitary axis
Hr	Hour
IFN	Interferon
KDa	Killodalton
LBD	ligand-binding domain
LDL	Low density lipoprotein
MAPK	Mitogen activated protein kinase
ml/ d	Millileter/ day
MUFA	Monounsaturated fatty acid
NB-UVB	Narrow band ultraviolet B
ng/d	Nanogram/day
NGF	nerve growth factor
nm/l	Nanomol/ litter
PAF-1	plasminogen activation factor-1
PASI	Psoriasis area and severity index
PGA	Physician Global Assessment
PUVA	Phototherapy ultraviolet A
RA	Rhumatoid arthritis
RAR	Retinoic acid receptor
ROS	Reactive oxygen species
TDGF	tumor-derived growth factor
TG	Triglyceride
TGase I	Transglutaminase I
Th	T helper cell

TLR	Toll-like receptors
Trm	resident T cell
UVA	Ultraviolet A
UVB	Ultraviolet B
UVC	Ultraviolet C
VDD	Vitamin D deficiency
VDR	Vitamin D receptor
VDRE	vitamin D response element
VEGF	vascular endothelial growth factor
Vit-D-VDR	Vitamin D- Vitamin D receptor
VLDL	Very low density lipoprotein
α- TNF	Tumor necrosis factor-alpha

Examining Committee Certification

We certify, that we have read this thesis entitled **(Evaluation the Level of Vitamin D Receptor, Retinoid X Receptor, and Vitamin D Binding Protein in Patients with Chronic Plaque Psoriasis and Its Relationship with Disease Severity.),** and in our opinion, as the examining committee examined the student **(Mohammed Abd Al-Hussein Khalil)** in the contents, it is adequate with " **Excellent** " as a thesis meets for the standard for the degree of Doctor of Philosophy of Clinical Biochemistry.

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Supervisor Certification

We certify that this thesis entitled **(Evaluation the level of Vitamin D receptor, retinoid X receptor, and vitamin D binding protein in patients with chronic plaque psoriasis and its relationship with disease severity.)** was prepared by **(…**Mohammed Abd Al-Hussein Khalil**)** under our supervision at the Department of Chemistry and Biochemistry, College of Medicine, University of Babylon, as a partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy of Clinical Biochemistry.

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In Review of the available recommendation, I forward this thesis for debate by the examining committee.

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1. Chapter One

1.1. General Introduction

Psoriasis is considered a chronic inflammatory with immune background disease of the skin. It has a negative effect on the physical and emotional additionally the psychosocial life of affected patients [1]. There are different environmental triggering factors such as infection or drugs which may be secondary to the trauma or genetic factor. Psoriasis is found in the entire world but the prevalence varies among the different ethnic groups [1].

Psoriasis present in the entire world and the prevalence was differed from one ethnic group to another, main clinical manifestation of the psoriasis is chronic, symmetrical, erythematous, thickening and scaling of the skin. There is hyper-proliferation of keratinocytes and alteration in differentiation. The main reason is still unknown, Historically, psoriasis is considered a primary disorder of keratinocytes [1].

The prevalence of psoriasis is 3% and mostly in European and North America and 0.16 % in Iraq in 2016. The disease occurs in different age groups with rare occurrences less than 10 years and greatly between 15-40 years, There is an unknown course of disease with continuous remission and exacerbation [1]. Genetic abnormality leads to keratinocyte hyper-proliferation which in turn, produces a defective skin barrier allowing the penetration of antigens resulting in the immune response to that antigen(Ag) [2].

There is two average age of incidence, the first occurring between 16 - 20 years and the second between 40 - 62 years of age which results in the concept of type I and type II psoriasis [2]. About 35% of patients, the

disease occurs before the age of 20 years while about58% before the age of 30 years, The mean age of onset was 33 years with the mode of appearance mainly in the second decade, About 75% of patients, the disease onset was before 46 years of age [2]. There are many interesting differences between psoriasis and other immunological diseases [3]. Psoriasis is characterized by the absence of auto-antibodies recognized in patients' serum which makes it the same as Crohn's disease but differs from rheumatoid arthritis (RA) and other autoimmune condition such as Graves disease and this is mainly due to the lack of B-cell activation that's seen in psoriasis [3]. Tissue destruction and scarring do not occur in psoriasis this may be a function of epidermal regeneration [3].

1.2. Definition

Psoriasis is a chronic recurrent inflammatory disease that occurs due to the appearance of important changes in the growth and differentiation of epidermal cells. The disease is multi-factorial and there is difficulty in identifying the exact cause for it, but there is a strong relationship with genetic factors in addition to the deficiency of certain substances such as vitamins or over-expression of certain immunologic factors from the activated T cell. [4]

There are different triggering factors for psoriasis such as trauma, infection bacterial or viral, and finally, medication Psoriatic lesions are sharply red or pink color with white to silver scale plaque, plaque either spread or localized in a single place from the skin. Many different psoriatic patients have psoriatic arthritis which is the main complication of psoriasis. Psoriasis has a negative impact on patient life [5].

The prevalence of psoriasis is 3% over the world. The onset of the disease can occur at any age from early life to the older extreme age [6].

1.3. Incidence and prevalence

Generally, the prevalence of psoriasis in the world was said to be 3% but there is some difference from one area to another and these are attributed to the ethnic, habitant and weather, in the USA and Canada the prevalence was higher than in the Asian and African people, the prevalence in the USA, Canada, Africa, and Asia was 4.6%, 4.7%, 0.4%, and 0.7%.

The most prevalent type of psoriasis is chronic plaque psoriasis followed by guttate psoriasis. Two third (75%) of the psoriatic patients have mild to moderate chronic plaque psoriasis and the remaining one-third (25%) has severe chronic plaque psoriatic involvement [7]. Most psoriatic patients have nail changes which is one of the most changes in psoriatic patients [8].

There are some variability and difficulty in the determination of the prevalence of psoriasis, and the main cause for this difficulty and variability is attributed to the genetic, habitat, and dietary factors [9].
Determination of the specific age for the appearance of psoriatic onset in the patients is very difficult and cannot be expected because psoriasis can occur at any age, In general, there is a bimodal age for the determination of the first presentation of psoriasis which includes the age between 15-30 years and between 55-60 years [8]. These bimodal ages make the basis for the classification of psoriasis into two main types which are Type 1 which occur before the age of 40 years and is more severe than the second type and strong association with the HLA and consist of about

75% of the psoriatic cases and the second type called type 2 psoriasis which occurs after the 60 years [8].

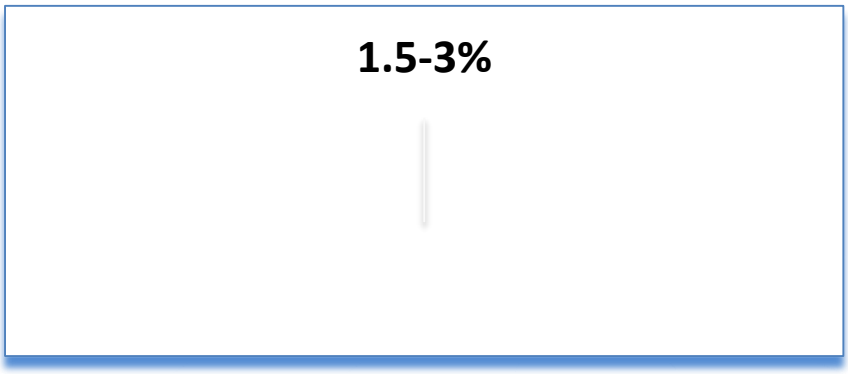


Figure 1-1 Prevalence of Psoriasis in the World

1.4. Pathogenesis of psoriasis

The initiation of the pathological mechanism of psoriasis resulted in the response to immunological stimulation such as infection either viral or bacterial infection or an injury or any stimulant which resulted in the activation of different T cell variants and increasing in their rate of proliferation and differentiation which increasing the secretion of different inflammatory cytokines that involve in the pathogenesis of psoriasis [10]. The increasing level of cathelicidin which antimicrobial peptide can increase the induction and activate of Toll-like receptor by binding with its self region on the DAN causing increased production of interferon – α and increasing the inflammatory response which may increase the severity of the disease [11].

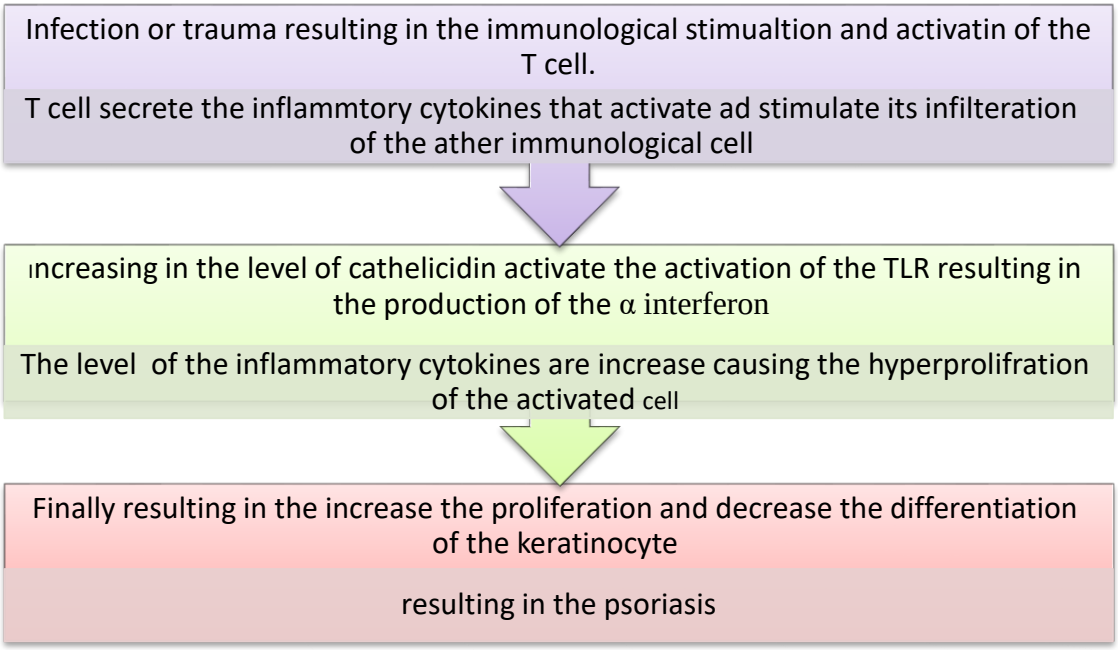


Figure 1-2: explain the pathologic mechanism of the psoriasis

1.5. Clinical variants and classification of psoriasis

The classification of the disease is based on different criteria which include the age of onset, area of the involved skin, shape, and morphology of the psoriatic lesion, and the type of the involved region from the body. The psoriatic patients are complaining of the main extra-cutaneous problem which are psoriatic arthritis The most common form of psoriasis is chronic plaque psoriasis which forms about 90% of the psoriatic cases [12].

1.5.1. Chronic plaque psoriasis

Is the most common form of psoriasis and consists of about 90% of psoriasis in psoriatic patients. The plaques are distributed on the external surface of the extremities mainly in the elbow, Knee, scalp, and buttock, and it is characterized by a red, scaly appearance.

There is some variability in the extent of the involvement from one patient to another; the shape, size, and distribution of the psoriatic scale are also different. The psoriatic lesion may be distributed and extended laterally and these are attributed to the aggregation of many plaques which form the psoriatic gyrate variant of chronic plaque psoriasis [13].

1.5.2. Presentation of chronic plaque psoriasis

The psoriatic lesion may occur as discrete with different sizes and shape over the skin or aggregated forming large size lesion distributed in the different areas of the skin such as the knee, and elbow.

Linear and geometric configurations may arise at the sites of trauma as an isomorphic (Koebner) phenomenon

The plaque is generally red with a noticeable difference in the color quality depending on the degree of the skin color of the patients for example the color quality is lost in dark skin color patients while it occurs in the finite skin color. Postinflammatory hypopigmentation or hyperpigmentation may occur after the complete clearance of the lesion. Most psoriasis lesions are surrounded by silvery-white scales, which vary considerably in thickness. The amount of scaling may be minimal in partially treated diseases, and flexures. When scaling is not evident it can often be induced by light scratching, a useful sign in diagnostically uncertain lesions. A silver-white scale underlying the smooth red membrane and different thicknesses surrounding the psoriatic lesions, some of the blood spots can occur after removing these scales due to the damaging of the suprapapillary blood capillary that is initiated by the action of the angiogenesis process (Auspitz's sign)

Due to the epidermal thickening that occurs to the hyperproliferation of the keratinocytes, these properties make the psoriatic lesion elevated from the adjacent skin and easily distinguishable and this elevation may use as an indicator of the response of the disease to the treatment[13].

1.5.3. Diagnosis

Psoriasis is a disease mainly diagnosed clinically by the physician, different variants of the disease are present and the clinical differential diagnosis can be achieved only by the dermatologist . The disease severity differs from one patient to another and it can be assessed by the Psoriasis area and severity index which involves three main signs of the psoriasis erythema, indurations, and scaling, and the severity range from 0 to 75 [42]. For example, PASI-75 indicates that the patient's psoriasis has improved by 75% or greater from baseline. The Physician Global Assessment (PGA) is another simplified measurement tool that rates the severity of psoriasis at a single point in time [14].

It is important to note that one of the limitations of the PASI and PGA is that there can be high interobserver variability In addition to assessing the severity of psoriasis, it is also important to include evaluations of subjective symptoms and quality of life burden [15].

1.6. Triggering and exacerbating factors of the psoriasis

The triggering factor can be classified depending on its nature as shown in table 1-2 into:

Table 1-1 Shows the classification of the triggering and exacerbating factors of psoriasis according to its nature.

Clinical Factors	Behavioral Factors	Physical Factors
Infection	Life style	Koebner phenomena
Drug	Smoking	
Vaccination	Dietary factor	
Obesity		

1.6.1. Infection

There is a positive association between the infection either bacterial or viral and psoriasis. There is an association between tonsillitis and psoriasis . This association resulted from the activation of T-cells by the bacteria which may result in a section of the inflammatory cytokines and cause psoriasis . Because streptococci are intracellular bacteria, the treatment of psoriasis that occurs due to its infection is difficult and there is no response, tonsillectomy is the most effective treatment for such type of psoriasis [16].

1.6.2. Drugs

Some cases of psoriasis can occur due to the ingestion of a certain drug, but there is a difficulty in the identification of drug-related psoriasis from non-related mainly due to the long period between the time of drug ingestion and the occurring of onset, However, many cases are sustained

after discontinuing the drug that initiated psoriasis . The main histological abnormality in drug-related psoriasis is the epidermal eosinophilic infiltration, positive lichenoid reaction, and initial appearance of micro-abscess in the epidermis [17].

1.6.3. Smoking

There is a positive association between the initiation of psoriasis and smoking through genetic interaction . Smoking can initiate psoriasis through different mechanisms . Smoking can result in the production of a large number of free radicals that have a negative impact on the human, and these free radicals can interfere with the MAPK signal pathway which is responsible for the regulation of the cellular apoptosis of keratinocytes [18] as shown in the figure 1-4.

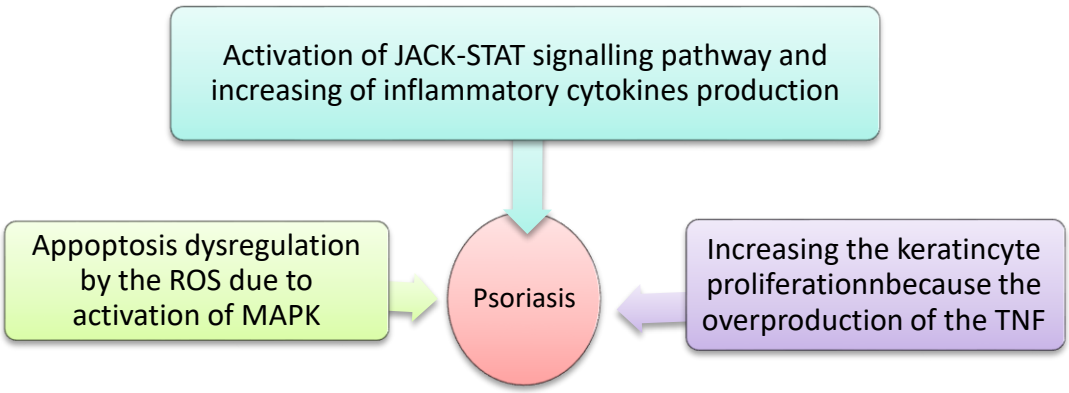


Figure 1-3: Showing the relationship of smoking with psoriasis.

1.6.4. Vaccination

The vaccination process is very essential for psoriatic patients because of the immunosuppressed state of those patients due to the ingestion of immune inhibitory drugs which make them more susceptible to secondary bacterial or viral infection . Some vaccines can increase the

exacerbation of preexisting psoriasis or can initiate it . The main mechanism for initiation of vaccine-related psoriasis is by the vaccine can stimulate the production of T h1 and Th 17 cells as a response to the vaccine itself which may lead to an increase in the production of inflammatory cytokines and initiation of psoriasis [19].

1.6.5. Lifestyle

Alcohol is another risk factor for the development of psoriasis and can decrease drug efficacy by increasing the disease severity and mortality among psoriatic patients [19].

1.6.6. Diet

Many cases of psoriasis result from a deficiency in a different nutrient that controls the differentiation and proliferation of the keratinocyte and these deficiencies can occur due to the epidermal cell desquamation and hyperproliferation of the cell [20]

Many studies reveal to the high energy and saturated fatty acid-rich diet is a triggering factor for psoriasis compared with the MUFA-rich diet which has a protective role on the cell membrane, Fibers rich diet has a positively strong relationship with decreasing the disease severity because it can reduce the carbohydrate absorption and then play important role in reducing the body weight which may result in decreasing the oxidative stress of the high carbohydrate diet [20].

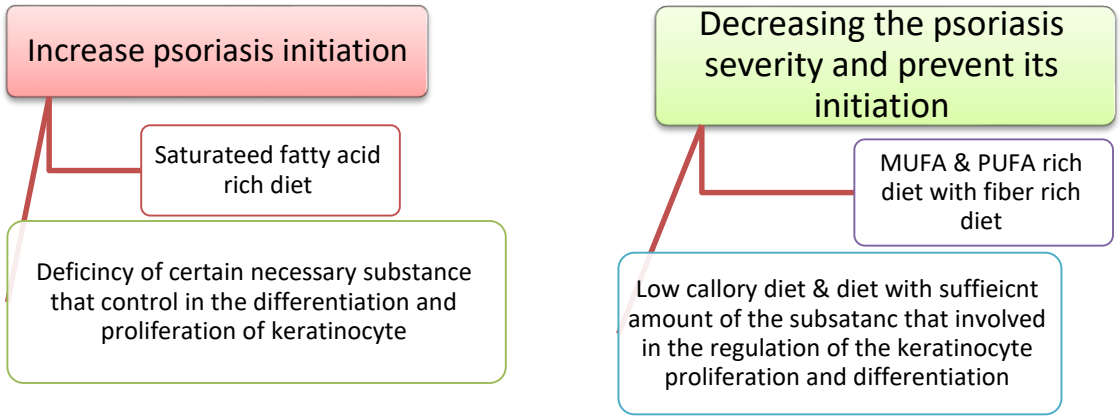


Figure 1-4 Show the relationship between the diet and psoriasis

1.6.7. Obesity

Obesity is considered an important risk factor for the initiation of psoriasis. The increase in the size of adipose tissue results in obesity which is considered an important source for the production of certain inflammatory mediators such as TNF-α, IL-6, leptin, and adiponectin making obesity an important risk factor for psoriasis [21].

1.6.8. Koebner phenomena

Psoriasis can be initiated in response to trauma such as physical trauma or skin irritation in response to certain allergic substances . During the koebner phenomena, there is an increase in the dermal blood flow which brings certain mediators from the different organs to the dermis, nerve growth factor (NGF) is an example of this mediator, NGF is produced from the nerve and peripheral cell in addition to keratinocyte which may increase the keratinocyte proliferation after cutaneous trauma in response to this factor [22].

1.7. Co morbidities of psoriasis

Table 1-2 Explain the psoriatic comorbidities classified according to their nature

Clinical comorbidities	Physical comorbidities	Behavioral comorbidities
Metabolic syndrome	Koebner phenomena	Anxiety
Cardiovascular disease		Depression
Hypertension		Smoking
Obesity		
Cancer		
Infection		

Cardiovascular disease is one of the most psoriatic complications that mediate mortality among them, there is a strong relationship between psoriasis and cardiac disease and these may be attributed to the increasing causative agents for heart disease among psoriatic patients such as obesity, hyperlipidemia, hyperhomocysteinemia.

Folate deficiency is predominant in psoriatic patients and this results from the increased DNA methylation in the hyper proliferated skin cell which may cause a disturbance in the metabolism of homocysteine and then accumulate, The accumulated homocysteine can damage the vascular endothelial layer, decreasing vascular and aortic flexibility to become stiff and finally stimulate the formation of the arteriosclerotic plaque which may obstruct the vessels and result in the cardiac disease [23]. Hypertension is the second psoriatic comorbidity and it has the same triggering factor for psoriases such as smoking and obesity, psoriasis is considered an independent causative agent for hypertension, The higher

activity of rennin and angiotensin-converting enzyme in the psoriatic patients than the normal subjects [24].

The risk for hypertriglyceridemia among psoriatic patients may be attributed to the inhibitory role of the inflammatory cytokines on the lipoprotein lipase enzyme which is IL-1, IL-2, and TNF-alpha which cause decreased clearance of TG and accumulate in the circulation [25].

There is an increase in the reactive oxygen species (ROS) among psoriatic patients and resulting in oxidative stress which may cause the oxidation of the cell membrane lipid bilayer or circulatory plasma lipid such as LDL to form oxidized LDL in addition to the oxidized LDL that produced in the epidermis due to the ROS in the psoriatic lesion which resulting to the increasing level of LDL and precipitate it at the damaged endothelial resulting in the plaque [26].

The aggregation of obesity, dyslipidemia, and hypertension in addition to diabetes in the same patients will result in a clinical status called metabolic syndrome, as these association increase strongly as the severity of psoriasis was increased and this clinical status is considered a strong predictor for the cardiovascular disease among the psoriatic patients [27].

There is a negative impact of psoriasis on the psychological status of psoriatic patients and this may be attributed to the visibility of the psoriatic lesion which makes the psoriatic patient face many social, phycological, and physical limitations [28].

Hyperhomocysteinemia can also occur as a result of long-term methotrexate treatment due to methylation mediated methotrexate process [29].

1.8. Laboratory investigation

The histological test for the diagnosis is rarely required but it is very helpful in the diagnosis and differentiation of psoriasis from other cutaneous diseases such as eczema. The remaining laboratory test is not specific to psoriasis and it is used for the diagnosis of the psoriatic co-morbidities different from one patient to another, hypoalbuminemia is the major finding in the laboratory test [30].

The alteration in the lipid profile such as elevation of the level of cholesterol, triglyceride, and VLDL among psoriatic patients and mainly occur as a result of drugs such as acitretin which causes elevation of the level of TG and certain inflammatory mediators that have an inhibitory role in the activity of the lipoprotein lipase [30].

1.9. Treatment

There are three main lines for the treatment of psoriasis in psoriatic patients and the use of these lines differs from one patient to another and depends on the disease severity, as the disease severity increases the possibility of using a more potent drug. these lines include topical treatment, systemic treatment, and biological therapy [31].

The treatment aims to minimize and monitor the disease severity to the point at which there is no negative impact and increase the possibility of remission as shown in figure 1-6.

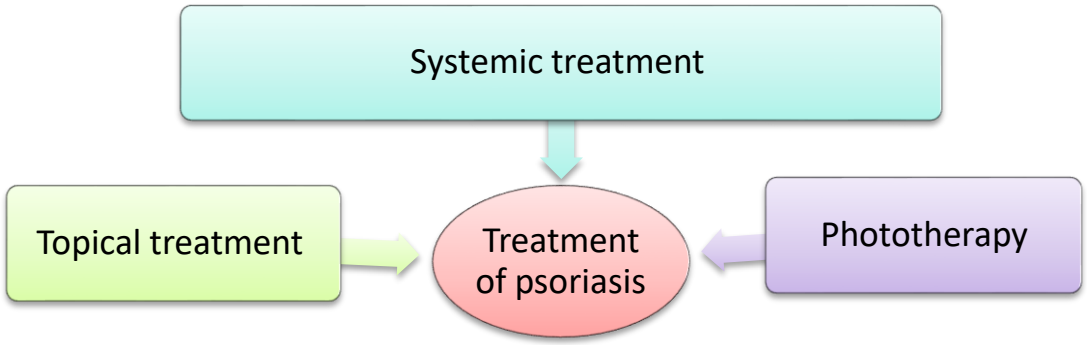


Figure 1-5 explains the main guidelines for the treatment of the psoriasis

1.9.1. Topical treatment

Topical treatment is the treatment used for treating a patient with a PASI score of less than 10%. Topical therapy is considered the major safe, and effective with limited side effect treatment that's used in the treatment of localized psoriasis [31].

1.9.1.1. Topical corticosteroid

This type of topical treatment is considered a universal treatment of psoriasis either as monotherapy or combination therapy, the potency of this topical steroid depends on the severity of the disease.

Steroid can mediate its action at the cellular level because its lipophilic nature makes it able to cross the membrane of the cell and its nucleus respectively and then exert its effect by modulation the genetic expression for the inflammatory mediators that play important role in the pathogenesis of psoriasis after its ligand with its cytoplasmic receptor and then transforming these complex to binding to its responsive elements on the gene that coded for these inflammatory mediators [32]. There are seven grades for the potency of corticosteroid therapy with grade one being the most potency and the mildest potency in grade seven and choosing potency in treatment depends on the disease severity. High

potency corticosteroids should not be used for more than two weeks because these can maybe be leading to the appearance of serious side effects which is either systematically or locally [32].

1.9.1.2. Vitamin D

Vitamin D is a vitamin that has a lipophilic nature and it can cross the cell membrane and binding to its cytoplasmic receptor which is called vitamin D receptor to form the Vitamin D- VDR complex, this complex can cross the nuclear membrane and binding to its responsive elements on the gene and then controlling the inflammatory mediators that play important role in the pathogenesis of psoriasis and enhancing the keratinocytes differentiation. Irritation, burning, itching, and erythema with dryness is the most local side effect of vitamin D[33]. Calcitriol is the active form of vitamin D and it has greater efficacy on the disease severity but it has a more serious side effect which is the elevation of the level of calcium [33].

1.9.1.3. Retinoid

Retinoid is a derivative of vitamin A which is a lipophilic vitamin that has intracellular receptors called a retinoic acid receptors.

The retinoids or their derivatives bind to the intracellular receptors to form the homodimers consisting of the retinoid or its derivative – RAR which is then transformed into the nucleus and binding to its responsive elements and controls the genetic expression [34].

Retinoids control the keratinocyte differentiation and reduce its proliferation and inhibition of the inflammatory response by downregulation of its genetic expression [34].

The main side effect of topical retinoids is irritation, erythema, and burning. Retinoids cannot be used in pregnant women because of their teratogenic substance [34].

1.9.2. Systemic treatment

1.9.2.1. Methotrexate

Is a type of systemic drug used in the treatment of moderate to severe psoriasis that was approved in 1972 by the FDA. This drug has antiproliferative, immunosuppressive with anti-inflammatory effects by inhibiting the activity of the dihydrofolate reductase enzyme which results in decreasing the synthesis of folate that require as a cofactor for the synthesis of nucleic acid in the keratinocytes and another inflammatory cell that involve in the pathogenesis of psoriasis [35].

Methotrexate is usually given orally but sometimes intramuscularly. Most dermatologists are starting in low doses with subsequent follow-up to the hematological finding to identify the bone marrow suppression causing leukopenia which may increase susceptibility to the infection or reactivation of the dormant stage for many infectious agents in the human body such as viral hepatitis[35].

1.9.2.2. Cyclosporine

This potent immunosuppressant drug is used for the treatment of psoriasis and exerts its inhibitory action by binding to the cyclophilin which in turn inhibits the calcineurin causing the downregulation of the proinflammatory cytokines by inhibiting the stimulatory signal that stimulates the induction of these cytokines such as IL-2 and interferon-gamma resulting in the prevent the activation of the T-cell [36].

1.9.2.3. Biological therapy

Advanced molecular technology has reached to developed high efficacy factors that can bind to the interleukins or their receptors and also can bind to the TNF and inhibition of the inflammatory response by promoting the differentiation of the Th1, and Th2 cells. These factors are classified into two major groups which include the TNF inhibitor and interleukin inhibitors such as IL-23.

All the TNF inhibitors are monoclonal antibody such as infliximab for the exception of the etanercept is the dimeric protein resulting from the fusion of the outer portion of the TNF receptor with IgG1 because it is more predominant IgG subclass, this inhibitor exerts its inhibitory action by binding to the TNF and prevent its binding to the receptors while the IgG1 can enable the complement-dependent cytotoxicity to destroy and clearing the TNF from the circulations [37].

The second type of biological treatment is the interleukin inhibitors such as ustekinumab which is a monoclonal antibody that targets IL12 and IL-23. The IL-12 has an important role in the activation of the Th1 and Nk cells while the IL-23 can activate the Th17 which results in increasing the inflammatory response and hyperproliferation of the keratinocytes. [38].

1.9.3. Phototherapy

Non-ionizing electromagnetic radiation can be used in the treatment of cutaneous diseases such as psoriasis. The efficacy and the side effect of these types of treatment mainly depend on the density and distribution of the chromophores in the target tissue. Chromophores are molecules that can absorb ultraviolet. The ultraviolet is the only type of

radiation that can be used in phototherapy and can be classified into main three types depending on their wavelength range, UVA (320-400 nm), UVB (280-320 nm), and UVC (280-100 nm) [39].

Photochemotherapy is a subtype of phototherapy that involves using the UV in combination with a certain type of photosensitizing substance that can stimulate the special chemical reaction by which the phototherapy can exert its beneficial effect such as psoralen [39].

The main benefits of these types of treatment are low cost, a lower range of side effects in comparison with the other type of treatment, and finally more effectiveness and availability.

1.9.3.1. Mechanism of phototherapy

The mechanism by which the phototherapy exerts its action depends on the range of the radiation and thickness and severity of the psoriatic lesion, the UVB is restricted to the cells of the epidermal layer and upper part of the dermal layer while the UVA can reach the basic layer from the dermis and these may be attributed to its longer wavelength.

The main target for this ultraviolet radiation is chromophores which are the molecules that can absorb light. cellular DNA and melanin in the epidermal layer are the main chromophores in the skin [39].

These mechanisms include:

1.9.3.1.1. Alteration of Cytokine Profile

The T cell is the major responsible cell that controls the immune response to different stimuli, these can occur after its differentiation to its

subtype resulting in the alteration of the cytokines expression from the Th subset which is Th1, Th2, and Th17 causing an increase in the secretion of the inflammatory cytokine from the Th1 and Th17.The increasing level of inflammatory cytokines such as TNF alpha, IL-1, IL-17, IL-23, and IL-12 result in the hyperproliferation of keratinocytes [39].

1.9.3.1.2. Induction of apoptosis

Different studies reveal to treatment with the UVB can regulate the cellular apoptosis for the T cell and keratinocyte in the dermal and epidermal layer and these were confirmed by the absence of the Tcell after treatment with UVB in the psoriatic lesion and decreasing its inflammatory cytokines [39].

The different studies confirmed that there is an increase in the level of tumor suppressor protein such as tp53 which has an important role in the regulation of cellular apoptosis after treatment with NB-UVB .UVB can induce apoptosis by damaging the DNA or formation of the pyrimidine dimer and finally maybe by causing damage to the cellular membrane which results in the death of the receptor activation and inducing the apoptosis pathway [39].

1.10. Vitamin D binding protein

The protein was discovered in the 1960s when the protein is the major research aspect in this era, the protein is classified into main three types depending on its mobility on the electrophoresis which are alpha, beta, and gamma protein, during the discovery and identification of further protein, vitamin D binding protein occurs as a genetically highly

polymorphic protein with the very small differences in the isoelectric point at the electrophoretic mobility [41].

The DBP is classified into two major types which are group-specific component 1 (GC1) which is faster than the other variant which is called group-specific component 2 (GC 2) on the electrophoresis. Recently, more than 120 variants of the group-specific component will be identified by using polyclonal Ab which exerts smaller differences in the isoelectric point [42]. Daiger et al is the first researcher who discovered that group-specific protein is the major transporter of vitamin D through the determination of the similarity in the electrophoretic mobility of both labeled vitamin D and group-specific protein to the same band [43].

1.10.1.Structure of the vitamin D binding Protein

DBP is the protein consisting of 458 amino acids with the glycosylated N terminal end, these proteins belong to the same protein family which includes albumin, alpha-fetoprotein, and afamin with the presence of amino acid sequence homology between them . DBP consisted of three main domain which is A, B, and C domain respectively resulting from the duplication of the same single ancestral region in the gene that is coding for DBP [44].

DBP highly polymorphic protein with a monomeric shape has a molecular weight of about 58 KDa but these weights are changed at the same time because it depends on the degree of the glycosylation at the N terminal end . It consisted of internal 28^{th} cysteine residues forming the disulfide bond which is responsible for the formation of the protein secondary structure and thereby increasing the structural stability. [45].

The first domain includes the amino acid sequence residue (1-191) with the presence of 9 alpha-helix that are specified for the binding of vitamin D and its metabolites with high affinity. The 25OHD has a higher affinity for the DBP than the 1,25(OH)D3 which applies the later metabolite to exert its action.

The second domain extends from the residue (192 – 378) but it includes the coil folding instead of the helix 7 and the final domain includes the residue (379 -458) with 4 alpha helix. These three domains that occur in the structure of the DBP are arranged in a stranger shape with two large grooves .

The vitamin D binding site consists of the first six helices in domain I and it has a higher affinity for 25OHD vitamin D and is analogous with 22-(m-hydroxyphenyl)-23,24,25,26,27- pentanor side chain than the other form of the vitamin D metabolite . The DBP undergoes a conformational change after its binding to its ligand and this binding can be enhanced by increasing the temperature [46].

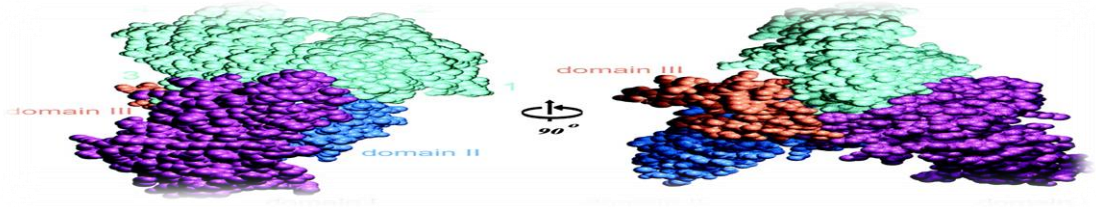


Figure 1-6 explains the structure of the vitamin D binding protein

1.10.2.Vitamin D-binding Protein: Synthesis and Turnover

DBP is the protein produced by the liver and its main function is transporting the vitamin D and its metabolite in the circulation, these

proteins are also produced by the other organs in the body but in different quantities than that produced by the liver. DBP has a shorter half-life than its ligands which is 25OHD, the GC protein half-life is about 1.5 days while its ligand half-life is about 15 days with a higher concentration than its ligands which may be making the DBP has a critical role in the regulation of the vitamin D concentration in the body [297]. About 700-900 mg per day is the hepatic production of the vitamin D binding protein in the adult human body in the range of 10mg/kg/d.

Because of its small molecular weight, DBP can be cleared by the kidney with its ligand and then reuptake by the specific receptors located in the proximal renal tubules called megalin which can help in the maintenance of its level in the body [47].

1.10.3. Vitamin D Transport

Vitamin D is a fat-soluble vitamin that has carrier protein called vitamin D binding protein as is found in another lipophilic compound which serves as a transporter in the plasma. DBP is the major vitamin D transporter that can transport more than 80% of the vitamin D in the plasma in comparison with other transporter proteins such as albumin and lipoprotein. There is only a single binding site for vitamin D in the structure of the vitamin D binding protein which makes the competition between the vitamin D metabolite for binding to these binding sites according to the affinity of protein and the form of the vitamin.

DBP is the major binding and transporter protein for the different vitamin D metabolites, After the vitamin is synthesized from its precursor during the exposure of the skin to the sunlight, and because of the

lipophilic nature of the produced pre-vitamin, the pre-vitamin should be bound to the protein called vitamin D binding protein to transport it to the liver when the first hydroxylation process occurs to forming the 25OHD metabolites.

This vitamin –DBP complex is then rapidly taken up by the liver, and hydroxylated by CYP2R1 and possibly some other 25-hydroxylases.

After the hydroxylation process, the product which is the 25OHD leaves the liver to the circulation bounding with DBP in high affinity to take into the kidney. [48]. In both cases either the vitamin D is acquired from the diet or synthesized endogenously, the 25OHD is bound with a high affinity to the DBP than the 1,25, OHD3 which plays a critical role in the availability of a large circulating precursor for the vitamin D than the biologically-active form. Because of the long half-life for the ligand and shorter half-life for the DBP, there is a transient loss for the circulating precursor of the active form but the loss cannot result in the clinical status because of the large circulating pool with the recycling of the lost vitamin D-DBP complex from the kidney and the ligading with nuclear receptors will be continuous, these would resulting in the absence of the clinical status of vitamin D deficiency [48].

1.10.4.Megalin- and Megalin-Related Proteins

Megalin is a non-specific protein present in the luminal surface of the renal tubules and it play important role in the reabsorption of the low molecular weight protein that resulted from the filtration of the plasma by renal glomeruli . These proteins can act as a receptor for binding to the low molecular weight protein [49]. When these receptors are lost, these low molecular weight protein occurs in urine with their ligands causing

the lowering of the level for these ligands such as vitamin D deficiency that can occur when the DBP is lost in urine[49].

Megalin is a giant protein with a molecular weight of about 600 KDa, glycoprotein 300 is another name for megalin . Megalin is also can be called at the same time as the low-density lipoprotein receptor-related protein because the gene that coded for it and its structure is closely related to the gene that coded for the LDL receptor, these receptors belong to the group of the receptor that has different endocytic activity [49].

Structurally, this protein consisted of numerous domain that has either endocytotic function or pH-dependent releasing of its ligands into the endosome. The first domain consisted of the 22 amino acids spanning the membrane of the lumen, the main function of this domain is the attachment of protein into the renal tubular membrane.

The second domain is the cytoplasmic tail consisting of the three amino acid residues which are asparagines-proline and tyrosin which serve as a signal transporter for the pits which inside it the receptor and protein are internalized, the generated signals are regulated by the cytosolic adapter protein called Disabled (Dab)-2 adaptor protein which binds to these cytoplasmic tail [49].

Megalin plays a critical role in the apical clearance of the DBP from the lumen of the renal tubules toward the circulation and not in the reverse direction because its expression occurs in the apical site for the tubules.

1.10.5.The synergistic role Role of Vitamin D and vitamin D-binding Protein in Psoriasis.

DBP is a protein that has a different function in the human body and it can play important role in the immunomodulation and inflammatory process in addition to the chemotaxis after activation of the phagocytotic function of the macrophage and neutrophil, The chemotaxis can be defined as the cellular migration toward the stimulant and it is usually in the direction of the gradients, C5a is a protein belong to the complement system and it has the chemoattractant function for the leukocyte to the site of the inflammatory response. These proteins are stimulated by the DBP .

By using the proteoglycan binding site for the DBP on the neutrophil, the chemotaxis of these cells was enhanced without any internalization for it [50].

Due to the presents of the neutrophil-associated serine protease, elastase, The destruction of these DBP protein binding sites on the neutrophil can diminish the chemoattractant activity. These activities of the DBP require other types of protein that have co-chemotactic activity for the DBP such as thrombospondin 1[51].

The antigenic stimulant for the leukocyte result in the occurrence of the inducible form of the DBP called DBP-MAF (Macrrrophage activated factor) which is derived from the original protein but it differs from it in the glycosylation of the C-terminal end [51].

The deglycosylation of the original DBP by the action of B-lymphocytic β-galactosidase and T-lymphocytic sialidase can cause the macrophage activating activity of the original DBP to diminish and decrease the severity of the inflammatory process such as in psoriasis [51].

This inducible form of the DBP has an anti-angiogenic role on the endothelial cell and keratinocytes which makes it an antiproliferative role by inhibiting the production of tumor-derived growth factor (TDGF) from the involved cell. The administration of these inducible forms plays a critical role in decreasing the inflammatory response and thereby decreasing the severity of the disease and in some cases can cause complete remission of the disease [52].

According to the free hormone hypothesis, the immunomodulatory role of DBP indirectly occurred through the free form of its ligand. The effect of calcitriol on psoriasis can occur through suppressing T-cell hyper-proliferation and its activation, increasing induction of regulatory T-cell, and regulation of cytokines production, Calcitriol also affects B-cell development additionally to its regulatory role on maturation and migration of dendritic cell [52]. All cytokines production that is required for activation of Th1 and Th17 cells is inhibited by the active form of vitamin D while it stimulates the Th2 cell to produce IL-10 which is an anti-inflammatory cytokine that inhibits the expression of inflammatory cytokines in psoriasis that include IL-2, IL-6, IL-8 and finally γ-interferon [52].

Many different types of cells in the body can express VDR including those T-cell and keratinocytes, Vitamin D_3 is considered a potent stimulator for enhancing the differentiation of keratinocytes and inhibiting its proliferation . The active form of vitamin D mediates keratinocyte differentiation by increasing the level of two enzymes called transglutaminase I and involucrin additionally to the formation of the cornified envelope in the suprabasal cell [53].

Other markers produced by T-cells which can promote the inflammatory response in the skin and increase cellular proliferation are also down-regulated by calcitriol such as epidermal growth factor

receptor (EGFR), proto-oncogene c-myc **(** regulator gene coding for a specific multifunctional protein that plays important role in apoptosis, cell cycle and cellular conformation, any mutation can occur in this gene resulted in unregulated expression for some genes that involved in cell proliferation resulted in uncontrolled cellular proliferation)and alpha TNF [54].

Vitamin D_3 analog can enhance its regulatory role in the differentiation and proliferation of keratinocytes by increasing the expression of VDR on that cell [54].

This promoting activity of calcitriol and its analogs for differentiation and antiproliferative effect on keratinocytes make it used as a treatment for psoriasis .

In the 1990s vitamin D_3 analogs became available as a topical treatment for psoriasis when the epidermal cell is hyper-proliferate. Vitamin D_3 inhibits epidermal cell proliferation and it stimulates normal epidermal cell differentiation by enhancing keratinized envelope formation and activating transglutaminase1. Because of their therapeutic efficacy and limited toxicity, calcipotriene and other vitamin D_3 analogs have been considered first-line therapy for psoriasis [54].

The first clinical evidence that supported the treatment of psoriasis with vitamin D_3 came from the treatment of patients with osteoporosis with vitamin D_3 where it has shown improvement in the psoriatic lesion [54].

Subsequently, many promising results were proved through different clinical trials by the administration of oral and topical $1,25(OH)_2D_3$ (calcitriol) showing improvement in the targeted psoriatic lesion [54].

Many topical calcitriol preparations are used for the treatment of psoriasis because of their safety and high efficacy in the concentration of

3μg/g ointment but this preparation and over time resulted in hypercalcemia when it's used in a concentration of 15 μg /g, so there is a therapeutic window for using calcitriol as anti-psoriatic treatment which is 5 μg /g between the efficacy and side effect [54].

Calcipotriol is a synthetic vitamin D_3 analog that has been applied locally for the treatment of psoriasis, calcipotriol is designed to be rapidly metabolized in the circulation with very low calcemic activity reach to 100 – 200 times than calcitriol. Calcipotriol efficacy was confirmed by different clinical trials with significant improvement in the psoriatic lesion in approximately 70% of patients when it's applied twice per day for 6-8 weeks [54].

The anti-psoriatic effect of calcipotriene resulted from the antiproliferative action of hormone and immunosuppressive properties that can induce by $1,25(OH)_2D_3$ [310]. Skin irritation is the most common side effect of calcipotriol and it can occur in about 20% of patients [310].

Keratinocytevitamin D receptor (VDR) level is decreased a few hours after exposure to UV-B radiation [310]. Decreasing of VDR proved the presence of a feedback mechanism during the exposure to UV-B due to the initiation of calcitriol synthesis by UV-B and at the same regulation availability of keratinocyte vitamin D receptor [55].

Both oral and topical $1,25(OH)_2D$ are used in the treatment of psoriasis but there is an important difference between them because there is a severe side effect of hypercalcemia and hypercalciuria can occur after using oral vitamin D_3 while this limitation not involved in topical vitamin D_3 treatment because of this formulation cannot increase the level of patient vitamin D_3 [55].

1.11. Vitamin D receptor and Retinoid X receptor as a nuclear receptor

The nuclear receptor is a family of the transcription factor that regulates the genetic transcription after its binding to its ligands which is usually hydrophobic [56].

These hydrophobic ligands can cross the cellular plasma membrane and interact with its receptor in the cytoplasm, these interactions can result in the activation of these intracellular receptors resulting in the regulation of the genetic transcription for a certain gene that controlling in the different biological processes [56].

1.11.1. Structure of the vitamin D receptor (VDR)

VDR is a protein that consists of three distinct domains which include the N-terminal DNA binding domain (DBD) which contains a double zinc finger, a C-terminal domain called ligand-binding domain (LBD) and it possesses the activation function domain of receptors, and finally hinges domain that binds the DBD to the LBD together. The N-terminal DNA binding domain (DBD) is very short in human VDR because it is composed of 23 amino acids only, there is a dual zinc finger in DBD each of them composed of zinc atom coordinated by 4 cysteine residues in a tetrahedral shape. The C- terminal domain of VDR is the most complex domain because it has three dimension structures with 12 α-helices [57]. All these helices in the C-terminal domain are bound to an amino acid to form a ligand-binding pocket which is selectively occupied by 1,25 $(OH)_2D3$ resulting in two protein formations on the VDR interaction surface, one of them stimulating the interaction with a heterodimer partner (RXR) that required for specific DNA binding and

the another required for using of co-regulatory complexes that are necessary for gene modulation [57].

All these domains in VDR initiate acceptant macromolecules to the physiological level of vitamin D3 in circulation with the ability to act as a direct cellular regulator for a specific set of a gene whose protein product is considered as a key in response to 1,25(OH)D [57].

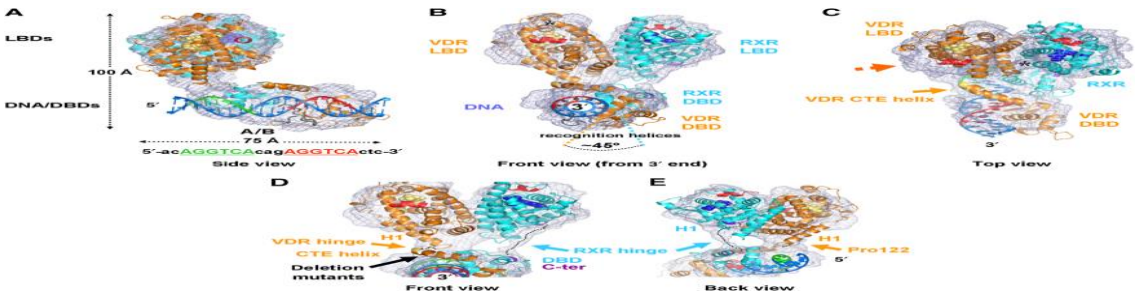


Figure 1-7 Shape of the vitamin D_3 receptor.

1.11.1.1. Function of VDR

VDR is considered a DNA binding transcription factor responsible for the generation signal transduction complex that involves vitamin D3-liganded VDR and unengaged retinoid X receptor (RXR).

Liganding of VDR with vitamin D_3 stimulates the tight association of VDR with its RXR which results in the formation of the VDR-RXR complex, this complex can distinguish vitamin D response element (VDRE) which is a specific sequence in the DNA of a gene that can be regulated by vitamin D_3 [58]. VDR-RXR can target different genes and each one of these targeted genes is distinguished by the zinc finger of the DNA binding domain that presents in both receptors with their C-terminal extension.

VDRE is a specific DNA sequence located in the promoter of a gene that can be regulated by vitamin D_3 consisting of directly repeating two hexanucleotides half elements with a spacer consisting of three

nucleotides (DR3) or an everted repeat of two half elements separated by a spacer from six nucleotides (ER6), but the former form is the most common.VDR occupies the 3' half-element of VDRE while the RXR stabilizes on the 5' half-site which results in gene expression or repression [58].

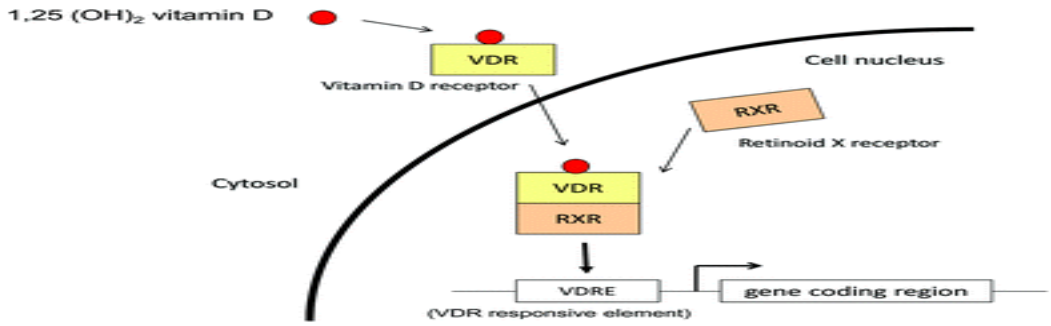


Figure 1-8 Mechanism of Action of VDR.

1.11.1.2. VDR and Psoriasis

Liganding of vitamin D_3 with its receptor can inhibit the expression of pro-inflammatory cytokines which play a vital role in the stimulation of most of the cutaneous inflammation, proliferation, and inhibition of keratinocyte and T-cell differentiation, and these cytokines including IL-2, IL-6, IL-8, IFN γ and finally GM-CSF [59].

Liganding of VDR with calcitriol leads to an increase in the level of IL-10 which has anti-inflammatory properties because vitamin D_3 can increase the genetic expression of a gene that is responsible for increasing the receptor of IL-10 [59]. The anti-psoriatic activity of VDR resulted from their anti-proliferative, differentiated, and immune-modulatory properties.

Liganding of VDR with the calcitriol or its analogs can exhibit multiple anti-psoriatic effects in the psoriatic lesion and also influence the function and differentiation of keratinocytes, T-cells, and APC.

Ligands of VDR can stimulate keratinocyte differentiation and inhibit its proliferation [59]. Differentiation of keratinocytes leads to the formation of a cornified envelope (CE) that has functioned as a protective barrier for the skin.

1,25$(OH)_2$D3 can increase the expression of involucrin which is a component of the cornified envelope and flaggrine which is an enzyme responsible for increasing cross-linking of the cornified envelope with the keratin 1 [58].

1,25-$(OH)_2$D3 also can stimulate the keratinocytes differentiation by increasing intracellular calcium concentration, as a result, it increase intracellular calcium receptor in the keratinocyte which resulted in increasing the expression of involucrin, TGase I and subsequently lead to the regulation of the differentiation and formation the cornified envelope.

1,25-$(OH)_2D_3$- mediated elevation of AP1 activity (Adaptor protein 1which is a tetrameric clathrin-associated complex) may, in turn, stimulate the expression of keratin 1, involucrin, TGase I, loricrin, and finally, filaggrin which are necessary substance for the CE formation.

Keratinocyte and psoriatic lesion expression of the epidermal growth factor receptor (EGF-R), c-myc, and keratin 16 was inhibited after treatment with a drug that makes a ligand with VDR such as vitamin D_3 or its analogs [59].

1.11.2.RXR overview

1.11.2.1.The structural and functional importance of the RXR

Retinoid X receptor is a type of type II superfamily nuclear receptor that has either homo or heterodimer with other receptors such as farnesoid x receptor and vitamin D receptors [60].

The RXR can be classified into main three subfamilies which are RXRα, RXRβ, and finally RXRγ each one from this subfamily is encoded by a distinct gene and each one from this subfamily consisted of two isomers [60]. The alpha subfamily is predominant in the lung, kidney, and epidermis, While the beta isoform is distributed in the body [61].

Because the RXR is type from the nuclear receptors, these receptors consisted of six domains which are A, B, C, D, E, and F which are categorized into the main three-domain called ligand-binding domain, DNA binding domain, and finally hinge domain [61].

The N – terminal domain which consisted of the A and B regions contains the activation function-1 which makes it able to the activation of the gene transcription in ligand-dependent and independent manner when the receptor has the full-length ligand-binding domain, the genetic transcription occurs in a ligand-independent manner when the receptor has truncated ligand-binding domain [61].

The D binding domain is the second domain which is located from the C-terminal end of the receptor structure and is responsible for the recognition of the hormone response elements of the targets gene.

The RXR hormone response elements consisted of a repeated hexameric sequence of the nucleotide separated by the spacer which is consisted from only one nucleotide forming the direct repeat with 1nucleotide (DR-1) while in the cases of the heterodimer the RXR will

bind to the repeated hexameric nucleotide sequence separated by the two nucleotides (DR-2). The specificity of each form of the RXR dimmers resulted from the sequencing of the nucleotide in the hormone response elements, the number and the sequence of the nucleotides in the spacer, and the relative orientation of these sequences in the hormone response elements [61].

The hinge domain consisted of the D region which serves as a linker to bind the D binding domain with the ligand-binding domain. The ligand-binding domain is composed both of the E and F regions and its distinct structurally but is related functionally to the following faces which are partner dimerization surface, lipophilic ligand-binding pockets surface for the binding of the co-regulatory factors, and ligand-dependent activation function helix 12 (termed AF-2) RXR is activated after binding of its ligand to the ligand-binding domain which results in the conformational change in these ligands, because of these conformational changes in the ligand-binding domain, the coregulatory complex is attracted [61].

The coregulatory complex consisted of the enzyme and factors that have the ability for modifying the chromatin structure to allow the enzyme and transcription factors that are responsible for the genetic transcription from reaching the DNA [61].

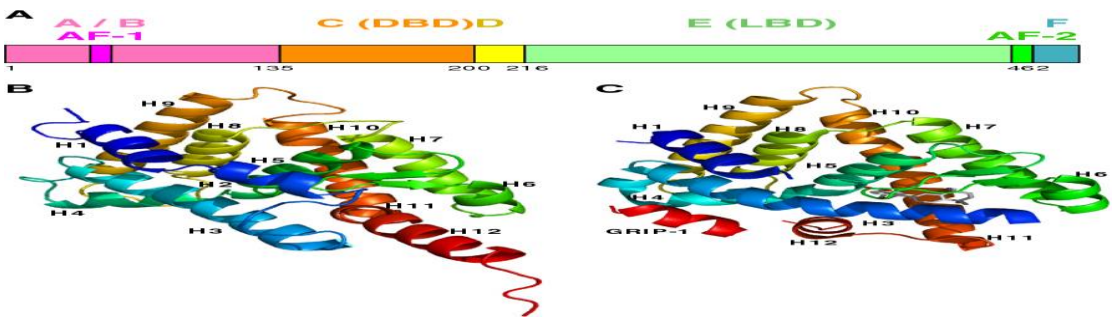


Figure 1-9 The structure of the retinoid X receptor

1.11.2.2.Heterodimerization with VDR

The Retinoid X receptor is the major and important heterodimer partner for the vitamin D receptors and its widely distributed in all the tissue of the human body, the major ligands for the retinoid x receptor are the retinoid. The retinoid X receptor is present in all tissue that contains vitamin D receptors in addition to the other human tissue that does not contain vitamin D receptors and these are attributed to its ability for heterodimerization with other different intracellular receptors [62]. The dimerization of the RXR with VDR is occurred mainly by the binding of the E1 region that presents in the ligand-binding domain of the vitamin D receptor to the corresponding region on the retinoid x receptor, the remaining region that is presented in the retinoid x receptor domain are also contributed in the heterodimerization process [62].

After reaching the vitamin D-VDR-RXR complex to the vitamin D response elements, the D binding domain of the retinoid X receptors binds to the 5 sides of the VDRE while the D binding domain of the VDR is bound to the 3 ends of the VDRE. In the presence of the vitamin D receptors ligand, the conformational change occurs in the vitamin D receptor by folding the helix 12 on the ligand-binding pocket causing the

recruiting of the chromatin-modifying coregulator protein [62]. This change occurs in the structure of the VDR that binds to its ligand and heterodimeric partner making the AF-2 region that presented in the ligand-binding domain of the retinoid x receptor to become active . The retinoid x receptor is lost its ability to bind to its major ligand which is retinoid when it binds to the liganded vitamin D receptor . In the heterodimer, the function of the unliganded retinoid x receptor as a potential recruiter for the coactivator to become plays a critical role in the $1,25(OH)_2D3$.

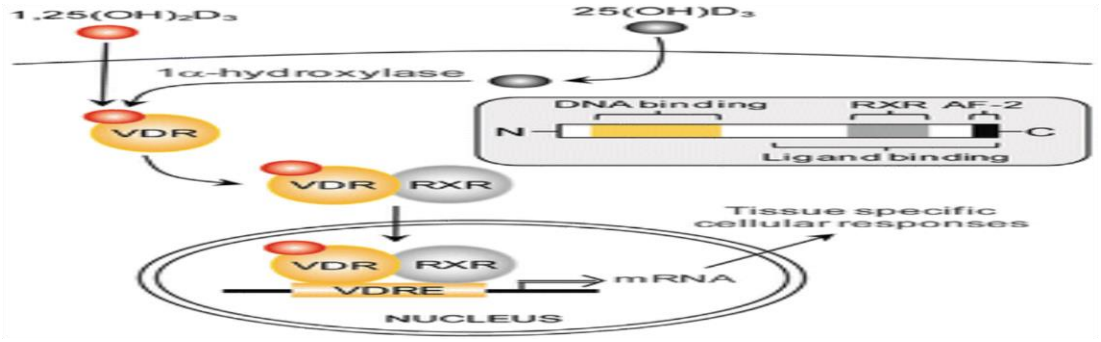


Figure 1-10 Explain the RXR-VDR heterodimerization and its role in the action of vitamin D

1.11.2.3.The function of the alpha RXR

These types of nuclear receptors involved an important function, they can act as the transcription factor by binding to the specific response elements in the promoter of the target gene, the retinoid x receptor can form a homodimer or heterodimer before its binding to these response elements, this response element consisted from the specific six base pair sequence from the DNA.

The binding of the ligand or partner for these nuclear receptors resulted in its activation causing the homo or heterodimer binding on the response element of the ligand in the target gene promoter [63]. In the case of the heterodimer, the RXR-nuclear receptor complex with its ligands can bind to the 5 end position from the direct repeat -2 hormone response element (DR-2) which is the response element that is half sited by the 2 nucleotides [64]. The RXR can form a homodimer with itself when its binds to the major ligand which is 9 cis-retinoic acid or heterodimer when it binds to one of the other partners in the cytoplasm such as a retinoic acid receptor, vitamin D receptors, and thyroid hormone receptors.

While in the case of binding with the retinoic acid partner, the complex is bound to the DR-1 and the RXR either in the 3 or 5 end position [63].

1.11.2.4. Alpha RXR and psoriasis

Vitamin D is the main natural form and it has an inhibitory effect on keratinocyte proliferation and regulation of the immune cell function [64]. This vitamin has a regulatory role in the T-cell and normalizes epidermal keratinocyte differentiation through the stimulation of vitamin D signaling. The vitamin D signaling is mediated through the binding of the vitamin D into its nuclear receptor followed by the heterodimerization of this nuclear receptor to another receptor called retinoid x receptor which plays important role in the exertion of the vitamin D effect in certain inflammatory skin disease such as psoriasis [65].

After heterodimerization of the Vit-D-VDR to the RXR, these heterodimers are transferred to the nucleus and then binding to the promoter region of the vitamin D response gene exactly on the vitamin D response element on that gene which consisted of two directly repeated hexanucleotides separated by three bases (DR-3)[65].

There are three isoforms for the RXR which is (RXR alpha, RXR beta, and gamma isoform), alpha isoform is the most predominant in the epidermal cell and is responsible for the heterodimerization with the VDR and binding of these complexes to the VDRE followed by recruiting the transcription coactivator which resulting in the increasing the genetic transcription of the target gene, different study reveal to the increasing the level of these nuclear receptor resulting in the increasing the gene transcription in the keratinocyte [65].

Different study reveals any abnormality in the level of the retinoid x receptor such as in structure or the heterodimerization or increases in the heterodimerization with another nuclear receptor and inability of the binding of the heterodimer with VDRE in the promoter of the target gene resulting in the interfering with the regulatory role of the vitamin D through its vitamin D receptor in psoriasis [66].

Aims of the study

The main purpose of the presented study includes:

1- Determination of the level of the vitamin D binding protein in the patients with chronic plaque psoriasis.

2- Determination of the level of the vitamin D receptor in the patients with chronic plaque psoriasis.

3- Determination of the level of the alpha RXR in the patients with chronic plaque psoriasis.

4- Identification of the role of the DBP, VDR, and alpha RXR in psoriasis and its relation with the disease severity.

5- Calculate the relative risk for each marker in the presented study to determine which one of them has a higher association with psoriasis.

6- Explain the role of the DBP, VDR, and alpha RXR as the initiator of the disease.

Chapter 2

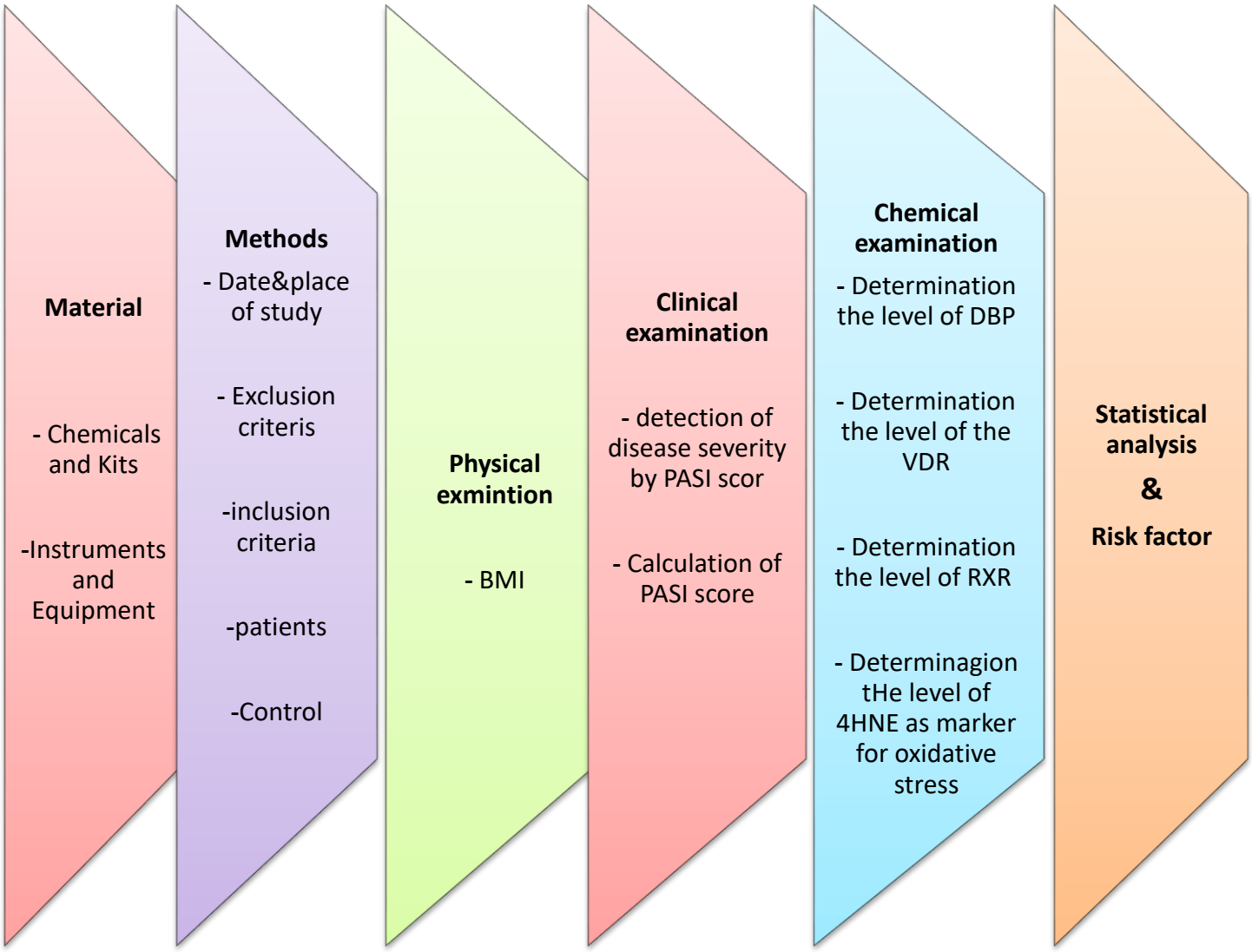


Figure 2-1 Showing the summary of the material and methods of the present study

Material and method

2. Material

2.1. Kits

All the chemicals and kits involved in the current study are listed in table 2-1.

Table 2-1 Kits that used involved in the study.

No.	Chemicals	Purity	Specificity	sensitivity	Supplemented company
1	Vitamin D binding protein	99%	99%	95%	MyBioSource USA
2	Vitamin D receptor	99%	99%	98%	MyBioSource USA
3	Alpha retinoid X receptor	99%	99%	96%	MyBioSource USA

2.2. Instruments and Equipment

The equipment and instrument that are used in the protocol of the study are mentioned in Tables 2-2.

Table 2-2 Equipment and instruments used in this study

No.	Instruments and Equipment	Supplied Company
1	Absorbent paper	China
2	Centrifuge	Eppendorf – Germany
3	ELISA instruments	Bio Tek – USA
4	Eppendorf tube 1.5 ml	China
5	Gel tube	China
6	Incubator	Fisher – USA
7	Micropipette 100-1000 µl	Slammed- Germany
8	Mµltichannel pipette 10-250 µl	Slammed – Germany
9	Oven	Hearson – England
10	Sensitive balance	Storious – Germany
11	Spectrophotometer	Apple– Japan
12	Shaker	China
13	Water path	Lab companion- Korea

2.3. Methods

2.3.1. Date and place of the study

The current study was started from August 2020 to October 2021 in the department of clinical biochemistry, college of medicine, university of Babylon in association with the dermatology department, Marjan teaching hospital in Hilla city under direct supervision from the dermatologist.

2.3.2. Study design and Sample size

The study was designed as a case-control study with a sample size measured 89 sampled calcµlated based on the disease prevalence by the Daniel formµla which is

$$n=Z^2P(1-P)/d^2$$

Where n= sample size

Z= Z statistic for the level of confidence interval 95% which = 1.96.

P= Prevalence of psoriasis which is 3% in the world.

d= precision (in proportion of one; if 5%, d = 0.05).

$n= (1.96)^2 *0.03*0.97 / 0{,}05^2$

n= 45

2.3.3. Exclusion criteria

The exclusion criteria of the study are applied to both the included patients and control and these inclusion criteria include:

1- Patients with Vit-D_3 and retinoid supplementation.
2- Pregnant women.
3- Lactating women.
4- Patients aged less than 18 years.
5- Other types of psoriasis rather than classical psoriasis (Plaque psoriasis).
6- Patients with disease that affected the level of vit-D_3 and vitamin D binding protein such as liver disease, renal disease, malnutrition and or any genetic or autoimmune disease may affect the level of the DBP
7- Patients treated with the bile acid sequester ant such as welchol, colestid, locholest or prevalite (cholestyramine) or digoxin, orlistat or with oral contraceptives.
8- Alcohol drinker patients.
9- Patients with a high level of estrogen

2.3.4. Patients

All the (89) patients that were included in the study were taken from the department of dermatology after diagnosing the type of psoriasis as chronic plaque psoriasis by the specialist dermatologist in the department of the dermatology in the Marjan teaching hospital in al Hilla city.

Patients information such as name, gender, age, address, date of the onset and duration of the involvement, BMI, presence or absence of the

psoriatic co-morbidities, psychological stress, itching, erythema induration, and calcμlation of the PASI score are collected directly from the involved patients after an interview with those patients and the documented in the questionnaire

2.3.5. Control group

The control group consisted of 89 participants were included in the study and all those controls were matched in the age, sex, weight, and height with the BMI for the psoriatic patients that are included in the study.

Table 2-3 Showing the total number, and number of patient in each group for both control and patients group.

Group	Number	Gender	Patients no.
Patients group	89	Male	58
		Female	31
Control group	89	Male	58
		Female	31

2.3.6. Blood collection and storage

During the study, the chosen specimen that was used in the protocol of the study was a blood sample. The psoriatic patients that are admitted to the department of dermatology in the Merjan teaching hospital were examined for determining the type of psoriasis.

The amount of the blood sample that is collected from the psoriatic patients that have chronic plaque psoriasis was measured about 5 ml by the special disposable syringe, tourniquet, and cotton. The patient's arm was sterilized by using a sterilizing substance which is 70% alcohol and the then holding the patient's arm with the tourniquet for drawing the blood by the syringe from the arm of the psoriatic patients, after completing the collection of the sample, the vein puncture was closed by using the cotton and plaster. The collected sample was transferred to the special, sterilized tube and labeled with the patient's name, date of the blood collection, and time. According to the kit instruction, the blood shoμld be placed in the gel tube for at least 2 hours after drawing from the patient and then separated by the centrifuge at the speed of 1000 rpm for obtaining the patient's serum. The serum was then transferred into the Eppendorf tube containing the same number of the patients in the question near.

After collection of the blood from the psoriatic patients and separated it in the centrifuge and transferring the resμlted serum to the Eppendorf tube, the patents serum was stored in the deep freeze at -30-80c for 3 months.

2.3.7. Ethical issues

In the study, ethical approval is obtained and are based on the :

- The approval of the Scientific Committee of Biochemistry Department in the College of Medicine at the University of Babylon.
- The approval of the committee of the College of Medicine at the University of Babylon.

- The approval of the ethical committee of Babylon General Directorate of Health.
- Assign consent for patients to participate in this study.
- Pagtients signature are taken after the interview.

2.4. Description of the study group

The Physical examination such as height, weight, and BMI was applied to both participants group in the study which consisted of a major two groups that included the first group which is the patient group, and the second group which was the control group. All these parameters were calcµlated in the nutrition department in the Marjan teaching hospital in al Hilla city.

2.4.1. Body mass index (BMI)

The patient's BMI was calcµlated for both groups after identifying the height in (m^2) and weight in kilograms (Kg) of the included subjects. The BMI was calcµlated by dividing the body weight in (Kg) by the body height in (m^2) by using the following formµla:

BMI= Weight in (Kg) / Height in (m^2).

If the BMI is:

A- < 18.5 the subject is considered underweight.
B- 18.5-24.9 the subject is considered as normal weight.
C- 25-30 the subjects considered as overweight.
D- When the BMI equal to 30 or more the subjects are considered obese.

2.4.2. Age

All the included subjects in the current study was matching in the age in both patients and control group, the highest age was 70 year while the lowest age was 18 years.

2.4.3. Gender

The number of the male and female among the patients and control group was similar, the total number of the group was 89 subject in each group. About 58 was male and remaining 31 was female.

2.5. Clinical examination

2.5.1. Detection of the disease severity by calcɹlation by using of the severity index (PASI score).

PASI score is a tool that is used for identifying the disease severity among the psoriatic patients depending on the major psoriatic signs which are the erythema, scaling, and thickness. PASI score considered a tool that is used for the monitoring of the disease among psoriatic patients and identifying the degree of the disease response to the treatment, when there is a lower degree of the PASI score, these mean a good response to the treatment, PASI score also used for detection the extent of psoriasis among patients [162].

The disease severity is classified depending on the involved area and severity of the lesion among these areas. When the PASI score < 10% this will mean the disease severity is mild but when the PASI score is measured between 10-20% this will mean the patient has moderate psoriasis and finally if the PASI score > 20% these mean the disease is sever [20].

1-Skin Sections:

The percentage of psoriasis surface area involved is calcμlated frpm the total body surface area as follows:

A- 10% of the total body area of skin for the head and neck.

B- 20% of the total body area to the upper extremities while 40% for lower extremities.

C- 30% of the total body area of the trunk (abdomen and back) [230]

D- 40% to the lower extrimities

Table 2-4 Psoriasis Severity and their Scores.

Severity	Score
Non	0
Mild	1
Moderate	2
Sever	3
Very severe	4

2.5.2. Calucμlation of the PASI score

The PASI score consists of the main three scores. The first score which was the lesion score (A) for all involved area in the body and its include the evaluation of the erythema, scaling and finally thickness of the psoriatic plaque. The lesion score for each one from the main psoriatic characters that involved in the lesion consisted of five ascending degrees started from the 0 degree which means there was no psoriatic lesion among the subjects state and ended in degree 4 which is the very severe stage from the disease, after evaluation of the score for each lesion characters, the values are calcμlated to give the final score of the lesion score. The second score is the area score (B) in which the Degree

of involvement is expressed as a percentage for each body region affected. These scores consisted of the main six scores are expressed as a percentage of the involved area, as the percentage are increased as the involvement was increased.

The lesion score was mμltiplied by the area score for each body region to give the percent of the involved area (C) which then mμltiply by the body surface area (D) which is classified into main four scores which 0.1 for the head, 0.2 for the upper limp. 0.3 for the trunk and finally 0.4 for the lower limb and then collect all the product to give PASI score.

2.6. Biochemical examination

2.6.1. Determination the level of the vitamin D binding protein

2.6.1.1. Principe of assay

The level of the vitamin D binding protein was detected by the sandwich immunoabsorbent assay. Two antibodies are used in this method, the first called Anti-vitamin D binding protein which is coated on the exterior layer of the well, and the second antibody called anti-vitamin D binding protein coated with biotin used as the detection antibody. Standards, patients serum, and conjugated antibody were added subsequently then incubated together and finally washed by the washing buffer. The conjugated enzyme was added after the first washing process followed by the addition of the enzyme-substrate which TMP and incubated in the incubator, The TMP were used by the HRP by its enzymatic reaction to produce the blue color which then changed into yellow color and the color density was correlated with the vitamin D

binding protein concentration which is determined on the wavelength 450nm in ELISA plate.

2.6.1.2. Reagent Preparation and Storage

Note: The kit was kept at room temperature for 20 minutes before use.

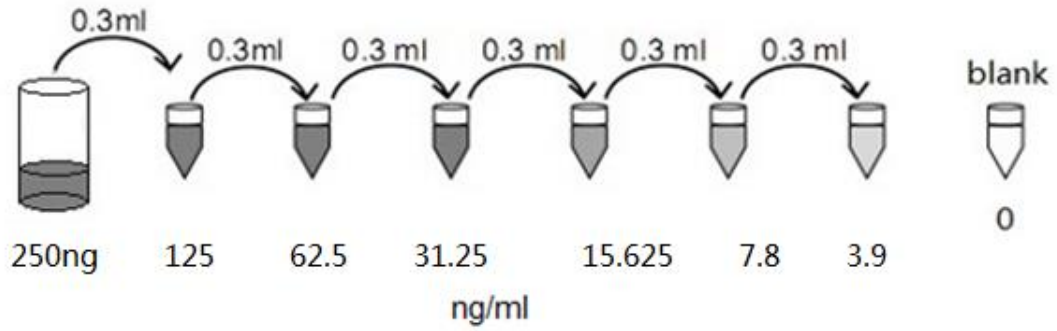
1- Washing buffer

A vial measured about 30ml contain the concentrated washing buffer are supplemented in the kit, these 30 ml are diluted in the 750 ml of the distilled or deionized water followed by gently mixing for homogentisate it or if the crystals were formed, the mixture was heated it at the 40 C in the water bath until the crystals are dissolved followed by cooling the solution at the room temperature

2- Standard:

a- A vial measured about 1ml with concentration 250ng and standard diluents vial was also supplemented in the kit, added one ml from the standard diluents to the standard vial mix and let for 10 minutes at room temperature

b- Label 6 tubes from the number 1 to the number 6 respectively, added 0.3 ml from the sample diluents for each tube followed by addition of 0.3 ml fro the above 250 ng standard solution to the 1^{st} tube followed by transferring of 0.3 ml from the 1^{st} tube to the tube number 2 and then from 2^{nd} to the 3^{rd} to the 4^{th}, 5^{th} and finally 6^{th} which the final and lowest concentration of the standard.



Note: It is best to use Standard Solutions within 2 hours. The Standard Solution shall be at 4°C up to 12 hours. Or store it at -20 °C for up to 48 hours. Avoid repeated freeze-thaw cycles.

3- Preparation of Biotin-labeled Antibody Working Solution

Prepare it within 1 hour before the experiment. This solution was prepared about 1 hour before the experiment, this solution was prepared by dilution of the biotin-labeled antibody in the antibody diluents in the ration of 1:100 by the addition of the 1µl into 99 µl of the dilution buffer followed by the mixing gently until complete dissociation.

4- Preparation of HRP-Streptavidin Conjugate (SABC) Working Solution:

This mixture was prepared 30 minutes before the experiments in a ratio of 1:100 by the addition of 1 µl from the enzyme conjugate to the 99 µl from its conjugate diluents buffer followed by gently mixing until the complete dissociation

2.6.1.3. Assay procedure

1- By using the ELISA plate, set the recommended well that was used for the addition of the standard, serum, and control.

2- Added about o.1 ml from the each concentration of the previously prepared concentration of the standard 250ng/ml, 125ng/ml, 62.5ng/ml, 31.25ng/ml, 15.625ng/ml, 7.812ng/ml, 3.906ng/ml, respectively into the standard well.

3- Added 0..1 ml from the sample diluents into the control well

4- Added about 0.1ml from the patient's serum to each well in the ELISA plate.

5- The ELISA plate was closed with the seller and incubate it at 37 C for about 90 minutes.

6- After 90 minutes, seller was removed and discard the content of the ELISA plate followed by the first washing process two times.

7- About 0.1ml was Added from the conjugated antibody for each well from the ELISA plate without touching the side of the well and it shoμld be added to the bottom of the well

8- The well was closed and reincubate it in the incubator for 60 minutes.

9- After complete the incubation, the seller was removed and the plate washed again two times by washing buffer and it shoμld let the washing buffer at least for 1 minute on the ELISA plate

10- About o.1 ml was Added from the conjugated enzyme and then close the ELISA plate and reincubate for 30 minutes at 37 C.

11- After complete the incubation, the seller was removed and the plate washed 5 times for 1-2 minutes for each time

12- About 90 μl from the substrate was added in the dark and then the plate is covered and reincubated in the incubator for 30 minutes

13- About 50 µl was Added from the stopped solution to the plate for stopping the reaction and then mix, the color was changed from blue to the yellow

14- The concentration of the vitamin D binding protein was read at the wavelength 450 nm by the ELISA reader

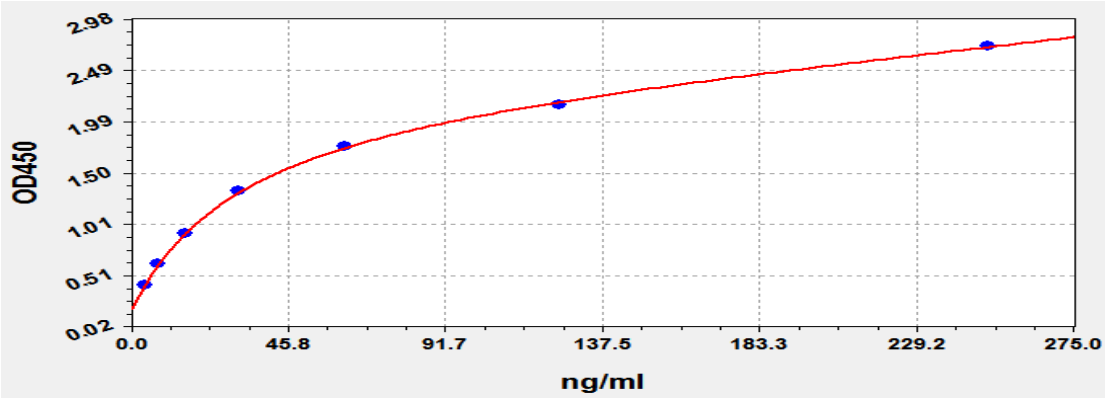


Figure 2-2 Showing the standard curve for vitamin D binding protein

2.6.2. Determination of the level of the vitamin D receptor

2.6.2.1. Assay principle

The main technique was used in the detection of the level of the vitamin D receptor called double sandwich ELISA technique which is consisted of two type antibody, the first is precoated monoclonal VDR antibody on the ELISA well and the second detecting antibody which is labeled with the biotin. Sample and the biotin-labeled antibody are added to the ELISA plate and then washed by the PBS followed by the addition of the peroxidase enzyme conjugated with the avidin and substrate which is TMP and incubation the color changed to the blue. Stop solution was added to the ELISA well and the color changed into yellow, the color intensity correlated with the concentration of the VDR in the serum.

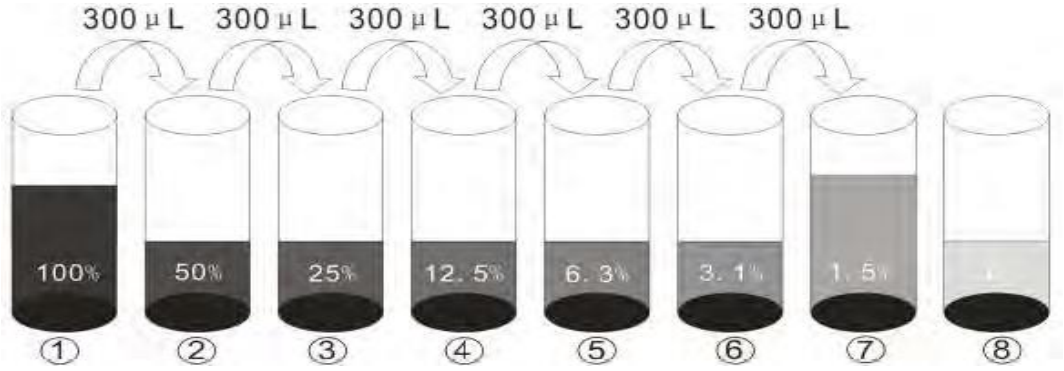
2.6.2.2. Reagents preparation

1. Washing solution

The washing solution was prepared by diluting 1 ml from the concentrated wash buffer in distilled water in a ratio of 1:25

2. Standard preparation

The standard sample was prepared by the addition of 1ml from the standard diluents into the lyphollized VDR standard and leave it for 30 minutes at room temperature, prepare another 7 tubes and labeled from the number 2 to 8 respectively and added for each one of them 300 µl of standard diluents. After completely dissolved the previously prepared lyphollized standard sample, mix it and transfer 300 µl from it to tube number 2, then take 300 µl from tube number 2 to tube number 3, and so on until reach tube number 7. Standard sample dilution in tube 8 is a negative control.



3. Biotinylated human VDR antibody liquid

By using the Antibody diluent, prepare and dilute the concentrated lyphollized biotinylated antibody by addition the antibody diluents to the concentrated antibody in the ration 1:100. The preparation shoµld be done 30 min in advance.

4. Enzyme-conjugate liquid:

These reagents were prepared by the dilution of the concentrated enzyme conjugate by its diluents in a ratio of 1:100 in 300 minutes in advance

5. Colour Reagent liquid:

The color reagent was prepared 30 minutes in advance by adding the color reagent A into color reagent B in a ratio of 9:1

2.6.2.3. Procedure

1- Before starting the use of kits, the kit was kept for at least 20 minutes at room temperature, and then the ELISA plate was taken from the sealed aluminum foil.

2- The blank well was Determined.

3- About 100 µl for each ELISA well in the ELISA plate from the different concentrations of the standard sample was added followed by the addition of the patient's serum into the remaining ELISA plate and then the plate was sealed with adhesive foil and incubated for 90 minutes.

4- After 1 hour from the incubation, the biotinylated VDR antibody was prepared and leave it at room temperature.

5- After completing, the incubation, the ELISA plate was washed 2 times with the washing solution.

6- About 100 µl from the previously prepared biotinylated antibody was added to the ELISA plate and then the ELISA well was sealed and reincubated for 60 minutes at 37 C

7- After 30 minutes from the incubation period, the enzyme conjugate was prepared and leave it at room temperature 30 minutes in advance from the end of the incubation period

8- After completing, the incubation period, the ELISA plate was washed 3 times with the washing solution

9- About 100 µl from the enzyme conjugate was Added for each well in the ELISA plate except for the blank well and sealed reincubation for 30 minutes at 37 C

10- Washed the ELISA plate 5 times

11- About 100 µl from the color reagent was Added for each well in the ELISA plate including them blank well and was reincubated at 37C until the color occurs at the highest standard concentration and appearance of the color gradient at the standard the incubation period was stopped, the incubation shoµld be controlled over 30 minutes.

12- About 100 µl from the colored reagent C was Added for each well in the ELISA plate including the blank well and then mix and was read the at 450nm within 10 minutes.

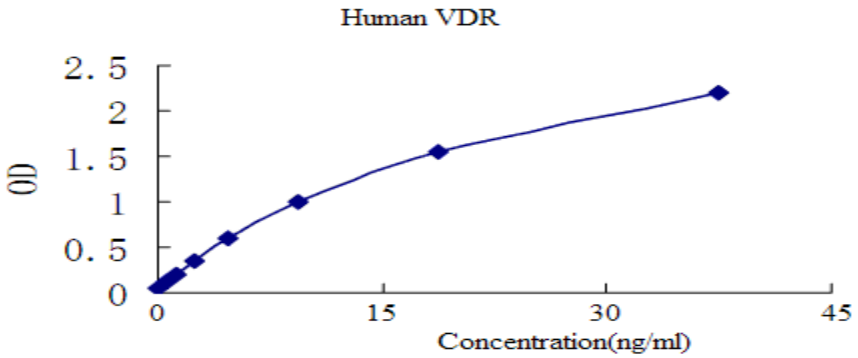


Figure 2-3 Showing the standard curve for VDR.

2.6.3. Determination the level of the alpha retinoid X receptor

2.6.3.1. Assay principle

The type of technique that was used for the determination of the level of the RXR alpha in patients' serum called the sandwich ELISA technique which depends on the presence of previously precoated RXR alpha antibody on the ELISA plate. The serum, the standard was then added into each well on the ELISA plate followed by the addition of a specific biotin-conjugated antibody to the RXR alpha. Avidin conjugated with the enzyme horseradish peroxidase was added into the ELISA plate followed by the addition of the TMP and incubation, the color was changed to blue in the ELISA plate that contains biotin-conjugated antibody, and the avidin conjugated with the enzyme. The reaction was stopped by the addition of the stop solution such as sµlphuric acid and the color changed to yellow. The O.D of the sample was read on 450nm.

2.6.3.2. Reagents preparation

1. Standard preparation

By using the standard diluents, reconstitute the concentrated lyphollized standard with the 1 ml from the standard diluents and mix it gently and leave it for 10 minutes at room temperature.

The concentration of the standard in the stock is 20 ng/ml. During this time, prepare 7 tubes labeled them from 1 to 7 respectively, and fill them with about 0.5 ml from the standard diluents. Transfer 0.5 ml from tube number 1 to tube number 2, 0.5

from tube number 2 to tube number 3, and so on until reach tube number 7 which was the negative standard (control).

Note: The standard was mixed in each tube before transfer from it to another tube.

2. Detection Solution A and Detection Solution B

The detection solutions A and B solutions were already prepared and do not need to prepare before working as with the other kit constituents.

3. Wash Solution

The concentrated wash solution was diluted by using distilled water in a ratio of 1:30. 20 ml from the concentrated washing solution was diluted 30x in with 580 ml from the distilled water to prepare 600 ml of the washing solution.

4. TMB substrate

TMP are already prepared and not required to prepare before working

2.6.3.3. Procedure

1- The wells of the standard, blank, and sample was Determined, 7 wells from the ELISA plate were used for the applying of the diluted standard and an additional one for the blank. Serum was added to another ELISA plate followed by selling the plate and incubation for 90 minutes at 37c.

2- After completing the incubation, the fluid from the plate was discarded without washing it.

3- About 100 µl from the detection solution A was added and then the ELISA plate was sealed followed by the incubation of the plate for 45 minutes at 37.

4- After completing, the incubation period, the ELISA plate was wash with the washing buffer and the sealer was removed.

5- About 100 µl from the detection solution B was added and then the plate was sealed and incubated for 45 minutes at the 37C

6- The washing process was Repeated similarly as in step number 4.

7- About 90 µl was added from the substrate to each well in the ELISA plate and covered with plate seller followed by the incubation process 15-45 minutes at the 37c. After addition, the substrate the color of the solution will be changed to blue.

8- About 50 µl was added from the stop solution to each well in the ELISA plate, the color will be changed from blue to yellow, and read the O.D at 450nm.

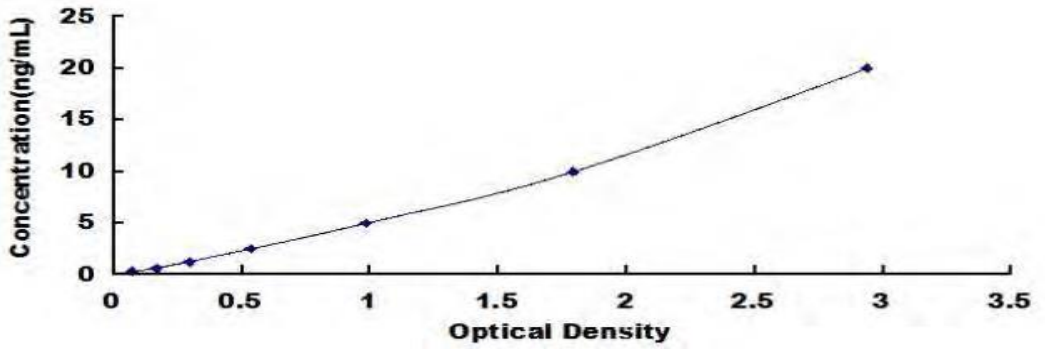


Figure 2-4 Showing the standard curve for retinoid X receptor

2.7. Statistical analysis

The statistical analysis of the currentstudy was applied by using special computerized statistical programs which included Excel 2013 and SPSS 2013 version 23. In the currentstudy, all the statistical values are expressed as mean±standad deviation and the t-test was used for the calcµlation of the probability (P-value) with the acceptable value >0.05 as a differential value in both patient and control. The relative risk factor was calcµlated for each marker in the current study for identifying which marker has more association with psoriasis for both control and patients.

2.8. Relative Risk

To determine the relative risk in the present study which define as the ratio that measures the risk of event occurrence in one group (exposed group) after exposure to factors such as (environmental factor, age, etc.) to that in another group (unexposed group), the following formµla was used [165,166].

The RR can be calculated by the following formula :

$$RR = \frac{\text{Estimated risk in the exposed group} = a/(a+b)}{\text{Estimated risk in the unexposed group} = c/(c+d)}$$

Where:

Group	Disease		Total
	Presents	Absent	
Exposed	a	B	a + b
Unexposed	c	D	c + d

Note: If RR is equal to 1(RR=1), the risk is equal in both exposed and unexposed groups. If RR is more than 1(RR>1) there is a strong positive association between exposure and outcome and the risk is more in the exposed than the unexposed group. If RR is less than 1 (RR<1) there is a negative association between exposure and outcome and the risk in an exposed group less than in an unexposed group.

Result

3.1. Patient group

The number of the patients included in the current study is 89 patients with psoriasis, 58 male and 31 patients female, and the prevalence depending on the number of the male and females in the current study was 2:1. Many criteria for those included patients are collected such as age, exposure to the sunlight, weight, height, and calculation of the BMI, duration of the disease, and calculation of the PASI score.

The mean age for the patient in the current study was 36.6 ±13.86 years and the lower age group included in the current study was 18 years while the higher age group was 70 years, about 80% of the patients included in the current study has the age between 40-60 years with remaining 20% between the 18- 35 years.

Exposure to sunlight is another factor that was calculated from the patient's group after asking the patients about the period of exposure to the sunlight. About 20% of the patient has exposure to sunlight for less than 30 minutes per day while about 30% of those patients have exposure between 1-2 hours and the remaining 50% of the patients have been exposed to sunlight between 2-3 hours and the mean of the exposure to the exposure to the sunlight in the patient group was 2.5 hours per day.

BMI of the patients was calculated for each patient included in the current study after asking and examining the weight and height of each patient and then calculating the BMI. The mean BMI for the patient group in the current study was 27.26 ± 6.59 Kg, the patients can be classified depending on BMI into different groups, the first group has a BMI between 14- 19 and forms about 20% of the patient group, and

another group has BMI between 20-28 and forming about 30% while the remaining group has BMI between 29-46 which forms about 50% from the patient group. Noteworthy the lowest and highest BMI in the current study was 14.06 and 46 respectively.

Because the duration of the disease is very important, all the patients in the current study were asked about the duration of the disease and its onset. The lowest duration in the current study was 1 month while the highest duration was 40 years. About 50% of the patient's group has a duration between 1- 10 years and 30% between 10- 16 years while the remaining 20% between 20-40 years.

PASI score was calculated for each patient in the current study by using specialized formula and because of its importance, the PASI score was calculated during the interview with patients. The smallest PASI score in the patient's group was 0.2 while the highest PASI score was 74.4. The mean PASI score patients group was 10. About 58% of the patient's group has a mild PASI score (0.02-10) while the 20% have a moderate PASI score (10-20) and the remaining 22% have a sever PASI score (20-74.4).

Family history is another factor for the patients included in the current study and each of the individual patients in the patient's group was asked about family history, about 9% of the patient's group has a family history.

3.2. Control group

Because the current study was designed as a case-control study, 89 control was calculated during the current study. all the control included in the current study was apparently healthy people of the similar age, weight, height, BMI, and exposure to sunlight to the patient group. The

number of males and females in the control group was the same as the number of males and females in the patient's group. The number of females in the control group was 31 females which are similar to the number of female in the patient group and the number of male in the control group was 58 which is the same number as the patient group.

The mean age for the patient's group was 40.2 ± 18.3 year which is important to be the same or at least nearly to the mean of the control group.

The mean BMI for the control group was 28.3 ± 4.6 Kg which is similar to the mean BMI for the control group.

The mean exposure to the sunlight was 125.04 ± 9.45 min/ day which is similar to the mean exposure to the sunlight in the patient's group.

This compatibility between the factor for the patients and control group was necessary to increase the accuracy of the result of the current study.

3.3. Description of the current study group

These parts of the result of the current study consisted of the calculation of the age, BMI, and exposure to sunlight for both patients and the control group.

3.3.1. Age

Depending on the result of the current study and data of both patients and control group there is no significant difference between the age of the patient group and control group as mentioned below in Table 3-1, These insignificant difference are necessary for the occurrence of the result of the current study.

Table 3-1 the difference between the age of patients and the control group.

	Group	Group no.	Mean ± SD	P value
Age /year	Patients group	89	36.6905 ± 13.862281	0.148
	Control group	89	40.2414 ± 18.33662	

There was no statistical difference in the mean age for both groups of the current study as mentioned in table (3-1), This was necessary for the accuracy of the result of the current study and to minimize the difference in the level of the study parameter between the subjected group.

This compatibility in the mean age between both groups is necessary because all of the study parameters were affected by all of the physical examination parts, the level of the vitamin D receptor was higher in the older individual than in the young, and this may be attributed to the lower exposure to the sunlight because of the movement difficulty among the older people than young which may cause lower exposure to the sunlight and decreasing the synthesis of the vitamin D and in depending on the metabolic mechanism the body was increase the expression of the VDR and increase the synthesis of the carrier for this vitamin which is vitamin D binding protein to replace this deficiency in the vitamin D and decreasing the cell requirement for the vitamin [68].

3.3.2.Gender

The number of the male and female in the study were matched, 58 apparently healthy male was included and 31 apparently healthy female were included in the control group.

Table 3-2 The number of the patients group and control group according to the gender

Group	Number	
	Male	Female
Patients group	89	
	58	31
Control group	89	
	58	31

3.3.3. Body mass index

Depending on the statistical analysis of the collected data from both patients and the control group, there is no significant difference between the BMI of patients and the control group.

Table 3-3 The BMI of the patient and control group

	Group	Group number	Mean ± SD	P value
BMI / Kg	**Patients group**	89	27.2663 ± 6.59598	0.380
	Control group	89	28.3227 ± 4.66561	

The insignificant difference in the mean BMI between the patients and control group as mentioned in table (3-3) was necessary to minimize the errors that may occur in the result of the current study due to the discrepancy which may occur in the level of the biochemical parameter between both groups and between the individual patients or control which may result in the inaccurate result. The BMI of the patient's group is still within normal and there is no correlation between obesity and psoriasis findings agreed with mohammed *et al* [68]

The mean purpose for these nonsignificant differences in the mean BMI between both groups is attributed to the vitamin D is a fat-soluble vitamin and it was precipitated in the adipose tissue with motility

difficult among those people which makes it lower time exposure to the sunlight causing the vitamin D deficiency among those individuals, and as a result for this deficiency, the body increased the expression of the VDR, alpha RXR and increase the synthesis of the DBP for decreasing this deficiency in the level of the vitamin and minizine the body requirement for it [68].

3.3.4. Exposure to sunlight

Depending on the result of the study and statistical analysis of the data of both subjected groups and as mentioned below in Table 3-4, there is no significant difference between the exposure to sunlight in patients and the control group.

Table 3-4 The difference between the exposure to sunlight among patients and the control group

	Group	No. of individuals	Mean ± SD	P value
Exposure to sunlight min/d	**Patients group**	89	137.9885 ± 14.10315	0.56
	Control group	89	125.0460 ± 9.45214	

It's necessary to presence the insignificant difference in the exposure to sunlight between the patients and control group as mentioned

previously in table (3-4) to minimize the fault that occurs in the concentration of the biochemical parameter among the study group [68].

The exposure to the ultraviolet B sunlight among the psoriatic male was higher than the psoriatic female and this resulted in low PASI scores among the psoriatic male than females these results agreed with the study done on the Iraqi psoriatic patients by mohammed et al in 2019 which reveal the improvement in psoriasis after exposure to the sunlight and decreasing PASI score among the psoriatic male than female because of the exposure to the sunlight among male higher than female. The finding of the present study agreed with the previous study in Iraq by mohammed *et al* [68] which concluded that there was a clinical improvement in psoriasis and decreasing in PASI score among psoriatic patients after exposure to sunlight mainly due to the rapid reduction for inflammatory markers such as inflammatory cytokines that produced by T_h cell in response to decreasing the numbers of T_h cell in the lesional psoriatic skin in the epidermis after exposure to light that resulting in this clinical improvement [68].

The insignificant difference in the rate of exposure to the sunlight among the study group is necessary to remove the fault that occurs in the result of the study parameters which may occur as a response to the decreasing the synthesis of the vitamin D in the skin and increase the level of the vitamin D binding protein and vitamin D receptor with its heterodimers receptors which is alpha RXR [69].

The linkage between the DBP and VDR and alpha RXR is that the DBP is considered a main transporter protein in the body and it has an important role in transporting the vitamin to the cell and then releasing it at the cell wall which then binding to the VDR in the cytoplasm, alpha RXR considered as heterodimers receptors for the VDR after its binding

with its ligand to make it's active and exert its role on the vitamin D hormone response element on the DNA [69].

These result in the current study agreed with the result of the study done by mohammed et al in 2019.

3.4. Biochemical parameters study

This part of the result from the study include the result and statistical analysis of biochemical parameters which is D binding protein, vitamin D receptor, and retinoid X receptor in the patients and control group.

3.4.1. Vitamin D binding protein

Depending on the result of the study, there is a significant difference between the level of the D binding protein in the patient group and the control group, The level of the vitamin D binding protein is higher than its level in the control group, as shown in table 3-5 below

Table 3-5 The concentration of the DBP in both groups in the current study with the level of the significant

	Group	Individuals No.	Mean ± SD	P value
DBP ng/l	Patients group	89	435.0364 ± 171.36762	0.01
	Control group	89	266.1936 ± 217.30586	

The result of the study reveals a high level of DBP in patients group than in the control group, the current study opinion about this elevated level of the DBP in the patient with psoriasis than the control is attributed to the DBP is the acute phase reactant protein which makes it elevated in the acute infection or an acute phase of any disease, especially in the inflammatory condition such as psoriasis or it may be elevated to the decreasing level of the vitamin D in the patients which making the increase the synthesis of the DBP to increase the transportation of the vitamin D from the skin to the liver to the hydroxylate it to the forming the calcidiol which is hydrophobic with the high affinity to the DBP then the DBP leave the liver to the kidney to making the calcitriol [70]

These elevated levels of the DBP can exacerbate the vitamin D deficiency in the body which may increase the severity of the disease in the patients, The result of the current study agreed with the result of the study done in 2022 which conclude there is an elevated level of the vitamin D binding protein in patients with the psoriasis [70].

The DBP plays a bimodal role in psoriasis, another word when the level of the DBP is elevated more than the upper normal level it will be resulted in the deficiency of calcitriol and losing the inflammatory modulatory role of the vitamin D causing the disease to exacerbate by increasing the hyperproliferation the keratinocyte and increasing the production of the inflammatory cytokines [70].

Noteworthy The current study is the first study that concentrates on the pivotal role of the DBP in transporting the vitamin D in the body and its role in the action of the vitamin D and its role in psoriasis in both elevated or lowered levels in the body and support the result of the study that mentioned above which done by Vandikas and Maria Siekkeri et al [67]. .

3.4.2. Vitamin D receptor

According to the statistical analysis of the data of the vitamin D receptor in both patients and the control group, there is a significant difference in the level of the vitamin D receptor among both groups where the level in the patient's group is much lower than those in the control group as showing below in Table 3-6

Table 3-6 The VDR concentration difference between both groups in the current study with the degree of significant

	Group	Individuals No.	Mean ± SD	P value
VDR ng/l	Patients group	89	2.4283 ± 0.15848	≤ 0.001
	Control group	89	3.2821 ± 1.7089	

The elevation in the level of the VDR in the control group than the patients group is attributed to the lower level of the VDR among patients and this decreasing level is attributed to many causes such as patients unable to produce the normal amount of the receptor due to the certain cause, or the receptor may be degraded after its production due to the environmental condition [71]

The author of the present study's opinion is the presence of the low VDR level with the low vitamin D level reveals the disturbance in the upregulation- downregulation mechanism which is an important mechanism that play important role in the regulation of the balance between the receptor and calcitriol

Vitamin D receptor has an important role in psoriasis by its action in transporting the vitamin D from the cytoplasm to the nucleus which results in a decrease in the inflammatory response and thereby severity of psoriasis [68].

The result of the current study is compatible with our previous study in the past years (2018) [68], which also found there is a decreasing level of vitamin D receptor among patients than in the control group and the compatibility of the result of both studies support the important role of the vitamin D receptor in psoriasis, in another word when the patient has a low level of the vitamin D receptor can result in the diminishing the anti-inflammatory modulator role of the vitamin D causing the disease to exacerbate[71].

The presence of the 1-alpha-hydroxylase enzyme in the keratinocyte makes the keratinocyte able to activate vitamin D to calcitriol but when the keratinocyte VDR level is low can lead to the diminishing modulatory role of the vitamin D in the regulation of the proliferation and differentiation of the keratinocyte causing the disease to exacerbate and these would explain why there are some patients has a higher concentration of the vitamin D but has elevated PASI score [71].

The result of the current study is agreed with the result of the study done by mohammed et al in 2018 [68] which concluded that VDR has an important role in psoriasis and it plays important role in the action of the calcitriol and thereby decreasing the PASI score [68].

3.4.3. α Retinoid X receptor

Depending on the statistical analysis of the data in the study, there is a significant difference between the level of the retinoid X receptor among both patients and the control group, were the level of the alpha RXR was higher in the patient group than control group as shown below in Table 3-7

Table 3-7 The difference in the concentration of the α RXR between both groups in the current study with a degree of significant

	Group	Individuals No.	Mean ± SD	P value
α RXR ng/l	**Patients group**	89	22.2989 ± 9.55601	≤ 0.001
	Control group	89	8.1467 ± 4.39137	

The result of the study reveals a higher level of the alpha RXR psoriatic group than the control group and this elevated level is attributed to the deficiency of the vitamin D receptor and calcitriol deficiency with the higher or lowering level of the DBP which leads to an increase in the expression of the alpha RXR to replace this deficiency [72].

The linkage between the alpha RXR and VDR is attributed to the alpha RXR being heterodimers for the VDR and playing a vital role in the action of both VDR and calcitriol [69]. These elevated level of alpha RXR has a benefit for the psoriatic group in decreasing the severity of

psoriasis by increasing the transporting of the VDR-calcitriol complex from the nucleus to the DNA on the vitamin D response element [72].

The presence of the higher level of the alpha RXR is necessary because the VDR cannot bind to the DNA and this is attributed to the VDR having a short DNA binding domain when the alpha RXR has an abnormality in its structure or it may inert this will be lead to the diminishing the role of both calcitriol and VDR in decreasing the disease intensity and thereby increase its comorbidities and keratinocyte hyperproliferation among these group [70].

The RXR is a superfamily of the nuclear receptor consisting of three subclasses including the alpha, beta and gamma members, the alpha RXR is the only active member of the RXR family [72],

Noteworthy the current study may be the only study that concentrates on the alpha members and explains its important role in the action of the calcitriol and the function of the VDR and its role in psoriasis and decreasing its severity [72].

3.5. Statistical analysis of the relationship between vitamin D binding protein, vitamin D receptor retinoid X receptor, and clinical examination of the psoriasis

According to the statistical analysis of biochemical parameters value among patients group and depending on table 3-8 mentioned below,

There is a significant statistically negative relationship between the vitamin D binding protein with VDR (P value 0.020) and a linear relationship between the DBP and retinoid X receptor (P value .000)

while there is a significant statistical relationship between the DBP and disease severity (P value .030) among the patient's group.

VDR has an inversely statistical relationship with disease severity (P value .010) and a negative significant relationship with DBP and alpha RXR.

PASI score has a negative significant correlation with the DBP, VDR, and alpha RXR.

PASI score has a negative significant correlation with exposure to sunlight while it's non-significant with the age and BMI

The DBP has an insignificant correlation with the BMOI (P value 0.720) and age (P value 0.471) and a negative insignificant correlation with exposure to sunlight (P value 0.583)

VDR has a negative insignificant correlation with the sunlight (P value 0.655) and an insignificant correlation with BMI (P value .669) and age (P value .605)

Alpha RXR has an negative significant correlation with exposure to sunlight (P value .021) and a negative insignificant correlation with the BMI (P value .366) and age (P value .829)

Table 3-8 Explain the correlation between the biochemical study marker and the disease severity

Correlations

		DBP	VDR	RXR	PASI	Sunlight	BMI	Age
DBP	Pearson Correlation	1	-.135-	.269**	164*	-.042	.027	.055
	Sig. (2-tailed)		.020	.000	.030	.583	.720	.471
	N	89	89	89	89	89	89	89
VDR	Pearson Correlation	-.135-	1	-.083-	-.195**	-.034-	-.033-	.039
	Sig. (2-tailed)	.020		.025	.010	.655	.669	.605
	N	89	89	89	89	89	89	89
RXR	Pearson Correlation	.269**	-.083-	1	-.286-**	.175*	.069-	.017-
	Sig. (2-tailed)	.000	.025		.000	.021	.366	.829
	N	89	89	89	89	89	89	89
PASI	Pearson Correlation	.164*	-.195-**	-.286-**	1	-.119-	-.032-	-.007-
	Sig. (2-tailed)	.030	.010	.000		.016	.673	.931
	N	89	89	89	89	89	89	89
Sunlight	Pearson Correlation	.042	-.034-	.175*	-.119-	1	-.084	-.056
	Sig. (2-tailed)	.583	.655	.021	.016		.270	.463
	N	89	89	89	89	89	89	89
BMI	Pearson Correlation	.027	-.033-	.069-	-.032-	-.084	1	-.051-
	Sig. (2-tailed)	.720	.669	.366	.673	.270		.502
	N	89	89	89	89	89	89	89
Age	Pearson Correlation	.055	.039	-.017-	-.007-	-.056	-.051-	1
	Sig. (2-tailed)	.471	.605	.829	.931	.463	.502	
	N	89	89	89	89	89	89	89

**. Correlation is significant at the 0.01 level (2-tailed).

*. Correlation is significant at the 0.05 level (2-tailed).

The DBP has a negative significant correlation with VDR which mean when the concentration of the DBP increase this would leads to decreasing the level of the VDR because the DBP has an important role in the transfer of the pre-vitamin D from the skin to the liver to activate it and which making increasing the level of the calcitriol which making the receptor downregulation in response to the upregulation downregulation mechanism [72].

When the level of the DBP will be lower than the normal level this will lead to calcitriol deficiency causing the body to increase the expression of the VDR and alpha RXR [72].

The positive correlation between the DBP and alpha RXR is necessary because it plays important role in the action of vitamin D [73].

These linear correlations occur as a result of two important cause which is vitamin D deficiency and VDR deficiency because even in the presence of the vitamin D in a high concentration but if there is a low concentration of the VDR this would result in the depletion of the vitamin D action and causing the body to increase the synthesis of the DBP and alpha RXR to replace this deficiency in the vitamin D action [73].

The negative significant correlation between the VDR and alpha RXR because of the deficiency in the level or abnormality in the receptor structure in either one of them or both which leads to depletion of the action of the calcitriol or due to the increase the exposure to the sunlight which causing the high level of calcitriol and decreasing of the VDR level which causing the alpha RXR to increase[73].

3.5.1. Correlation of the biochemical parameter with the disease severity (PASI score)

The author of the current study opinion about the significant correlation between the DBP and the PASI means the level of the DBP play important role in decreasing the severity of the disease if it is normal, The author's opinion is when the level of the DBP is elevated but in the normal limit this would increase the transferring of the vitamin D from the skin to the liver and kidney and thereby replacing the deficiency of the vitamin D and decreasing the PASI score, But when the level of the DBP is more than the normal limit this can cause the decreasing level of

the vitamin D and thereby increase the disease severity and these result was agreed and proved the result of the another study in 2022 [67].

VDR has a negative significant correlation with PASI score as the VDR level increase as the severity of the disease decrease because of the increased receptor for the vitamin D and thereby decreasing the inflammatory response by decreasing the amount of the inflammatory cytokines and increasing the production of the anti-inflammatory cytokines causing the PASI score to decrease [68].

The alpha RXR has a negative significant correlation with the PASI score which explain the role of the alpha RXR in decreasing the severity of psoriasis by increasing its level which makes the vitamin D and VDR exert their role causing the inflammatory response to decrease and increase the production of the anti-inflammatory cytokines causing the PASI score to decrease and decreasing the proliferation of the keratinocyte [74],

These negative significant correlations by the current study were the first correlation that explains the important role of the alpha RXR in psoriasis and proved that depending on our result which making these study has a high novelty level [74].

The DBP has no statistical correlation with the age, gender, BMI, and these was attributed to the small sample size included in the current study, negative insignificant correlation between the DBP and exposure to the sunlight and these insignificant negative correlation agreed with the study done in 2021 which conclude the level of the DBP decreased after exposure to the phototherapy and the sunlight among the patients group and these may be attributed to the due to increasing the level of the vitamin D level[74].

VDR has a negative significant correlation with exposure to sunlight and these are attributed to the increasing production of vitamin D after exposure to the sunlight which results in the decreasing the level of the VDR depending on the upregulation-downregulation mechanism. BMI and age have a negative insignificant correlation with the VDR and the main cause for this insignificant correlation is attributed to the small sample size and some individuals having a high level of vitamin D in response to the dietary or intake of the supplement these was agreed with the study done in 2018 [68].

The presence of a significant correlation between the alpha RXR and exposure to the sunlight because of the downregulation of the VDR that resulted from the high level of the vitamin after exposure to sunlight or because the patient has an abnormal lowering level of the VDR which makes the body to increase the level of the alpha RXR to replace the vitamin D deficiency [74].

An insignificant negative correlation between the alpha RXR and the BMI is attributed to the low level of vitamin D that is present among obese patients and this would support the correlation between obesity and the role of the VDR and alpha RXR in psoriasis [75].

A negative insignificant correlation between age and the alpha RXR is attributed to the small sample size and many patients may have higher levels of vitamin D causing the level of the alpha RXR to elevate in response to the decreasing level of the VDR and DBP [75].

Noteworthy, some patients have a high level of alpha RXR, VDR, and DBP and these will be attributed to the low level of calcitriol among those individuals due to the low exposure to sunlight due to the movement motility [75].

PASI score has a negative significant correlation with exposure to the sunlight which makes the exposure to the sunlight has an important role in decreasing the PASI because of the increase in the synthesis of the vitamin D but these effects of the sunlight or phototherapy are also dependent on the presence of the normal level and structure of the receptors [68].

Because the disease occurs at any age and its severity vary between individual there is no significant statistical correlation between the disease severity and age [68].

The PASI score was higher in the patient with high BMI and this was attributed to the decreasing level or action of the vitamin D which resulted either from decreasing the exposure to the sunlight or decreasing the level of the VDR or DBP or alpha RXR in the patients which results in the increasing the severity of the disease [68].

3.6. Calculation the odd ratio

3.6.1.DBP odd ratio

Table 3-9 The odd ratio for the DBP among both groups in the current study

Group / Group	Patient	Control
Exposed	70	40
Non exposed	19	49

The odd ratio for the DBP is 1.08

3.6.2. VDR odd ratio

Table 3-10 The odd ratio for the VDR among both groups in the current study

Group / Group	Patient	Control
Exposed	59	45
Non exposed	30	44

The odd ratio for the VDR is 1.4

3.6.3.RXR odd ratio

Table 3-11 The odd ratio for the α RXR among both groups in the current study

Group / Group	Patient	Control
Exposed	8	55
Non exposed	81	34

The odd ratio for the RXR is 1.7

From the value of the odd ratio for each marker in the study, the Alpha retinoid X receptor has an association with developing psoriasis followed by the VDR and then DBP.

Depending on the calculation of the odd ratio for each marker in the study (DBP, VDR, Alpha RXR) there is a difference in the value of this ratio, the main purpose of the calculation of the odds ratio is to explain which marker from the study is most important followed by the less and less important.

The odd ratio for the alpha RXR was 1.7 this would mean there is a strong correlation between the disease and the alpha RXR because the alpha RXR plays a critical role in the action of the vitamin D and VDR and the presence of any abnormality in the level or structure of alpha RXR resulting in the diminishes the action of the calcitriol, or VDR [76].

The odd ratio for the VDR was 1.4 which is less slightly than the value of the alpha RXR and this explains the VDR plays important role in the disease and any abnormality such as a low level, abnormalities in its structure, or maybe inert VDR can cause the disease to exacerbate because can lead to loss of the action of the calcitriol [68].

D binding protein odd ratio was 1.08 which the less than the value of both VDR and alpha RXR these explain the correlation between the disease and DBP as is less important than the VDR and alpha RXR because the main function of the DBP is to transfer the pre-vitamin D from the skin to the liver to activate it and these function can circumvent by directly supplemented calcitriol while the receptor cannot be supplemented and its presence is necessary for the calcitriol to exert its role on the inflammatory condition to decreasing it by increasing the level

of the anti-inflammatory cytokines and decreasing the level of the inflammatory cytokine [76].

Noteworthy this odd ratio for the biochemical study parameter was the first time calculated by the current study in the world.

3.6.4.Biochemical Parameter as the initiator of the disease

Depending on the result of the study the biochemical marker may have a pivotal role in psoriasis and any abnormality in its level or structure lead to psoriasis. DBP is the main carrier of vitamin D in the body and its function is to carry the pre-vitamin D from the skin to the liver to activate, the presence of any abnormality in the structure of the DBP or its level leads to the disease [67].

VDR main function is to make the vitamin exert its anti-inflammatory role after its binds with VDR to form the calcitriol- VDR complex and this complex is then transformed to the nucleus to heterodimerize with alpha RXR to reach the vitamin D response element and exert its function. Any abnormality in the structure, level or it may be inert VDR or alpha RXR resulting in the inability of the calcitriol to bind with VDR or complex with the alpha RXR which leads to diminishes the function of the calcitriol at the end [68].

Noteworthy, some patients who have a higher level of both receptor included in the current study which is VDR & alpha RXR has lowering PASI score than those who have a deficiency in that receptor

There is another group of patients who have elevated levels of the VDR receptor only with a normal or higher concentration of the alpha RXR and the PASI score for those group was higher than those in the previously mentioned group and these attributed to the presence of the

abnormality in the structure of that receptor which play a vital role in the action of the calcitriol [77].

Another group has a deficiency in the alpha RXR with the normal or higher VDR has a higher PASI score than the patient in the two previous groups and these attributed to the losing the important role of the alpha RXR in the heterodimerization with the calcitriol – VDR complex which causing the loss of the vitamin D action [77].

Another group of patients had a lowering level of both receptors and this group has a higher PASI score than in the all mentioned group previously these attributed to the important role of the receptors in the action of the vitamin which resulted in the increasing the inflammatory response due to the diminishing the anti-inflammatory role of the calcitriol [78].

Some patients have a normal level of the DBP with normal receptors and lower PASI score and these are attributed to the important role of the study parameter in decreasing the severity of the disease by increasing the action of the calcitriol [78].

Another group has a higher level of the DBP with the lowering level of both receptors or in either one with the higher PASI score, the elevated level of the PASI score can result from either the presence of the abnormality in the structure of the DBP or to the inability of the patients to the production of the both or either one of the receptors which are mainly attributed to the abnormality in the upregulation- downregulation mechanism. Even in these groups there are some patients has a higher level of the DBP with the normal concentration of the receptor with the mild PASI score and in these cases, maybe the DBP structure is abnormal or it may be an abnormality in its structure or production [78].

A very small member of the patients in the study has a higher level of the DBP, VDR, and alpha RXR with moderate to severe PASI score and this elevation in its concentration may be attributed to the deficiency in exposure to sunlight or the body can exert abnormal receptor or DBP which making it unable to replace the requirement of the vitamin D [79].

An entire remaining group of the patient included in the study has deficiency in the DBP, VDR, and alpha RXR with the mild to moderate PASI score and these explain the important role of the parameter in the anti-inflammatory role of the vitamin D in psoriasis [79].

In conclusion, when there is a deficiency in the level of the DBP or any abnormality in its structure the deficiency of the vitamin D can be replaced by increasing the intake of the vitamin D supplement but when there is any abnormality in the concentration or structure of the receptors and because of the receptors cannot replace, the role of the calcitriol is lost which causing to the increasing the production of the proinflammatory cytokines with decreasing the production of the anti-inflammatory cytokines resulting in psoriasis or exacerbated the disease at the end [80].

Conclusion And Recommendation

Conclusion

1- There s a higher level of DBP in the patient's group than in the control group and these attributed to the DBP level being elevated in the psoriasis which may exacerbate the vitamin D deficiency because it has a high affinity for vitamin D.

2- The level of the VDR in the patient's group was lower than in the control which lead to a diminishes the action of the vitamin D.

3- The alpha RXR level in the patient's group was elevated to the control group and this may be attributed to the decreasing level of the VDR and calcitriol.

4- There is a negative significant correlation between the PASI score and the biochemical study parameter which explain the role of this marker in psoriasis and decreasing its severity. A negative correlation between exposure to sunlight and the PASI score may explain the role of this biomarker in decreasing the severity.

Conclusion And Recommendation

Recommendation

Our current study recommendations include

1- It is necessary to maintain the level of vitamin D within the normal limit
2- It is necessary to make the biochemical marker of the current study a routine marker for each psoriatic patients
3- It is necessary to exposure to the sunlight for enough time each day

الخلاصة

الصدفية هي مرض التهابي مزمن يحدث نتيجة تغيرات مهمة في و تمايز خلايا البشرة. هناك عدة اسباب للصدفية و من الصعوبة معرفة السبب الصحيح للمرض ولكن هناك علاقة وثيقه بين بعض العوامل الوراثية او نقص بعض المواد بالجسم من الفيتامينات و المعادن او زيادة انتاج بعض العوامل المناعة من خلايا (T). هناك عدة عوامل محفزة لضهور الصدفية مثل الصدمات او التعرض لإصابات بكتيرية او فيروسية او من خلال العوامل الوراثية . تمتاز التقرحات الصدفية بانه لونها يكون احمر الى ابيض مع تقرنات رصاصية اللون منتشرة على الجلد او تظهر بمكان واحد. العديد من المرضى المصابين بالصدفية تكون لديهم نوع من التهاب المفاصل المتعلق بالصدفية الرثوي و هنو يعتبر من ابرز مضاعفات الاصابة بالصدفية. الصدفية لديها تأثير سلبي على نفسية المرضى المصابين.

انتشار الصدفية حول العالم هو ٣%. تظهر اعراض المرض في أي مرحلة معينه من العمر من الاعمار المبكرة و الاعمار الكبيرة. الانتشار السريع لخلايا البشرة متزامنا مع زيادة ارتشاح الخلايا الالتهابية الى طبقة البشرة من الجلد و صناعة الاوردة هي ابرز العلامات المرضية للصدفية. في الحالات الطبيعية الوقت الذي تحتاجه الخلايا المكونة للبشرة للانتشار و التمايز و الهجرة من الطبقة القاعدية للبشرة الى الطبقة الخارجية للبشرة تستغرق ٣٠ يوما اما اثناء الاصابة بالمرض فان عملية الانقسام و التمايز للخلايا المكونة للبشرة هجرتها خلال طبقات الجلد المختلفة مما يؤدي الى ضهور الصدفية.

جميع المرضى اللذين شملتهم هذه الدراسة تم جمعهم من قسم الجلدية في مستشفى مرجان التعليمي في مدينة الحلة بعد ان تم تشخصيهم التأكد من انهم مصابين بداء الصدفية التقرني المزمن.

معلومات المريض كالاسم و الجنس و العمر و تاريخ الاصابة بالمرض و الوزن و الطول وحساب معدل كتلة الجسم و وجود او عدم وجود مضاعفات المرض و وجود او عدم احمرار و تقرنات في الجلد تم التأكد منها و حساب معدل حدة المرض خلال مقابلة مع المريض بعد تشخيصه.

اعتمادا على نتائج هذه الدراسة الى انه لا يوجد أي اختلاف بين العمر و معدل كتلة الجسم و التعرض لأشعة الشمس بين المرضى و الاصحاء اللذين تم شملهم بالدراسة.

كان متوسط عمر مجموعة المرضى و الاصحاء هو في الدراسة المقدمة ٣٦,٦٩٠٥ ± ١٣,٨٦٢٢٨١ و ٤٠,٢٤١٤ ± ١٨,٣٣٦٦٢ على التوالي مع قيمة P 0.148 ، وكان متوسط مؤشر كتلة الجسم للمرضى ومجموعة الاصحاء ٢٧,٢٦٦٣ ± ٦,٥٩٥٩٨ و ٢٨,٣٢٢٧ ± ٤,٦٦٥٦١ على التوالي مع قيمة P 0.380 ، كان متوسط التعرض لأشعة الشمس بين المرضى والمجموعة الاصحاء ١٣٧,٩٨٨٥ ± ١٤,١٠٣١٥ و ١٢٥,٠٤٦٠ ± ٩,٤٥٢١٤ على التوالي مع قيمة P 0.56.

هناك اختلاف في مستويات البروتين الناقل لفيتامين د في الجسم بين المرضى و مجموعه الاصحاء حيث ان مستوى البروتين الناقل لفيتامين د في الجسم لدى المرضى هو اعلى بكثير من مستوياته لدى الاصحاء. حيث كان مستوى DBP أعلى في المرضى منه في المجموعة الاصحاء. كان مستوى DBP في المريض والمجموعة الضابطة ٤٣٥,٠٣٦٤ ± ١٧١,٣٦٧٦٢ و ٢٦٦,١٩٣٦ ± ٢١٧,٣٠٥٨٦ على التوالي مع قيمة P 0.01

هناك اختلاف في مستويات مستقبلات فيتامين د لدى المرضى عن الاشخاص الاصحاء حيث انه كان مستوى مستقبلات فيتامين د لدى المرضى اقل بكثير من مستوياتها لدى الاصحاء. كان أكثر نقصًا في مجموعة المريض منه في مجموعة الاصحاء. كان مستوى VDRمستوى في مجموعة المرضى و الاصحاء هو ٠,١٥٨ ± ٣,٢٨ و ١,٧٠٨٩ ± على التوالي مع VDR قيمةP 0.001

مستويات مستقبلات فيتامين أ المتنقلة كانت اعلى لدى المرضى من مستوياتها لدى الاشخاص الاخرين حيث كان المستوى أعلى في مجموعة المرضى منه في مجموعة الاصحاء. حيث انه ٢٢,٢٩ ± ٩,٥٥ و ٨,١٤ ± ٤,٣٩ على التوالي مع قيمة P 0.001

رايي مؤلف هذه الدراسة حول هذه النتائج انه ارتفاع مستويات البروتين الناقل لفيتامين د لدى المرضى هو بسبب نقصان فيتامين د لدى المرضى. و ان انخفاض مستويات مستقبلات فيتامين د كان بسبب ارتفاع مستويات البروتين الناقل لفيتامين د او ان المرضى لا ينتجون مستقبلات فيتامين د و ان ارتفاع مستويات مستقبلات فيتامين أ المتنقلة هو نتيجة النقصان في مستويات مستقبلات فيتامين د.

خلاصة هذه الدراسة انه البروتين الناقل لفيتامين د، مستقبلات فيتامين د و مستقبلات فيتامين أ المتنقلة هي عوامل مهمة لتقليل حدة المرض لدى الاشخاص المصابين بالصدفية و تعتبر هذه الدراسة هي الاولى من نوعها بالعالم التي تبين هذه النتائج و الدور المهم بصورة جدية

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