ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

Depigmentation Therapy for Vitiligo: Results from a National Survey

Dissertation submitted to

Libyan International Medical University

In partial fulfillment of the requirements

For the award of the degree of

Bachelor of Pharmacy

BY

Makpula Abdelaziz Tarhuni

Aisha Ali Derbash

Under The Guidance of

Dr. Salma Bukhatwa

Declaration

This is to certify that research work embodied in this thesis entitled

"Depigmentation therapy of vitiligo: Results from a national survey"

has been carried out by us under supervision of Dr. Salma Bukhatwa.

Makpula Abdelaziz Tarhuni .....

Aisha Ali Derbash .....

Abstract

Vitiligo is a chronicpigmentarydisorderthat causes the loss of skin color in blotches. Vitiligo happens because of destruction of melanocytes. Classification of vitiligo according to distribution of the lesions is of practical importance usually for assessing the prognosis of the disease. Repigmentation therapyfor vitiligo includes corticosteroids, topical and oral antioxidants, topical calcineurin inhibitors, phototherapy and laser therapy. Depigmentation therapy can be beneficial as alternative therapy for extensive vitiligo patients. Depigmentation therapyof vitiligo includes hydroquinone, monobenzene, mequinol, butylphen, laser therapy and cryotherapy. Recent studies revealed that Laser and cryotherapy as depigmentation therapy for vitiligo if compared with MBEH cream treatment, laser therapy and cryotherapy are faster and more targeted methods that are capable ofdestroyingmelanocytes selectively on one specific area. But up till now the controlled trialsas depigmenting treatment in vitiligo are lacking.
Main objectives of this study were to demonstrate clinical pattern of vitiligo in Libya and to report response of Libyan patients and also dermatologists, towards depigmentation therapy. For this purpose a total of 104 patients from 6 Libyan cities were interviewed using a structured questionnaire.

According to current study, females were more affected by vitiligo compared to males. About 90% of patients are receiving a repigmentation therapy of vitiligo noting that almost 30% of study sample, their vitiligo covers more than 50% of total BSA. Among the 33% of patients who believed in depigmentation, there were only 6.06% patients who experienced depigmentation therapy using 20% monobenzene cream. There have been 67% of patients who did not believe in depigmentation for many reasons including the worry about side effects and success rate of depigmentation therapy.

This study revealed that an acceptable percentage of vitiligo patients in Libya believe in depigmentation therapy even though only few patients have been treated by depigmentation using 20% monobenzone cream noting that results were excellent and without any side effects.MBEH is not available in Libya and in most if not all of the Arab countries. Libyan dermatologists agreed the importance of depigmentation therapy but none of them suggested any solution for the unavailability of 20% MBEH cream for their patients in Libya.

Acknowledgment

Ourgratitude and praise are to Allah who enlightened all our paths in life and showed us his mercy and kindness.

We would like to sincerely thank**Dr. Salma Bukhatwa** who facilitated all procedures to complete the project and also on patience and her efforts exerted for the success of this project.
We are indebted to our great and dearly beloved family specially **Dad** and **Mom** who was always looked out for our future and for being there when we need them. Big thankful to our **Brothers** and **Sisters** who supported us when we were feeling down and who were with us in our times of happiness.

Thanks also are extended to the Libyan International Medical University administration and all the teachers who exerted all efforts for the sake of science and educational process throughout the five years of our study.
We are indebted also to our colleagues for their continued encouragement and for being always nearby when needed.

We also thank all the people helped us and encouraged us to complete this piece of work.

Thank you all.

Table of Contents

Table of Figures

Table of tables

List of abbreviations

4-MP	4-methoxyphenol
4-TBP	4-Tertioart Butyl Plenol
AA	Alopecia Areata
BSA	Body Surface Area
CAT	Catalase
CIs	Calcineurin Inhibitors
CML	Chronic Myeloid Leukemia
CSs	Corticosteroids
DNA	Deoxyribonucleic Acid
DPCP	Diphenylcyclopropenone
FDA	Food Drug Admistretion
GV	Generalized Vitiligo
HLA	Human Leukocyte Antigen

HMB	Human Melanoma Black
HQ	Hydroquinone
LT	Laser Therapy
MBEH	Monobenzyl Ether Hydroquinone
MEL	Monochromatic Excimer Laser
MSR	Methionine Sulfoxide Reductase
NBUVB	Narrow Band Ultraviolet B
NLRP1	Family Pyrin Domain Containing 1
NSV	Non Segmental Vitiligo
PTPN22	Protein Tyrosine Phosphatase (Non-receptor type 22)
PUVA	Psoralen plus UVA
QSA	Q-Switched Alexandrite
QSR	Q-Switched Ruby
RNA	Ribonucleic Acid
ROS	Reactive Oxygen Species
SCSs	Systemic Corticosteroids
SOD	Superoxide Dismutase
SV	Segmental Vitiligo.
TCIs	Topical Calcineurin Inhibitors
TCSs	Topical Corticosteroids
TNFa	Tumor Necrosis Factor-Alfa
UV	Ultraviolet
UVA	Ultraviolet A
UVB	Ultraviolet B
XBP1	X-Box Binding Protein 1

Chapter I
Introduction
General introduction

Vitiligois a chronicpigmentary disorderthat causes the loss of skin color in blotches. Vitiligo happens because of destruction ofmelanocytes (cells responsible for producing the skin pigmentation [melanin]) (Fig1.1).

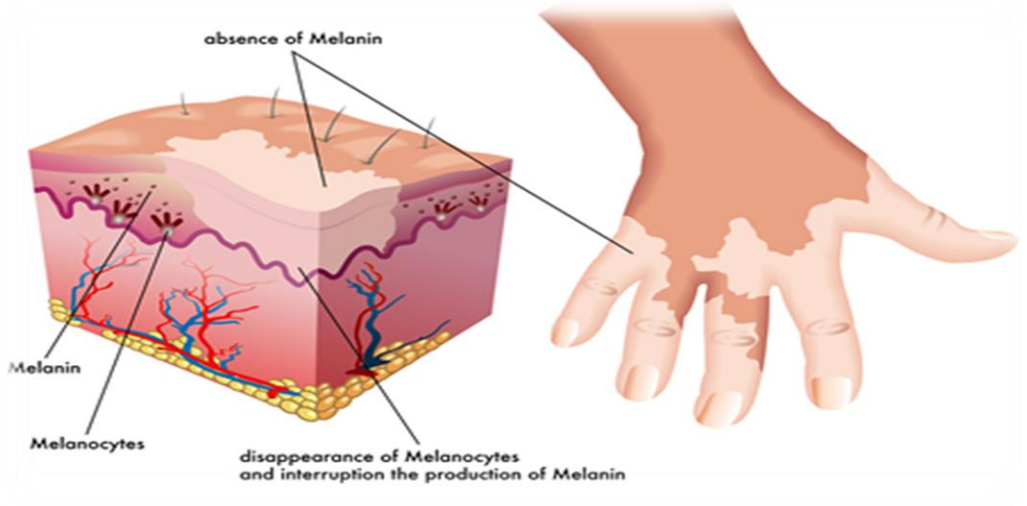


Figure 1.1: Skin layers from vitiligo patient
Adopted fromhttps://www.vitix.co.uk/what-is-vitiligo.html

Types of vitiligo:-

There aretwo important typesofvitiligo; non-segmental vitiligo (NSV),and segmental vitiligo (SV).

Non-segmental of vitiligo:-

NSV is the most common type of vitiligo and occurs in up to 90% of the people who have this disorder.In NSV, the patches often become visible equally on both sides of the body, with some kind of symmetry. These unusual symmetrical patches most commonly appear on skin that is exposed daily to the sun, such as the face, neck, and hands, but it also appears on these other areas; backs of the hands,arms,eyes,knees,elbows,feet and mouth.

Sub-categories of NSV:-

- Generalized vitiligo (GV): The most familiar pattern, it has no specific area or size when the white patches start occurring.
- Acrofacial vitiligo: This type of vitiligo is considered only when the appearance is mostly on the fingers or toes.
- Mucosal vitiligo: The appearance of the depigmentation generally around the mucous membranes and lips.

- Universal vitiligo: It is very rare since depigmentation has to cover most of the body
- Focal vitiligo: One or a few scattered white patches in a small certain areas. It is often noticeable in young children.

Segmental vitiligo:-

SV has a different form of appearance. This condition spreads more rapidly, but is considered more constant and stable than NSV. It is much less common, and it affects only about 10% of people with this condition, but unpredictably, SV is more noticeable in the early ages affecting about 30% of the children that have been diagnosed with vitiligo. SV is non-symmetrical and usually tends to affect dorsalroots of the spine. It is more stable, less erratic, andresponds well to topical treatments.[1]

Pathophysiology of vitiligo:-

Vitiligo is a multifactorial polygenic disorder with a complex pathogenesis. It is related to both genetic and non-genetic factors. Although several theories have been proposed about the pathogenesis of vitiligo, the precise cause remains unknown. Generally agreed upon principles are an absence of functional melanocytes in vitiligo skin and a loss of histochemically recognized melanocytes, owing to their destruction. However, the destruction is most likely a slow process resulting in a progressive decrease of melanocytes. Theories regarding destruction ofmelanocytes include autoimmune mechanisms,cytotoxic mechanisms, an intrinsic defect of melanocytes, oxidant-antioxidant mechanisms, and neural mechanisms.[2] other factors may involve in the etiology of vitiligo, these include; stressful events, harmto the skin due to a critical sunburn or cut, exposure to certain chemicals or viruses.

Autoimmune destruction of melanocytes:-

The autoimmune theory proposes alteration in humoral and cellular immunity in the destruction of melanocytes of vitiligo. Thyroid disorders, particularly Hashimoto thyroiditis and Graves disease; other endocrinopathies, such as Addison disease and diabetes mellitus; and alopecia areata; pernicious anemia; inflammatory bowel disease; psoriasis; and autoimmune polyglandular syndrome are all associated with vitiligo. The most convincing evidence of an autoimmune pathogenesis is the presence of circulating antibodies in patients with vitiligo.[3]

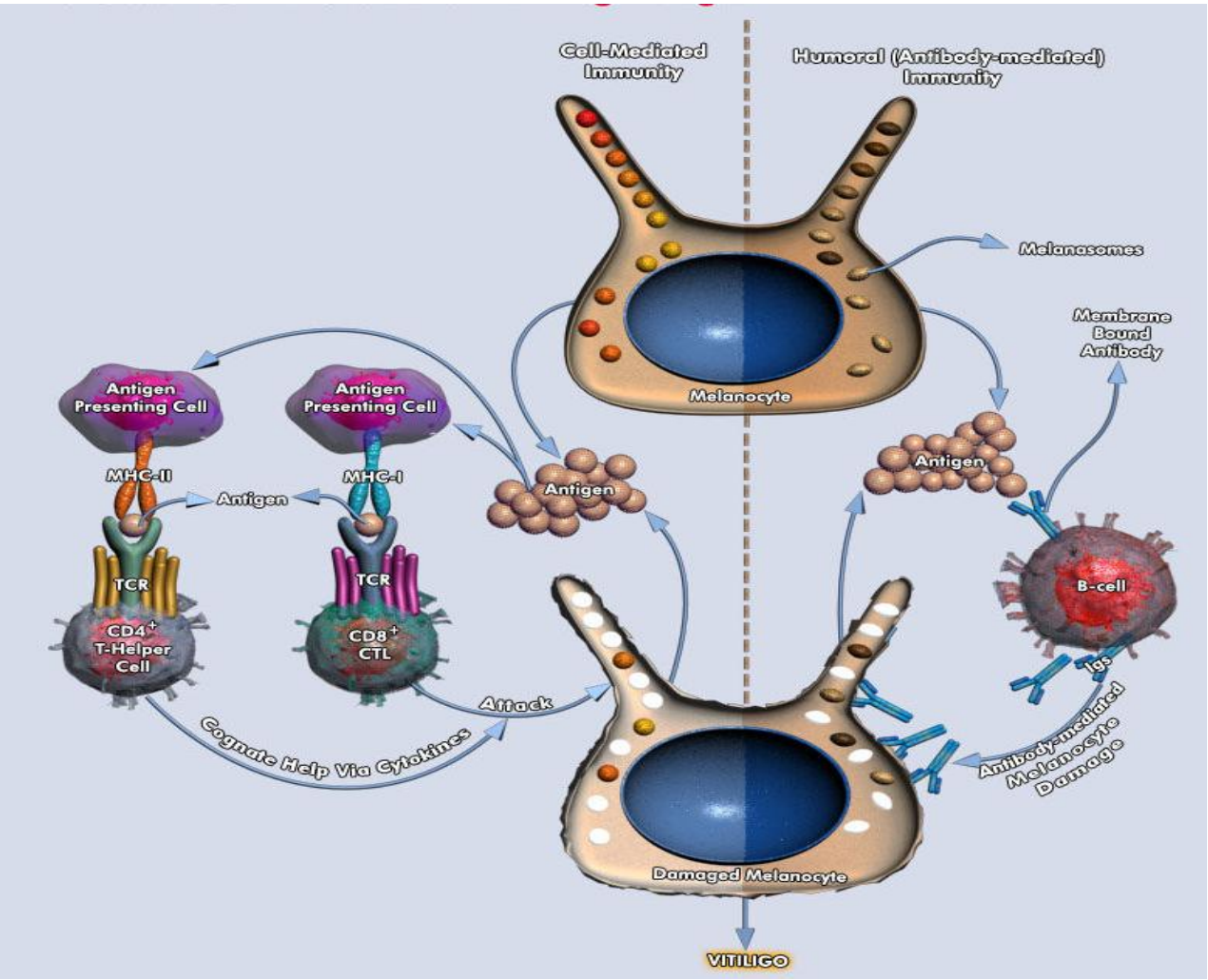
In addition to the involvement of humoral immune mechanisms in the pathogenesis of vitiligo, strong evidence indicates involvement of cellular immunity in vitiligo. Destruction of melanocytes may be directly mediated by auto reactive $CD8^+$ T cells. Activated $CD8^+$ T cells have been demonstrated in perilesionalvitiligo skin. In addition, melanocyte-specific T cells have been detected in peripheral blood of patients with autoimmune vitiligo(Fig 1.2).[4,5]

Intrinsic defect of melanocytes

Vitiligo melanocytes may have an intrinsic defect leading to melanocytes death. These melanocytes demonstrate various abnormalities, including abnormal, rough endoplasmic reticulum and incompetent synthesis and processing of melanocytes. In addition, homing-receptor dysregulation has also been detected. Early apoptosis of melanocytes has also been suggested as a cause of reduced melanocyte survival; however, subsequent investigation found that the relative apoptosis susceptibility of vitiligo melanocytes was comparable with that of normal control pigment cells.[6]

Disturbance in oxidant-antioxidant system in vitiligo:-

Oxidative stress may also play an essential role in thepathogenesis of vitiligo. Studies suggest thataccumulation of free radicals toxic to melanocytes leads to their destruction. Because patients with vitiligo exhibit a characteristic yellow/green or bluish fluorescence in clinically



affectedskin, this led to the discovery that the fluorescence is due to accumulation of two different oxidizedpteridines. The overproduction of pteridines led to the discovery of a metabolic defect in tetrahydrobiopterinhomeostasis in patients with vitiligo, which results in the accumulation of melanocytotoxic hydrogen peroxide.[7]

Figure 1.2: Autoimmune mechanism causing vitiligo:-
Adopted from https://www.qiagen.com/ly/products/genes%20and%20pathways/pathway%20details/?pwid=47

Because oxidative stress has been suggested to be the initial pathogenic event in melanocyte degeneration, several studies have been conducted to evaluate this theory. Recent investigations set out to evaluate the role of oxidative stress by measuring levels of the antioxidant enzymes superoxide dismutase (SOD) and catalase (CAT) in lesional and normal skin of patients with vitiligo and in the skin of normal control subjects. They concluded oxidative stress is increased in vitiligo, as indicated by high levels of SOD and low levels of CAT in the skin of vitiligo patients.[8]

Neural theory:-

Case reports describe patients afflicted with a nerve injury who also have vitiligo have hypopigmentation or depigmentation in denervated areas. Additionally, SV frequently occurs in a dermatomal pattern, which suggests that certain chemical mediators are released from nerve endings that affect melanin production. Further, sweating and vasoconstriction are increased in depigmented patches ofvitiligo, implying an increase in adrenergic activity. Finally, increased urinary excretion of homovanillic acid and vanilmandelic acid (neurometabolites) has been documented in patients with vitiligo. This may be a secondary or primary phenomenon.[9]

In summary, although the ultimate cause of vitiligo is not completely known, this condition does not reflect simple melanocyte loss, but possible immunologic alterations and other molecular defects leading to pigment cell destruction; however, melanocytes may be present in depigmented skin after years of onset and may still respond to medical therapy under appropriate stimulation.

Genetics of vitiligo:-

Vitiligo is characterized by incompletepenetrance, multiple susceptibility loci, and genetic heterogeneity. [10]The inheritance of vitiligo may involve genes associated with the biosynthesis of melanin, a response to oxidative stress, and regulation of autoimmunity. [11]

Vitiligo susceptibility genes:-

These four confirmed GV susceptibility loci - *HLA*, *PTPN22*, *NLRP1* and *XBP1* -all encode important immunoregulatory proteins, lending support to the autoimmune hypothesis of GVpathogenesis. [12-15]

Epidemiology of vitiligo:-

The prevalence of vitiligo is likely less than1%, but varies based on region. Females usually acquire the disease earlierthan males.The published prevalence of vitiligo is 0.5% to1%.[16]Large studies in China, India, and Denmark,have found the prevalence to be 0.093%, 0.005%, and0.38%, respectively.Gujarat, India is consideredto have the highest prevalence in the world, at about8.8%.Men and women are equally affected,but women are more likely to seek treatment.[11,17-21,]The mean age of onset is earlier in those with apositive family history,which ranges from 7.7% tomore than 50%.[11,22-25]Vitiligo is significantly moreprevalent in young women (30 years of age) thanyoung men.The peak in females occurs inthe first decade of life. Male peak prevalence is in thefifth decade of life. Vitiligo is more frequently diagnosedin spring and summer (64.4%).[18,26-29].

Signs and symptoms of vitiligo:-

Vitiligo lesions are characterized as follows:

- White or hypopigmented
- Usually well demarcated
- Round, oval, or linear in shape
- Borders may be convex [7]
- Range from millimeters to centimeters in size
- Enlarge centrifugally over time at an unpredictable rate

Initial lesions occur most frequently on the face (Fig 1.3),hands (Fig 1.4),forearms, andfeet favoring a perioral and periocular distribution.[30]

Diagnosis of vitiligo

Diagnosis of vitiligo generally is made on the basis of clinical findings, by white patch appearance on the body; a special tool called a woods lamp might be used. This lamp uses UV light in a dark room to illuminate areas of damaged skin that would otherwise be hard to see with the naked eye. There is a few questions can be asked to the patient about his medical history, also may do a blood test to check for thyroid problems and diabetes, since they can increase the risk of vitiligo.

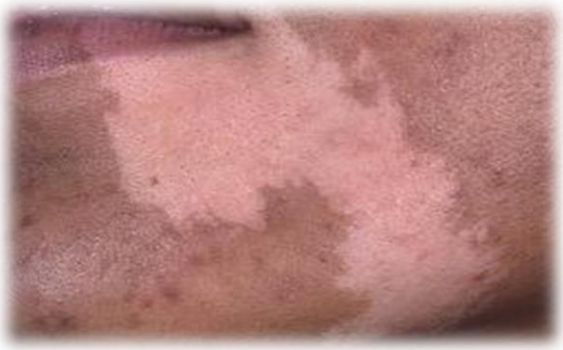


Figure 1.3: Vitiligo affecting the face (lips and under the lips)
Adopted fromhttp://www.skinsight.com/adult/vitiligo.htm

Figure 1.4: Vitiligo affected on hand
Adopted from http://www.nature.com/nchembio/journal/v10/n7/fig_tab/nchembio.1548_F6.html

Histopathology of vitiligo:-

Histopathologic evaluation may help differentiate vitiligo from other disorders in ambiguous cases. [31]Although biopsy is occasionally helpful fordifferentiatingvitiligo from other hypopigmentry disorders.Microscopic examination ofinvolved skin shows a complete absence of melanocytes in association with a total loss of epidermal pigmentation. Superficial perivascular

and perifollicular lymphocytic infiltrates may be observed at the margin of vitiliginous lesions, consistent with a cell-mediated process destroying melanocytes.

Other documentedhistologic findings include the following:

- Degenerative changes in keratinocytes and melanocytes in the border lesions and adjacent skin.
- Increased numbers of Langerhans cells.
- Epidermal vacuolization.
- Thickening of the basement membrane.

Loss of pigment and melanocytes in the epidermis is highlighted by Fontana-Masson staining and immunohistochemistry testing.[32,33]

Interestingly histopathologic evaluation of biopsy is not commonly used for the diagnosis of vitiligo in Libya.

Treatment of vitiligo:-

Counseling vitiligo patients on therapeuticoptions should create realistic expectations.Understanding the factorsthat may affect a patient'sprognosis and response totreatment is essential for success.[34]

Corticosteroids:-

Corticosteroids (CSs) are commonly used as a first-line andadjunctivetherapy for the treatment ofvitiligo. Their efficacy is attributed to modulation ofthe immune response. Studies have shown an abundance ofinflammatory cells in vitiligo, with apredominance of macrophages and T cells.[35,36]Elevated autoantibodyand complement-mediatedmelanocyte destructionhas also been reported.Treatment with CSs decreases this destruction,andappears to induce melanocyte repopulation andmelanin production in vitiliginous skin.[37,38]

Systemic corticosteroids:-

Although systemic CSs (SCSs) are currently notconsidered conventional treatment for vitiligo, but they canbe effective through inducingimmunosuppression.There are few large studies that have evaluated theirsafety and efficacy. It may beenfound that administeringmethylprednisolone 8 mg/kg, or dexamethasone 10 mg/kg intravenouslyin patients with GVled tocessation of disease progressionand repigmentation.[39,40]Oral pulse dose steroids achieve the highest repigmentation response with narrowbandUVB (NBUVB) light phototherapy compared to both psoralen plus UVA (PUVA) and broadband UVB phototherapies. [41]

Topical Corticosteroids:-

Placebo studies have supported the efficacy oftopical CSs (TCSs)monotherapy.Higher response rates are seen inchildren compared to adults,and head and neck lesionstend to have the greatest response to treatment.[42-44]

Topical and oral antioxidants:-

Topical and oral antioxidants may have a role in protecting melanocytes from destruction by reactive oxygen species (ROS). Vitamin E, vitamin C, alphalipoic acid, ginkgo biloba, topical catalase, SOD, and polypodium leucotomos have been used in vitiligo.Oxidative stress

has recently beenimplicated in the pathogenesis of vitiligo.Methioninesulfoxide reductase (MSR) in dietary supplementation; an important reducingagent in repairing damage caused by ROS, is less active and present in loweramounts in patients with vitiligo. Lower MSR increasesmelanocyte sensitivity to oxidative stress andultimately leads to greater cell death. [45]

Topical calcineurin inhibitors:-

Topical calcineurin inhibitors(TCIs) provide similarto slightly inferior results compared toTCSs. TCIsenhance the effect oflight/laser.TCIs therapyis consideredsafe for short-term or intermittentlong-term use. Calcineurin inhibitors (CIs) are advantageous intreating vitiligo because they have immunomodulatoryeffects without the side effect profile of CSs.Calcineurin is an intracellular protein in lymphocytes and dendritic cells. When activated, it acts as a transcription factor for cytokines, such as interleukin-2 (IL-2) and tumor necrosis factor-alfa(TNFα).Compared to healthy controls, patients with vitiligo have elevated IL-10, TNFa, andinterferon-gamma.[46]

Phototherapy:-

UV lighthas been used to treat patientswith vitiligo since the 1800s. The exact mechanismof action is unknown; it is believed to have bothimmunosuppressive and melanocyte stimulatoryeffects. In vitro studies have shown that bothUVA and UVB phototherapies promote melanocytemigration and proliferation, promote a favorableenvironment for melanocyte growth, and inhibitautoimmunity. [47- 49]

Ultraviolet A:-

UVA phototherapy is almost alwaysgiven inconjunction with the photo sensitizer psoralen.PUVAphototherapyinduces hypertrophy of melanocytesand hyperactive melanosomes.
It also stimulates melanocytes in hair follicles, induceskeratinocyte release of factors that simulatemelanocyte growth, and may reduce the presence of vitiligo-associated melanocyte antigens on melanocyte membranes.[50,51]
PUVA is approved by the FDA for the treatment ofvitiligo (Fig 1.5),but high doses of UVA alone (15 J/cm^2)induce repigmentation of more than 60% in half ofsubjects.Enhanced withpsoralen, response ratesare as high as 78% and up to 100% for head and necklesions in more thanhalf of subjects. Lesions on theextremities are less responsive.[52-54]

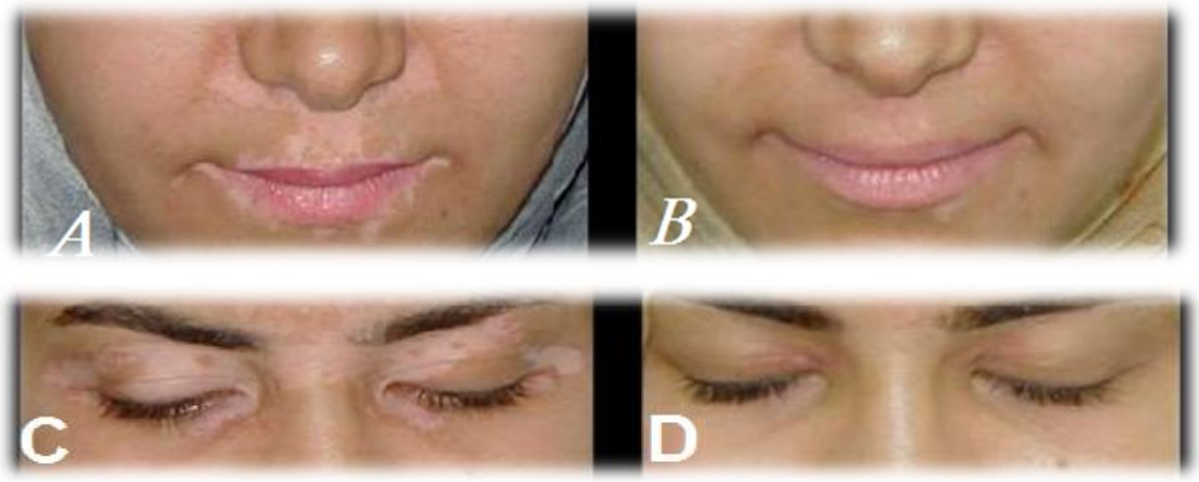


Figure 1.5: (A)(C) patient has vitiligo before using psoralen Ultraviolet A (PUVA) for treatment, (B)(D) patient after using (PUVA) for treatment
Adopted from http://www.laskinmd.com/psoriasisvitiligolosangeles.html

Ultraviolet B:-
In the past decade, 311-nm NBUVB phototherapy has superseded PUVA phototherapy in the treatmentof vitiligo because it was shown to be clinically more effective. NBUVB induces tyrosinase, an enzyme crucial to melanin production, and increases the presentation of HMB on the surface of melanosomes.[55] NBUVB currently representsthe phototherapy of choice for active and ⁄or widespreadvitiligo. Side effects are less frequent than in PUVAtherapy and efficacy is at least equivalent.[56,57]

In 94th Annual Meeting of the British Association of Dermatologist, Glasgow 2014,Dr. Tsui Ling (Salford) presented the updated BAD/British PhotodermatologyGroup guidelines for psoralen and PUVA.[58] A comparison of therelative efficacy andsafety ofNBUVB, conventional oraltreatments and biologics found that the best treatment variesbetween diseases. However, manycenters have disposed oftheir PUVA equipment because of the potential for carcinogenicity.NBUVB is more efficacious than PUVA forvitiligo, but recent evidence suggests that a xenon monochloridemonochromatic excimer lamp that generates a monochromaticwavelength of 308 nm may be even more effective.[59]In an Indian study of 400 patients, 19.5% had an excellentresponse (> 75% repigmentation after 1–10 sessions) and38.0% had a good response (> 75% repigmentation in 10–20sessions).[60]

Laser therapy:-

Laser therapy (LT) for vitiligo is a relatively new treatmentthat has gained popularity in thelast decade.The mechanism of action is thought to be similar to conventional light therapy, but lasers allow targetedtreatment, less total body irradiation, and less impact on healthy skin. The best studied and most used laser therapy for vitiligo is theMonochromatic Excimer Laser(MEL), a nonablative technology that emits light in the UV range. The specific wavelength depends on the halogen and noble gas source. Compared to NBUVB phototherapy, MEL hasbetter clinical outcomes.Foundexcellent repigmentation rates(Fig 1.6).[61,62]

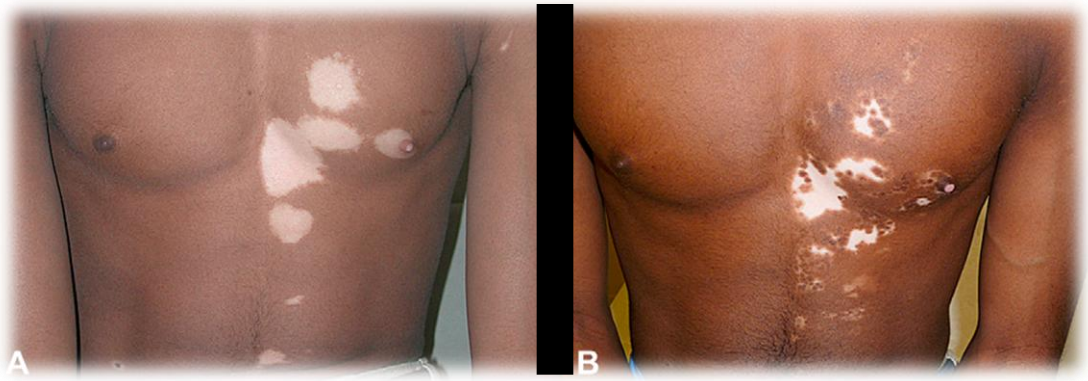


Figure 1.6: An African American patient on thrice weekly excimer laser therapy (A) before and (B) 23 months into treatment [63]

How effective are different types of treatment protocols for vitiligo, remains a big question. Can patient a complete cure?

Depigmentation therapy:-

Depigmentation may be beneficial to patientswho fail repigmentation therapy orhave extensive disease;completerepigmentation in all patients is far to be achieved. When satisfactoryresults are unattainable, depigmentation therapy may provide more desirable cosmetic outcomes.Topical agents that depigment normal skin include hydroquinone and monobenzyl ether of hydroquinone (MBEH). Their mechanism of action is not entirely clear, but studies have shown that both induce melanocyte death.[64-67]

Generally, depigmentation therapy can be considered ifvitiligoaffectsmore than 60% to 80% of the body. A recent survey, however,showed variation among dermatologists, where 32% of dermatologistswere in favour of depigmentation whenvitiligo affectsmore than 75% ofthe bodywhile 42% dermatologists were infavour of depigmentationwhen vitiligo affects more than 50% ofthe body.[68] Expert consensus recommends that patient selection isimportant in depigmentation treatment.

In general, depigmentationis undertaken only when the patient has more than 50% pigmentloss in their skin because of vitiligo, or when the depigmentation isextensive in the cosmetically sensitive areas of the hands and face.Depigmentation is not recommendedforchildren.[69]In patients with extensive areas ofdepigmentation and/or disfiguringlesions on the face who do not respond to repigmentationtherapies (e.g. phototherapy), thendepigmentation can be usefulbecause complete repigmentation may never occur even after longperiods of phototherapy.[70]Sometimes subtotal repigmentationmay be achieved after patients undergo 150–200 sessions of PUVA therapy with or without adjuvanttherapy but there is always a possibility of depigmentation after cessationofPUVA therapy.[71]Therefore, complete depigmentation rather than repigmentation therapy is recommendedby manyinvestigators in case of universal vitiligo. During and uponcompletion of the depigmentationtherapy, patients are permanentlyat risk for acquiring sunburn. Patients should therefore beadvised to minimize sun exposure and to apply broad-spectrumsunscreens because recurrence of the pigment is also observedwithin a few weeks of discontinuing successful depigmentationtherapy on sun-exposed sites.[66,70]

Agents for depigmentation:-

Hydroquinone:-

Topical application of hydroquinone (HQ) produces a reversible depigmentation of the skin by inhibition of the enzymatic oxidation of tyrosine to 3,4-dihydroxyphenylalanine and suppression of other melanocyte metabolic processes.Exposure to sunlight or UV light will cause repigmentation of bleached areas. Chemically, HQ is $C_6H_6O_2$ and has a molecular weight of (110.11). The chemical name of HQ is 1,4 dihydroxybenzene, and the structural formula of HQ is shown below (Fig 1.7).

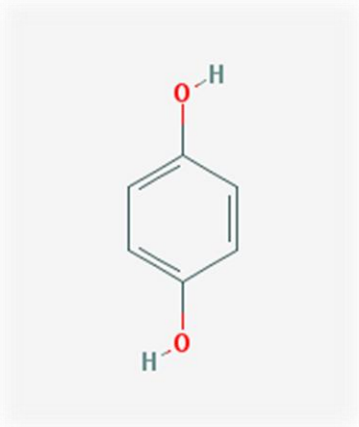


Figure 1.7: Chemical structural formula of Hydroquinon (HQ)
Adopted from.https://pubchem.ncbi.nlm.nih.gov/compound/785#section=2D-Structure

Pharmaceutical dosage form of HQ:-

Each gram of**HQ 4% cream** contains 40 mg HQ, in a cream base of alcohol, capryloyl glycine, C13-14 isoparaffin, glycerin, glycolic acid, kojic acid, laureth-7, lecithin, polyacrylamide, purified water, *Simmondsia Chinensis* (Jojoba) seed oil, sodium hydroxide, squalane and xanthan gum. [72]

Side effects of HQ:-

The use of HQ cream may lead to a mild skin irritation and sensitization (e.g., burning, erythema, stinging).[72]

Monobenzyl ether hydroquinone:-

Monobenzyl ether hydroquinone (MBEH), is the mainstay of depigmentation therapy. MBEH is a topical product used for the treatment of pigmentary disorders.[73,74]

MBEH has trade name asmonobenzone® occurs as a white,almost tasteless crystalline powder, soluble in alcohol and practically insoluble in water. Chemically, monobenzone is designated as p-(benzyloxy) phenol. Its empirical formula is $C_{13}H_{12}O_2$ with molecular weight(200.24) and structural formula as shown below (Fig 1.8).

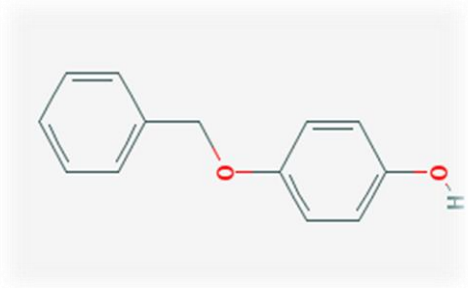


Figure 1.8: Chemical structural formula of monobenzyl ether hydroquinone (MBEH):-
Adopted from.https://pubchem.ncbi.nlm.nih.gov/compound/7638#section=2D-Structure

Pharmaceutical dosage forms of MBEH:-

MBEH is applied topically as a 20% cream. Each gram of thecream contains 200 mg of monobenzone USP, in a water-washable base consisting of purified water USP, cetyl alcohol NF, propylene glycol USP, sodium lauryl sulfate NF and white wax NF.[75]

A thin layer of cream should be applied uniformly and rubbed into the pigmented area two to three times daily. Prolonged exposure to sunlight should be avoided during treatment, or a sunscreen should be used, as exposure to sunlight reduces the depigmenting effect. Depigmentation is usually obtained after 1-4 months of treatment. After 4 months of treatment without success, the drug should be discontinued. when the desired degree of depigmentation is obtaind, monobenzone should be applied as often as needed to maintain depigmentation (usually only two times weekly).[67]

Recently, Hariharan et al. reported that MBEH couldinduce cell death without activating the caspase cascadeor DNA fragmentation.[76]Riley postulated that MBEH diffuses into melanosomes of pigment cells where the enzyme tyrosinase converts it to toxic species such as quinones, which react with essential cellular macromolecules such as proteins and DNA and cause melanocyte death.[77-79]Depigmentation induced by MBEH is generally irreversible and is associated histologically with loss of melanosomes and, eventually, loss of melanocytes.[80,81]

Side-effects of MBEH:-

Although the use of MBEH may lead to a satisfying degree ofdepigmentation in most patients, some disadvantages such as skinirritation, contact dermatitis and ocular side effects have also beenreported. In addition, exogenous ochronosis is also reportedas a potential complication after application of MBEH in manycases. In some cases MBEH resistance and recurrence of thepigment were also observed because of intense sun exposure.Therefore, the use of MBEH has been restricted in the Netherlands,since 1990. [66,71,82-88]Repigmentation in previously successfully depigmented skinhas previously been reported as an occasional problem afterthe end of MBEH therapy.[82,89]Thiswas triggered by sun exposure in almost all of the patients, reinforcing the need for sun protection in these patients afterthe end of treatment.[90]

However, MBEH is presently the only depigmenting agent approved by the U.S. Food and Drug Administration (FDA) for use in patients with extensive vitiligo. [91]

Mequinol or 4-methoxyphenol:-

This compound is a phenol derivative and also known as ‘p-hydroxyanisole’ or 'monomethylether of hydroquinone'. The compound has been shown to have melanocytotoxic properties that are comparable with those of MBEH.4-methoxyphenol (4-MP) has molecular formula$C_7H_8O_2$with Molecular weight124.13722 g/moland structure formula as shown below(Fig 1.9). [92]

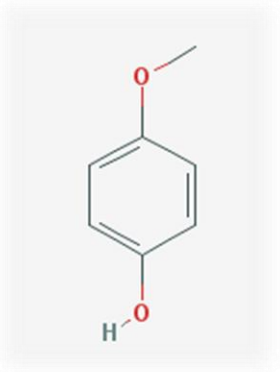


Figure 1.9: Chemical structural formula of 4 methoxyphenol (4-MP)
Adopted from. http://pubchem.ncbi.nlm.nih.gov/compound/4-Methoxyphenol#section=Top

Pharmaceutical dosage formof 4-MP:-

4-MP is applied topically as a cream. The effectiveness of 4-MP wassignificantly correlated with the duration of use of the cream; the longer the cream was used, the better the results. However, compared with MBEH cream, a disadvantage of 4-MP was the longer time prior to the onset of visible depigmentation (between 4 and 12 months), whereas it was previously reported that depigmentation with MBEH may already be evident after 1 month.[66,77,93,94]
Melanocytes in the hair follicles may also be affected by 4-MPin a dose-response fashion, but because of their deeper localization, these melanocytes in comparison with epidermal melanocytesare less susceptible to the compound.[78]

Side-effects of 4-MP:-

Mild burning or itching, irregular leucoderma and skin irritationwere reported with 4-MP. Patients should be advised that pigmentmay return and protection from sunlight is necessary.[66,85,95]

Butylphen or4- Tertioart butyl plenol:-

The depigmentation process that follows exposure to phenolic agent butylphen (4-TBP) is better understood.*In vitro* studies have shown that 4-TBP is specifically cytotoxic to melanocytes. Though 4-TBP is a tyrosine analog that binds the catalytic site of the tyrosinase enzyme and act as a competitive inhibitor of tyrosinase. Thecytotoxic effectswerefound to be independent of tyrosinase activity. 4-TBP is shown to activate apoptosis in melanocytes.[96,97]In summary, exposure to MBEH or 4-TBP has profoundly different consequences for melanocyte physiology and activates different death pathways. [76] 4-TBP has the empirical formula $C_{10}H_{14}O$with molecular weight150.21756 g/mol.[98] and structural formula as shown below (Fig 1.10).

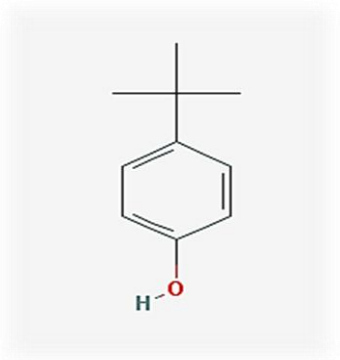


Figure 1.10: Chemical structural formula of Butylphen or 4- Tertioart butyl plenol (4-TBP)
Adopted from. https://pubchem.ncbi.nlm.nih.gov/compound/4-tert-Butylphenol

Eighty-eight percent phenol solution:-
This solution is inexpensive and applied topically for chemical peelings. Protein coagulation is observed in the epidermis immediately after application of 88% phenol solution. It can penetrate deep in to the tissue, down to upper reticular dermis. All phenolcompounds have toxicity towardsmelanocytes. Eighty-eight per cent phenol solution can be considered asa therapeutic option to eliminate residual normally pigmentedareas in patients with generalized vitiligo.[99,100]

Side-effects of 88% phenol solution:-
Generally, 88% phenol solution does not produce any complicationsin experienced hands. However, sometimes 88% phenolsolution produces complications such as non-aesthetic scar formation, dyschromia and development of herpetic eczema. High-dosephenol usage is toxic, so it should not be applied over largeareas.[101] Phenol exerts marked corrosive action on any tissue itcontacts when ingested, inhaled or brought into contact with theskin. Its cellular uptake is both rapid and passive because of itslipophilic character and signs of systemic toxicity develop soonafter exposure. Phenol's main target organs are liver, kidney, respiratoryand cardiovascular systems. Cardiovascular shock, cardiacarrhythmias and bradycardia, as well as metabolic acidosis havebeen reported within 6 hours of skin peeling procedures with phenol.[102] Repigmentation may occur if patients do not protect themselvesproperly from UVradiation.[103] Transient or definitehypo-pigmentation after application of phenol is due to thedevelopment of a melanocytic incapacity to normally synthesizedmelanin.[104] On the other hand, other depigmenting agents, such asHQ and MBEH, destruct the melanocytes. It is reportedthatHQ causes depigmentation because of decreasedtyrosinase activity by 90% and reversible inhibition of cellularmetabolism by affecting both DNA and RNA synthesis of melanocytes.[105]Additionally, unlike hydroquinone, MBEHalmost alwayscauses a nearby irreversible depigmentation of skin. It has beensuggested that the mechanism of depigmentation by MBEHinvolves selective melanocytic destruction through free radical formationand competitive inhibition of the tyrosinase enzyme system.[106]

Depigmentation using laserstherapy:-
Depigmentation therapy using laserscan be applied in cases where patients do not respond well to the depigmentation cream or in areas, especially the face, where rapid depigmentation required.[70] Lasers have proven to be highly effective in selectively targeting melanocytes for destruction, thus causing depigmentation. The Q-switched ruby (QSR, 694 nm) and Q-switched alexandrite (QSA,755 nm) lasers are known to induce selective photothermolysis of pigmented

lesions because their wavelengths are between 600 nm and 800 nm, which are absorbed more easily by melanin than by haemoglobin. Light emitted by both lasers iswell absorbed bymelanin.[107,108]

QSR laser:-

The QSR laser selectively targetsmelanosomes and destroysmelanocytes and keratinocytes. Because of the QSR laser effects,the more tan skin is, the more therapeutic the effects become. Tanning can induce activation of melanocytes in normal pigmentedareas and these activated melanocytes are the target ofselective photothermolysis performed with pigment-specific lasers. Therefore, QSR laser therapy after tanning can induce permanentdamage in activated melanocyte-containing structures.[109,110] There aremany advantages to depigmentation therapy with QSR laser: thetherapeutic effects are fast and safe, the duration of treatment isshort; and the area to be depigmented can be large, as comparedwith depigmentation performed using a bleaching agent. Anadditional advantage of the QSR laser is that its beam reduces therisk of scar formation on the skin (Fig 1.11).[66,70,111,112]

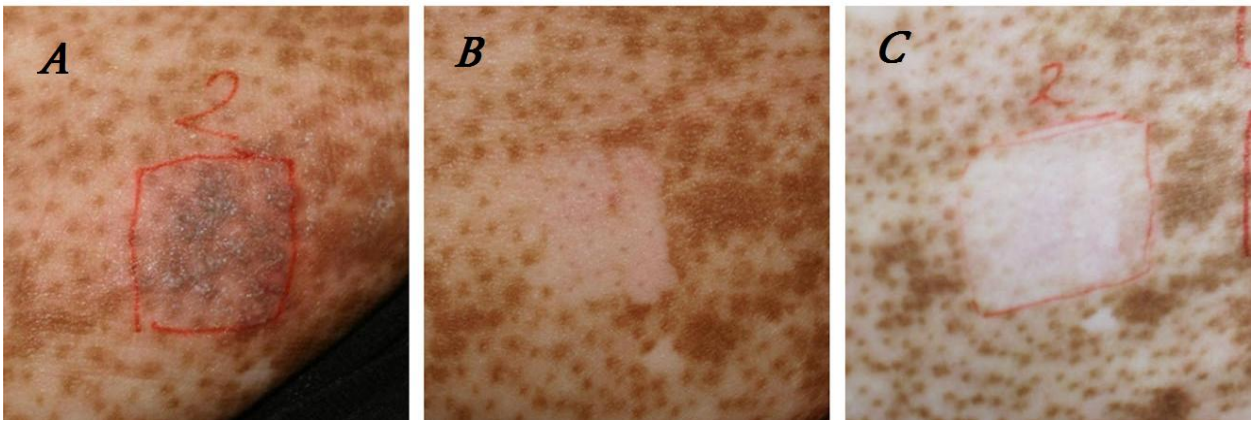


Figure 1.3: Skin reaction on test area directly after laser treatment (a), after 7 weeks (b) and 15 months of follow-up (c)[113]

QSA laser:-

The QSA laser has shown efficacy intreating both naturally occurring pigmented lesions and exogenouspigment. [114,115] The QSA therapy is safe, simple and effective intreating recalcitrant pigmentation after depigmentation therapy invitiligo patients. The QSA laser is advantageous over the QSR laserbecause it has a faster pulse frequency, which allows for morerapid therapy. In addition, it also has a higher wavelength of755 nm, as compared with the 694nm QSR laser, which facilitatesgreater tissue penetration and improves results.[67]

Side-effects of laser therapy

The main disadvantage of this therapy is that sometimes localanesthesia is required because it may be painful to the patients.Therefore, this treatment is only possible in the clinic, rendering it an expensive therapy.[66] The QSR laser therapy may fail in permanentlyremoving pigmented patches and after several months oftreatment both epidermal repigmentation and an increased number of dermal macrophages have been observed.[67,116]

Cryotherapy:-

Cryotherapy is suitable for very small lesions and cannot be utilized if the surface area of pigmented lesions is more than a few centimeters. Cryotherapy has been suggested to depigment

MBEH-resistant skin.[71,74] Cryosurgery is melanocytotoxic and can easily kill melanocytes. Cryosurgery was used to remove normally pigmented patches in patients with universal vitiligo.[71,117].

Cryotherapy has numerous advantages over other modalities. Preparation time is short and treatment requires no other expensive supplies or injectable anaesthesia. In addition, the risk of infection is low and wound care is minimal.[118] This technique is used to remove many melanocytic lesions including simple or solar lentigines, junctional nevi and cafe´-au-lait spots without causing permanent damage to other cell structures or scarring in vitiligo patients.[117] This method is simple, easy to perform, safe, efficacious and cost effective.[119] Therefore, it may be superior to other medical and surgical methods. Depigmentation developed by cryotherapy ispermanent, not scar forming, if performed by experienced dermatologists. The technique yields excellent cosmetic results (Fig 1.12).[71]

Side-effects of cryotherapy:-

If cryotherapy is performed aggressively, it can lead to permanentscarring. Cryotherapy should be used by experienced persondelicately to avoid side effects.[71, 120]

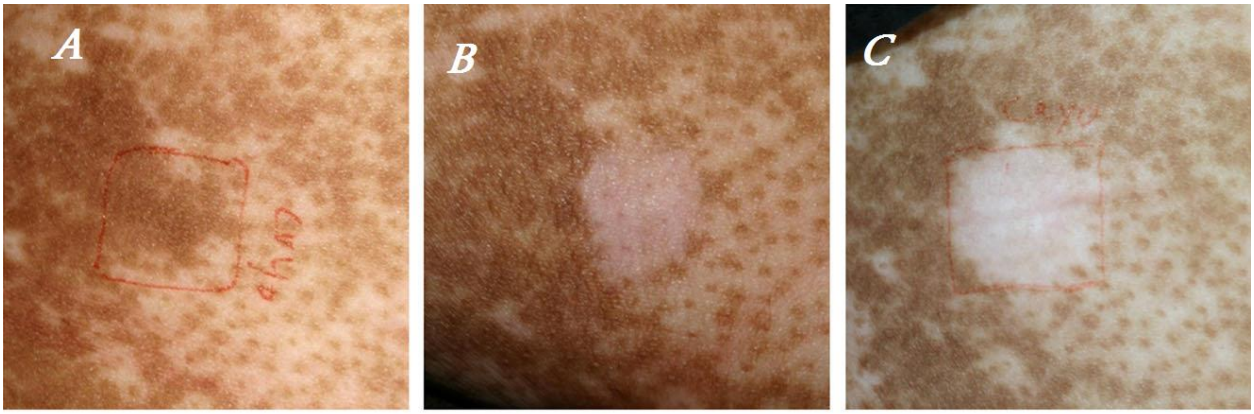


Figure 1.4: Test areas cryotherapy before (a), after 7 weeks (b) and 15 months of follow-up (c)[113]

This is an important finding as laser therapy is far more expensive compared to cryotherapy. However, side effects were only found after cryotherapy although they were in general reversible.[113]Future research with this treatment set-up may lead to new insights in depigmentation therapy of vitiligo. More controlled studies are required to confirm these findings.[113]

AlGhamdi and Kumar showed that MBEH is the most safe and effective depigmenting agent comparing to other depimanting agents (4-MP, 88% phenol solution, laser, cryotherapy, etc.). [121] Howevera recent study in 2015 has indicated that in contrast to MBEH cream treatment, laser therapy and cryotherapy are fast and targeted methods, capable of destroying melanocytes selectively on one specific area.[122] Up till now, controlled trials comparing laser and cryotherapy as depigmenting treatment for vitiligo are lacking.

Potential depigmenting agents:-

Imatinib:-

Imatinib is used for the treatment of leukaemia. Vitiligo-likedepigmentation of the skin was observed as a side-effect of treatmentwith this drug in several patients with chronic myeloid leukaemia (CML). Skin hypo-pigmentation was noted in fivepatients with CML who were treated with imatinib mesylate (Table 1.1).[123]

Imiquimod:-

Imiquimodis a low molecular weight imidazoquinolinamines, haspotent antiviral and antitumour properties and approved from FDA, USA,[124]but during treatment permanent post inflammatoryhypo-pigmentation has been reported as a side-effect (Table 1.1).[125]

Diphencyprone:-

Diphencyprone or diphenylcyclopropenone (DPCP) is used totreat dermatological conditions resulting from an altered immunologicalstate, such as extensive alopecia areata (AA). AA has been treated with DPCP since 1976 without serious adverse events, except for the induction of hypo- or depigmentation (Table 1.1).[126]

Table 1.1: Potential depigmenting agents

No	Name	Mechanism of action	Dose / concentration	Onset of depigmentation	Side effects
1	Imatinib	Tyrosine kinase inhibitor	800 mg/day for 6 months by thesystemic route	4 weeks	Periorbital edema, fluid retention, nausea, emesis, diarrhea and Myelosuppression.[127]
2	Imiquimod	Stimulation of the innate immune response and cell-mediated adaptive immunity	5% cream	3 months	Burning, itching and pain at the target site.[128,129]
3	Diphencyprone	Immuno-modulatory action	0.0001%	10.5 months	Local eczema with blistering, regional lymphadenopathy and contacturticarial.[130]

Vitiligo causes appearance of white patches on the skin. These patches may impair cosmetic appearance and psychological functions.[131]Emotional impact of this condition on patient and their families is severe.[132]Patient with extensive and resistant vitiligo may benefit from depigmentation therapy rether than regaining pigmentation in the vitiligo skin. [66]MBEH the only depigmentation agent approved by the U.S.FDA. The decision to treat vitiligo usually depends on patient's desire and motivation. Previous local studies concerned with vitiligo treatment amongLibyan patients raised many important questions that need to be answered.

Current study is the only study concerned with vitiligo among Libyan patients since long time. Currentstudy tries to discuss the potential availability of depigmenting agents in Libya giving a chance for patients with universal vitiligo to be treated.

Objectives of this study

1. Discuss potentially available depigmenting agents and their success in treatment of vitiligo. This has been discussed already in previous chapter of this thesis.
2. Demonstrate clinical pattern of vitiligo in Libya.
3. For the first time, report response of Libyan patients and dermatologists regarding depigmentation therapy.

Chapter II
Materials and Methods:-
Data collection:-
All outpatients presenting to patient dermatology clinics, with vitiligo and during the periodJuly to September 2015in Benghazi, Almarj, Algouba, Dernah, Ejdabya and Tripoli were reviewed (Table 2.1). Also, some patientswere interviewed outside the clinics. The total number of patients interviewed was 104 (Table 2.1).

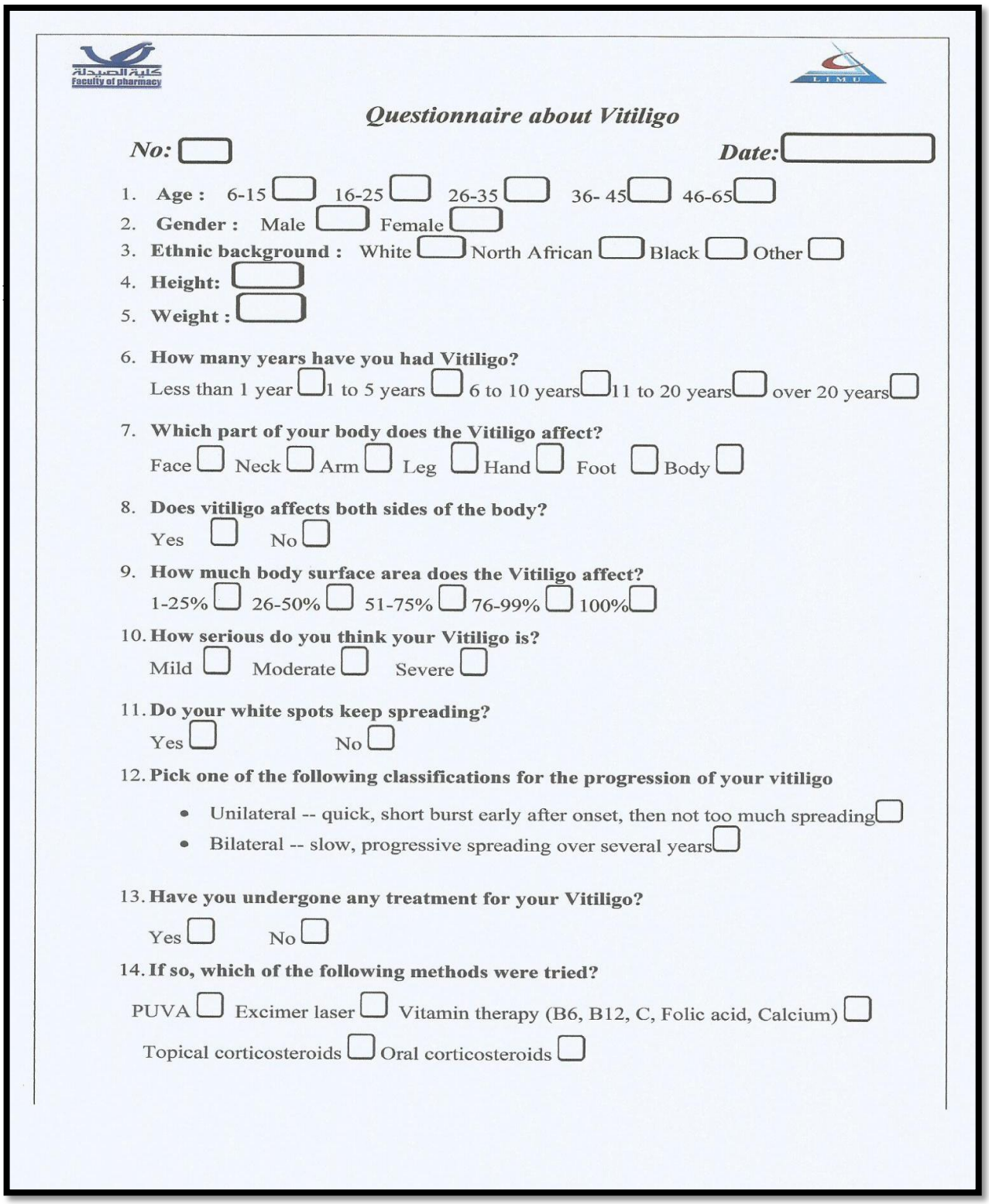
Table 2.1: Distribution of patients participated in the study among Libyan cities

City	Number of patients
Benghazi	45
Almarj	15
Algouba	1
Derna	4
Ajdabia	3
Triopli	36
Total	104

Data collection tool:-
A detailed clinical history including: age, gender, ethnic background, height, weight, history, places of lesion, extent of prevalence of the disease in the body,different classifications for the progression of vitiligo, kind of treatment used and its effectiveness, alternative treatment, coexistent autoimmune disease and response of the patient to the depigmentation therapies as successful alternative treatment.All information wascollected by the investigators with the aid of a structured questionnaire (Fig 2.1- 2.2).

Another questionnaire with a limited number of questions was used to get opinion of dermatologists regarding depigmentation therapy (Fig 2.3).

Data analysis:-
All data was presented as Mean ± SEM (n) or percent (%) as required. Excel was used for statistical of analysis of resultsfor drawing graphs.



كلية الصيدلة
Faculty of pharmacy

LIMU

Questionnaire about Vitiligo

No: ☐ ***Date:*** ☐

1. **Age :** 6-15 ☐ 16-25 ☐ 26-35 ☐ 36- 45 ☐ 46-65 ☐
2. **Gender :** Male ☐ Female ☐
3. **Ethnic background :** White ☐ North African ☐ Black ☐ Other ☐
4. **Height:** ☐
5. **Weight :** ☐
6. **How many years have you had Vitiligo?**
   Less than 1 year ☐ 1 to 5 years ☐ 6 to 10 years ☐ 11 to 20 years ☐ over 20 years ☐
7. **Which part of your body does the Vitiligo affect?**
   Face ☐ Neck ☐ Arm ☐ Leg ☐ Hand ☐ Foot ☐ Body ☐
8. **Does vitiligo affects both sides of the body?**
   Yes ☐ No ☐
9. **How much body surface area does the Vitiligo affect?**
   1-25% ☐ 26-50% ☐ 51-75% ☐ 76-99% ☐ 100% ☐
10. **How serious do you think your Vitiligo is?**
    Mild ☐ Moderate ☐ Severe ☐
11. **Do your white spots keep spreading?**
    Yes ☐ No ☐
12. **Pick one of the following classifications for the progression of your vitiligo**
    - Unilateral -- quick, short burst early after onset, then not too much spreading ☐
    - Bilateral -- slow, progressive spreading over several years ☐
13. **Have you undergone any treatment for your Vitiligo?**
    Yes ☐ No ☐
14. **If so, which of the following methods were tried?**
    PUVA ☐ Excimer laser ☐ Vitamin therapy (B6, B12, C, Folic acid, Calcium) ☐
    Topical corticosteroids ☐ Oral corticosteroids ☐

15. **After this treatment, what was the status of your Vitiligo?**

No improvement ☐ Slight improvement ☐

Significant improvement ☐

Complete repigmentation of vitiliginous lesions ☐

16. **Have you tried any alternative treatment (medicine)?**

Yes, ☐

No ☐

17. **Do you have any autoimmune disease?**

Yes ☐ if yes, which disease? _____

No ☐

18. **Has any doctor told you, there is no therapy for Vitiligo?**

Yes ☐

No ☐

19. **Do you have any family member who has Vitiligo?**

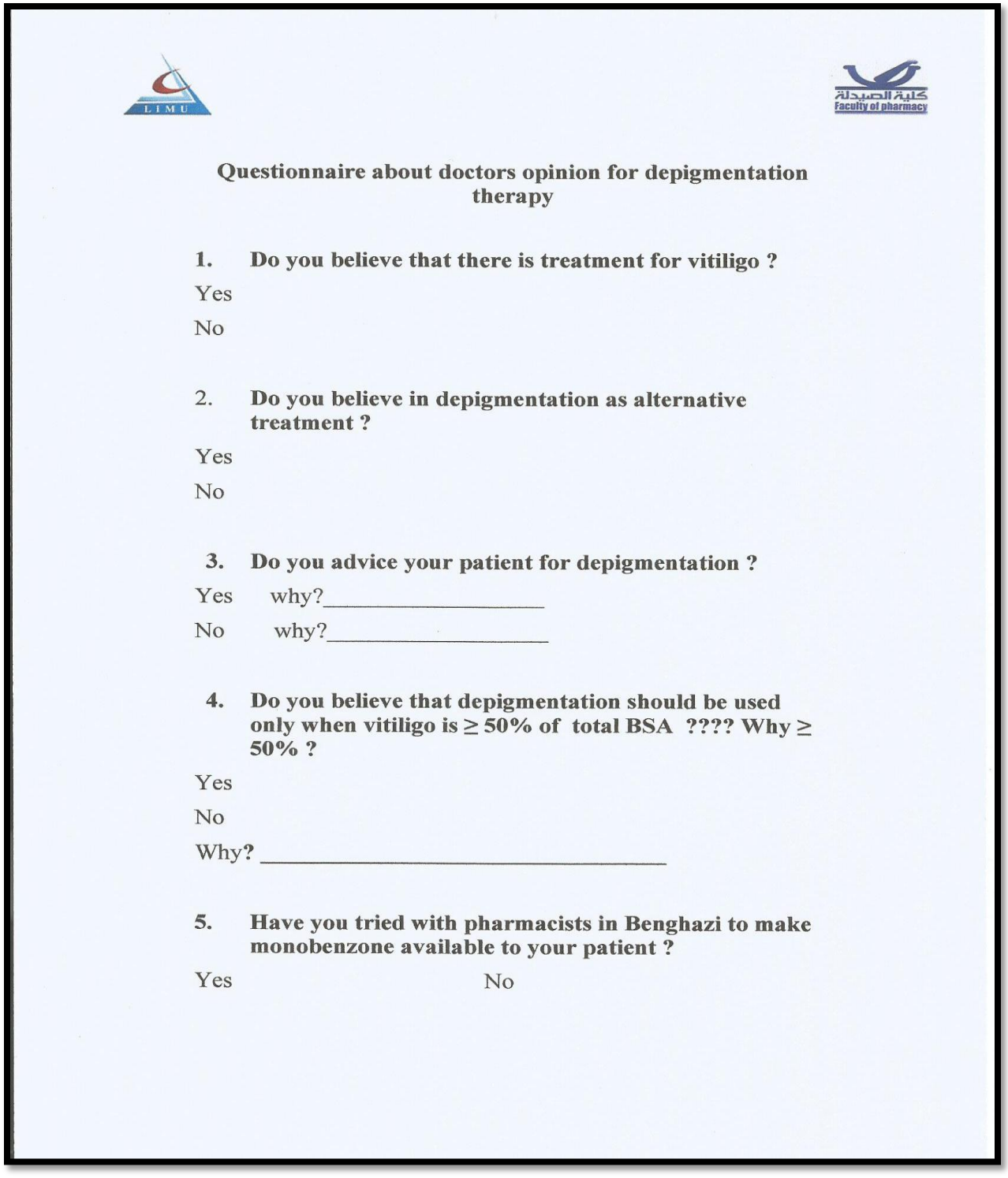
Yes ☐ if so, in which relative? _____

No ☐

20. **Do you think depigmentation all your body could be a successful alternative treatment?**

Yes ☐ Why did not you go through it untill now? _____

No ☐ Why? _____



Questionnaire about doctors opinion for depigmentation therapy

1. **Do you believe that there is treatment for vitiligo ?**

Yes

No

2. **Do you believe in depigmentation as alternative treatment ?**

Yes

No

3. **Do you advice your patient for depigmentation ?**

Yes why?_____

No why?_____

4. **Do you believe that depigmentation should be used only when vitiligo is ≥ 50% of total BSA ???? Why ≥ 50% ?**

Yes

No

Why? _____

5. **Have you tried with pharmacists in Benghazi to make monobenzone available to your patient ?**

Yes No

Figure 2.3: Questionnaire used for interviewing

Chapter III
Results
Baseline characteristics of study subjects:-

Men represented 42.31% (n=44) of study sample while women represented 57.69% (n=60) of total (n=104) study sample (Table3.1).Patients with white ethnicity represented 35.29% (n=36) of study sample, patients with North African ethnicity represented 58.82% (n=60) of study sample, patients black ethnicity represented 3.92% (n=4) of study sample and patients with other ethnicities represented 1.96% (n=3) of study sample (Table 3.1).Patients with age within the range 6-15 years represented 14.56% (n=15) of study sample, patients with age within the range 16-25 years represented 26.21% (n=27) of study sample,patients with age within the range 26-35 years represented 27.18% (n=28) of study sample, patients with age within the range 36-45 years represented 29.13% (n=30) of study sample and patients with age within the range 46-65 years represented 2.91% (n=3) of study sample (Table 3.1).

Mean height (cm) ± SEM of men equals to167±2.39 (n=41) while mean height (cm) ± SEM of women equals to 157.24±2.54 (n=46) (Table 3.1).Mean weight (kg) ± SEM of men equals to 71.86±2.86 (n=42) while mean weight (kg) ± SEM of women equals to 66.57±2.08 (n=58) (Table 3.1).Mean BMI (kg/cm^2) ± SEM of men equals to 25.76±0.97 (n=40) while mean BMI ± SEM of women equals to 27.43±1.25 (n=45) (Table 3.1).

Patients having vitiligo for < 1 year represented 13.59% (n=14) of study sample, patients having vitiligo for 1-5 years represented 24.27% (n=25) of study sample, patients having vitiligo for 6-10 years represented 28.16% (n=29) of study sample, patients having vitiligo for 11-20 years represented 17.48% (n=18) of study sample and patients having vitiligo for > 20 years represented 16.50% (n=17) of study sample(Table 3.1).

Patients rated their vitiligo as mild, represented 28.16% (n=29) of study sample, patientsrated their vitiligo as moderate, represented 46.60% (n=48) of study sample and patients rated their vitiligo as severe, represented 25.24% (n=26) of study sample (Table 3.1).

Patients believed that there is no improvement in their case following treatmentrepresented 18.56% (n=18) of study sample, patients believed that there is only a slight improvement in their case following treatment represented 62.89% (n=61) of study sample, patients believed that there is a significant improvement in their case following treatment represented 16.49% (n=16) of study sample and patients believed that there is a complete repigmentation of vitiligo in their case following treatment represented 2.06% (n=2) of study sample (Table 3.1).

Table 3.1: Baseline characteristics of study subjects

	Men			Women			Total
	Mean ± SEM	n	%	Mean ± SEM	n	%	n (%)
Gender	_____	44	42.31	_____	60	57.69	104 (100)
Ethnicity							
White	-----	11	26.19	------	25	41.67	36 (35.29)
North African	------	29	69.05	-------	31	51.67	60 (58.82)
Black	-----	0	---	-----	4	6.67	4 (3.92)
Other	------	2	4.76	--------	0	0	2 (1.96)
Age (years)							
6_15	------	10	9.71	-------	5	4.85	15 (14.56)
16_25	-------	8	7.77	------	19	18.45	27 (26.21)
26_35	--------	14	13.59	-------	14	13.59	28 (27.18)
36_45	-------	10	9.71	------	20	19.42	30 (29.13)
46_65	-------	1	0.97	-------	2	1.94	3 (2.91)
Height (cm)	167.68 ±2.39	41	-----	157.24±2.54	46	------	87
Weight (kg)	71.86 ±2.86	42	------	66.57±2.08	58	------	100
BMI (Kg/cm^2)	25.76 ± 0.97	40	----	27.43 ± 1.25	45	----	85
Period having vitiligo (years)							
< 1	------	7	16.28	------	7	11.67	14 (13.59)
1_5	------	13	30.23	------	12	20	25 (24.27)
6_10	------	9	20.93	-------	20	33.33	29 (28.16)
11_20	-----	8	18.60	-------	10	16.67	18 (17.48)
>20	-------	6	13.95	--------	11	18.33	17 (16.50)
Patient opinion about severity							
Mild	--------	12	28.57	-------	17	27.82	29 (28.16)
Moderate	-------	19	45.24	-------	29	47.54	48 (46.60)
Severe	--------	11	26.19	-------	15	24.59	26(25.24)
Patient opinion about status following treatment							
No improvement	----------	10	25	---------	8	14.4	18(18.56)
Slight improvement	-------	23	57.5	--------	38	66.67	61 (62.89)
Significantimprovement	--------	6	15	--------	10	17.54	16 (16.49)
Complete regimentation of vitiligo	--------	1	2.5	----------	1	1.75	2 (2.06)

Distribution of the lesions according to the affected site:-

Distribution of the lesions according to the affected site is shownin (Table.3.2).
The affected areas in men were; upper and lower extremities [32.69%(n=34)], upper limbs [25.96%(n=27)], lower limbs [25.00%(n=26)], face [22.11%(n=23)], abdomen [19.23%(n=20)] and neck [14.42%(n=15)].

The affected areas in women were; upper limbs [39.41%(n=41)], upper and lower extremities [38.46%(n=40)], abdomen [32.69%(n=34)], lower limbs [31.73%(n=33)], face [31.73%(n=33)] and neck [15.38%(n=16)].

Table 3.2: Distribution of the lesions according to the affected site

Site		Men n(%)	Woman n(%)
Face		23 (22.11)	33 (31.73)
	alone	2 (1.92)	3 (2.88)
	with other area	21 (20.19)	30 (28.85)
Neck		15 (14.42)	16 (15.38)
	alone	1 (0.96)	0 (0)
	with other area	14 (13.46)	16 (15.38)
Upper limbs		27 (25.96)	41 (39.41)
	alone	6 (5.77)	7 (6.73)
	with other area	21 (20.19)	34 (32.69)
Lower limbs		26 (25.00)	33 (31.73)
	alone	0 (0)	0 (0)
	with other area	26 (25.00)	33 (31.73)
Upper & lower extremities		34 (32.69)	40 (38.46)
	alone	7 (6.73)	7 (6.73)
	with other area	27 (25.96)	33 (31.73)
Abdomen		20 (19.23)	34 (32.69)
	alone	6 (5.77)	15 (14.42)
	with other area	14 (13.46)	19 (18.27)

- Upper limb (abdomen, neck, face, arms and hands).
- Lower limb (abdomen, legs and feet).
- Upper and lower extremities(arms, hands, legs and feet).
- Abdomen (chest, stomach, and back).

Distribution of vitiligo according to affected side of the body:-

Patients with unilateral and one side of spreading vitiligo represented 26.92% (n=7) of study sample while patients with unilateral and two side of spreading vitiligo represented 73.07% (n=19) of study sample and patients with bilateral and one side of spreading vitiligo represented 26.08% (n=18) of study sample while patients with bilateral and two side of spreading vitiligo represented 73.91% (n=51) of study sample (Table 3.3).

Table 3.3: Distribution of vitiligo according to affected side of the body

	Unilateral	%	Bilateral	%
One side	7	26.92%	18	26.08%
Two side	19	73.07%	51	73.91%

Distribution of vitiligo according to spreadability of white spots:-

Patients with spreadable white spots represented 70.87% (n=73) of study sample while patients with non- spreadable white spots represented 29.13% (n=30) of study sample (Fig 3.1).

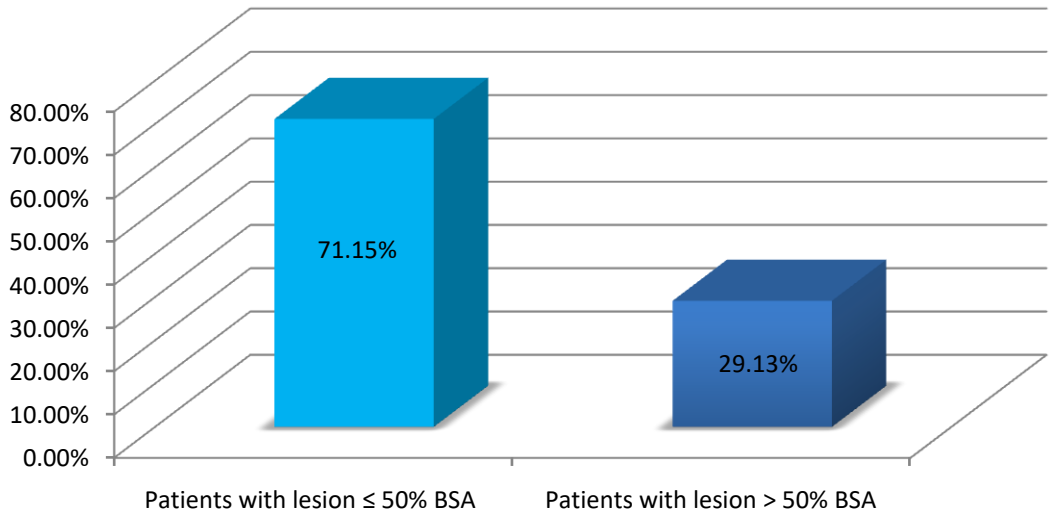


Figure 3.1: Distribution of vitiligo according to spreadability of white spots

Distribution of vitiligo according to bodysurface area affected by lesions:-

Patients with more than 50% of BSA affected with vitiligo represented 29.13% (n=30) of study sample. Meanwhile, patients with ≤ 50% of BSA affected with vitiligo represented 71.15% (n=74) of study sample (Fig 3.2).

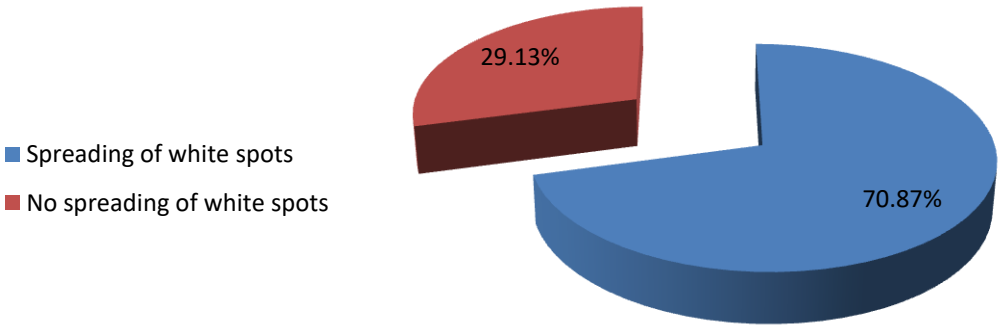


Figure 3.2: Patients with >50% BSA affected by vitiligo

Vitiligo treatment:-

Patientsreceiving treatment for vitiligo currently represented 92.16% (n=94) of study sample, patients not taking any treatment currently represented 4.90% (n=5) of study sample, patients received treatment for vitiligo at certain point their life represented 2.94% (n=3) of study sample (Fig3.3).

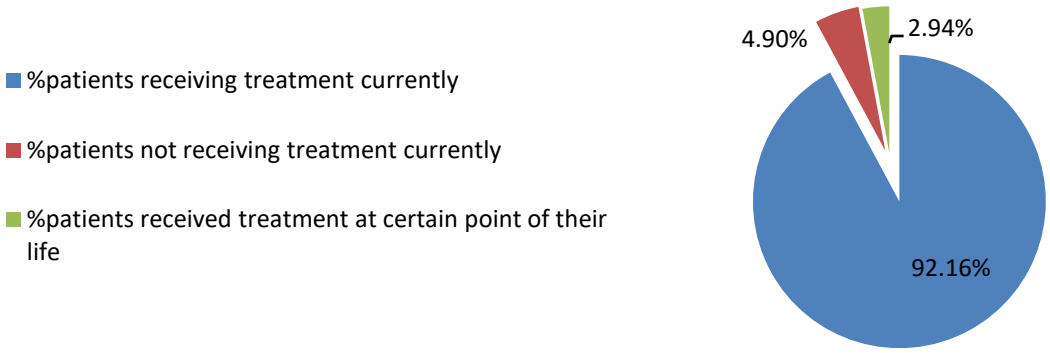


Figure 3.3: Percentage of patients receiving vitiligo treatment

Patients received PUVA, PUVA+ steroids, steroids, steroids + vitamin, laser, PUVA + steroids + vitamin, steroids + vitamin + UVB, PUVA + vitamin and vitamin alone represented 34.69%,16.32%, 11.22%, 9.18%, 7.14%, 5.10%,4.08%,3.06% and 3.06% respectively(Fig 3.4).

Patients received vitamin + laser, steroids + vitamin + laser, PUVA + vitamin + laser, vitamin + UVB, PUVA + steroids + vitamin + laser and PUVA+vitamin + some other unknown treatment represented 1.02%, 1.02%, 1.02%, 1.02%, 1.02% and 1.02% respectively (Fig 3.4).

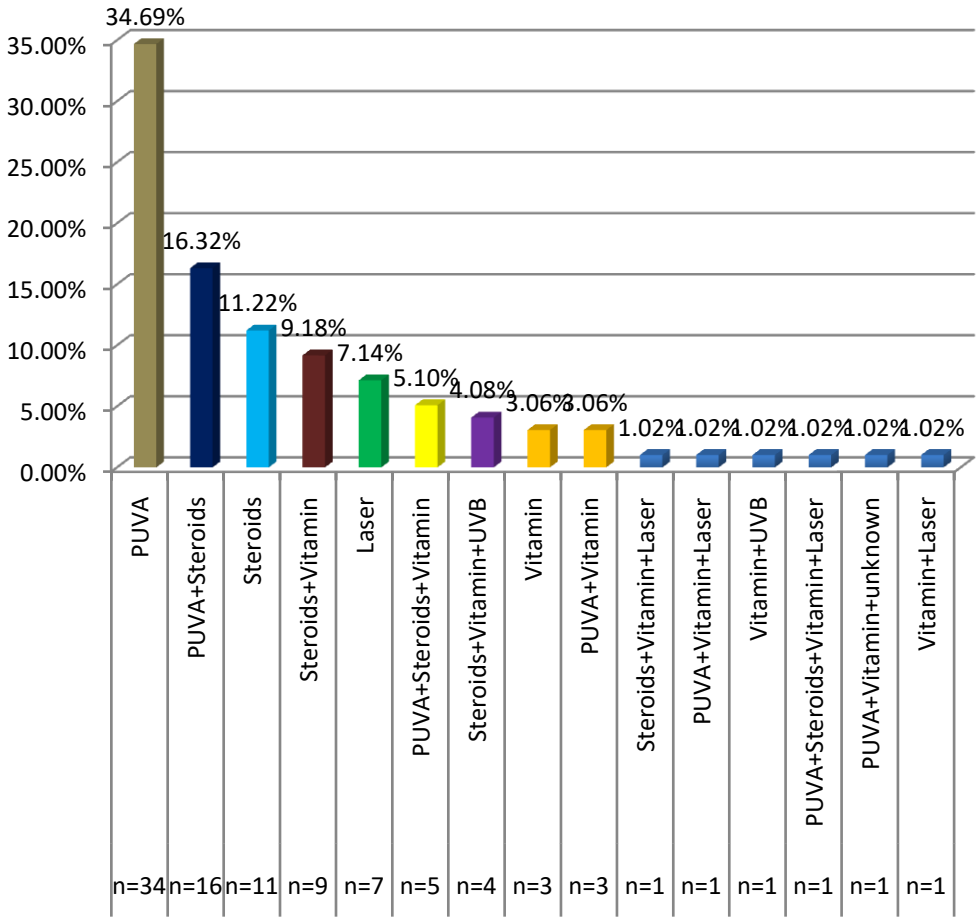


Figure 3.4: Protocols for vitiligo treatment

Patients using alternative treatment represented 25.49% (n=26) of study sample; meanwhile patients not using alternative treatment represented 74.51% (n=76) of study sample (Fig 3.5).

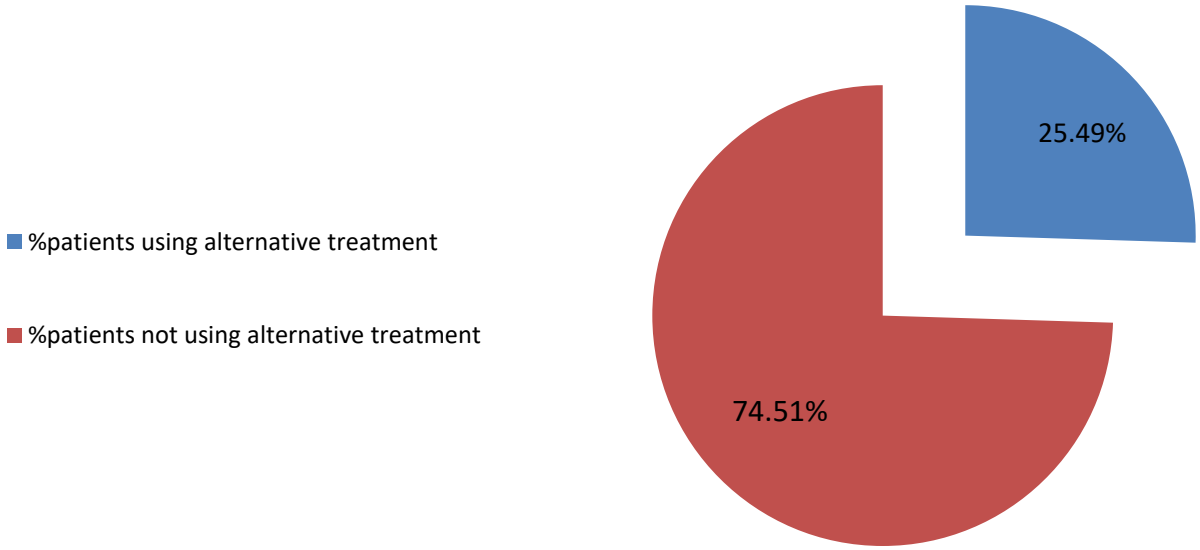


Figure 3.5: Use of alternative treatment for vitiligo

Autoimmune diseases

Patients with history of autoimmune disease represented 11.65% (n=12) of study sample, meanwhile patients with no history of autoimmune disease represented 88.35% (n=91) of study sample (Fig3.6).

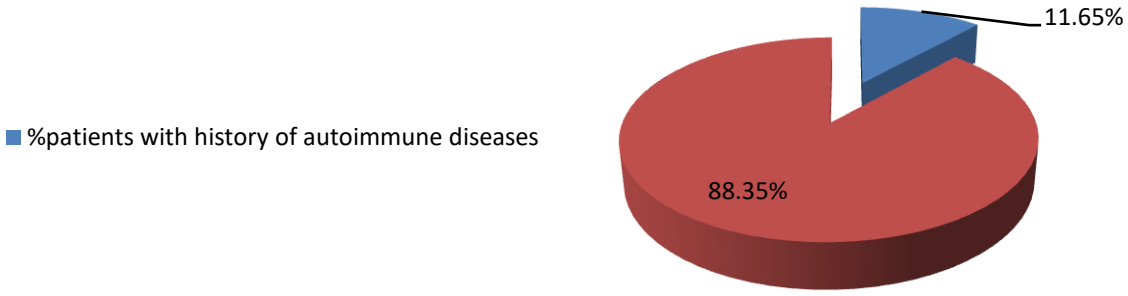


Figure 3.6: History of autoimmune diseases

Patients with history of thyroid represented 50% of study sample, patients with history of diabetes represented 30% of study sample and patients with history ofHCV(+) and HIV represented 10% each out of study sample (Fig 3.7).

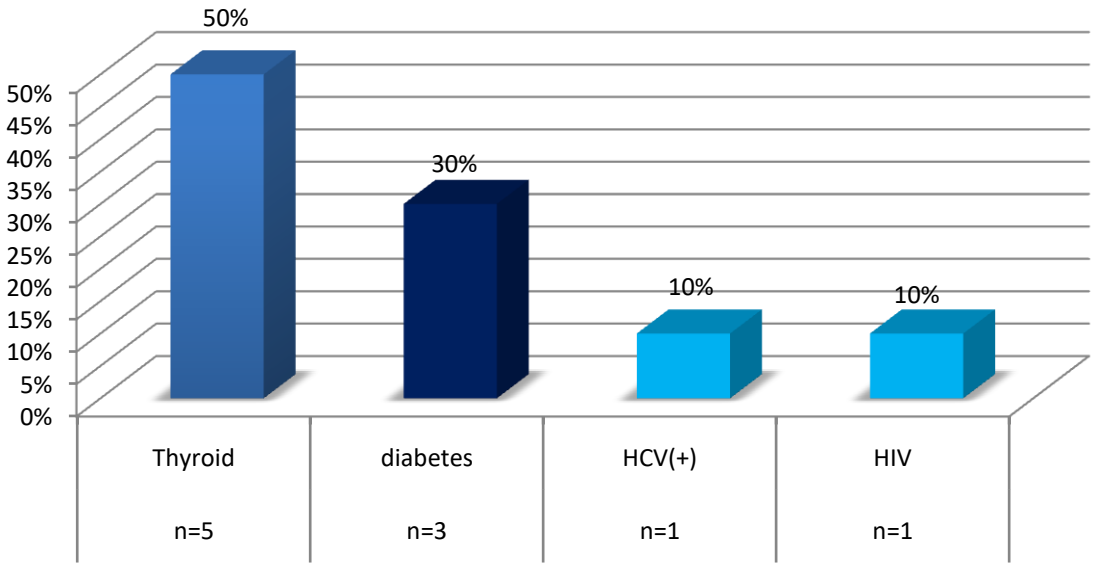


Figure 3.7: Types of autoimmune diseases among study sample

Family history of vitiligo

Patients with family history of vitiligo represented 34.95% (n=36) of study sample, meanwhile patients with no family history of vitiligo represented 65.05% (n=67) of study sample (Fig 3.8).

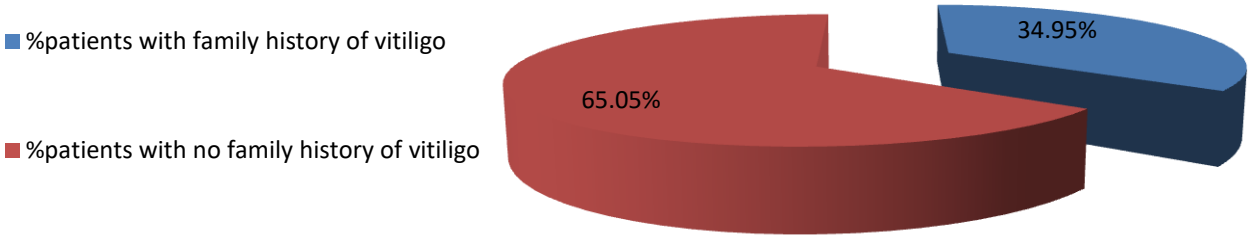


Figure 3.8: Family history of vitiligo among study sample

Patients with family history from father, mother, brother, sister, son or daughter side represented 69.44% (n=25) of study sample, patients with family history from uncle, aunt, grandparents or grandfather of dad side represented 22.22% (n=8) of study sample, patients with family history from wife or husband represented 5.56% (n=2) of study sample and patients with family history by maternal uncle's wife represented 2.78% (n=1) of study sample (Fig 3.9).

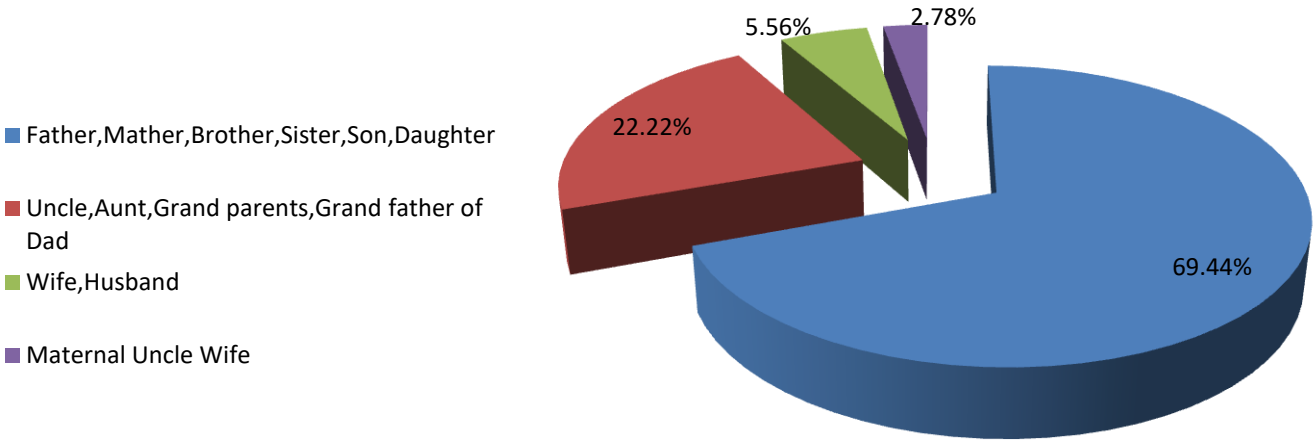


Figure 3.9: Detailed family history of vitiligo among study sample

Depigmentation:-

Patients believe in depigmentation represented 33% (n=33) of study sample, meanwhile patients not believe in depigmentation represented 67% (n=67) of study sample (Fig 3.10).

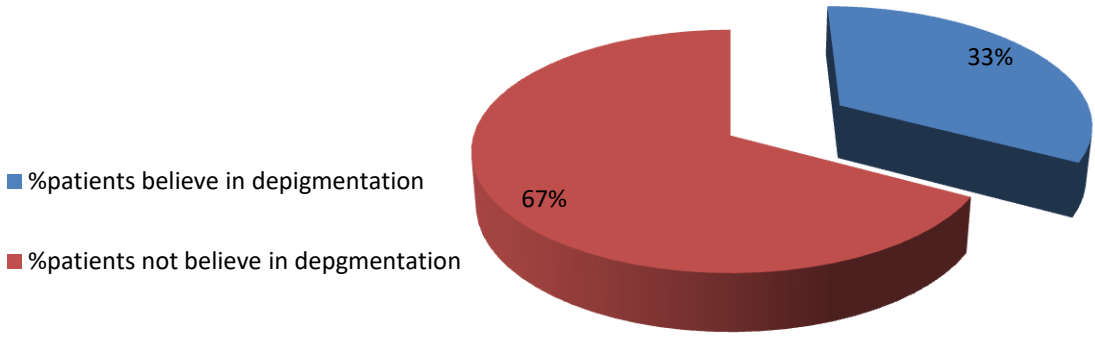


Figure 3.10: Patients believe in depigmentation

Among the 33% patients who believe in depigmentation; the patients who believe in depigmentation and did it already represented 6.06% (n=2) of study sample, patients believe in depigmentation but did not experience this type oftreatment for different reasonsrepresented 93.93% of study sample. These reason are presented in(Table 3.4).

Table 3.4: Positive opinions of vitiligo patients regarding depigmentation therapy

Table	YES	n	%
[A]	[YES] experienced depigmentation	2	6.06
[B]	[YES] but did not undergo depigmentation for the following reason:		
	Unavailability of treatment in Libya	5	15.15
	Cost	10	30.30
	Not advised by their doctors	4	12.12
	Vitiligo less than 50%	6	18.18
	Afraid of the success rate	1	3.03
	Other	5	15.15
Total of [B]		31	93.93

Out of whole study sample, 67% patients did not believe in depigmentation for many reasons.About 40% of study sample were worried about side effects and rest of sample presented other reasons as shown below (Table 3.5).

Table 3.5: Negative opinions of vitiligo patients regarding depigmentation therapy

NO for the following reason	n	%
Not advised by their doctors	3	7.69
Vitiligo less than 50%	5	12.82
Afraid of the success rate	4	10.26
Afraid of side effects	17	43.59
Other	6	15.39
The need to completetreatment	4	10.26
Total	39	100

Chapter IV
Discussion and Conclusion

Discussion:-

It is of great importance to understand the importance of depigmentation therapy for special cases of vitiligo instead of keep going on with repigmentation therapy without progress.
Assessing the prevalence of using depigmentation therapy among Libyan patients affected with vitiligo that is covering more than 50% of their BSA, could be the core of this study affected. Overall data of this therapy study was collected from different Libyan cities during July-September 2015.

According to this study, females were more affected by vitiligo compared to males. Most of patients reported with the disease were within the age range 16-45 years, but still vitiligo was reported with a high percentage in children of 6-15 years old. This is in agreement with reports from othercountries.[133]

Genetics plays an important role with the disease which the study sample gives 34.95% of patients has a family history of vitiligo which is in agreement with the fact that the genetics one of the important reasons of vitiligo disease.[10]

Duration of the disease varied from months to > 20 years with 6-10 years duration as the highest which is higher than that presented by a previous study conducted in Libya during 1985. [134]

All patients were able to evaluate and describe their case as mild, moderate or severe, but most of patients believe that the treatment they are receiving can result in only slight improvement in their case. We are not sure if this is the case or may be the shortage in health care facilities in Libya is the main reason for this. This may be supported by the fact that in spite of the wide variety of treatment protocols used among study sample, more than quarter of patients were trying to find a solution by using alternative medicine.

Classification of vitiligo according to distribution of the lesions is of practical importance usually for assessing the prognosis of the disease. Involvement of different regions of body showed variation in different patients and some difference between men and women. Even though the maximally involved areas in both sexes were upper and lower extremities and upper limbs. This is in agreement with previous studies in Libya.[134] Also the minimally involved area in both sexes was the neck. Our study did not report mucous membranes, mainly oral and genital area,which used to be the least affected areas in previous studies. This may explain appearance of neck in this study as the least affected area.

About 90% of patients are receiving a repigmentation therapy of vitiligo noting that almost 30% of study sample, their vitiligo covers more than 50% of total BSA. According to this study only two patients received depigmentation therapy and this actually was done outside Libya practically in one European country.

Among the 33% of patients who believe in depigmentation there were only 6.06% patients who experienced depigmentation therapy using20% monobenzone cream. Drug was delivered from United Kingdom, as it is not available in Arab countries in general and in Libya especially. Otherwise there was 93.93% of patients who believe in depigmentation but did not undergo depigmentation therapy for many reasons; 15.15% of patients confirmed the unavailability of such type of therapy in Libya, 30.30% of patients complained the high cost of the drug, 12.12% of patients said that they did not have a advice from their doctors, 18.18% of patients have vitiligo that covers less than 50% of total BSA, only 3.03% of patients admitted that they are worried about the success rate of depigmentation therapy and 15.15% of patients had other different reasons as for example they did not hear about it before or that such type of therapy needs a long time before it gives positive results.

According to this study there was 67% of patients did not believe in depigmentation for many reasons; 7.69% of patients did not receive advice from their doctors, 12.82% of patients have vitiligo that cover less than 50% of total BSA, 10.26% of patients were worried about the success rate of depigmentation therapy, 10.26% of patients thinkthat they need to continue with repigmentation therapy, 15.39% of patients preferred to not tell the reason and nearly half of patients were scared of side effects of depigmentation therapy and they even started to list such side effects as for example: effect on hair, general appearance of patients following depimentation …. etc.

With 19.42% of patients admitted that doctors informed them that there is no treatment of vitiligo, in addition to the fact that loads of patients admitted that they have never been advised by their doctors or even informed about suitability of depigmentation to their case, adding to this many wrong information patients possess about side effects of monobenzone cream. For all of this we decided to get doctors opinion regarding depigmentation therapy especially.Questionnaire used for this purpose presented in chapter II of this thesis (Fig 3.3)

All doctors(n=10) admitted that there is a treatment for vitiligo via repigmentation if lesions coverless than 50% of total BSA and if the cause of the disease is not genetic. All doctors also admitted that they believe in the benefit of depigmentation therapy if lesions cover more than 50% of total BSA or if lesions are affecting badly both face and hands of patients with bad influence on the psychology of the patients. Even dermatologist would like to prescribe monobenzone 20% as depigmenting agent to their patients but unfortunately the drug is not prepared in any health facility in Libya. There have been some old trails to prepare hydroquinone 20% cream, but unfortunately and for unknown reasons, activity of preparation was much less than expected and we could not find anylogic explanations for this. We believe that more clinical research evaluating vitiligo in Libya should be alone in the future to clarify loads of questions raised in this paper(Table 4.1).

Table 4.1: Doctors opinions about vitiligo treatment and depigmentation therapy

	Yes	%	No	%
Do you believe that there is treatment for vitiligo	10	100	--	--
Do you believe in depigmentation as alternative treatment	10	100	--	--
Do you advice your patient for depigmentation	10	100	--	--
Do you believe that depigmentation should be used only when vitiligo is ≥ 50% of total BSA ???? Why ≥ 50%?	10	100	--	--
Have you tried with pharmacists in Benghazito make monobenzone available to your patient	-	-	10	100

The overall results of this survey reflect a variation in opinion and practice for the management of vitiligo by dermatologists in Libya.

Conclusion:-

To the best of our knowledge, this is the first study in Libya that discussed depigmentation as a therapy for vitiligo and reported the patients' responses to such type of therapy. MBEH is presently the only depigmenting agent approved by the U.S. FDA for use in extensive vitiligo patients. This study revealed that an acceptable percentageof vitiligo patients in Libya believe in depigmentation therapy even though only few patients have been treated by depigmentation using20% monobenzone creamnoting that results were excellent and without any side effects.Many patients refused depigmentation as a therapy because they thoughtthatit produces loads of side effects, which may indicate poor patient education by the health care providers.MBEH is not available in Libya and in most if not all of the Arab countries. Libyan dermatologists agreed the importance of depigmentation therapy but none of them suggested any solution for the unavailability of MBEH 20% cream for their patients in Libya.

Difficulties while doing this work:-

- The bad security situation in Benghazi resulted in a reduced number of working clinics in the city and consequently this was reflected on the small number of patients included in the study.
- Uncooperative doctors (dermatologists).

Limitations of this study:-

- Results of this study do not necessarily indicate the prevalence of the disease in Libya.
- Limited number of patients included in the study.

References:-

1. Medical News Today, (2014). "what is vitiligo?" Retrieved 29.05.2015, from http://www.medicalnewstoday.com/articles/245081.php .
2. Le poole IC, Luiten RM. Autoimmune etiology of generalized vitiligo. Curr Dir Autoimmun. 2008; 10:227-43.
3. Toussaint S, Kamino H. Noninfectous papular and squamous diseases. In: Elder D, Elenitas R, Jaworsky D, Johnson B J*Lever's Histopathology of the Skin*. Philadelphia, Pa: Lippincot-Raven; 1997:154-5.

4. Ongenae K, Van Geel N, Naeyaert JM. Evidence for an autoimmune pathogenesis of vitiligo.*Pigment Cell Res*. Apr 2003;16(2):90-100.
5. Zhang BX, Lin M, Qi XY, Zhang RX, Wei ZD, Zhu J, Man MQ, Tu CX. Characterization of circulating CD8+T cells expressing skin homing and cytotoxic molecules in active non-segmental vitiligo. *Eur J Dermatol.* Jun 19 2013.
6. van den Wijngaard RM, Aten J, Scheepmaker A, Le poole IC, Tigger AJ, Westerhof W, Das PK. Expression and modulation of apoptosis regulatory molecules in human melanocytes: significance in vitiligo. *Br J Dermatol.* Sep 2000;143(3):573-81.
7. Ortonne J. Vitiligo and other disorders of Hypopigmentation. In: Bolognia J, Jorizzo J, Rapini R, eds. *Dermatology.* Vol 1. 2nd. Spain: Elsevier; 2008:65.
8. 8.Sravani PV, Babu NK, Gopal KV, Rao GR, Rao AR, Moorthy B. Determination of oxidative stress in vitiligo by measuring superoxide dismutase and catalase levels in vitiliginous and non-vitiliginous skin. *Indian J Dermatol Venereol Leprol*. May-Jun 2009;75(3):268-71.
9. Kovacs SO. Vitiligo. *J Am Acad Dermatol.* May 1998;38(5 Pt 1):647-66; quiz 667-8.
10. Spritz RA. The genetics of generalized *vitiligo.Curr Dir Autoimmun.* 2008;10:244-57.
11. Halder R, Taliaferro S. Vitiligo. In: Wolff K, Goldsmith L, Katz S, Gilchrest B, Paller A, Lefell D, eds. *Fitzpatrick's Dermatology in General Medicine*. Vol 1. 7th ed. New York, NY: McGraw-Hill; 2008:72.
12. Spritz RA: Shared genetic relationships underlying generalized vitiligo and autoimmune thyroid disease. Thyroid2010, 20:745-754.
13. Birlea SA, Jin Y, Bennett DC, Wallace MR, Herbstman DM, McCormack WT,Kemp EH, Gawkrodger DJ, Weetman AP, Picardo M, Leone G, Taieb A, Jouary T,Ezzedine K, van Geel N, Lambert J, Overbeck A, Fain PR, Spritz RA:Comprehensive analysis of candidate genes for generalized vitiligosupports XBP1, FOXP3, and TSLP. J Investig Dermatol, in press.
14. Jin Y, Mailloux CM, Gowan K, Riccardi SL, LaBerge G, Bennett DC, Fain PR,Spritz RA: NALP1in vitiligo-associated multiple autoimmune disease.N Engl J Med2007, 356:1216-1225.
15. Ren Y, Yang S, Xu S, Gao M, Huang W, Gao T, Fang Q, Quan C, Zhang C, Sun L,Liang Y, Han J, Wang Z, Zhang F, Zhou Y, Liu J, Zhang X: Genetic variation of promoter sequence modulates XBP1 expression and genetic risk forvitiligo. PLoS Genet 2009, 5:e1000523.
16. Taieb A, Picardo M. The definition and assessment of vitiligo:a consensus report of the Vitiligo European Task Force.Pigment Cell Res 2007;20:27-35.
17. Lu T, Gao T, Wang A, Jin Y, Li Q, Li C. Vitiligo prevalence study in Shaanxi Province, China. Int J Dermatol 2007;46:47-51.
18. Das SK, Majumder PP, Chakraborty R, Majumdar TK, Haldar B.Studies on vitiligo. I. Epidemiological profile in Calcutta,India. Genet Epidemiol 1985;2:71-8.
19. Dwivedi M, Laddha NC, Shajil EM, Shah BJ, Begum R. The ACEgene I/ D polymorphism is not associated with generalizedvitiligo susceptibility in Gujarat population. Pigment CellMelanoma Res 2008;21:407-8.
20. Dogra S, Parsad D, Handa S, Kanwar AJ. Late onset vitiligo: astudy of 182 patients. Int J Dermatol 2005;44:193-6.
21. Onunu AN, Kubeyinje EP. Vitiligo in the Nigerian African: astudy of 351 patients in Benin City, Nigeria. Int J Dermatol2003;42:800-2.

22. Zhang Z, Xu SX, Zhang FY, Yin XY, Yang S, Xiao FL, Du WH, Wang JF, Lv YM, Tang HY, Zhangxy J. The analysis of genetics and associated autoimmune diseases in Chinese vitiligo patients. Arch Dermatol Res 2009;301:167-73.
23. Alkhateeb A, Fain PR, Thody A, Bennett DC, Spritz RA.Epidemiology of vitiligo and associated autoimmune diseasesin Caucasian probands and their families. Pigment CellRes 2003;16:208-14.
24. Sehgal VN, Rege VL, Mascarenhas F, Kharangate VN. Clinicalpattern of vitiligo amongst Indians. J Dermatol 1976;3:49-53.
25. Al Robaee AA. Assessment of quality of life in Saudi patientswith vitiligo in a medical school in Qassim province, SaudiArabia. Saudi Med J 2007;28:1414-7.
26. Kyriakis KP, Palamaras I, Tsele E, Michailides C, Terzoudi S.Case detection rates of vitiligo by gender and age. Int JDermatol 2009;48:328-9.
27. Handa S, Dogra S. Epidemiology of childhood vitiligo: a studyof 625 patients from north India. Pediatr Dermatol 2003;20:207-10.
28. Jaisankar TJ, Baruah MC, Garg BR. Vitiligo in children. Int JDermatol 1992;31:621-3.
29. Tamer E, Ilhan MN, Polat M, Lenk N, Alli N. Prevalence of skindiseases among pediatric patients in Turkey. J Dermatol2008;35:413-8.
30. Medscape, (2014) "Vitiligo" Retrieved 8.6.2015, from http://emedicine.medscape.com/article/1068962-overview#showall .
31. Montes LF, Abulafia J, Wilborn WH, Hyde BM, Montes CM.Value of histopathology in vitiligo. Int J Dermatol 2003;42:57-61.
32. McKee P, Calonje E, Granter S, eds. Disorders of Pigmentation. *Pathology of the Skin withClinical Correlations*. 3rd ed. China: Elsevier Mosby; 2005. Vol 2: 993-7.
33. Moellmann G, Klein-Angerer S, Scollay DA, Nordlund JJ, Lerner AB. Extracellular granular material and degeneration of keratinocytes in the normally pigmented epidermis of patients with vitiligo. *J Invest Dermatol*. 1982 Nov. 79(5):321-30.
34. Dave S, Thappa DM, Dsouza M. Clinical predictors of outcomein vitiligo. Indian J Dermatol Venereol Leprol 2002;68:323-5.
35. Badri AM, Todd PM, Garioch JJ, Gudgeon JE, Stewart DG, Goudie RB. An immunohistological study of cutaneouslymphocytes in vitiligo. J Pathol 1993;170:149-55.
36. Van den Wijngaard R, Wankowicz-Kalinska A, Le Poole C,Tigges B, Westerhof W, Das P. Local immune response inskin of generalized vitiligo patients. Destruction of melanocytesis associated with the prominent presence ofCLA+ T cells at the perilesional site. Lab Invest 2000;80:1299-309.
37. Hann SK, Kim HI, Im S, Park YK, Cui J, Bystryn JC. The changeof melanocyte cytotoxicity after systemic steroid treatmentin vitiligo patients. J Dermatol Sci 1993;6:201-5.
38. Bleehen SS. The treatment of vitiligo with topical corticosteroids.Light and electronmicroscopic studies. Br J Dermatol1976;94(suppl 12):43-50.
39. Seiter S, Ugurel S, Tilgen W, Reinhold U. Use of high-dosemethylprednisolone pulse therapy in patients with progressiveand stable vitiligo. Int J Dermatol 2000;39:624-7.
40. Radakovic-Fijan S, Furnsinn-Friedl AM, Honigsmann H, TanewA. Oral dexamethasone pulse treatment for vitiligo. J AmAcad Dermatol 2001;44:814-7.
41. Rath N, Kar HK, Sabhnani S. An open labeled, comparativeclinical study on efficacy and tolerability of oral minipulse ofsteroid (OMP) alone, OMP with PUVA and broad / narrow band UVB phototherapy in progressive vitiligo. Indian JDermatol Venereol Leprol 2008;74:357-60.

42. Kandil E. Treatment of vitiligo with 0-1 per cent betamethasone17-valerate in isopropyl alcohol—a double-blind trial.Br J Dermatol 1974;91:457-60.
43. Cockayne SE, Messenger AG, Gawkrodger DJ. Vitiligo treatedwith topical corticosteroids: children with head and neckinvolvement respond well. J Am Acad Dermatol 2002;46:964-5.
44. Kumari J. Vitiligo treated with topical clobetasol propionate. Arch Dermatol 1984;120:6315.
45. Zhou Z, Li CY, Li K, Wang T, Zhang B, Gao TW. Decreasedmethionine sulphoxide reductase A expression rendersmelanocytes more sensitive to oxidative stress: a possible
46. cause for melanocyte loss in vitiligo. Br J Dermatol 2009;161:504-9.
47. Castro AP. Calcineurin inhibitors in the treatment of allergicdermatitis. J Pediatr (Rio J) 2006;82(5 suppl):S166-72.
48. Wu CS, Lan CC, Wang LF, Chen GS, Wu CS, Yu HS. Effects ofpsoralen plus ultraviolet A irradiation on cultured epidermalcells in vitro and patients with vitiligo in vivo. Br J Dermatol 2007;156:122-9.
49. Abdel-Naser MB, Hann SK, Bystryn JC. Oral psoralen with UVA therapy releases circulating growth factor(s) that stimulatescell proliferation. Arch Dermatol 1997;133:1530-3.
50. Ponsonby AL, Lucas RM, van der Mei IA. UVR, vitamin D andthree autoimmune diseases—multiple sclerosis, type 1 diabetes,rheumatoid arthritis. Photochem Photobiol 2005;81:1267-75.
51. Ortonne JP, MacDonald DM, Micoud A, Thivolet J. PUVAinducedrepigmentation of vitiligo: a histochemical (split-DOPA) and ultrastructural study. Br J Dermatol 1979;101:1-12.
52. Kao CH, Yu HS. Comparison of the effect of 8-methoxypsoralen (8-MOP) plus UVA (PUVA) on human melanocytes in vitiligo vulgaris and in vitro. J Invest Dermatol1992; 98:734-40.
53. El-Mofty M, Mostafa W, Youssef R, El-Fangary M, Elramly AZ,Mahgoub D, et al. Ultraviolet A in vitiligo. PhotodermatolPhotoimmunol Photomed 2006;22:214-6.
54. Hann SK, Cho MY, Im S, Park YK. Treatment of vitiligo withoral 5-methoxypsoralen. J Dermatol 1991;18:324-9.
55. Lassus A, Halme K, Eskelinen A, Ranki A, Puska P, Salo O.Treatment of vitiligo with oral methoxsalen and UVA. Photodermatol1984;1:170-3.
56. De Francesco V, Stinco G, Laspina S, Parlangeli ME, Mariuzzi L,Patrone P. Immunohistochemical study before and afternarrow band (311 nm) UVB treatment in vitiligo. Eur JDermatol 2008;18:292-6.
57. Bhatnagar A, Kanwar AJ, Parsad D. Comparison of systemic PUVAand NB-UVB in the treatment of vitiligo: an open prospectivestudy. J Eur Acad Dermatol Venereol 2007; 21:638-42.
58. Dawe RS, Cameron H, Yule S et al. A randomized controlled trialof narrowband ultraviolet B vs. bath-psoralen plus ultraviolet Aphotochemotherapy for psoriasis. Br J Dermatol 2003; 148:1194–204.
59. Ling TC, Clayton TH, Crawley J et al. British Association of Dermatologistsand British Photodermatology Group guidelines for thesafe and effective use of psoralen combined with ultraviolet Atherapy 2014. Br J Dermatol 2014; 171(Suppl. 1):59.
60. Park KK, Liao W, Murase JE. A review of monochromatic excimerlight in vitiligo. Br J Dermatol 2012; 167:468–78.
61. Shah A, Shah B. Indian experience of a 308 nm monochromatic excimerlamp in 400 cases of vitiligo. Br J Dermatol 2014; 171(Suppl. 1):62.

62. Spencer JM, Hadi SM. The excimer lasers. J Drugs Dermatol2004;3:522-5.
63. Hong SB, Park HH, Lee MH. Short-term effects of 308-nmxenon-chloride excimer laser and narrow-band ultraviolet Bin the treatment of vitiligo: a comparative study. J KoreanMed Sci 2005;20:273-8.
64. Lesley M. Felsten, Ali Alikhan and Vesna Petronic-Rosic,Vitiligo: A comprehensive overviewPart II: Treatment options and approach to treatment. J American Academy of Dermatology. September 2011; 65:493-514
65. Grojean MF, Thivolet J, Perrot H. Acquired leukomelanoderma caused by topical depigmenting agents. Ann DermatolVenereol 1982;109:641-7.
66. Hariharan V, Klarquist J, Reust MJ, Koshoffer A, McKee MD,Boissy RE, et al. Monobenzyl ether of hydroquinone and4-tertiary butyl phenol activate markedly different physiological responses in melanocytes: relevance to skin depigmentation.J Invest Dermatol 2010;130:211-20.
67. Njoo MD, Vodegel RM, Westerhof W. Depigmentation therapy invitiligo universalis with topical 4-methoxyphenol and the Qswitchedruby laser. J American Academy Dermatol 2000; 42:760–769.
68. Rao J, Fitzpatrick RE. Use of the Q-switched 755-nm alexandritelaser to treat recalcitrant pigment after depigmentation therapy forvitiligo. Dermatol Surg 2004; 30:1043–1045.
69. AlGhamdi KM. A survey of vitiligo management among dermatologistsin Saudi Arabia. J Eur Acad Dermatol Venereol 2009; 23: 1282–1288.
70. Gawkrodger DJ, Ormerod AD, Shaw L Mauri SI, Whitton ME, Watts MJ, Anstey AV, Ingham J, Young K. Guideline for the diagnosis and management of vitiligo. Br J Dermatol 2008; 159: 1051–1076.
71. Kim YJ, Chung BS, Choi KC. Depigmentation therapy withQ-switched ruby laser after tanning in vitiligo universalis. DermatolSurg 2001; 27: 969–970.
72. Radmanesh M. Depigmentation of the normally pigmented patches inuniversal vitiligo patients by cryotherapy. J Eur Acad DermatolVenereol 2000; 14: 149–152.
73. Drug.com, (2015). "Hydroquinone cream" Retrieved 14.09.2015. fromhttp://www.drugs.com/pro/hydroquinone-cream.html
74. Jean L, Lapia BK, Somma S. Depigmentation therapy. Dermatol Ther,2001; 14: 29–34.
75. Kasraee B, Fallahi MR, Ardekani GS, Ebrahimis, Doroudchi G, Omrani GR, Handjani F, Amini M, Tandeh N, Haddadi m, Nikbakbakh M, Jahanbanis T, Song O, Saurat JH. Retinoic acid synergistically enhances the melanocytotoxic and depigmenting effects of monobenzylether of hydroquinone in black guinea pig skin. Experimental Dermatol 2006; 15: 509–514.
76. Drug.com, (2015). "Benoquin" Retrieved 10.08.2015. fromhttp://www.drugs.com/pro/benoquin.html.
77. Hariharan V, Klarquist J, Reust MJ, Koshoffer A, Mckee MD, Boissy RE and Le Poole C. Monobenzyl ether ofhydroquinone and 4-tertiary butyl phenol activate markedly differentphysiological responses in melanocytes: relevance to skin depigmentation.J Invest Dermatol 2010; 130: 211–220.
78. Riley PA. Mechanism of pigment cell toxicity produced by hydroxyanisole.J Pathol 1970; 101: 163–169.
79. Riley PA. Hydroxyanisole depigmentation: in vivo studies. J Pathol1969; 97: 185–191.
80. Grojean MF, Thivolet J, Perrot H. Acquired leukomelanoderma causedby topical depigmenting agents. Ann Dermatol Venereol 1982; 109:641–647.
81. Snell RS. Monobenzylether of hydroquinone.Arch Dermatol 1964; 90:63–70.

82. Frenk E. Depigmentation par agents chimiques. Bull Soc Fr DermatolSyphiligr 1971; 78:153–156.
83. Oakley AM. Rapid repigmentation after depigmentation therapy:vitiligo treated with monobenzyl ether of hydroquinone. Australas JDermatol 1996; 37: 96–98.
84. Freedberg IM, Eisen AZ, Wolff K et al. Hypomelanosis and hypermelanoses.In Mosher DB, Fitzpatrick TB, Ortonne JP, Hori Y, eds. Fitzpatrick'sDermatology in General Medicine, 5th edn. McGraw-Hill, NewYork, 1999: 945–960.
85. Hedges TR III, Kenyon KR, Hanninen LA, Mosher DB. Corneal andconjunctival effects of monobenzone in patients with vitiligo.ArchOphthalmol 1983; 101: 64–68.
86. Nordlund JJ, Forget B, Kirkwood J, Lerner AB. Dermatitis producedby applications of monobenzone in patients with active vitiligo. ArchDermatol 1985; 121: 1141–1144.
87. Bandyopadhyay D. Topical treatment of melasma. Indian J Dermatol2009; 54: 303–309.
88. Charlı´n R, Barcaui CB, Kac BK, Soares DB, Rabello-Fonseca R,Azulay-Abulafia L. Hydroquinone-induced exogenous ochronosis: areport of four cases and usefulness of dermoscopy. Int J Dermatol2008; 47: 19–23.
89. Ortonne JP, Passeron T. Melanin pigmentary disorders: treatmentupdate. Dermatol Clin 2005; 23: 209–226.
90. Dorsey CS. Dermatitic and pigmentary reactions to monobenzylether of hydroquinone: report of two cases. Arch Dermatol 1960;81:245–8.
91. E.S.T.Tan, R. sarkany. Topical monobenzyl ether of hydroquinone is an effective and safe treatment for depigmentation of extensive vitiligo in the medium term: a retrospective cohort study of 53 cases. J British Journal of Dermatology (2015) 172, pp1662–1664
92. AlGhamdi KM, Kumar A. Depigmentation therapies for normalskin in vitiligo universalis. J Eur Acad Dermatol Venereol 2011;25:749–57.
93. Pubchem. (2015). "4-Methoxyphenol" Retrieved 28.09.2015,from http://pubchem.ncbi.nlm.nih.gov/compound/4-Methoxyphenol#section=Top
94. Mosher DB, Parrish JA, Fitzpatrick TB. Monobenzylether of hydroquinone:a retrospective study of treatment of 18 vitiligo patients and areview of literature. Br J Dermatol 1977; 97: 669–679.
95. Reynolds JEF. Dermatological agents: mequinol. In Reynolds JEF, ed.The Extra Pharmacopoeia.Martindale, 29th edn. The PharmaceuticalPress, London, 1989: 1612-b, 923.
96. Boyle J, Kennedy CTC. Leucoderma induced by monomethylether ofhydroquinone. Clin Exp Dermatol 1985; 10: 154–158.
97. Yang F, Boissy RE. Effect of 4-tertiary butylphenol on the tyrosinase activity in human melanocytes. Pigment Cell Res 1999; 12:237-45.
98. Yang F, Sarangarajan R, Le Poole IC, Medrano EE, Boissy RE. The cytotoxicity and apoptosis induced by 4-trtiary butylphenol in human melanocyte are indepenndent of tyrosinase activity. J Invest Dermatol 2000; 114:157-64.
99. Pubchem.(2015) "4-tert-Butylphenol"Retrieved 30.09.2015,from https://pubchem.ncbi.nlm.nih.gov/compound/4-tert-Butylphenol
100. Brody HJ. Chemical Peeling and Resurfacing, 2nd ed. Mosby, Missouri,1997: 137-153.
101. Moy LS, Kotler R, Lesser T. The histologic evaluation of pulsed carbondioxide laser resurfacing versus phenol chemical peels in vivo. DermatolSurg 1999; 25: 597–600.
102. Coleman KM, Coleman WP. Complication. In Rubin MG, Dover JS,Alam M, eds. Chemical Peels, 1st edn. Elsevier & Saunders, Philadelphia,2006: 171–185.

103. Agency for Toxic Substances and Disease Registry (ATSDR), (1998).Toxicological Profile for Phenol.US Department of Health and Human Services,AtlantaRetrieved 1.10.2015.fromhttp://www.atsdr.cdc.gov/ToxProfiles/TP.asp?id=148&tid=27
104. Zanini M. Depigmentation therapy for generalized vitiligo with topical88% phenol solution. An Bras Dermatol 2005; 80: 415–416.
105. Stuzin JM, Baker TJ, Gordon HL. Treatment of photoaging: facialchemical peeling (phenol and trichloroaceticacid) and dermabrasion.Clin Plast Surg 1993; 20: 9–25.
106. Parvez S, Kang M, Chung HS et al. Survey and mechanism of skindepigmenting and lightening agents. Phytother Res 2006; 20: 921–934.
107. Lyon CC, Beck MH. Contact hypersensitivity to monobenzyl ether ofhydroquinone used to treat vitiligo. Contact Derm 1998; 39: 132–133.
108. Nelson JS, Applebaum J. Treatment of superficial cutaneous pigmentedlesions by melanin-specific selective photothermolysis using theQ-switched ruby laser. Ann Plast Surg 1992; 29: 231–237.
109. Leuenberger ML, Mulas MW, Hata TR. Comparison of the Q-switchedalexandrite, Nd:YAG and ruby lasers in treating blue-black tattoos.Dermatol Surg 1999; 25: 10–14.
110. Anderson R, Parrish J. Selective photothermolysis: precise microsurgeryby selective absorption of pulsed irradiation. Science 1983; 220:524–527.
111. Spicer MS, Goldberg DJ. Lasers in dermatology. J Am Acad Dermatol1996; 34: 1–25.
112. Thissen M, Westerhof W. Laser treatment for further depigmentationin vitiligo. Int J Dermatol 1997; 36: 386–388.
113. Alster TS. Q-switched alexandrite laser treatment (755 nm) of professional and amateur tattoos. J Am Acad Dermatol 1995; 33: 69–73.
114. Van Geel, L. Depaepe and R. Speeckaert. Laser (755 nm) and cryotherapy as depigmentation treatments for vitiligo: a comparative studyN. European Academy of Dermatology and Venereology 2015, 29, 1121–1127
115. Reda AM, Taha IR, Riad HA. Clinical and histological effect of a singletreatment of normal mode alexandrite (755 nm) laser on small melanocyticnevi.J Cutan Laser Ther 1999; 1:209–215.
116. Lam AY, Wong DS, Lam LK, Ho WS, Chan HH. A retrospective study on the efficacy and complications of Q-switched alexandrite laser in the treatment of acquired bilateral nevus of Ota-like macules. Dermatol Surg 2001; 27:937–942.
117. Taylor CR, Anderson RR. Ineffective treatment of refractory melasmaand post-inflammatory hyperpigmentation by Q-switched ruby laser.J Dermatol Surg Oncol 1994; 20: 592–597.
118. Kuflik GE. Cryosurgery updated. J Am Acad Dermatol 1994; 31: 925–944.
119. Guidelines of care for cryosurgery. American Academy of DermatologyCommittee on guidelines of care. J Am Acad Dermatol 1994; 31:648–653.
120. Mirshams M, Daneshpazhooh M, Mirshekari A, Taheri A, Mansoori P, Hekmet S. Cryotherapy in the treatment of pyogenic granuloma. JEADV 2006; 20: 788–790.
121. Elton RF. Complications of cutaneous cryosurgery. J Am Acad Dermatol1983; 8: 513–519.
122. K.M. AlGhamdi and A. Kumar. Depigmentation therapies for normal skin in vitiligo universalis. J European Academy of Dermatology and Venereology2011, 25, 749–757.

123. N. van Geel, L. Depaepe and R. Speeckaert. Laser (755 nm) and cryotherapy as depigmentationtreatments for vitiligo: a comparative study. J European Academy of Dermatology and Venereology2015, 29, 1121–1127.
124. Cerchione C, Fabbricini R, Pane F, Luciano L. Vitiligo-like lesions inan adult patient treated with Imatinib mesylate. Leuk Res 2009; 33:e104–e105.
125. US Food and Drug Administration. Aldara (imiquimod) 5% cream(online 14 July 2004). [WWW document]. URL http://www.fda.gov/.
126. Miller RL, Gerster JF, Owens ML, Slade HB, Tomai MA. Imiquimod applied topically: a novel immune response modifier and new class of drug. Int J Immunopharmacol 1992; 21: 1–14.
127. Schuttlaar ML, Hamstra JJ, Plinck EPB Peereboom, Wynia JD, Vuzevski VD, Mulder PG, Oranje AP. Alopecia areata in children: treatment with diphencyprone. Br J Dermatol 1996; 135: 581–585.
128. Tsao AS, Kantarjian H, Cortes JO, Brien S, Talpaz M. Imatinib mesylate causes hypopigmentation in the skin. Cancer 2003; 98: 2483–2487.
129. Senel E, Seckin D. Imiquimod-induced vitiligo-like depigmentation.Indian J Dermatol Venereol Leprol 2007; 73: 423.
130. Sriprakash K, Godbolt A. Vitiligo-like depigmentation induced byimiquimod treatment of superficial basal cell carcinoma. Australas JDermatol 2009; 50: 211–213.
131. Pan JY, Theng C, Lee J, Goh BK. Vitiligo as an adverse reaction totopical diphencyprone. Ann Acad Med 2009; 38: 276–277.
132. Y.J. Nordlund, R.E. Boissy, V.J. Hearing, R.A. King, J.P. Ortonne (Eds.), The pigmentary system: physiology and pathophysiology, Oxford University Press, Oxford (1998), pp. 513–551.
133. Porter J, Beuf AH, Nordlund JJ and Lerner AB.Psychological reaction to chronic skin disorders: a study of patients with vitiligo. Gen Hosp Psychiatry1979 Apr;1(1):73-77.
134. Rita VV, Bhumi BP, Arvind HC, Malay JM and Abhishek PP. A Clinical study of vitiligo in rural set up of gujarat. Indian J community med. 2014;39(3):143-146.
135. Singh M, Singh G, Kanwar A J and Belhaj M S. Clinical pattern of vitiligo in Libya. J International of dermatology. Banaras hindu university, India, 1985;24:233-235.