Sudan University of Science and Technology
College of Graduate Studies

Clinical Evaluation of Using Low Level Laser and Incoherent Light Source in the Treatment of Vitiligo

التقييم السريري باستخدام الليزر منخفض القدرة والضوء غير المترابط في علاج البهاق

A Thesis Submitted to the College of Graduate Studies in Fulfillment of the Requirements for the Philosophy Degree in Laser Applications in Dermatology

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DEDICATION

For my family, who offered me unconditional love and support throughout the course of this thesis...

ACKNOWLEDGMENT

I wish to introduce my deep gratitude and utmost thanks to laser institute, college of graduate studies, Sudan University of Science and Technology, and special thanks to Professor Nafie A. Almuslet whose hard and faithful efforts have helped me to complete my study in such a valuable way.

I am greatly thankful to Dr. Shaza Mohammed Yousif for her continuous support and valuable efforts which stands behind my success in this clinical study.

I am also indebted to the group of patients who agreed to come for treatment and follow-up.

ABSTRACT

Vitiligo is an acquired pigmentary disorder of the skin and mucous membranes that is characterized by circumscribed, depigmented macules and patches. Low-Level Laser Therapy (LLLT) is the use of low power visible and near-infrared monochromatic laser to enhance the body's natural healing processes.

The aim of the study is to evaluate new ways for treatment of vitiligo using low level laser (675 nm) and conventional light, then to compare the results of both of them.

This is a prospective clinical descriptive intervention study conducted during the period from Oct 2009 to Jan 2015. The study was conducted at the Institute of Laser, Sudan University of Science and Technology. Thirty nine patients were involved in this study, seventeen of them received incoherent light treatment, and twenty two of them received laser treatment.

Thirty nine patients were treated by the Omega Xp Mobile low level laser in this study. Two probes of the Omega Xp device were used, the first probe emitting red laser in a wavelength of 675 nm and 30 mW power, and the other probe containing cluster of 60 light emitting diodes (LED) combined together and emitting incoherent light of different wavelengths (660 nm, 810 nm , 940 nm, 880 nm, 850 nm) at once.

The majority of patients treated by incoherent light (64.71%) showed good repigmentation response, while the majority of patients treated by laser (50.00%) showed very good repigmentation response. Five patients showed delayed repigmentation response after stopping the treatment.

From our study, we can conclude that the low level laser probe of 675 nm and the 60 diode cluster probe are very effective and safe in treatment of vitiligo. It's recommended to use an incoherent light for the treatment of focal and generalized vitiligo, while a laser for treatment of acrofacial and focal vitiligo.

المستخلص

البهاق هو اضطراب صباغي مكتسب للجلد والأغشية المخاطية والذي يتميز بالإحاطة و البقع واللطخات ناقصة الصباغ. العلاج بالليزر منخفض المستوى هو استخدام الليزر المرئي منخفض القدرة والليزر أحادي اللون القريب من الأشعة تحت الحمراء لتعزيز عمليات الشفاء الطبيعية في الجسم.

الهدف من هذه الدراسة هو تقييم طرق جديدة لعلاج البهاق باستخدام ليزر واطئ القدرة بطول موجي 675 نانومتر والضوء التقليدي غير المترابط، ثم مقارنة نتائج كل منهما.

هذه الدراسة عبارة عن دراسة استباقية ، وصفية سريرية ، تدخلية ،أجريت خلال الفترة من أكتوبر 2009م إلى يناير 2015م، وقد أجريت هذه الدراسة في معهد الليزر ، جامعة السودان للعلوم والتكنولوجيا، ولقد شارك تسعة وثلاثون مريضا في هذه الدراسة ، سبعة عشر منهم تلقوا العلاج بالضوء غير المتماسك ، واثنين وعشرون منهم تلقوا العلاج بالليزر.

تسعة وثلاثون مريضا تم علاجهم في هذه الدراسة بالجهاز نوع إكس بي أوميغا واطئ القدرة، حيث تم استخدام مجسين من هذا الجهاز، المجس الأول يبعث الليزر الأحمر بطول موجي 675 نانوميتر وقدرة مقدارها 30 ملي واط، والمجس الآخر يحتوي على مجموعة من 60 ثنائياً باعثاً للضوء مجتمعة سويةً والتي ينبعث منها ضوء غير مترابط لأطوال موجية مختلفة (660 نانومتر، 810 نانومتر، 940 نانومتر، 880 نانومتر، 850 نانومتر) في آن واحد.

وأظهر الغالبية من المرضى الذين عولجوا بالضوء غير المترابط (64.71%) استجابة عودة تصبغ جيدة، بينما أظهر الغالبية من المرضى الذين عولجوا بالليزر (50.00%) استجابة عودة تصبغ جيدة جداً، وأظهر خمسة مرضى استجابة عودة تصبغ مؤجلة وذلك بعد التوقف عن العلاج.

من دراستنا يمكننا أن نستنتج أن ليزر واطئ القدرة (أوميغا إكس بي) بطول موجي 675 نانومتر و مجس مجموعة ال60 ثنائياً فعالين جداً وآمنين في علاج البهاق، كما ينصح باستخدام الضوء غير المتماسك لعلاج البهاق البؤري والعام، واستخدام الليزر لعلاج البهاق الوجهي و البؤري.

LIST OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

Abbreviation	Meaning
ACTH	Adrenocorticotropic Hormone
ANSI	American National Standards Institute
ATP	Adenosine Triphosphate
bFGF	Basic Fibroblast Growth Factor
CAT	Catalase
CD8+ T cells	T cells with CD8 on the surface, which are immunosuppressive and suppress mitogen-induced and antigen-specific antibody production, and require CD4 cell cooperation
CI	Confidence Interval
CO_2	Carbon Dioxide
CW	Continuous Waves
DNA	Deoxyribonucleic Acid
DOS	Density of Optical States
FDA	Food and Drug Administration
FGF2	Fibroblast Growth Factor
GaAlAs	Gallium-Aluminum-Arsenide
HeNe	Helium Neon
HLAs	Human Leukocyte Antigens
HSP701A	An intronless gene on chromosome 6p21.3 that encodes a member of the heat shock 70 (HSP70) family which, in conjunction with other heat shock proteins, stabilises extant proteins against aggregation and mediates the folding of newly translated proteins in the cytosol and in organelles. HSPA1A is a component of the so-called CatSper complex.
IGF-1	Insulin-Like Growth Factor 1
IR	Infrared
JAK	Janus Kinase
LASER	Light Amplification by Stimulated Emission of Radiation
LED	Light Emitting Diode
LLLT	Low-Level Laser Therapy
MEL	Monochromatic Excimer Light
mRNA	Messenger Ribonucleic Acid
MSH	Melanocyte-Stimulating Hormone
NBUVB	Narrow-Band Ultraviolet B
Nd:YAG	Neodymium-Doped Yttrium Aluminium Garnet
NF-kB	Nuclear Factor Kappa Beta
NGF	Nerve Growth Factor

NIR	Near Infrared
NSV	Nonsegmental Vitiligo
PDGF	Platelet-Derived Growth Factor
PGA	Physician's Global Assessment
PKC	Protein Kinase C
PUVA	Psoralen Ultra-Violet A
ROS	Reactive Oxygen Species
RSTL	Relaxed Skin Tension Lines
SOD	Superoxide Dismutase
TNF-alpha	Tumor Necrosing Factor α
T_R	Thermal Relaxation Time
USSR	Union of Soviet Socialist Republics
UV	Ultraviolet

CHAPTER ONE

Introduction and Basic Concepts

CHAPTER ONE

Introduction and Basic Concepts

1.1. Introduction:

Vitiligo is an acquired pigmentary disorder of the skin and mucous membranes that is characterized by circumscribed, depigmented patches surrounded by normal or hyperpigmented skin. The condition is frequently associated with disorders of autoimmune origin, with thyroid abnormalities being the most common. Vitiligo lesions are characterized as: white or hypopigmented, usually well demarcated, round, oval, or linear in shape, borders may be convex, range from millimeters to centimeters in size, and enlarge centrifugally over time at an unpredictable rate (Ortonne, 2008).

Initial lesions occur most frequently on the face, chest, hands, forearms, and feet, favoring a perioral and periocular distribution. The Clinical variants are: trichrome vitiligo, marginal inflammatory vitiligo, quadrichrome vitiligo, blue vitiligo, and koebner phenomenon. Vitiligo can be classified as: localized , generalized, and universal. Localized vitiligo can exist in the following forms: focal, segmental, and mucosal. Generalized vitiligo can manifest as the following: Acrofacial, Vulgaris, and Mixed.

Universal vitiligo results incomplete or nearly complete depigmentation. It is often associated with multiple endocrinopathy syndrome. Although the diagnosis of vitiligo generally is made on the basis of clinical findings, biopsy is occasionally helpful for differentiating vitiligo from other hypopigmentary disorders. Ultra microscopic examination of involved 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 documented histologic findings include the following: degenerative changes in keratinocytes and melanocytes in the border lesions and adjacent skin, increased numbers of Langerhans cells, epidermal vacuolization, and thickening of the basement membrane. Loss of pigment and melanocytes in the epidermis is highlighted by Fontana-Masson staining and immunohistochemistry testing (McKee, Calonje and and Granter, 2005; Moellmann et al., 1982).

The nonsurgical treatments are: systemic phototherapy (Matz and Tur, 2007), laser therapy, steroid therapy (Lotti et al., 2008), topical therapies, depigmentation therapy, and micro-pigmentation. The basic types of repigmentation surgery are (van Geel, Ongenae and Naeyaert, 2001; Rusfianti and Wirohadidjodjo, 2006): noncultured epidermal suspensions, thin dermoepidermal grafts, suction epidermal grafting, and punch minigrafting.

1.2. Aim of the Study:

This study is an attempt to provide new ways for treatment of vitiligo using Diode laser 675 nm and conventional light, then to compare the results of both of them.

1.3. Justifications:

The justifications for doing this study are:

- The incidence of vitiligo is ~ 1% of world population.
- There is no satisfactory method of treatment till now.
- No similar study worldwide was done in black skin using Diode laser and conventional light for treating Vitiligo.
- Diode laser and conventional light are non ionizing radiation compared to UV light which is used to treat patients of vitiligo.

1.4. Objectives:

1.4.1. General Objective:

To introduce a new method for the treatment of vitiligo.

1.4.2. Specific Objectives:

1. Using of non ionizing radiation (red laser and infrared light) with low power instead of Psoralen + Ultraviolet A (PUVA) in the treatment of vitiligo to protect the patient against the risk of skin cancer.
2. No need for psoralen uptake in the treatment of vitiligo with laser and light to protect the patient from sunburn, gastric irritation and eye damage caused by psoralen.
3. Reduction of the total period needed for the treatment of vitiligo patients compared to conventional therapies.
4. Reduce the side effects developed upon using conventional therapies by the usage of laser and non-coherent light therapy.

1.5. Rationale:

Low level laser in the range of red and infrared levels approved having beneficial biological effects in the living tissues which used in the treatment of different medical conditions including, for example, treatments of diabetic wounds, vascular ulcers, burns, surgical wounds , but nobody tried to use it in the treatment of vitiligo. This study tried to use incoherent light and low level laser to enhance melanocytes from a surrounding normal skin to multiply and migrate to vitiligo patches to cover it. Up to our knowledge, there was just one clinical study tried to use a low level laser in the treatment of vitiligo, but they didn’t success to get encouraging results.

1.6. The Structure of the Thesis:

Chapter one contained theoretical background (introduction and basic concepts), while chapter two illustrated the laser application in treating Vitiligo, that is a collection of information related to the subject.

Materials and methods are included in chapter three. The aim of chapter three was to illustrate the details of using the Diode red laser and incoherent light therapy in the treatment of vitiligo.

Chapter four covered the results and discussion. The aims of chapter four were to present the results of the treatment, discussing the advantages and disadvantages of each type of light used, and present the conclusions and future work.

1.7. Skin Anatomy:

1.7.1. Overview:

1.7.1.1. Functions of the Skin:

The skin covers the entire external surface of the human body and is the principal site of interaction with the surrounding world. It serves as a protective barrier that prevents internal tissues from exposure to trauma, ultraviolet (UV) radiation, temperature extremes, toxins, and bacteria. Other important functions include sensory perception, immunologic surveillance, thermoregulation, and control of insensible fluid loss.

1.7.1.2. Layers of the Skin:

The integument consists of 2 mutually dependent layers, the epidermis and dermis, which rest on a fatty subcutaneous layer, the panniculus adiposus, (See figure (1-1)). The epidermis is derived primarily from surface ectoderm but is colonized by pigment-containing melanocytes of neural crest origin, antigen-processing Langerhans cells of bone marrow origin (dendritic cells), and pressure-sensing Merkel cells of neural crest origin. The dermis is derived

primarily from mesoderm and contains collagen, elastic fibers, blood vessels, sensory structures, and fibroblasts (Carlson, 1994).

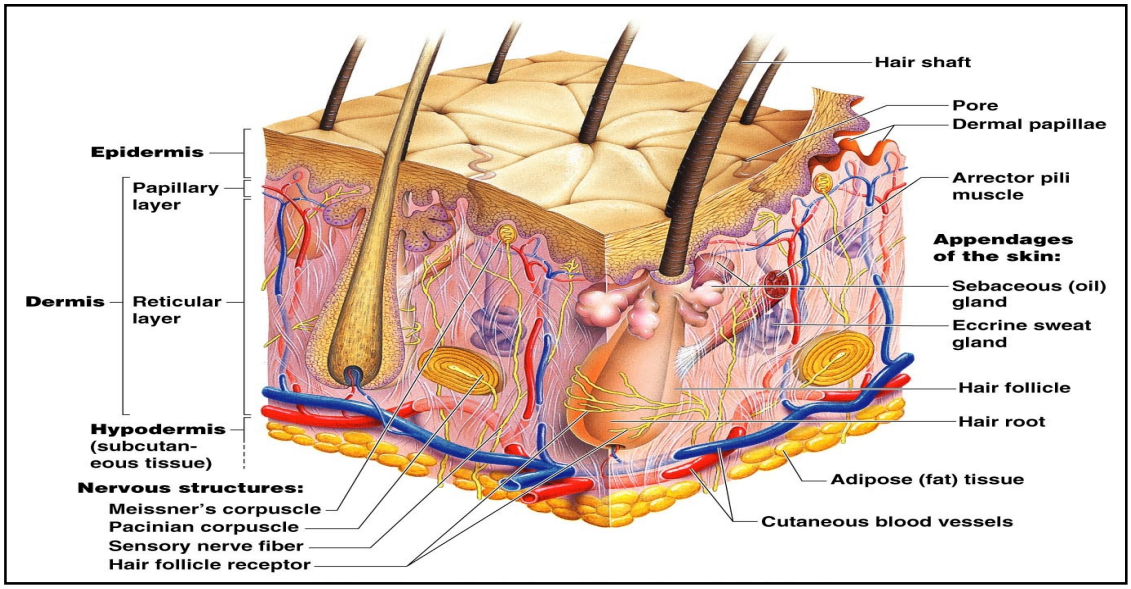

Figure (1-1): Skin Anatomy.

During the fourth week of embryologic development, the single cell thick ectoderm and underlying mesoderm begin to proliferate and differentiate. The specialized structures formed by the skin, including teeth, hair, hair follicles, fingernails, toenails, sebaceous glands, sweat glands, apocrine glands, and mammary glands also begin to appear during this period in development.

1.7.1.3. Appendages of Skin:

Teeth, hair, and hair follicles are formed by the epidermis and dermis in concert, while fingernails and toenails are formed by the epidermis alone. Hair follicles, sebaceous glands, sweat glands, apocrine glands, and mammary glands are considered epidermal glands or epidermal appendages, because they develop as downgrowths or diverticula of the epidermis into the dermis (Carlson, 1994; Moore and Persuad, 1998).

The definitive multi-layered skin is present at birth, but skin is a dynamic organ that undergoes continuous changes throughout life as outer layers are shed and replaced by inner layers. Skin also varies in thickness among anatomic location, sex, and age of the individual. This varying thickness

primarily represents a difference in dermal thickness, as epidermal thickness is rather constant throughout life and from one anatomic location to another. Skin is thickest on the palms and soles of the feet (1.5 mm thick), while the thinnest skin is found on the eyelids (0.02 mm thick) and in the postauricular region (0.05 mm thick).

Male skin is characteristically thicker than female skin in all anatomic locations. Children have relatively thin skin, which progressively thickens until the fourth or fifth decade of life when it begins to thin. This thinning is also primarily a dermal change, with loss of elastic fibers, epithelial appendages, and ground substance (Burns et al., 2004).

1.7.2. Epidermis:

1.7.2.1. Keratinocytes:

The epidermis is a stratified, squamous epithelium that consists primarily of keratinocytes in progressive stages of differentiation from deeper to more superficial layers. The named layers of the epidermis include the stratum germinativum, stratum spinosum, stratum granulosum, and stratum corneum plus two dendritic cells, melanocytes cells and Langerhans cells (Carlson, 1994).

The epidermis contains no blood vessels and is entirely dependent on the underlying dermis for nutrient delivery and waste disposal via diffusion through the dermoepidermal junction.

The stratum germinativum, or the basal layer, is immediately superficial to the dermoepidermal junction. This single cell layer of keratinocytes is attached to the basement membrane via hemidesmosomes. And it considered that the main cell of the basal cell layer that play a role in keratinization (keratin).

As keratinocytes divide and differentiate, they move from this deeper layer to the more superficial layers. Once they reach the stratum corneum, they are

fully differentiated keratinocytes devoid of nuclei and are subsequently shed in the process of epidermal turnover. Cells of the stratum corneum are the largest and most abundant of the epidermis. This layer ranges in thickness from 15-100 or more cells depending on anatomic location and is the primary protective barrier from the external environment.

1.7.2.2. Melanocytes:

Melanocytes, derived from neural crest cells, primarily function to produce a pigment, melanin, which absorbs solar energy from the sun and protects the skin from the harmful effects of UV radiation. Melanin accumulates in organelles termed melanosomes that are incorporated into dendrites anchoring the melanosome to the surrounding keratinocytes. Ultimately, the melanosomes are transferred via phagocytosis to the adjacent keratinocytes where they remain as granules. Melanocytes are found in the basal layer of the epidermis as well as in hair follicles, the retina, uveal tract, and leptomeninges. These cells are the sites of origin of melanoma.

In areas exposed to the sun, the ratio of melanocytes to keratinocytes in basal cell layers is approximately 1:10. In areas not exposed to solar radiation, the ratio may be as small as 1:36. Absolute numbers of epidermal melanosomes units are the same among the sexes and various races. Differing pigmentation among individuals is related to melanosome size rather than cell number. Sun exposure, melanocyte-stimulating hormone (MSH), adrenocorticotropic hormone (ACTH), estrogens, and progesterones stimulate melanin production. With aging, a decline is observed in the number of melanocytes populating the skin of an individual. Since these cells are of neural crest origin, they have no ability to reproduce.

1.7.2.3. Langerhans Cells:

Langerhans cells originate from the bone marrow (reticulo endothelial system) and are found in the basal, spinous, and granular layers of the epidermis. They serve as antigen-presenting cells. They are capable of

ingestion antigens, processing them into small peptide fragments, binding them with major histocompatibility complexes, and subsequently presenting them to lymphocytes for activation of the immune system. An example of activation of this component of the immune system is contact hypersensitivity (Burns et al., 2004).

1.7.2.4. Merkel Cells:

Merkel cells, also derived from neural crest cells, are found on the volar aspect of digits, in nail beds, on the genitalia, and in other areas of the skin. These cells are specialized in the perception of light touch.

1.7.3. Dermoepidermal Junction:

The dermoepidermal junction is an undulating basement membrane that adheres the epidermis to the dermis. It is composed of two layers, the lamina lucida and lamina densa. The lamina lucida is thinner and lies directly beneath the basal layer of epidermal keratinocytes. The thicker lamina densa is in direct contact with the underlying dermis. These structures are the target of immunologic injury in bullous pemphigoid and epidermolysis bullosa.

Dermal papillae from the papillary dermis contain a plexus of capillaries and lymphatics oriented perpendicular to the skin surface. These fingerlike projections are surrounded by similar projections of the epidermis. This highly irregular junction greatly increases the surface area over which oxygen, nutrients, and waste products are exchanged between the dermis and the avascular epidermis (Carlson, 1994).

1.7.4. Dermis:

The primary function of the dermis is to sustain and support the epidermis. The dermis is a more complex structure and is composed of two layers, the more superficial papillary dermis and the deeper reticular dermis. The papillary dermis is thinner, consisting of loose connective tissue containing capillaries, elastic fibers, reticular fibers, and some collagen. The reticular

dermis consists of a thicker layer of dense connective tissue containing larger blood vessels, closely interlaced elastic fibers, and coarse bundles of collagen fibers arranged in layers parallel to the surface (Carlson, 1994).

The reticular layer also contains fibroblasts, mast cells, nerve endings, lymphatics, and epidermal appendages. Surrounding the components of the dermis is the gel-like ground substance, composed of mucopolysaccharides (primarily hyaluronic acid), chondroitin sulfates, and glycoproteins. The deep surface of the dermis is highly irregular and borders the subcutaneous layer, the panniculus adiposus, which additionally cushions the skin.

1.7.4.1. Fibroblasts:

The fibroblast is the major cell type of the dermis. These cells produce and secrete procollagen and elastic fibers. Procollagen is terminally cleaved by proteolytic enzymes into collagen that aggregates and becomes cross-linked. These tightly cross-linked collagen fibers provide tensile strength and resistance to shear and other mechanical forces.

a) Collagen makes up 70% of the weight of the dermis, primarily Type I (85% of the total collagen) and Type III (15% of the total collagen).
b) Elastic fibers constitute less than 1% of the weight of the dermis, but they play an enormous functional role by resisting deformational forces and returning the skin to its resting shape (elasticity) (Carlson, 1994).

1.7.5. Epidermal Appendages:

Epidermal appendages are intradermal epithelial structures lined with epithelial cells with the potential for division and differentiation. These are important as a source of epithelial cells, which accomplish reepithelialization should the overlying epidermis be removed or destroyed in situations such as partial thickness burns, abrasions, or split-thickness skin graft harvesting. Epidermal appendages include the following:

- Sebaceous glands.

- Sweat glands (eccrine).
- Apocrine glands.
- Mammary glands.
- Hair follicles.

They often are found deep within the dermis and in the face may even lie in the subcutaneous fat beneath the dermis. This accounts for the remarkable ability of the face to reepithelialize even the deepest cutaneous wounds (Carlson, 1994).

1.7.5.1. Sebaceous Glands:

Sebaceous glands, or holocrine glands, are found over the entire surface of the body except the palms, soles, and dorsum of the feet. They are largest and most concentrated in the face, scalp, sternum, and back, where they are the sites of origin of acne. The normal function of sebaceous glands is to produce and secrete sebum, a group of complex oils that include triglycerides and fatty acid breakdown products, wax esters, squalene, cholesterol esters, and cholesterol. Sebum lubricates the skin to protect it against friction and makes the skin more impervious to moisture.

1.7.5.2. Sweat Glands:

Sweat glands, or eccrine glands, are found over the entire surface of the body except the vermillion border of the lips, the external ear canal, the nail beds, the labia minora, and the glans penis and the inner aspect of the prepuce. They are most concentrated in the palms and soles and the axillae.

Each gland consists of a coiled secretory intradermal portion that connects to the epidermis via a relatively straight distal duct. The normal function of the sweat gland is to produce sweat, which cools the body by evaporation. The thermoregulatory center in the hypothalamus controls sweat gland activity through sympathetic nerve fibers that innervate the sweat glands. Sweat

excretion is triggered when core body temperature reaches or exceeds a set point.

1.7.5.3. Apocrine and Mammary Glands:

Apocrine glands are similar in structure, but not identical, to eccrine glands. They are found in the axillae, in the anogenital region, and, as modified glands, in the external ear canal (ceruminous glands), the eyelid (Moll's glands), and the breast (mammary glands). They produce odor and do not function prior to puberty, which means they probably smell unpleasant and serve a sexual attraction function. The mammary gland is considered a modified and highly specialized type of apocrine gland.

1.7.5.4. Hair Follicles:

Hair follicles are complex structures formed by the epidermis and dermis, (See figure (1-2)). They are found over the entire surface of the body except the soles of the feet, palms, glans penis, clitoris, labia minora, mucocutaneous junction, and portions of the fingers and toes. Sebaceous glands often open into the hair follicle rather than directly onto the skin surface, and the entire complex is termed the pilosebaceous unit (Carlson, 1994; Poblet, Jiménez and Ortega, 2004; Prost-Squarcioni, 2006).

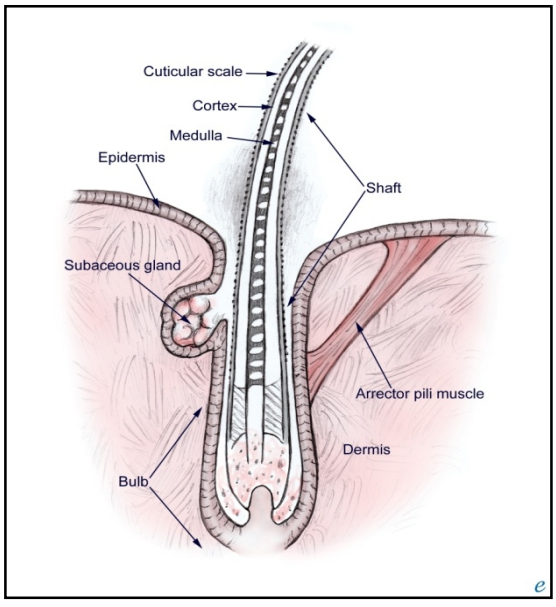

Figure (1-2): Anatomy of Hair Follicle.

Caucasian hair follicles are oriented obliquely to the skin surface, whereas the hair follicles of black persons are oriented almost parallel to the skin surface. Asian persons have vertically oriented follicles that produce straight hairs. These anatomic variations are an important consideration in avoiding alopecia when making incisions in the scalp.

The base of the hair follicle, or hair bulb, lies deep within the dermis and, in the face, may actually lie in the subcutaneous fat. This accounts for the remarkable ability of the face to re-epithelialize even the deepest cutaneous wounds. A band of smooth muscle, the arrector pili, connects the deep portion of the follicle to the superficial dermis. Contraction of this muscle, under control of the sympathetic nervous system, causes the follicle to assume a more vertical orientation.

Hair growth exhibits a cyclical pattern. The anagen phase is the growth phase, whereas the telogen phase is the resting state. The transition between anagen and telogen is termed the catagen phase. Phases vary in length according to anatomic location, and the length of the anagen phase is proportional to the length of the hair produced. At any one time at an anatomic location, follicles are found in all 3 phases of hair growth. This is extremely important for laser hair removal, because follicles in the anagen phase are susceptible to destruction, whereas resting follicles are more resistant. This explains why multiple treatments of an area may be necessary to ensure adequate hair removal.

1.7.6. Cutaneous Blood Supply:

Cutaneous vessels ultimately arise from underlying named source vessels. Each source vessel supplies a 3-dimensional vascular territory from bone to skin termed an angiosome. Adjacent angiosomes have vascular connections via reduced caliber (choke) vessels or similar caliber (true) anastomotic vessels. The cutaneous vessels originate either directly from the source

arteries (septocutaneous or fasciocutaneous perforators) or as terminal branches of muscular vessels (musculocutaneous perforators).

During their course to the skin, the cutaneous vessels travel within or adjacent to the connective tissue framework and supply branches to each tissue with which they come into close contact (bone, muscle, fascia, nerve, fat). They emerge from the deep fascia in the vicinity of the intermuscular or intramuscular septa or near tendons and travel toward the skin, where they form extensive subdermal and dermal plexuses. The dermis contains horizontally arranged superficial and deep plexuses, which are interconnected via communicating vessels oriented perpendicular to the skin surface. Cutaneous vessels ultimately anastomose with other cutaneous vessels to form a continuous vascular network within the skin. Clinically, this extensive horizontal network of vessels allows for random skin flap survival (Taylor and Pan, 1998; Lamberty and Cormack, 1990; McGregor and Morgan, 1963).

1.7.6.1. Thermoregulation:

In addition to the skin's natural heat conductivity and loss of heat from the evaporation of sweat, convection from cutaneous vessels is a vital component of thermoregulation. Cutaneous blood flow is 10-20 times that required for essential oxygenation and metabolism, and large amounts of heat can be exchanged through the regulation of cutaneous blood flow. The thermoregulatory center in the hypothalamus controls vasoconstriction and vasodilatation of cutaneous vessels through the sympathetic nervous system.

1.7.7. Lymphatics:

Skin lymphatics parallel the blood supply and function to conserve plasma proteins and scavenge foreign material, antigenic substances, and bacteria. Blind-ended lymphatic capillaries arise within the interstitial spaces of the dermal papillae. These unvalved, superficial dermal vessels drain into valved deep dermal and subdermal plexuses. These then coalesce to form larger lymphatic channels, which course through numerous filtering lymph nodes on

their way to join the venous circulation near the subclavian vein – internal jugular vein junction bilaterally.

1.7.8. Skin Innervation:

Sensory perception is critically important in the avoidance of pressure, mechanical or traumatic forces, and extremes of temperature. Numerous specialized structures are present in the skin to detect various stimuli. As previously mentioned, Merkel cells of the epidermis detect light touch. Meissner corpuscles also detect light touch. These are found in the dermal papillae and are most concentrated in the fingertips. Pacini corpuscles are found deep within the dermis or even in the subcutaneous tissue. These structures are specialized to detect pressure.

Pain is transmitted through naked nerve endings located in the basal layer of the epidermis. Krause bulbs detect cold, whereas Ruffini corpuscles detect heat. Heat, cold, and proprioception also are located in the superficial dermis. Cutaneous nerves follow the route of blood vessels to the skin. The area supplied by a single spinal nerve, or a single segment of the spinal cord, is termed a dermatome. Adjacent dermatomes may overlap considerably, which is important to note when performing field blocks with local anesthesia (Carlson, 1994; Morris and Gibbins, 1997).

1.7.9. Surface Anatomy:

Lines and creases are evident over major and minor joints. Skin contraction produces wrinkles and creases that lie perpendicular to the underlying muscular vector force. Relaxed skin tension lines (RSTL), however, are formed during relaxation and often follow a different direction than age and contracting wrinkles. (See figure (1-3)) Relaxed skin tension lines are created by the natural tension on the skin from underlying structures (Fongo, Ferraris and Bocca, 1966).

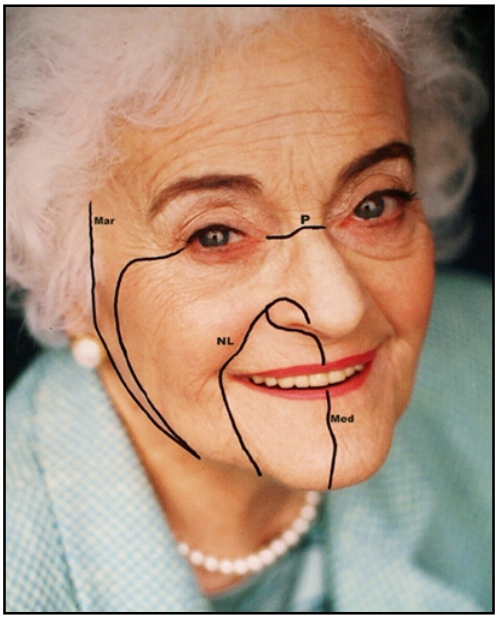

Figure (1-3): Four Main Facial Lines Show the Direction of Relaxed Skin Tension Lines.

Papillary ridges on the tips of the digits of the hands and feet and the surface of palms and soles are often used for personal identification. These are also known as friction ridges, since they assist in the ability to grasp. They are formed during fetal development and are unique to each individual, including identical twins. This distinct pattern does not change with aging. Stratum mucosum composes the outer surface of the ridges with underlying dermal papillae. Sweat pores are usually located at the top of the ridges (Ashbaugh, 1999).

1.7.10. Skin Phototype:

The amount of melanin pigment in the skin determines an individual's skin color (skin phototype). Skin pigment can be inherited genetically (constitutive melanin pigment) or can be acquired (inducible melanin pigment) through various diseases. Hormonal changes during pregnancy can also vary the amount of pigmentation.

The Fitzpatrick Scale is used to classify skin complexion and response to UV exposure (See table (1-1) below). This classification is based on a personal history of sunburning and suntanning (Goldman et al., 2008). This

classification is used clinically for evaluation of skin phototype before resurfacing procedures and is important for predicting outcomes and adverse effects.

Table (1-1): The Fitzpatrick Scale

Skin Type	Color	Features
I	White or freckled skin	Always burns, never tans
II	White skin	Burns easily, tans poorly
III	Olive skin	Mild burn, gradually tans
IV	Light brown skin	Burns minimally, tans easily
V	Dark brown skin	Rarely burns, tans easily
VI	Black skin	Never burns, always tans

1.7.11. Anatomy of Aging Skin:

Aging skin changes include thinning, skin laxity, fragility, and fine wrinkles. Sun-exposed areas demonstrate additional photoaging changes, including dyspigmentation, premature wrinkling, telangiectasia, and actinic elastosis. Cutaneous aging is characterized by intrinsic and extrinsic processes. Intrinsic, or chronologic, aging is a genetically determined and inevitable process in skin, including photoprotected skin. Intrinsic aging naturally occurs and is exacerbated by extrinsic aging, which is environmentally induced.

Aging at the cellular level is thought to be related to cellular senescence, specifically, the shortening of telomeres (the terminal portions of chromosomes) with each cell cycle. Telomere shortening ultimately results in cell-cycle arrest or apoptosis once a critical length is reached.

Preventable environmental factors that amplify intrinsic aging include sun exposure, smoking, diet, and poliotion. Long-term UV-A radiation exposure

accelerates intrinsic aging via the formation of reactive oxygen species (ROS). ROS lead to inflammatory cytokines and the up-regulation of matrix metalloproteinases, which result in the breakdown of collagen. UV-B radiation can also contribute to this aging process by causing direct deoxyribonucleic acid (DNA) mutations.

Histopathologically, aging is manifest as flattening (atrophy) of the dermal-epidermal junction, resulting in decreased nutrient transfer between the layers, heliodermatitis or chronic inflammation, elongated and collapsed fibroblasts, disorganized collagen fibrils with overall decrease in collagen levels, and the accumulation of abnormal elastin-containing material termed solar elastosis (Rabe et al., 2006; Baumann, 2007).

1.8. Vitiligo:

1.8.1. Overview:

1.8.1.1. Background:

Vitiligo is an acquired pigmentary disorder of the skin and mucous membranes, and it is characterized by circumscribed depigmented patches. Vitiligo is a progressive disorder in which some or all of the melanocytes in the affected skin are selectively destroyed. Vitiligo affects ~1% of the world population, and the average age of onset is 20 years. Figure (1-4) shows examples of vitiligo in the hands.

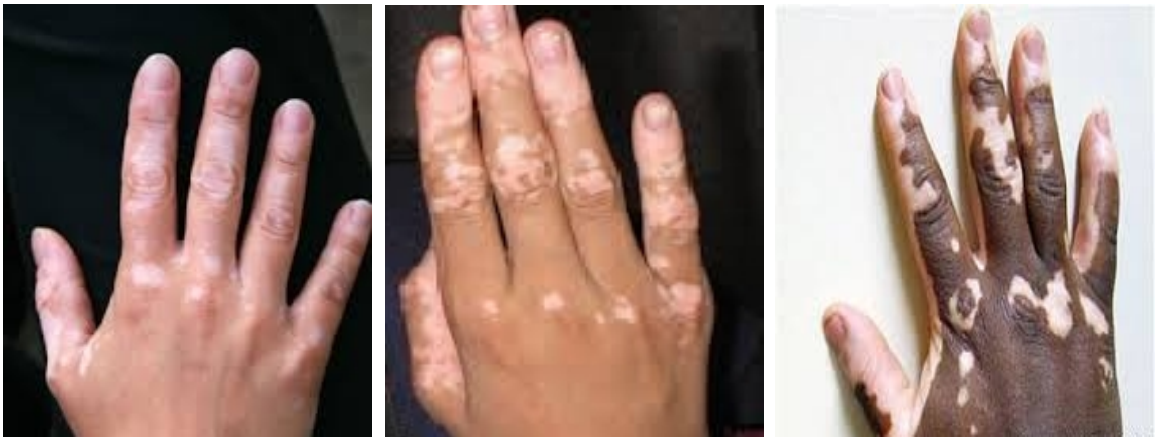

Figure (1-4): Examples of Vitiligo in the Hands.

1.8.1.2. Pathophysiology:

Vitiligo is a multifactorial polygenic disorder with a complex pathogenesis. It is related to both genetic and nongenetic 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 of melanocytes include autoimmune mechanisms, cytotoxic mechanisms, an intrinsic defect of melanocytes, oxidant antioxidant mechanisms, and neural mechanisms (Le Poole and Luiten, 2008).

1.8.1.2.1. Autoimmune Destruction of Melanocytes:

The autoimmune theory proposes alteration in humoral and cellular immunity in the destruction of melanocytes of vitiligo.

The most convincing evidence of an autoimmune pathogenesis is the presence of circulating antibodies in patients with vitiligo (Toussaint and Kamino, 1997). The role of humoral immunity is further supported by the observation that melanocytes are destroyed in healthy skin engrafted onto nude mice injected with vitiligo patient sera (Schallreuter, Wood and Pittelkow, 1994).

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 autoreactive CD8+ T cells. Activated CD8+ T cells have been demonstrated in perilesional vitiligo skin. In addition, melanocyte-specific T cells have been detected in peripheral blood of patients with autoimmune vitiligo (Ongenae, Van Geel and Naeyaert, 2003; Zhang et al., 2013).

1.8.1.2.2. Intrinsic Defect of Melanocytes:

Vitiligo melanocytes may have an intrinsic defect leading to melanocyte death (van den Wijngaard, Aten and Scheepmaker, 2000).

1.8.1.2.3. Disturbance in Oxidant-Antioxidant System in Vitiligo:

Oxidant stress may also play an essential role in the pathogenesis of vitiligo. Studies suggest that accumulation of free radicals toxic to melanocytes leads to their destruction (Ortonne, 2008).

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 (Sravani et al., 2009).

1.8.1.2.4. Neural Theory:

Segmental vitiligo frequently occurs in a dermatomal pattern, which suggests that certain chemical mediators are released from nerve endings that affect melanin production (Kovacs, 1998).

1.8.1.2.5. Genetics of Vitiligo:

Vitiligo is characterized by incomplete penetrance, multiple susceptibility loci, and genetic heterogeneity (Spritz, 2008). The inheritance of vitiligo may involve genes associated with the biosynthesis of melanin, a response to oxidative stress, and regulation of autoimmunity (Halder and Taliaferro, 2008).

Human leukocyte antigens (HLAs) may be associated, but not in a consistent manner (Birlea et al., 2009). The age of onset has a genetic component (Jin, Birlea and Fain, 2011).

1.8.1.3. Frequency:

1.8.1.3.1. United States:

In the United States, the relative rate of vitiligo is 1%.

1.8.1.3.2. International:

Vitiligo is relatively common, with a rate of 1-2%. Approximately 30% of vitiligo cases occur with a familial clustering of cases.

1.8.1.4. Mortality/Morbidity:

1.8.1.4.1. Sex:

A female preponderance has been reported for vitiligo, but it is not statistically significant and the discrepancy has been attributed to an increase in reporting of cosmetic concerns by female patients.

1.8.1.4.2. Age:

Vitiligo may appear at any time from birth to senescence, although the onset is most commonly observed in persons aged 10-30 years.

Vitiligo rarely is seen in infancy or old age. Nearly all cases of vitiligo are acquired relatively early in life.

The average age of onset for vitiligo is approximately 20 years. The age of onset is unlikely to vary between the sexes.

Heightened concern about the appearance of the skin may contribute to an early awareness of vitiligo among females.

1.8.2. Clinical Presentation:

1.8.2.1. History:

The most common form of vitiligo is an amelanotic macule or patch surrounded by healthy skin. The macules are chalk or milk-white in color, and

lesions are well demarcated. The lesions are not readily apparent in lightly pigmented individuals; however, they are easily distinguishable with a Wood lamp examination.

1.8.2.2. Physical:

Vitiligo manifests as acquired white or hypopigmented macules or patches. The lesions are usually well demarcated, and they are round, oval, or linear in shape. The borders may be convex (Ortonne, 2008).

Vitiligo lesions may be localized or generalized. The most common sites of vitiligo involvement are the face, neck, scalp, bony prominences, extensor forearm, ventral wrists, dorsal hands, and digital phalanges.

1.8.2.3. Clinical Variants:

- Trichrome vitiligo has an intermediate zone of hypochromia located between the achromic center and the peripheral unaffected skin. The natural evolution of the hypopigmented areas is progression to full depigmentation. This results in three shades of color—brown, tan, and white—in the same patient, as in figure (1-5) below.

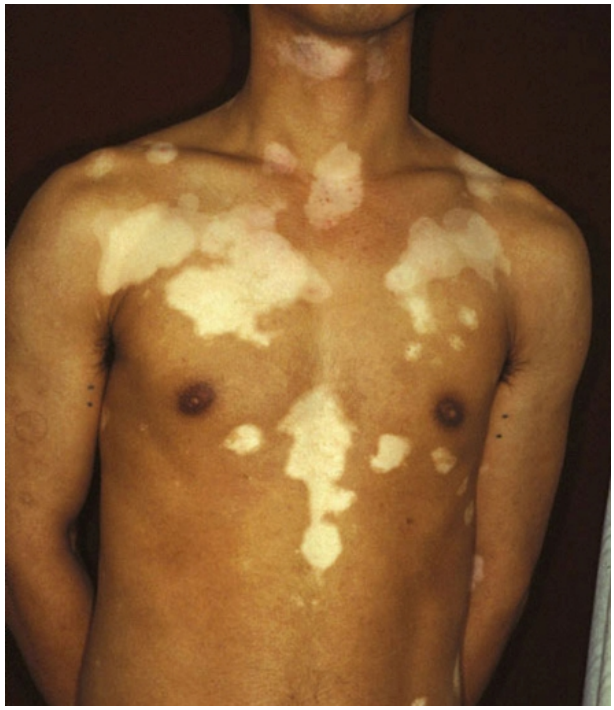

Figure (1-5): Trichrome Vitiligo.

- Marginal inflammatory vitiligo results in a red, raised border, which is present from the onset of vitiligo (in rare cases) or which may appear several months or years after the initial onset. A mild pruritus may be present, as in figure (1-6) below.

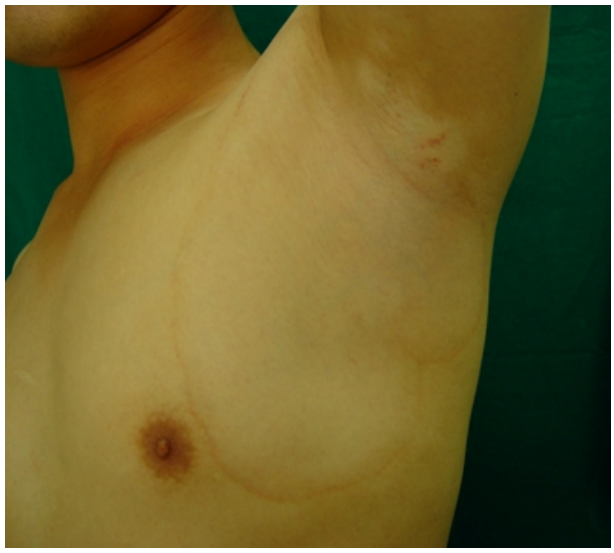

Figure (1-6): Marginal Inflammatory Vitiligo.

- Quadrichrome vitiligo is another variant of vitiligo, which reflects the presence of a fourth color (ie, dark brown) at sites of perifollicular repigmentation. A case of pentachrome vitiligo with 5 shades of color has also been described (Kovacs, 1998).

- Blue vitiligo results in blue coloration of vitiligo macules. This type has been observed in a patient with postinflammatory hyperpigmentation who then developed vitiligo.

- Koebner phenomenon is defined as the development of vitiligo in sites of specific trauma, such as a cut, burn, or abrasion. Minimum injury is required for Koebner phenomenon to occur.

1.8.2.4. Clinical Classifications of Vitiligo:

The most widely used classification of vitiligo is localized, generalized, and universal types and is based on the distribution, as follows:

a) Localized Vitiligo:

- Focal: This type is characterized by one or more macules in one area, most commonly in the distribution of the trigeminal nerve.
- Segmental: This type manifests as one or more macules in a dermatomal or quasidermatomal pattern. It occurs most commonly in children. More than half the patients with segmental vitiligo have patches of white hair or poliosis. This type of vitiligo is not associated with thyroid or other autoimmune disorders.
- Mucosal: Mucous membranes alone are affected.

b) Generalized Vitiligo:

- Acrofacial: Depigmentation occurs on the distal fingers and periorificial areas.
- Vulgaris: This is characterized by scattered patches that are widely distributed.
- Mixed: Acrofacial and vulgaris vitiligo occur in combination, or segmental and acrofacial vitiligo and/or vulgaris involvement are noted in combination.

c) Universal Vitiligo:

This is complete or nearly complete depigmentation. It is often associated with multiple endocrinopathy syndrome.

1.8.2.5. Classification of Vitiligo by Progression, Prognosis, and Treatment:

When progression, prognosis, and treatment are considered, vitiligo can be classified into two major clinical types: segmental and nonsegmental, as demonstrated in figures (1-7) and (1-8) below.

a) Segmental:

This usually has an onset early in life and rapidly spreads in the affected area. The course of segmental vitiligo can arrest, and depigmented patches can persist unchanged for the life of the patient.

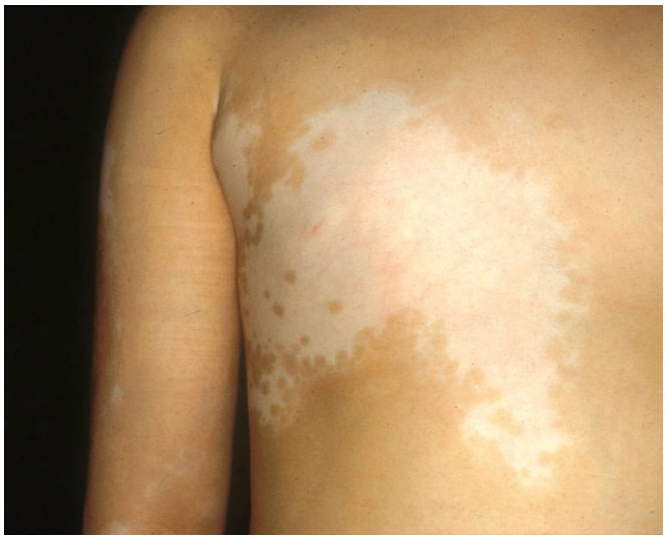

Figure (1-7): Segmental Vitiligo.

b) Nonsegmental:

This type includes all types of vitiligo, except segmental vitiligo (Hann, 2004).

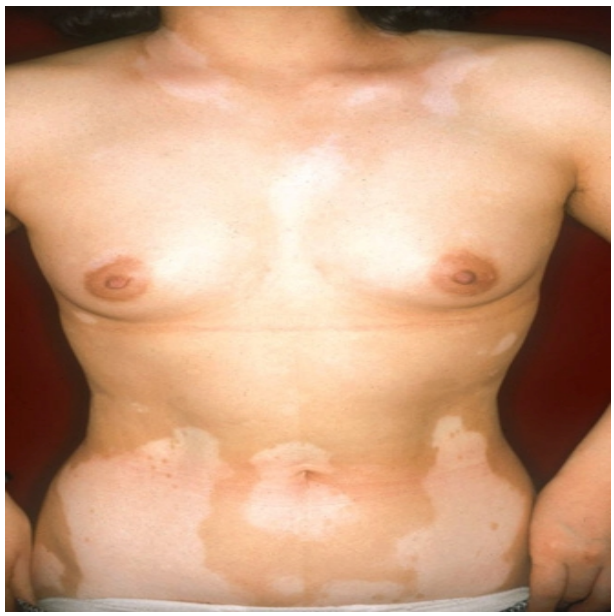

Figure (1-8): Nonsegmental Vitiligo.

A single-center study of 213 patients aged 17 years or younger with segmental or nonsegmental vitiligo found that nonsegmental vitiligo was more strongly linked than segmental vitiligo to markers of autoimmunity or inflammation such as halo naevi and thyroid antibodies; patients with nonsegmental vitiligo were also more likely to have a family history of vitiligo or autoimmunity (Ezzedine, Diallo and Leaute-Labreze, 2011).

1.8.3. Diagnostic Considerations:

1.8.3.1. Vitiligo and Ocular Disease:

Alezzandrini syndrome includes facial vitiligo, poliosis, deafness, and unilateral visual changes. The affected eye has decreased visual acuity and an atrophic iris (Ortonne, 2008).

1.8.3.2. Vitiligo and Autoimmune Disorders:

Vitiligo is frequently associated with disorders of autoimmune origin, with thyroid abnormalities being the most common (Yang et al., 2009).

Patients with autoimmune polyendocrinopathy candidiasis-ectodermal dystrophy have an increased prevalence of vitiligo. In this genetic syndrome, autoantibodies cause destruction of endocrine cells (Rashtak and Pittelkow, 2008).

Pediatric patients with a positive family history of vitiligo show an earlier age of disease onset (Pajvani, Ahmad and Wiley, 2006).

1.8.3.3. Vitiligo and Auditory Abnormalities:

Melanin may play a significant role in the establishment and/or maintenance of the structure and function of the auditory system and may modulate the transduction of the auditory stimuli by the inner ear (Aydogan et al., 2006; Ardiç et al., 1998).

1.8.3.4. Vitiligo and Melanoma:

Vitiligo like depigmentation can occur in patients with malignant melanoma and is believed to result from a T-cell–mediated reaction to antigenic melanoma cells and cross-reactivity to healthy melanocytes (Matz and Tur, 2007; Gul et al., 2007).

1.8.3.5. Differential Diagnoses:

- Albinism.
- Alezzandrini Syndrome.
- Chemical leukoderma.
- Idiopathic Guttate Hypomelanosis.
- Leprosy.
- Malignant Melanoma.
- Mycosis fungoides mimicking vitiligo.
- Nevus Anemicus.
- Onchocerciasis (River Blindness).
- Piebaldism.
- Pityriasis Alba.
- Postinflammatory depigmentation.
- Prior treatment with corticosteroids.
- Scleroderma.
- Tinea Versicolor.
- Treponematosis.
- Tuberous Sclerosis.

- Vogt-Koyanagi-Harada Syndrome.
- Waardenburg Syndrome.

1.8.4. Workup:

1.8.4.1. Laboratory Studies:

Although the diagnosis of vitiligo generally is made on the basis of clinical findings, biopsy is occasionally helpful for differentiating vitiligo from other hypopigmentary disorders.

1.8.4.2. Other Tests:

Vitiligo is diagnosed by means of inspection with a Wood lamp.

1.8.4.3. Histologic Findings:

Microscopic examination of involved skin shows a complete absence of melanocytes in association with a total loss of epidermal pigmentation (McKee, Calonje and and Granter, 2005; Moellmann et al., 1982).

1.8.5. Treatment and Management:

1.8.5.1. Medical Care:

No single therapy for vitiligo produces predictably good results in all patients; the response to therapy is highly variable (Schallreuter, Bahadoran and Picardo, 2008).

1.8.5.1.1. Systemic Phototherapy:

Systemic phototherapy induces cosmetically satisfactory repigmentation in up to 70% of patients with early or localized disease (Matz and Tur, 2007). Narrow-band UV-B phototherapy is widely used and produces good clinical results (Elgoweini and Nour El Din, 2009).

UV-B narrow-band microphototherapy is therapy targeting the specific small lesions (Menchini, Lotti and Tsoureli-Nikita, 2004).

Psoralen photochemotherapy involves the use of psoralens combined with UV-A light. Psoralens can be applied either topically or orally, followed by exposure to artificial UV light or natural sunlight. Vitiligo on the back of the hands and feet is highly resistant to therapy.

1.8.5.1.2. Laser Therapy:

Another innovation is therapy with an excimer laser, which produces monochromatic rays at 308 nm to treat limited, stable patches of vitiligo. This new treatment is an efficacious, safe, and well-tolerated treatment for vitiligo when limited to less than 30% of the body surface. However, therapy is expensive. Localized lesions of vitiligo are treated twice weekly for an average of 24-48 sessions.

According to studies from 2004 and 2007, combination treatment with 0.1% tacrolimus ointment plus the 308-nm excimer laser is superior to 308-nm excimer laser monotherapy for the treatment of UV-resistant vitiliginous lesions (Passeron, Ostovari and Zakaria, 2004; Lotti, Prignano and Buggiani, 2007).

A retrospective chart and photographic review of 80 patients concluded that segmental vitiligo has a better repigmentation response with excimer laser treatment used at earlier stages of the disease (Do et al., 2011). The study also concluded that long-term use and high cumulative UV energy of the excimer laser had better response.

1.8.5.1.3. Steroid Therapy:

Systemic steroids (prednisone) have been used, although prolonged use and their toxicity are undesirable (Lotti et al., 2008).

1.8.5.1.4. Topical Therapies:

Topical tacrolimus ointment (0.03% or 0.1%) is an effective alternative therapy for vitiligo. On the face, narrow-band UV-B works better if combined

with pimecrolimus 1% cream rather than used alone (Esfandiarpour et al., 2009; Njoo and Westerhof, 2004).

A 2009 study out of Kerman Medical University in Iran showed that a combination of pimecrolimus 1% cream and microdermabrasion enhanced response time and repigmentation rates in children with vitiligo (Farajzadeh et al., 2009).

Vitamin D analogs, particularly calcipotriol and tacalcitol, have been used as topical therapeutic agents in vitiligo (Birlea, Costin and Norris, 2008).

Use of khellin 4% ointment and monochromatic excimer light (MEL) 308 nm has been investigated (Saraceno et al., 2009).

1.8.5.1.5. Depigmentation Therapy:

If vitiligo is widespread and attempts at repigmentation do not produce satisfactory results, depigmentation may be attempted in selected patients (Grau and Silverberg, 2013; Chimento et al., 2008). This depigmentation is done by the use of either of hydroquinone 20% twice per week for six months.

1.8.5.2. Surgical Care:

Surgical alternatives exist for the treatment of vitiligo (Falabella, 2005).

Five basic methods for repigmentation surgery have been described, as follows (van Geel, Ongenae and Naeyaert, 2001; Rusfianti and Wirohadidjodjo, 2006):

- Noncultured epidermal suspensions (van Geel et al., 2010).
- Thin dermoepidermal grafts.
- Suction epidermal grafting.
- Punch minigrafting.

- Cultured epidermis with melanocytes or cultured melanocyte suspensions (Falabella, 2005).

Micropigmentation is another option (McGovern, Bolognia and Leffell, 1999). Long-term results of 2-mm punch grafts in patients with generalized vitiligo and segmental vitiligo were assessed (Fongers et al., 2009).

1.8.5.3. Consultations:

Consultation with an ophthalmologist is warranted. Additionally, psychological needs must be addressed on a continual basis with appropriate referrals to mental health specialists (Kovacs, 1998).

1.8.6. Medication:

1.8.6.1. Corticosteroids:

Corticosteroids have anti-inflammatory properties and cause profound and varied metabolic effects.

1.8.6.2. Psoralens:

These agents are used with UV-A exposure for the treatment of localized or generalized vitiligo.

1.8.6.3. Immunomodulator:

Immunomodulators suppress the activity of the immune system.

1.8.6.4. Vitamins:

Vitamin D analogs may regulate skin cell production and differentiation.

1.9. Laser Basics:

A laser is a device that emits light through a process of optical amplification based on the stimulated emission of electromagnetic radiation. The term "laser" originated as an acronym for "light amplification by stimulated emission of radiation" (Gordon Gould, 1959).

A laser differs from other sources of light because it emits light coherently. Spatial coherence allows a laser to be focused to a tight spot, enabling applications like laser cutting and lithography. Spatial coherence also allows a laser beam to stay narrow over long distances (collimation), enabling applications such as laser pointers. Lasers can also have high temporal coherence which allows them to have a very narrow spectrum, i.e., they only emit a single color of light. Temporal coherence can be used to produce pulses of light—as short as a femtosecond.

Lasers have many important applications. They are used in common consumer devices such as optical disk drives, laser printers, and barcode scanners. Lasers are used for both fiber-optic and free-space optical communication. They are used in medicine for laser surgery and various skin treatments, and in industry for cutting and welding materials. They are used in military and law enforcement devices for marking targets and measuring range and speed. Laser lighting displays use laser light as an entertainment medium.

1.9.1. Laser History:

In 1917, Albert Einstein established the theoretical foundations for the laser and the maser in the paper Zur Quantentheorie der Strahlung (On the Quantum Theory of Radiation) via a re-derivation of Max Planck's law of radiation, conceptually based upon probability coefficients (Einstein coefficients) for the absorption, spontaneous emission, and stimulated emission of electromagnetic radiation.

In 1928, Rudolf W. Ladenburg confirmed the existence of the phenomena of stimulated emission and negative absorption (Steen, 1998). In 1939, Valentin A. Fabrikant predicted the use of stimulated emission to amplify "short" waves. In 1947, Willis E. Lamb and R. C. Retherford found apparent stimulated emission in hydrogen spectra and effected the first demonstration of stimulated emission. In 1950, Alfred Kastler (Nobel Prize for Physics

1966) proposed the method of optical pumping, experimentally confirmed, two years later, by Brossel, Kastler, and Winter (Waller, 1966).

In 1953, Charles Hard Townes and graduate students James P. Gordon and Herbert J. Zeiger produced the first microwave amplifier, a device operating on similar principles to the laser, but amplifying microwave radiation rather than infrared or visible radiation. In 1955, Prokhorov and Basov suggested optical pumping of a multi-level system as a method for obtaining the population inversion, later a main method of laser pumping (Townes and Charles, 1999). At a conference in 1959, Gordon Gould published the term LASER in the paper The LASER, Light Amplification by Stimulated Emission of Radiation (Gordon Gould, 1959; Chu and Townes, 2003).

On May 16, 1960, Theodore H. Maiman operated the first functioning laser (Maiman, 1960; Townes and Charles, 2008), at Hughes Research Laboratories, Malibu, California. Later in 1960, the Iranian physicist Ali Javan, and William R. Bennett, and Donald Herriott, constructed the first gas laser, using helium and neon that was capable of continuous operation in the infrared.

In 1962, Robert N. Hall demonstrated the first laser diode device, made of gallium arsenide and emitted at 850 nm the near-infrared band of the spectrum. Later, in 1962, Nick Holonyak, Jr. demonstrated the first semiconductor laser with a visible emission. In 1970, Zhores Alferov, in the USSR, and Izuo Hayashi and Morton Panish of Bell Telephone Laboratories also independently developed room-temperature, continual-operation diode lasers, using the heterojunction structure.

1.9.2. Laser System Components:

Laser device is consisted of at least three components:-

- Gain medium that can amplify light by means of the basic process of stimulated emission.

- Pump source, which creates a population inversion in the gain medium.
- Two mirrors that form a resonator or optical cavity in which light is trapped, traveling back and forth between the mirrors, figure (1-9) (Trager, 2007).

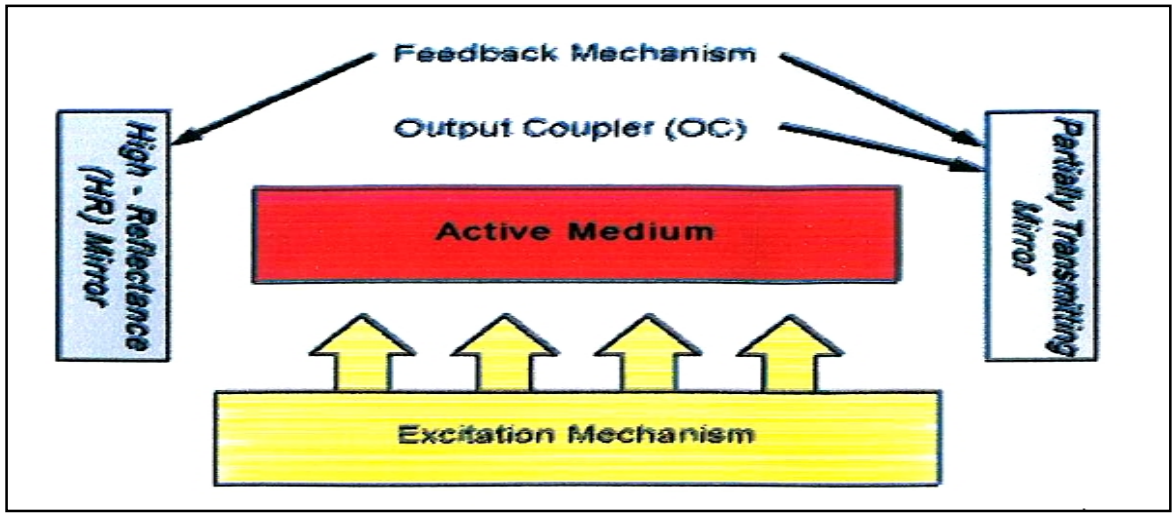

Figure (1-9): Laser System Components.

1.9.3. Lasing Action:

The light generated by stimulated emission is very similar to the input signal in terms of wavelength, phase, and polarization. This gives laser light its characteristic coherence, and allows it to maintain the uniform polarization and often monochromaticity established by the optical cavity design.The beam in the cavity and the output beam of the laser, when travelling in free space (or a homogeneous medium) rather than waveguides (as in an optical fiber laser), can be approximated as a Gaussian beam in most lasers; such beams exhibit the minimum divergence for a given diameter.

However some high power lasers may be multimode, with the transverse modes often approximated using Hermite–Gaussian or Laguerre-Gaussian functions. It has been shown that unstable laser resonators (not used in most lasers) produce fractal shaped beams (Karman et al., 1999).

1.9.4. Characteristics of Laser Light:

Laser generates light that has all the characteristics of ordinary light, However there are three important distinct characteristics of laser light that make it different from other ordinary light. These characteristics are (Weber, 1991):

1.9.4.1. Monochromaticity:

The laser light is monochromatic means colored. Thus laser light is monochromatic light means that laser light is only a single color light. The monochromaticity is the major characteristic of laser making it different from ordinary light.

1.9.4.2. Directionality:

Another important characteristic of laser is that laser light does not spread out or diverge, but it stays together in a beam. We know that when light travels then it tends to spread out, this spreading of light is called divergence and the angle at which the light spreads is called angle of divergence. Divergence is more in ordinary light as compared to laser light. Therefore the angle of divergence will be greater in ordinary light case while will be lesser in laser light case.

1.9.4.3. Coherence:

The 3rd important characteristic of laser light that makes it unique is the coherence. Coherence means that the light waves are in phase. Laser light is much more coherent than ordinary light. It is impossible to detect this property with our eyes and therefore its important is some time over lacked.

The incoherent waves have no relationship to each other. They don't start at the same point in time and space, nor do they have same wavelength (Weber, 1991).

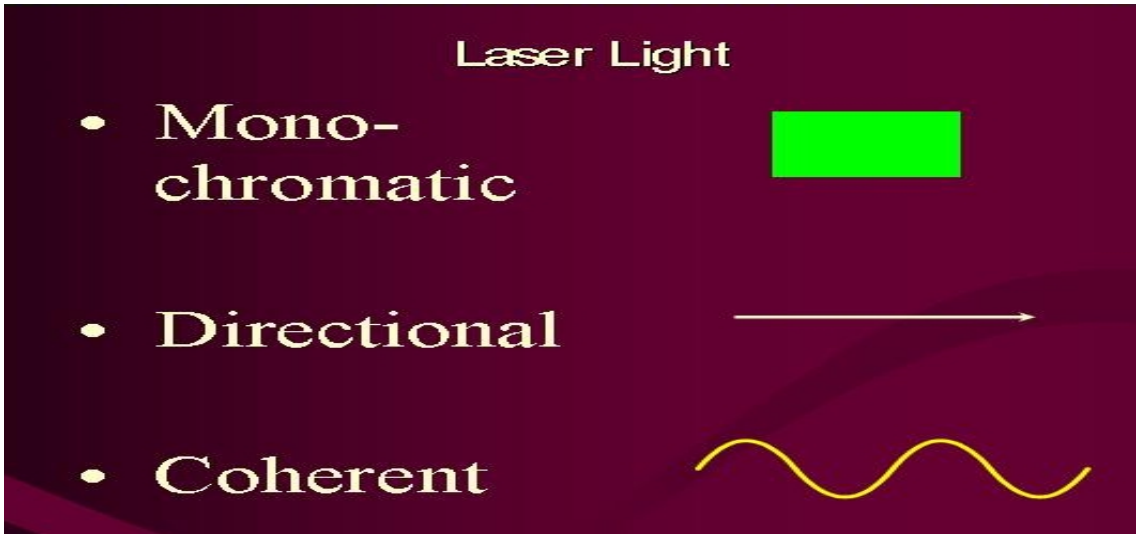

Figure (1-10): Characteristics of Laser Light.

1.9.5. Laser Parameters:

- The wavelength: is defined as the distance between the crests of each wave and that determines the functional properties of the laser energy. Electromagnetic radiation with long wavelengths, measured in meters, is commonly used for radio and television broadcasting. Wavelengths in the 0.4-0.7um range from the visible light of the spectrum. Ultraviolet rays, X-rays and gamma are forms of electromagnetic radiation with the wavelengths shorter than the visible light (Polanyi, 1983).
- Amplitude: is the height of the wave with the maximum displacement from the zero position.
- Frequency: is the number of waves passing in a given point per second and is expressed in cycles per second. The shorter the wavelength, the higher the frequency, and the higher is the energy.
- Velocity: it is a constant and is equal to 186000 miles per second.
- Energy: the capacity to do work and is usually calculated as power multiplied by time of laser light exposed on the skin surface. The unit of measurement is a joule.

- Power: is the rate of flow of energy. This is calculated as energy divided by the time of application. The unit of measurement is the Watt. One Watt equals one joule per second (Polanyi, 1983).

- Power density: is the rate of energy delivery per unit of tissue area. This is expressed in Watts per square centimeter. Power density is determined by the power divided by the surface area of the beam or the spot size. It should be noted that the increase in the power output vaporizes tissues rapidly and reduction in the spot size will increase the energy and accelerates more tissue interaction.

- Fluence: is the energy density and calculated as the total energy divided by the cross sectional area of the beam. It is expressed as joules per square centimeter. It is calculated as the laser power multiplied by time of exposure over an area of spot size in cm^2. If the heat is deposited at a rate faster than the thermal relaxation time, there will be minimal conduction of heat to the surrounding structure.

- Spot size: Large spot size allows for smoother, more uniform vaporization of tissues. The spot size is controlled by focusing or defocusing of the lenses. Simply by moving the hand piece towards and near the skin decreases the spot size, or away from the target increases the spot size. The smaller the spot size the greater tendency to create uneven ridges, furrows and bleeding. Larger spot size causes decrease in peripheral thermal damage.

- Thermal relaxation time: This is the time required for the heated tissues to lose half of its heat through diffusion. It is the time needed for adjacent tissue to cool during laser surgery. This is very important and has a direct relation to tissue destruction.

1.9.6. Laser Tissue Interaction:

Incident light energy will interact with a medium that is denser than air, in one of four ways. These can be listed as follows:

- Transmission: in this way, the beam enters the medium, but there is no interaction between the incident beam and the medium. The beam will emerge distally, unchanged or partially refracted.
- Scattering: there is some interaction, but this is insufficient to cause complete attenuation of the beam. Scattering will cause some diminution of light energy with distance, together with a distortion in the beam, whereby rays proceed in an uncontrolled direction through the medium. Back-scattering of the laser beam can occur as it hits the tissue; this is seen most in short wavelengths, e.g. diode, Nd:YAG (greater than or equal to50% back-scatter).
- Reflection: the density of the medium, or angle of incidence being less than the refractive angle, results in a total reflection of the beam. In true reflection, the incident and emergence angles will be the same or, if the medium interface is rough or non-homogenous, some scattering may occur.
- Absorption: the incident energy of the beam is attenuated by the medium and transferred into another form (Ball, 1995), (See figure (1-11)).

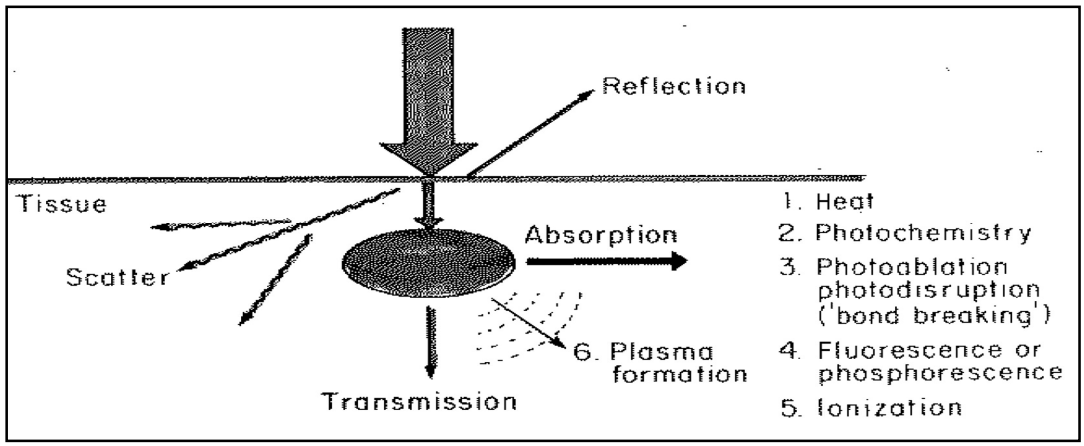

Figure (1-11): Laser Tissue Interaction.

1.9.7. Interactions Mechanisms:

At moderate irradiances and exposures roughly longer than seconds (laser) light, mainly in the visible or ultraviolet (UV) region produces photochemical reactions.

1.9.7.1. Photothermal:

At exposure times around milliseconds to a second and irradiance up to 10 W/cm^2 photocoagulation can be achieved as a therapeutic effect. This interaction results from a thermal induced process associated with protein denaturation, (See figure (1-12)).

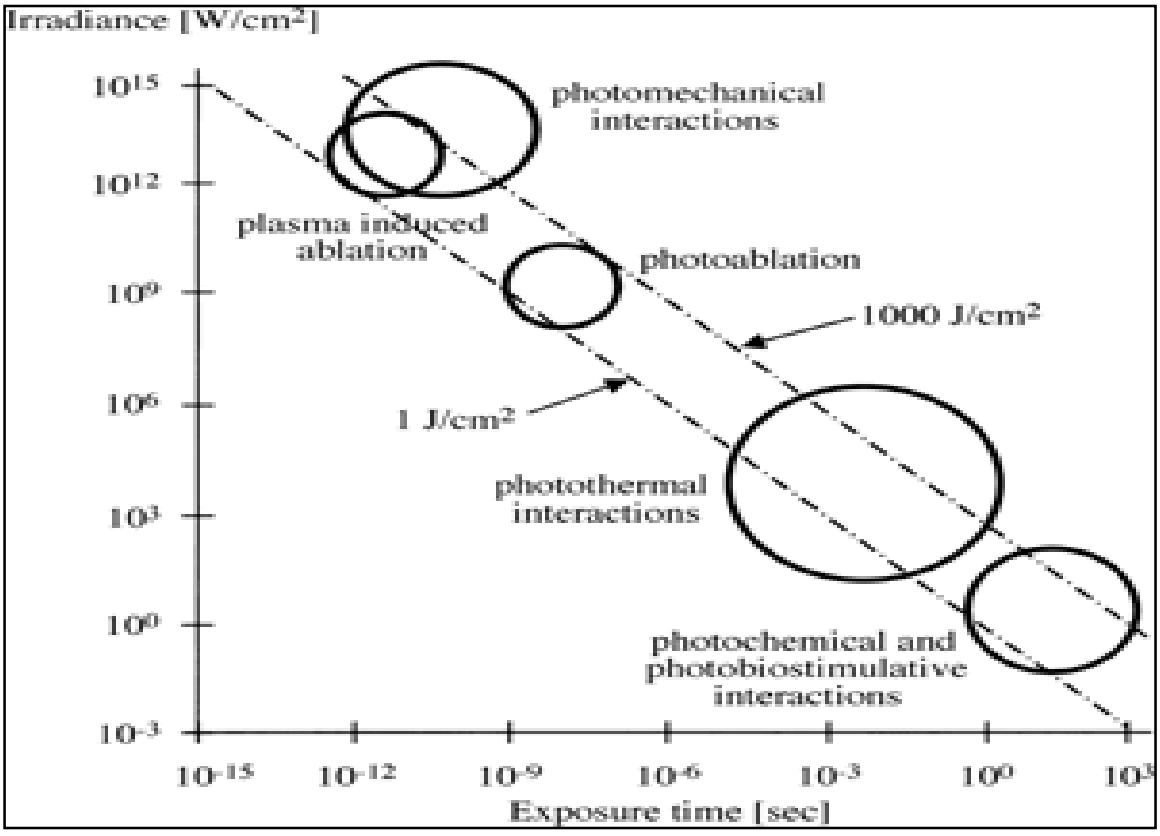

Figure (1-12): Interactions Mechanism.

1.9.7.2. Photochemical:

Historically one of the first surgical laser applications was tissue cutting and removal at relatively high laser-beam intensities with exposure times of milliseconds to seconds, resulting in the rapid deposition of heat and subsequent vaporization of the water inside the tissue.

1.9.7.3. Photomechanical:

This mechanism happen when the pulse duration of the laser is equal or shorter than the Thermal Relaxation Time. The Thermal Relaxation Time (T_R) of a laser heated region of tissue is the time required for the peak temperature to diffuse over the distance of the optical penetration depth δ of the laser light (Marjorie et al., 2009).

1.9.7.4. Photodisruption:

It is plasma-mediated ablation, or optical breakdown. It relies on the nonlinear absorption of laser energy in the target achieved when the material specific radiant exposure is exceeded. Fundamentally, optical breakdown is characterized by three successive major events: plasma formation, shock wave generation and cavitations.

The plasma is a highly ionized state of matter, and can be generated by laser pulses (from femtosecond to few nanoseconds) of relatively low energy but high peak power. It has been shown that shortening the pulse duration from nanoseconds to femtoseconds decreases the threshold for plasma formation and reduces mechanical effects.

1.9.8. Types of Laser:

According to the active media of lasers, we can classify laser machines into:

1.9.8.1. Gas Lasers:

Gas lasers are widely available in almost all power (milliwatts to megawatts) and wavelengths (ultraviolet {UV}- infrared {IR}) and can be operated in pulsed and continuous modes. Based on the nature of active media, there are three types of gas lasers viz atomic, ionic, and molecular.

Most of the gas lasers are pumped by electrical discharge. Electrons in the discharge tube are accelerated by electric field between the electrodes. These accelerated electrons collide with atoms, ions, or molecules in the active

media and induce transition to higher energy levels to achieve the condition of population inversion and stimulated emission (Weber, 1991).

1.9.8.1.1. CO_2 Laser:

The carbon dioxide laser (CO_2 laser) was one of the earliest gas lasers to be developed (invented by Kumar Patel of Bell Labs in 1964), and is still one of the most useful (Patel, 1964).

CO_2 lasers are frequently used in industrial applications for cutting and welding (Andreeta et al., 2011). They are also very useful in surgical procedures because water (which makes up most biological tissue) absorbs this frequency of light very well. Some examples of medical uses are laser surgery and skin resurfacing ("laser facelifts", which essentially consist of vaporizing the skin to promote collagen formation) (Barton and Fritz, 2014).

1.9.8.1.2. Nitrogen Laser:

This laser operates in the ultraviolet region at 337nm wavelength. Here, upper electronic level has a shorter lifetime compared to the lower one, hence continuous waves (CW) operation cannot be achieved, but pulsed operation with narrow pulse width is possible (Luchini and Motz, 1990).

1.9.8.1.3. Excimer Lasers:

Excimer lasers are a special sort of gas laser powered by an electric discharge in which the lasing medium is an excimer, or more precisely an exciplex in existing designs (Schuocker, 1998).

1.9.8.1.4. Helium –neon lasers:

The best-known and most widely used HeNe laser operates at a wavelength of 632.8 nm in the red part of the visible spectrum (Willet, 1974).

1.9.8.2. Chemical Lasers:

Chemical lasers are powered by a chemical reaction permitting a large amount of energy to be released quickly.

1.9.8.3. Solid State Lasers:

Solid-state lasers use a crystalline or glass rod which is "doped" with ions that provide the required energy states (Stewen, Larionov and Giesen, 2000).

A ruby laser is a solid-state laser that uses a synthetic ruby crystal as its gain medium (Maiman, 1960).

1.9.8.3.1. Nd:YAG Lasers:

Nd:YAG (neodymium-doped yttrium aluminium garnet; Nd:$Y_3Al_5O_{12}$) is a crystal that is used as a lasing medium for solid-state lasers. The dopant, triply ionized neodymium, Nd(III), typically replaces a small fraction (1%) of the yttrium ions in the host crystal structure of the yttrium aluminium garnet (YAG), since the two ions are of similar size. It is the neodymium ion which provides the lasing activity in the crystal. Laser operation of Nd:YAG was first demonstrated by J. E. Geusic et al. at Bell Laboratories in 1964 (Geusic, Marcos and Van Uitert, 1964).

1.9.8.3.2. Photonic Crystal Lasers:

Photonic crystal lasers are lasers based on nano-structures that provide the mode confinement and the density of optical states (DOS) structure required for the feedback to take place. They are typical micrometre-sized and tunable on the bands of the photonic crystals (Wu, 2004).

1.9.8.3.3. Semiconductor Lasers:

Semiconductor lasers are diodes which are electrically pumped. Recombination of electrons and holes created by the applied current introduces optical gain. Reflection from the ends of the crystal form an optical

resonator, although the resonator can be external to the semiconductor in some designs. Commercial laser diodes emit at wavelengths from 375 nm to 3500 nm (Hanel Photonics, 2014).

1.9.8.4. Dye Lasers:

Dye lasers use an organic dye as the gain medium. The wide gain spectrum of available dyes, or mixtures of dyes, allows these lasers to be highly tunable, or to produce very short-duration pulses (on the order of a few femtoseconds). Although these tunable lasers are mainly known in their liquid form, researchers have also demonstrated narrow-line width tunable emission in dispersive oscillator configurations incorporating solid-state dye gain media (Duarte, 2003; Ifflander, 2001).

1.9.9. Laser Classifications According to Safety:

The safety classes in the "old system" of classification were established in the United States through consensus standards (ANSI Z136.1) and federal and state regulations. Classification is also dependent on the wavelength and on whether the laser is pulsed or continuous (International Electrotechnical Commission, 2007).

1.9.9.1. Class I:

Inherently safe; no possibility of eye damage. Output power may be 0.5 mW.

1.9.9.2. Class IIa:

The blink reflex of the human eye (aversion response) will prevent eye damage, unless the person deliberately stares into the beam for an extended period. Output power may be up to 1 mW.

1.9.9.3. Class IIb:

A region in the low-power end of Class II where the laser requires in excess of 1,000 seconds of continuous viewing to produce a burn to the retina.

Commercial laser scanners are in this subclass. Output power may be 1.5 mW.

1.9.9.4. Class IIIa:

Lasers in this class are mostly dangerous. Direct contact with the eye for over two minutes may cause serious damage to the retina. Output power does not exceed 5 mW (International Electrotechnical Commission, 2007).

1.9.9.5. Class IIIb:

Lasers in this class may cause damage if the beam enters the eye directly. This generally applies to lasers powered from 5–500 mW. Lasers in this category can cause permanent eye damage with exposures of 1/100th of a second or less depending on the strength of the laser.

1.9.9.6. Class IV:

Lasers in this class have output powers of more than 500 mW in the beam and may cause severe, permanent damage to eye or skin without being magnified by optics of eye or instrumentation (International Electrotechnical Commission, 2007).

1.9.10. Laser Safety Guidelines:

Many scientists involved with lasers agree on the following guidelines:

- Everyone who uses a laser should be aware of the risks. This awareness is not just a matter of time spent with lasers; to the contrary, long-term dealing with invisible risks (such as from infrared laser beams) tends to reduce risk awareness, rather than to sharpen it (Institute of Technology, 1998).
- Optical experiments should be carried out on an optical table with all laser beams travelling in the horizontal plane only, and all beams should be stopped at the edges of the table. Users should never put their eyes at the

level of the horizontal plane where the beams are in case of reflected beams that leave the table.

- Watches and other jewelry that might enter the optical plane should not be allowed in the laboratory. All non-optical objects that are close to the optical plane should have a matte finish in order to prevent specular reflections.
- Adequate eye protection should always be required for everyone in the room if there is a significant risk for eye injury.
- High-intensity beams that can cause fire or skin damage (mainly from class 4 and ultraviolet lasers) and that are not frequently modified should be guided through opaque tubes.
- Alignment of beams and optical components should be performed at a reduced beam power whenever possible.

1.9.11. Low Level Laser Therapy:

Low-Level Laser Therapy (LLLT) is the use of low power visible and near-infrared monochromatic laser to enhance the body's natural healing processes. The laser source is placed in contact with the tissue, allowing the light energy (photons) to penetrate tissue where it interacts to increase circulation and help restore normal cellular function. LLLT does not break the skin as do surgical lasers.

The Food and Drug Administration (FDA) approved LLLT (with this approval came a new classification of therapeutic devices, referred to as NHN), known by many other names, such as cold laser, non-thermal laser, soft laser, biostimulation laser, low-intensity laser, and low-power laser therapy, as an effective method for temporary pain relief. The LLLT is the type of laser most commonly used in your doctor of chiropractic's office (Center for Device and Radiological Health, 2013).

The low-energy laser pulses can be adjusted to penetrate more deeply and more aggressively into the skin tissue, depending on the condition and goals of treatment. The light energy, which can be delivered by either a large device that emits multiple laser panels at once or a hand-held device for smaller targeted areas, will pass through the skin layers to reach the cells and tissue causing the pain and inflammation. The laser device is held against the skin over the area being treated. The light energy is absorbed and converted to biochemical energy, which stimulates the cells. That activates the natural healing process of the cells, which reduces pain, increases blood flow, and stimulates repair of the tissue (Enwemeka et al., 2005; Cancer orgnization, 2014).

LLLT is integrated with mainstream medicine with ongoing research to determine where there is a demonstrable effect. Areas of dispute include the ideal location of treatment (specifically whether LLLT is more appropriately used over nerves versus joints (Brosseau et al., 2005)), dose, wavelength, timing, pulsing and duration (Huang et al., 2009). The effects of LLLT appear to be limited to a specified set of wavelengths of laser, and administering LLLT below the dose range does not appear to be effective (Bjordal et al., 2008; Bjordal et al., 2003).

Despite a lack of consensus over its scientific validity, specific test and protocols for LLLT suggest it may be mildly effective, but in most cases no better than placebo, in relieving short-term pain for rheumatoid arthritis (Brosseau et al., 2005), osteoarthritis (Jamtvedt et al., 2007), acute and chronic neck pain (Chow et al., 2009), tendinopathy (Bjordal et al., 2008; Tumilty et al., 2010), and possibly chronic joint disorders (Bjordal et al., 2003).

1.9.11.1. History:

In 1967 a few years after the first working laser was invented, Endre Mester in Semmelweis University in Budapest, Hungary experimented with the

effects of lasers on skin cancer. While applying lasers to the backs of shaven mice, he noticed that the shaved hair grew back more quickly on the treated group than the untreated group (Mester, Szende and Tota, 1967).

1.9.11.2. Mechanism of Action of LLLT:

LLL supply energy to the body in the form of none thermal photons of light. The effects are thought to be mediated by photochemical reaction that alters cell membrane permeability, leading to increase messenger ribonucleic acid (mRNA) synthesis and cell proliferation. Its effects are not due to heat as in surgical laser but due to biochemical stimulation. These include augmentation of cellular adenosine triphosphate (ATP) levels, manipulation of inducible nitric oxide synthase activity, suppression of inflammatory cytokines such as tumor necrosing factor α (TNF-alpha), interleukin beta , interleukin -6, and interleukin-8.

Upregulation of growth factor production such as PDGF, IGF-1, nerve growth factor (NGF), and fibroblast growth factor (FGF2), alteration of mitochondrial membrane potential due to chromophores found in the mitochondrial respiratory chain as reviewed in stimulation of protein kinase C (PKC) activation, manipulation of NF-kB activation, direct bacterio-toxic effect mediated by induction of reactive oxygen species (ROS), modification of extra cellular matrix components, inhibition of apoptosis, stimulation of mast cell degranulation and upregulation of heat shock proteins (Hu et al., 2007; Aimbire et al., 2008).

1.9.11.3. Usage of LLLT:

1.9.11.3.1. Pain Relief:

Before recommending LLLT treatment, a provider must diagnose the condition to confirm that the issue is pain from a neuromusculoskeletal condition caused by aging, injury, or genetics and that there is no disqualifying condition or contraindication for laser use. For example, if the

back has visible lesions on the skin, it must first be confirmed that they are not cancerous before the patient can undergo laser therapy. Pregnant women also are not good candidates for LLLT; the effects on unborn children are unknown.

LLLT has various protocols for different conditions. The treatment provider must know the exact location, cause, degree, and frequency of back pain in order to select the appropriate protocol for optimum performance. LLLT is used to help heal wounds (Woodruff et al., 2003) and to treat many types of musculoskeletal injuries (Chow et al., 2008) and disorders. As research continues, the use of lasers is expanding. LLLT is used to treat both acute and chronic pain.

1.9.11.3.2. Targeting Inflammation:

For inflammation, laser therapy causes the smaller arteries and lymph vessels of the body to increase in size, which is called vasodilation. Vasodilation allows inflammation, swelling, and edema to be cleared away from injury sites more effectively. Vasodilation in lymph nodes promotes lymphatic drainage, which also aids in the healing process. Bruises are often resolved faster due to this effect.

The benefits of LLLT appear to be cumulative, it may take several treatments for the results to become evident. The total number of treatments needed depends on the condition being treated, the severity of the condition, and each patient's individual response.

1.9.11.3.3. Sports Injuries:

Doctors of chiropractic also use lasers to treat the weekend athlete with common sports injuries such as plantar fasciitis, hamstring pulls, and various muscular strains. The laser can help any level of athlete heal faster and with a more positive outcome (Leal Junior et al., 2008).

1.9.11.3.4. Other Usages of LLLT in Dermatology:

Low level lasers therapy (LLLT) is used for different cosmotological and skin diseases. It is used for treatment of fine wrinkles, acne scars, hair growth bio-stimulation, and stretch marks. LLLT is used for the treatment of diabetic ulcers, vascular ulcers, pressure sores, burn of the skin of second and third degree, and a treatment of different skin pathologies including cutaneus leishmaniases, keloids, psoriases, eczema, acne vulgaris, herpis simplix, warts, and verrucae vulgaris.

CHAPTER TWO

Laser Applications in Treating Vitiligo

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2.1. Introduction:

Lasers and light sources have become more commonplace in the treatment of dermatologic medical diseases. There are many lasers used for treating vitiligo as phototherapy, which are different in types, wavelengths, and fluencies used. Lasers are used as a unique treatment of vitiligo or used in combination with other medical treatments. Vitiligo also treated by incoherent light in the region of UV-A and UV-B. Over the last fifteen years, the field of laser and light therapies has continued to grow and expanded with the appearance of new laser and light sources. What has become clear is that a significant understanding of lasers and light sources is required for optimum use of this technology (Goldberg, 2005).

A basic understanding of laser physics is also fundamental to obtain good laser treatments and widen the laser applications in medicine as general and in dermatology as specific. Laser safety and minimizing risk to patients is at least as important as understanding of laser physics. When those concepts are understood, skin laser and light technology can be safely and successfully used for variety of purposes.

There are two types of lasers, surgical lasers (high power lasers), and low level lasers (LLL). The surgical lasers depends upon thermal effects on the biological tissues while low level lasers have bio-stimulatory effects. The physiological effects of low level laser therapy (LLLT) include, improvement of cell metabolism, stimulate collagen production, improved blood circulation and vasodilatation, anti-inflammatory and anti-oedematus effects, stimulate wound healing, stimulate the immune system, stimulate nerve function, relieves acute and chronic pain (Omega company, 2006; Savai et al., 2008; Saygun et al., 2008; Schwartz et al., 2002; Yousefi-Nooraie, 2008).

2.2. Mechanism of Action of LLLT in Vitiligo:

The cellular responses observed in vitro after LLLT can be broadly classed under increases in metabolism, migration, proliferation, and increases in synthesis and secretion of various proteins. Many studies report effects on more than one of these parameters. Yu et al. reported on cultured keratinocytes and fibroblasts that were irradiated with 0.5-1.5 J/cm2 HeNe laser (632.8 nm). They found a significant increase in basic fibroblast growth factor (bFGF) release from both keratinocytes and fibroblasts, and a significant increase in nerve growth factor release from keratinocytes. Medium from laser irradiated keratinocytes stimulated [3H]thymidine uptake, and the proliferation of cultured melanocytes. Furthermore, melanocyte migration was enhanced either directly by HeNe laser or indirectly by the medium derived from HeNe laser (632.8 nm) treated keratinocytes (Yu et al., 2003).

The presence of cellular responses to LLLT at molecular level was also demonstrated. Normal human fibroblasts were exposed for 3 days to 0.88J/cm2 of 628 nm light from a light emitting diode. Gene expression profiles upon irradiation were examined using a cDNA microarray containing 9982 human genes. 111 genes were found to be affected by light. All genes from the antioxidant related category and genes related to energy metabolism and respiratory chain were upregulated. Most of the genes related to cell proliferation were upregulated too. Amongst genes related to apoptosis and stress response, some genes such as JAK binding protein were upregulated, others such as HSP701A, caspase 6 and stress-induced phosphoprotein were downregulated. It was suggested that LLLT stimulates cell growth directly by regulating the expression of specific genes, as well as indirectly by regulating the expression of the genes related to DNA synthesis and repair, and cell metabolism (Zhang et al., 2003).

2.3. Considerations of Using LLLT:

- Wavelength: This is probably the parameter where there is most agreement in the LLLT community. Wavelengths in the 600-700 nm range are chosen for treating superficial tissue, and wavelengths between 780 and 950 nm are chosen for deeper-seated tissues, due to longer optical penetration distances through tissue. Wavelengths between 700 and 770 nm are not considered to have much activity. Some devices combine a red wavelength with a NIR wavelength on the basis that the combination of two wavelengths can have additive effects, and can also allow the device to be more broadly utilized to treat more diseases. There is of course much more work to be done to define what is the optimum wavelength for the different indications for which LLLT is employed (Hamblin, 2008).
- Laser vs. non-coherent light: One of the most topical and widely discussed issues in the LLLT clinical community is whether the coherence and monochromatic nature of laser radiation have additional benefits, as compared with more broad-band light from a conventional light source or LED with the same center wavelength and intensity. Two aspects of this problem must be distinguished: the coherence of light itself and the coherence of the interaction of light with matter (biomolecules, tissues). The latter interaction produces the phenomenon known as laser speckle, which has been postulated to play a role in the photobiomodulation interaction with cells and subcellular organelles. It is difficult to design an experiment to directly compare coherent laser light with non-coherent non-laser light for the following reason. Laser light is almost always monochromatic with a bandwidth of 1 nm or less, and it is very difficult to generate light from any other source (even an LED) that has a bandwidth narrower than 10-20 nm, therefore it will be uncertain if observed differences are due to coherent versus non-coherent light, or due to monochromatic versus narrow bandwidth light (Hamblin, 2008).

- Dose: Because of the possible existence of a biphasic dose response curve referred to above, choosing the correct dosage of light (in terms of energy density) for any specific medical condition is difficult. In addition there has been some confusion in the literature about the delivered fluence when the light spot is small. If 5J of light is given to a spot of 5 mm2, the fluence is 100 J/cm2, which is nominally the same fluence as 100 J/cm2 delivered to 10 cm2, but the total energy delivered in the latter case is 200 times greater. The dose of light that is used depends on the pathology being treated, and in particular upon how deep the light is thought to need to penetrate into the tissue. Doses that are frequently used in the red wavelengths for fairly superficial diseases tend to be in the region of 4 J/cm2 with a range of 1-10 J/cm2. Doses of the NIR wavelengths that tend to be employed for deeper-seated disorders can be higher than these values, i.e., in the 10-50 J/cm2 range. The light treatment is usually repeated either every day or every other day, and a course of treatment can last for periods around two weeks (Hamblin, 2008).
- Pulsed or CW: There have been some reports that pulse structure is an important factor in LLLT; for instance Ueda et al. found better effects using 1 or 2 Hz pulses than 8 Hz or CW 830 nm laser on rat bone cells, but the underlying mechanism for this effect is unclear (Ueda and Shimizu, 2001; Ueda and Shimizu, 2003).
- Polarization status: There are some claims that polarized light has better effects in LLLT applications than otherwise identical non-polarized light (or even 90-degree rotated polarized light) (Ribeiro et al., 2004). However, it is known that polarized light is rapidly scrambled in highly scattering media such as tissue (probably in the first few hundred μm), and it therefore seems highly unlikely that polarization could play a role, except for superficial applications to the upper layers of the skin.
- Systemic effects: Although LLLT is mostly applied to localized diseases and its effect is often considered to be restricted to the irradiated area, there are reports of systemic effects of LLLT acting at a site distant from

the illumination (Moshkovska and Mayberry, 2005; Santana-Blank, Rodriguez-Santana and Santana-Rodriguez, 2005). It is well known that UV light can have systemic effects (Kripke, 1994), and it has been proposed that red and NIR light can also have systemic effects. These have been proposed to be mediated by soluble mediators such as endorphins and serotonin. There is a whole field known as laser acupuncture (Whittaker, 2004) in which the stimulation of specific acupuncture points by a focused laser beam is proposed to have similar effects at distant locations to the more well known needle acupuncture techniques.

2.4. Excimer Lasers:

The xenon chloride excimer laser emits a wavelength of 308 nm. The excimer laser has some useful technical characteristics like an articulated arm and variable spot size. It is possible to selectively turn the beam of light and to treat only the involved area, sparing healthy skin. In vitiligo, this selectivity limits the unwanted hyperpigmentation of non involved skin, which is commonly observed with the other phototherapies. The articulated arm makes it possible to treat body regions that are otherwise difficult to reach (eg, folds and mucosa). Disadvantages include the fact that the limited spot size means that large surfaces (>20% of total surface body area) cannot be treated and the purchase and maintenance costs of these devices remain quite expensive (Passeron and Ortonne, 2006).

In 2006, Hadi et al performed a retrospective chart review of 97 patients (a total of 221 vitiligo patches) with chronic stable vitiligo treated with the excimer laser and concluded that it is an effective and safe modality for the treatment of vitiligo, with good results achieved in a relatively short duration of time. Lesions on the face responded better than lesions elsewhere (Hadi, Tinio and Al-Ghaithi, 2006).

The aim of this study was to study the effectiveness of the 308-nm xenon chloride excimer laser in the treatment of vitiligo and to determine factors that favor a good response to treatment.

Targeted phototherapy using the 308-nm xenon chloride excimer laser represents an effective therapy for the management of vitiligo. However, studies on a large number of patients are few despite the increasing use of the excimer laser to treat patients with vitiligo.

Out of 221 vitiligo patches treated, 50.6% showed 75% pigmentation or more, 25.5% achieved 100% pigmentation of their patches, and 64.3% showed 50% pigmentation or more. Lesions on the face responded better than lesions elsewhere.

The study concluded that, the 308-nm xenon chloride excimer laser is an effective and safe modality for the treatment of vitiligo, with good results achieved in a relatively short duration of time.

In 2006, Passeron and Ortonne performed a review of 10 studies on the use of the 308-nm excimer laser for psoriasis and vitiligo. For vitiligo, low fluencies (from 50 to 200 mJ/cm2) were used in 1 to 3 sessions a week for 1 to 6 months, depending on the study. The number of plaques with repigmentation at the end of the treatment was high (57%–100%). On average, 20% to 30% of treated plaques reach more than 75% repigmentation, but some series report conflicting results (from 0% to 75%). The clinical response to the treatment is especially dependent of the localization of the lesions. UV-resistant areas (eg, extremities and bony prominences) were more difficult to treat (Passeron and Ortonne, 2006).

In 2004, Passeron et al examined the efficacy of combined treatment with 0.1% tacrolimus ointment plus 308 nm excimer laser versus excimer laser monotherapy. They concluded that the combined therapy was only superior to the excimer laser monotherapy for the treatment of UV-resistant vitiliginous lesions (Passeron, Ostovari and Zakaria, 2004).

In 2007, Goldinger et al did a prospective (left-right) comparative, single blinded study to compare the effectiveness of the excimer laser and the combination of the excimer laser with topical calcipotriol in the treatment of vitiligo. They showed no significant difference in overall repigmentation between the two groups (Goldinger, Dummer and Schmid, 2007).

A large variety of therapeutic agents are being used for the treatment of vitiligo, but treatment remains a challenge. Recently, monochromatic phototherapies such as 311-nm narrowband ultraviolet B therapy and 308-nm xenon chloride excimer laser have been reported to be an effective and safe therapeutic option in children and adult patients with vitiligo. Single reports stipulate that the addition of topically applied calcipotriol to phototherapy increases its effectiveness.

The purpose of this pilot study was to determine if the addition of topical calcipotriol increases the efficacy of the 308-nm xenon chloride excimer in the treatment of vitiligo.

Ten patients with vitiligo with essentially bilateral symmetrical lesions were enrolled in this prospective right/left comparative, single-blinded trial conducted over a 15-month period. All patients received 308-nm XeCl excimer laser therapy three times weekly. Calcipotriol ointment (Daivonex) was applied to lesions on one side of the body twice daily.

After 24 treatments (8 weeks), nine patients were evaluated. Eight patients showed evidence of repigmentation on both body sides, with no significant difference between the body side treated with calcipotriol and excimer laser and the side treated with excimer laser alone. The mean repigmentation rate was 22.4% (1-37%).

The study concluded that, the addition of calcipotriol ointment to 308-nm xenon chloride excimer laser phototherapy does not significantly enhance its efficacy. Small additive effects must be investigated in a larger trial.

In 2008, Sassi et al did a randomized, controlled trial to compare the effectiveness of the 308 nm excimer laser alone or in combination with topical hydrocortisone 17-butyrate cream in patients with vitiligo, unresponsive to previous treatment with topical steroids or narrowband ultraviolet B phototherapy (Sassi, Cazzaniga and Tessari, 2008).

Consecutive patients aged 18-75 years with nonsegmental vitiligo localized on the face and/or neck lacking response to previous conventional treatment were eligible. In total, 84 patients (44 women and 40 men, mean age 44 years) were randomized to 308-nm excimer laser phototherapy twice weekly alone or in combination with topical hydrocortisone 17-butyrate cream twice daily for three periods of 3 weeks followed by a 1-week steroid-free interval. The primary outcome was a reduction of at least 75% of the overall lesional areas as judged by automatic image analysis on reflected UV photographs, conducted blind to treatment assignment, at 12 weeks compared with baseline. Secondary outcomes were clearance, and improvements on Physician's Global Assessment (PGA) and Skindex-29 scores (Sassi, Cazzaniga and Tessari, 2008).

A total of 76 (90%) patients completed the study. In an intention-to-treat analysis, seven [16.6%; 95% confidence interval (CI) 5.3-27.8%] patients in the excimer monotherapy arm and 18 (42.8%; 95% CI 27.8-57.8%) in the combination arm showed > or = 75% reduction of vitiligo lesions at 12 weeks (chi^2 test 6.89, P = 0.0087). Clearance was observed in two (4.7%; 95% CI 1.6-11.2%) and nine (21.4%; 95% CI 9.0-33.8%) patients, respectively (Fisher's exact test P = 0.04). A significant difference also emerged for PGA scores, while no difference was documented for Skindex-29.

The study concluded that, recalcitrant vitiligo of the face and neck may benefit from the combination of excimer laser phototherapy with topical hydrocortisone 17-butyrate cream.

In 2005, Seok –Beom Hong et al performed a study to compare the clinical efficacy of a short-term intervention of 308-nm excimer laser with that of narrow-band UVB (NBUVB) phototherapy for vitiligo patients to see the early response. Twenty-three symmetrically patterned patches of vitiligo on 8 patients were selected. Vitiligo patches on one side of the body were treated 2 times per week for a maximum of 20 treatments with the excimer laser, and NBUVB phototherapy was used on patches on the other side (Hong, Park and Lee, 2005).

Improvement (repigmentation) was assessed on a visual scale via serial photographs taken every five treatments and scored as follows: 0, ≤1% improvement; 1, ≤25% improvement; 2, 26-50% improvement; 3, 51-75% improvement; and 4, ≥75% improvement. At five treatments, the excimer laser-treated patches had an average score of 0.26, compared with 0.04 for patches treated with NBUVB phototherapy. A slightly higher repigmentation ($p>0.05$) in the excimer treated area was thus observed. At 10, 15, or 20 treatments, the differences between the average scores were significant: 0.83, 1.17, and 1.39 for the excimer-treated patches, and 0.17, 0.30, and 0.74 for the NBUVB phototherapy-treated areas ($p<0.05$).

The study concluded that, the 308-nm excimer laser appears to be more effective than NBUVB phototherapy, as it produces more rapid and profound repigmentation.

In 2011, John A. Mouzakis et al performed a study to support the use of excimer laser in treating vitiligo especially of the face. Patients with extensive facial depigmentation were treated with excimer laser twice weekly and calcipotriene daily until they developed significant repigmentation. Evaluation and treatment was performed at the Veterans Affairs outpatient dermatology clinic in Tampa, Florida. Three patients with Fitzpatrick skin types IV to VI were selected. These patients had failed a variety of topical

treatments including steroids and calcipotriene, but were light naïve prior to beginning the study (John et al., 2011).

The primary outcome measure employed was percent repigmentation by visual estimation. The average dose of radiation, number of treatments, and weeks of therapy were also recorded. All three patients experienced greater than 75 percent repigmentation of their facial vitiligo over a treatment course from 10 to 20 weeks.

The study concluded that the excimer laser is a viable treatment for vitiligo and may yield results more expeditiously than other commonly utilized therapies. The rapid response may be correlated with skin type, but a more extensive study needs to be undertaken to further evaluate this correlation.

In 2004, Suhail M. et al performed a study to evaluate the effectiveness of the new 308-nm excimer laser for the treatment of vitiligo. A retrospective chart review of thirty-two patients with 55 spots of vitiligo were enrolled; a population-based sample was studied that included men and women, adults and children, with different ethnic backgrounds. The treatment was started with the lowest dose, which is 100 mJ/cm2 (comparable to one minimal erythema dose value and one multiplier). Depending on Fitzpatrick skin type, the dose was raised gradually in a stepwise fashion. In skin types I to II, the same does was repeated twice before going up to avoid burns. Patients were treated for 30 sessions, or 75% repigmentation, whichever comes first (Hadi et al., 2004).

Overall 55 spots were treated: 29 (52.8%) had 75% pigmentation or greater, and 35 (63.7%) had 50% pigmentation or greater. The best results were on the face: of the 21 spots treated 15 (71.5%) had 75% pigmentation, and 16 (76.2%) had 50% pigmentation or greater. Other areas (neck, extremities, trunk, and genitals) had moderate response in comparison to the face. The least response was on the hands and feet; of the 5 spots treated only 20% showed 50% pigmentation or more.

The study concluded that Slightly more than 50% of the patients tested showed 75% or more pigmentation of their lesions, after 30 treatments or less; most of the responders had Fitzpatrick skin type III and above. All the untreated patches (controls) remained unchanged. This demonstrates that the 308-nm excimer laser is an effective method of treatment for vitiligo.

The excimer laser is a promising therapy for vitiligo, with good clinical results, especially for the treatment of vitiligo lesions on the face.

2.5. Helium –Neon Lasers:

In 2003, Yu Hs et al performed a study to determine the theoretical basis and clinical evidence for the effectiveness of helium-neon lasers in treating vitiligo. Cultured keratinocytes and fibroblasts were irradiated with 0.5-1.5 J per cm^2 helium-neon laser radiation. The effects of the helium-neon laser on melanocyte growth and proliferation were investigated. The results of this in vitro study revealed a significant increase in basic fibroblast growth factor release from both keratinocytes and fibroblasts and a significant increase in nerve growth factor release from keratinocytes (HS. et al., 2003).

Medium from helium-neon laser irradiated keratinocytes stimulated [3H] thymidine uptake and proliferation of cultured melanocytes. Furthermore, melanocyte migration was enhanced either directly by helium-neon laser irradiation or indirectly by the medium derived from helium-neon laser treated keratinocytes. Thirty patients with segmental-type vitiligo on the head and/or neck were enrolled in this study. Helium-neon laser light was administered locally at 3.0 J per cm2 with point stimulation once or twice weekly. The percentage of repigmented area was used for clinical evaluation of effectiveness. After an average of 16 treatment sessions, initial repigmentation was noticed. Marked repigmentation (>50%) was observed in 60% of patients with successive treatments. Basic fibroblast growth factor is a putative melanocyte growth factor, whereas nerve growth factor is a paracrine

factor for melanocyte survival in the skin. Both nerve growth factor and basic fibroblast growth factor stimulate melanocyte migration.

The study concluded that helium-neon laser irradiation clearly stimulates melanocyte migration and proliferation and mitogen release for melanocyte growth and may also rescue damaged melanocytes, therefore providing a microenvironment for inducing repigmentation in vitiligo.

In 2008, Wu CS et al perform a study using a continues wave He-Ne laser with an average power output of 1 mW .It was designed for point stimulation (irradiation point by point) once or twice a week , and the irradiation fluence for each treatment point was 3 J/cm^2 . Initial depigmentation was noticed after an average of 17 He-Ne laser therapy sessions. Marked repigmentation (more than 50%) was observed in 60% of patients following successive treatment and three patients (7.5%) showed 100% recovery. They are considered to be safe and no obvious adverse effects were found (Wu et al., 2008).

In 2011, Ataie L performed a study using 630 nm GaAlAs laser (20 mW , 1 J/cm^2) twice a week for maximum of 24 treatments. Reduction in surface area of vitiligo patches were seen ranged between 25% - 75% (Ataie, 2011).

In 2010, Yu W-t et al performed a study used continues wave low energy GaAlAs laser at 635 nm, 3 J/cm^2, (24 sessions), showed greater than 25% repigmentation of the patches (W-t, Hs and CH-SH, 2010).

2.6. Carbon Dioxide Lasers:

In 1997, Keith Allen Knoell et al performed a study to evaluate the treatment of vitiligo with ultrapulse carbon dioxide laser. Six patients (average age, 39 years) with diffuse vitiligo (average duration, 18 years) were divided into 2 groups: those who were receiving but had not responded to PUVA therapy (Keith, Avraham and Sandy, 1997). Vitiligo lesions were treated with the ultrapulse carbon dioxide laser and concomitant PUVA treatments in 2 patients who did not respond to PUVA therapy alone in the hope that growth

factors created by superficial epidermal destruction would potentiate the effect of PUVA locally. These results were compared with those seen in 4 other patients who also received ultrapulse carbon dioxide laser treatment of vitiligo macules who were not receiving PUVA therapy. Only laser-treated lesions in patients receiving PUVA, who did not respond previously to PUVA therapy alone, showed re-pigmentation.

In 2012, Shin J et al performed a study to investigate the effects of fractional carbon dioxide (CO_2) laser therapy followed by systemic narrowband ultraviolet B (NB-UVB) phototherapy on nonsegmental vitiligo (NSV) as a prospective and randomized left-right comparative study.

Ten patients with NSV who presented symmetrical vitiligo lesions with no further improvement despite more than 1 year of conventional treatment were enrolled. Two sessions of half-body fractional CO_2 laser therapy were performed at a 2-month interval. NB-UVB phototherapy was then administered to the entire body 5 days after each fractional laser treatment twice a week, increasing the dose incrementally by 15% at each session. Objective clinical assessments were made by two blinded dermatologists using a quartile grading scale, and the patients' overall satisfaction was evaluated using a 10-point visual analogue scale.

Two months after the last treatment, mean improvement scores, assessed by physicians, were significantly higher for those treated with half-body fractional CO_2 laser therapy followed by NB-UVB phototherapy, compared with those treated with NB-UVB alone (P=0·034). In addition, according to subjective assessment, the half-body laser treatment followed by NB-UVB showed significantly higher improvements compared with NB-UVB treatment alone (P=0·023). Noticeable adverse events, such as infection, scarring and Koebner phenomenon, were not found in any patient.

The study concluded that fractional CO_2 laser therapy followed by NB-UVB phototherapy could be used effectively and safely as an alternative modality for the treatment of refractory vitiligo.

In 2014, Hélou J et al performed a study to investigate the effects of fractional CO_2 laser followed by systemic sun exposure on non-segmental vitiligo (NSV). Ten patients presenting refractory NSV were enrolled in this study. The patients underwent three sessions, one month apart, of fractional CO_2 laser therapy on the affected areas of the skin (L-group). Five days after each laser treatment, patients were asked to expose themselves to the sun for 2 hours on a daily basis. Objective and subjective clinical assessments were performed at the beginning and at the end of the treatment. The L-group was then compared to a control group (C-group) that consisted of vitiligo lesions in the same patients but with sun exposure as the exclusive therapy (Hélou et al., 2014).

Compared to the C-group, the L-group showed better improvement in both objective and subjective assessments. There were no noticeable adverse events in terms of scarring and Koebner phenomenon among others.

The study concluded that all patients treated with both, laser sessions and sun exposure, improved their chronic NSV lesions. Improvement was less significant in patients who exhibited vitiligo lesions over articular surfaces such as elbows and underarms. The best results were observed in vitiligo plaques located on the face, neck and legs. Consequently, fractional CO_2 laser followed by sun exposure could be considered as an alternative modality for the treatment of refractory vitiligo, especially in sunny regions.

2.7. Incoherent Light Sources:

To my knowledge, no researches were done for the treatment of vitiligo using incoherent visible and infrared light sources.

CHAPTER THREE

Materials and Methods

CHAPTER THREE

Materials and Methods

3.1. Introduction:

This chapter, concentrate on the materials and methods used in this study which includes the study design, patients, patients record , description of the laser and incoherent light sources used in this study, the light parameters , and clinical assessments.

3.2. Study Design:

This is a prospective clinical descriptive intervention study conducted during the period from Oct 2009 to Jan 2015.

3.3. Study Area:

The study was conducted at the Institute of Laser, Sudan University of Science and Technology, and applied in the private clinic at Asia hospital.

3.4. Study Population:

This study was conducted on forty eight Sudanese patients. Nine patients were excluded from the study due to their disease activity shown before and during the study period, so thirty nine patients completed the treatment in this study. The sample size was randomly chosen from the patients with vitiligo who attended Asia hospital, Omdurman locality, Khartoum state.

3.5. The Materials:

3.5.1 The Laser Medical System:

The Omega Xp Mobile laser system was used in this study. The Xp Mobile is more traditionally manufactured in steel for robustness. It is lighter, more compact and is provided in a padded carry case.

Figure (3-1) shows a photograph of this device, and table (3-1) lists its characteristics.

Figure (3-1): Omega Xp Mobile Laser Medical System.

Extremely versatile, the Xp Mobile accepts the complete range of Omega's interchangeable probes, allowing treatment of everything from superficial wound healing to deeper muscular penetration and acupuncture point stimulation. The flexibility to enter repeatable treatment times in 5 second increments is offered via an intuitive menu system and the unit then automatically calculates the resultant energy density 'dose' to be delivered.

Table (3-1): Omega Xp Mobile Characteristics.

Classification	3 B Laser
Medium	GaAIAs
Size	H:140 x D:125 x W:220mm
Weight	3.02 kilos
Pulsing Frequencies	2.5 Hz, 10 Hz, 20 Hz, 73 Hz, 146 Hz, 700 Hz,

	1 kHz, 5 khz, 10 kHz
Battery Life	Cluster Probe 4 hours; Single probe 10 hours
Rechargeable	Metal Nickel Hydryde 4.1 Ah

The Mobile Xp is also has Straightforward and helpful menu system, Automatic probe recognition, Automatic calculation of surface energy density, Nine pulsing frequency options, Multi-pulse mode, Automatic repeat treatment time, Total treatment time record, Precise laser beam power output measurement, Visible warning when probe capable of emitting or in use, Acupuncture point finder, High performance 4Ah Metal Nickel Hydryde battery built in, Wipe-clean membrane fascia, and Padded carry case and stand included.

3.5.2. Omega Laser Probes:

The Omega Laser system used in this work involves one control unit, and several probes.

3.5.2.1. Visible Red Laser Probe (675 nm):

The specifications of this probe are listed in table (3-2).

Figure (3-2): 675 nm Laser Single Probe.

Table (3-2): 675 nm Laser Single Probe Characteristics.

Wavelength	675 nm
Coherence	Laser
NOHD	120 mm
Beam Divergence	9× 38 degrees Typ
Power output	30 mW
Laser Class	3 B
Lasing Medium	GaAlAs

3.5.2.2. 820 nm 50 mW Infrared Laser Probe:

The specifications of this probe are listed in table (3-3).

Table (3-3): 820 nm 50 mW Infrared Laser Probe Characteristics.

Wavelength	820 nm
Coherence	Laser
NOHD	155 mm
Beam Divergence	25× 10 degrees Typ
Power output	50 mW
Laser Class	3 B
Lasing Medium	GaAlAs

3.5.2.3. 60 Diode Cluster Probe (Incoherent Light):

This probe contains 60 LEDs emitting different wavelengths cover the visible and NIR range. Its specifications are listed in table (3-4).

Figure (3-3): 60 Diode Cluster Probe None Laser (Incoherent Light) Diodes.

Table (3-4): 60 Diode Cluster Probe None Laser (Incoherent Light) Diodes Characteristics.

Wavelengths	24 × 660 nm 10 mW LED's 12 × 810 nm 20 mW LED's 12 × 940 nm 20 mW LED's 6 × 880 nm 20 mW LED's 6 × 850 nm 20 mW LED's
Coherence	Incoherent
Power Density	0.100 W/cm^2

3.5.2.4. 46 Diode Cluster Probe:

This probe contains one laser diode and 45 non laser diodes emitting different wavelengths cover the visible and NIR range. Its specifications are listed in table (3-5).

Table (3-5): 46 Diode Cluster Probe Characteristics.

Characteristics	Laser Diode	Non Laser Diodes
Wavelengths	820 nm	10 × 660 nm 15 mW LED's 10 × 950 nm 15 mW LED's 10 × 870 nm 25 mW LED's 10 × 880 nm 25 mW LED's 5 × 940 nm 25 mW LED's
Power output	15 Mw	-
NOHD	215 mm	-
Laser Class	3 B	-
Lasing Medium	GaAlAs	GaAlAs
Coherence	Laser	Incoherent

The Xp Mobile offers a range of nine pulsing frequency options and the ability to set three different pulsing repetition rates within one treatment through the multi-pulse facility. This affords the practitioner pulse variation capability without having to reset. This control unit has a built-in beam tester which gives an accurate reading of the single probe outputs, meaning that the user can always check that the equipment is working properly – so important when invisible wavelengths are in use.

The Xp Mobile also includes an acupuncture point finder, which gives a continuous electronic reading indicating the proximity of acupuncture points. The micro-current conductivity measurement which is used to identify such points can also locate trigger points making it an indispensable tool for physical therapists. This feature utilises a purpose-designed hand probe and acupuncture probe tip, both of which are included at no additional cost when this control unit is purchased with an appropriate probe.

This unit is designed so that it can be used in the carry case, and placing the bag's strap over the shoulder or around the waist can leave the clinician's hands free for treatment. When used in the clinic the separate stand provided positions the unit with complete stability and visibility on any flat surface.

In our study, we used only two probes, the visible red laser probe (675 nm) and 60 diode cluster probe (incoherent light).

3.6. The Patients:

Thirty nine patients were involved in this study, seventeen of them received incoherent light treatment, and twenty two of them received laser treatment. The treatment procedure was described to the patients and all of them were signed a consent for the treatment details.

3.6.1. Inclusion Criteria:

- All sites of vitiligo.
- Different sizes of vitiligo patches.
- Patients with skin phototype IV-VI.
- All age groups.
- Both sexes.

3.6.2. Exclusion Criteria:

- Patients who received other treatment for vitiligo in a period less than 3 months.
- Patients who have any type of malignancy.
- Pregnant and lactating ladies.
- Patients who had disease activity before and during the period of treatment.

3.6.3. Patient's Record:

A record was designed and filled for each patient with vitiligo included in this study. The record contained:

1. Name, age, sex, skin type, history, date, examination notes and results of investigations.
2. Duration of the lesions.
3. Site of the lesions.
4. Followup photographs.
5. Laser and incoherent light parameters used for each patch.
6. Notes about the response to the treatment.
7. Consent forms for laser treatment.

3.6.4. The Patient's Photographs:

Photographs were taken for each patient showing:

- Lesions before treatment.
- The same lesions at the end of the treatment.

3.6.5. Ethical and Legal Considerations:

All patients agreed and signed a consent form. The consent form is written in simple Arabic language.

3.6.6. Evaluation of the Clinical Response:

The area of the patches was measured before and after treatment by specific rulers. According to the degree of reduction in the area of each patch and the clinical observation, the clinical evaluation after treatment was as follows:

- 75-100% reduction of the surface area = Excellent.

- 50-74 % reduction of the surface area = Very Good.
- 25-49% reduction of the surface area = Good.
- Less than 25% reduction of the surface area = Poor.
- 0% reduction of the surface area = Bad.

3.6.7. Clinical Safety:

The suitability of the patients for laser treatment was assessed and documented before commencing treatment and the treatment records were provided for that purpose. A part for the requirements to protect the eye for the potential ocular hazards, all cautions were designed to protect the clinician as much as the patients, and in many cases were based on caution rather than any proven medical concern.

3.6.8. Safety Precautions of the Omega Xp Laser System:

1. The user must always connect the power supply cable with the key switch in the off position.
2. The laser must be off before disconnecting the cable.
3. The user must never put fingers or metal objects in the laser apertures to avoid damage to the laser diodes.
4. In the event of danger, the switch must be down by means of the red button.
5. The apparatus must not be used by unauthorized personnel.

3.6.9. Laser Room Precautions:

- All warning signs were made clear and put in the appropriate locations.
- Reflective objects were strictly prohibited in the operating room, the walls were made rough, the windows were closed and any opaque materials were not allowed.

- When the laser system is in use, the restricted room entrance is obeyed.

3.6.10. Other Materials:

- **Yamidine:** Povidine –Iodine 10% which is usually used as an antiseptic tincture for skin against bacteria, was applied two minutes pre-operatively as a dye to cover the white patches by an amber colour before applying laser and incoherent light treatment, (See figure (3-4)).

Figure (3-4): Yamidine.

In this study, Yamidine tincture gives the skin an amber colour which simulates the colour of melanin which absorbs red and infrared light, that means it was used in this study to help the chromophore in the white patches to absorb red laser and incoherent red and infrared light and initiate the biochemical effects of the low level laser and incoherent light. Up to our knowledge, this is the first time that yamidine tincture is used as a chromophore applied in human skin, (See figure 3-5).

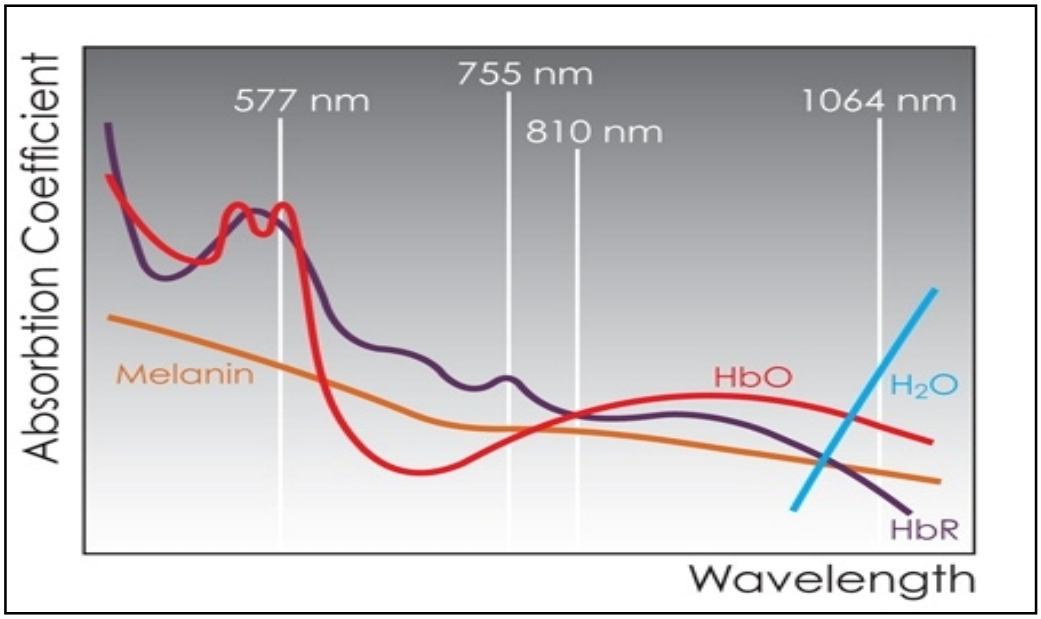

Figure (3-5): Absorbtion Coefficient of Different Skin Chromophores.

3.7. The Methods:

3.7.1. Preparations of Patients:

The steps done for preparation of the patient for the laser treatment were as follows:

- Patient was asked about his personnel data and medical history to be recorded in the laser therapy form.
- Patient has to fill the consent form.
- Initial photographs were taken showing the patient patches from a suitable distance.
- The white patches were stained by yamidine dye two minutes before the starting the treatment.

3.7.2. Treatment Apparatus Settings:

Two probes of the Omega xp device were used , the first probe emitting red laser in a wavelength of 675 nm, and the other probe containing cluster of 60 light (incoherent) emitting diodes (LED) combined together and emitting light

of different wavelengths (660 nm, 810 nm , 940 nm, 880 nm, 850 nm) at once.

The parameters used for laser probe were:

- **Fluence:** 2.4 J/cm^2**.**
- **Exposure time:** 10 sec.
- **Pulse Mode:** Multi pulse mode, 10 Hz, 2.5 Hz, and 20 Hz, respectively, which were divided equally over 10 seconds automatically by the device.
- **Application Direction:** Contact mode was used to cover the whole patch by the cluster probe while a single laser probe was used in contact with the borders of the patch in points.

The parameters that were used for the cluster probe:

- **Fluence:** 2 J/cm^2**.**
- **Exposure time:** 20 sec.
- **Pulse Mode:** Multi pulse mode, 10 Hz, 2.5 Hz, and 20 Hz, respectively.
- **Application direction:** Contact mode was used to cover the whole patch by the cluster probe.

3.7.3. Post –Treatment Care:

No need for post – Treatment care except washing the dye by soap.

3.7.4. Frequency of Sessions:

Once per week.

3.7.5. The Follow-Up:

All patients have been followed up starting from day one till the end of treatment every two weeks.

CHAPTER FOUR

Results and Discussion

CHAPTER FOUR

Results and Discussion

4.1 Introduction:

This chapter covers the results of the treatment and discusses the advantages and disadvantages of treatment of vitiligo for thirty nine patients by Low level laser and incoherent light.

4.2. Patients Data:

4.2.1. Patients Age:

Ages of patients were ranged from 15 to 68 years. Three patients (7.69%) were 15 years old and less. Seventeen patients (43.59%) were between 16-25 years old. Twelve patients (30.77%) were between 26-35 years old. Three patients (7.69%) were between 36-45 years old. Three patients (7.69%) were between 46-55 years old. And One patient (2.56%) was between 56-65 years old (See table 4-1).

Table (4-1): Patients Age.

Age (year)	Frequency	Percentage (%)
< = 15.00	3	7.69
16.00 - 25.00	17	43.59
26.00 - 35.00	12	30.77
36.00 - 45.00	3	7.69
46.00 - 55.00	3	7.69
56.00 and more	1	2.56
Total	**39**	**100**

4.2.2. Patients Sex:

Seventeen patients (43.59%) were males, and twenty two patients (56.41%) were females, (Figure 4-1).

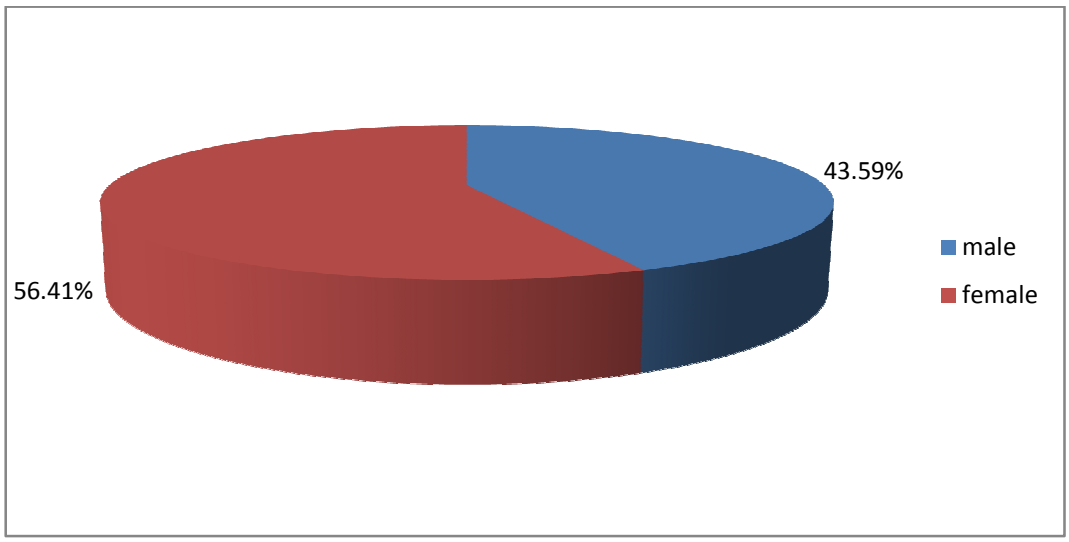

Figure (4-1): Patients Sex.

4.2.3. Duration of Vitiligo:

The duration of vitiligo for eleven patients (28.21%) was less than one year, for seven patients (17.95%) was between 1-2 years, for three patients (7.69%) was between 3-4 years, for four patients (10.26%) was between 5-6 years, for five patients (12.82%) was between 7-8 years, for four patients (10.26%) was between 9-10 years, for four patients (10.26%) was between 13-14 years, and for one patient (2.56%) was 20 years (Table 4-2).

Table (4-2): Duration of Vitiligo.

Duration	Frequency	Percentage (%)
Less than one year	11	28.21
1-2 years	7	17.95
3-4 years	3	7.69
5-6 years	4	10.26
7-8 years	5	12.82

9-10 years	4	10.26
13-14 years	4	10.26
15-20 years	1	2.56
Total	**39**	**100**

4.2.4. Stage of Vitiligo before Starting the Treatment:

The stage of vitiligo before starting the treatment for thirty seven patients (94.87%) was stable, and for two patients (5.13%) was complete healing and then relapse (unstable), (Figure 4-2).

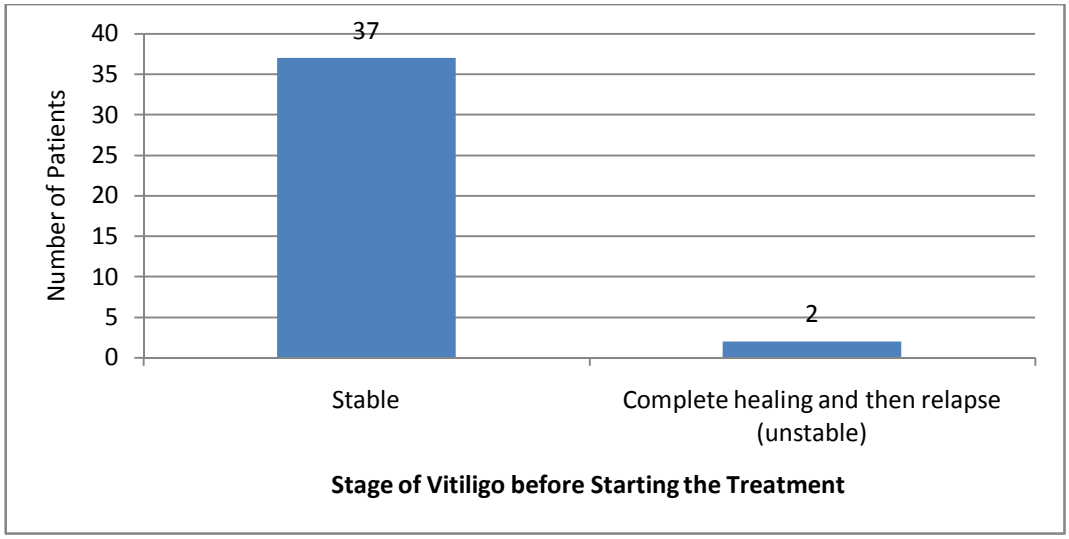

Figure (4-2): Stage of Vitiligo before Starting the Treatment.

4.2.5. Classification of Vitiligo Type:

The type of vitiligo for twelve patients (30.77%) was focal, for three patients (7.69%) was segmental, for fourteen patients (35.90%) was acrofacial, and for ten patient (25.64%) was generalized, (Table 4-3).

Table (4-3): Classification of Vitiligo Type.

Classification	Frequency	Percentage (%)
Focal vitiligo	12	30.77
Segmental vitiligo	3	7.69

Acrofacial vitiligo	14	35.90
Vitiligo vulgaris (generalized)	10	25.64
Total	**39**	**100**

4.3. Total Number of Treatment Sessions:

The total number of sessions for two patients (5.13%) was 5 sessions, for nineteen patients (48.72%) was between 6-15, for fourteen patients (35.90%) was between 16-25, for one patients (2.56%) was 40 sessions, and for three patients (7.69%) was 46 and more, (Figure 4-3).

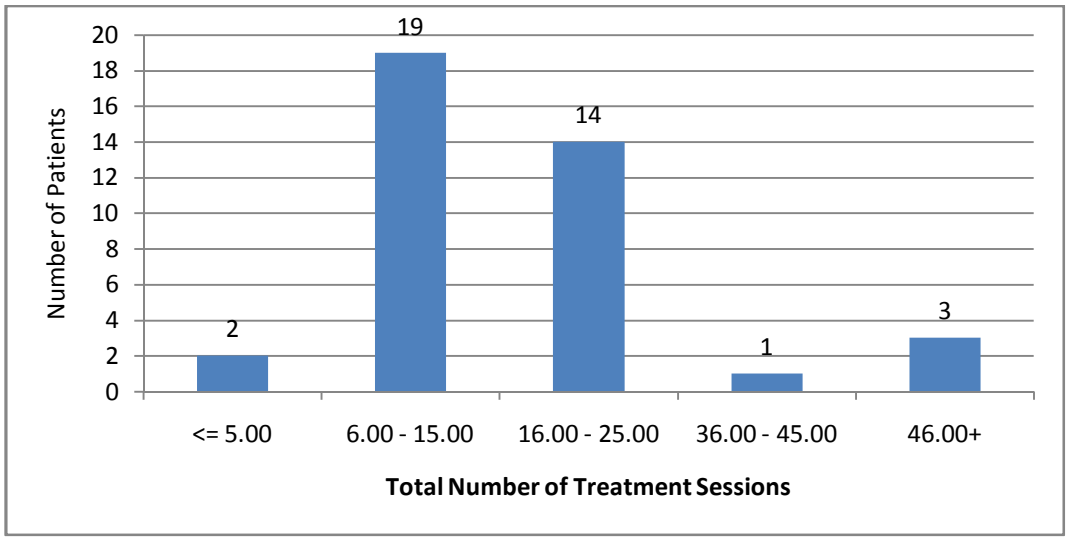

Figure (4-3): Total Number of Treatment Sessions.

4.4. Initial Repigmentation Response for Incoherent Light vs. Laser Treatment:

The initial repigmentation response for all patients who received incoherent light treatment (17 patients) was from the first session (100.00%), while for those who received laser treatment (22 patients), 20 patients of them showed response from first session (90.91%), and 2 patients of them showed no response from first session (9.09%), (figure 4-4).

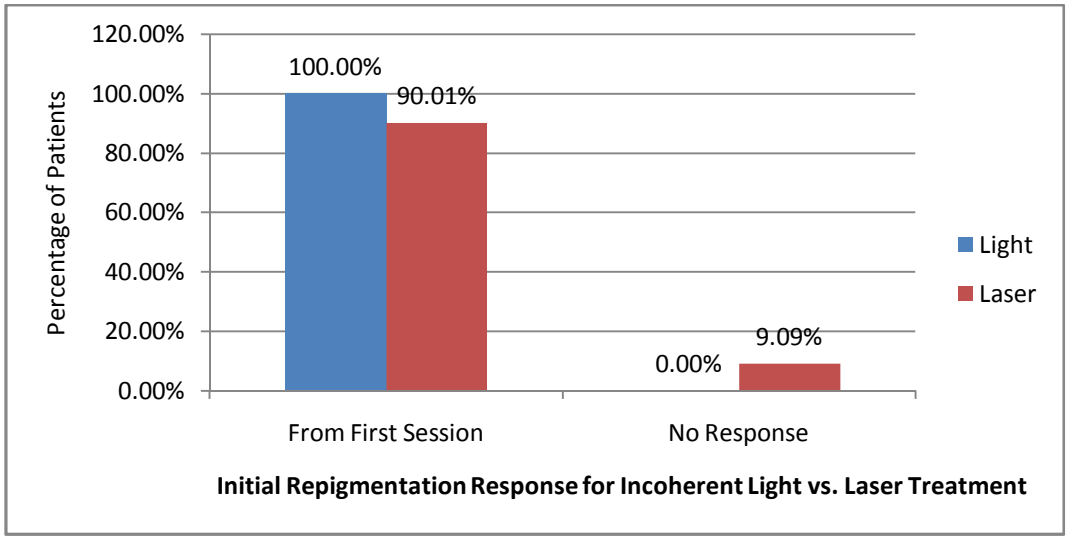

Figure (4-4): Initial Repigmentation Response for Incoherent Light vs. Laser Treatment.

4.5. Repigmentation Response for Incoherent Light Treatment:

The total sizes of patches before and after incoherent light treatment are listed in table 4-4.

Table (4-4): Total Size of Patches before and after Incoherent Light Treatment.

Patient S/N	Total size of patches before treatment(mm^2)	No. of sessions	Total size of patches at the end of last session (mm^2)	The percentage of reduction in patches (%)	Treatment response
41	55.154	55	30.478	44.74	Good
5	394.527	14	377.925	4.21	Poor
42	108.383	10	71.342	34.18	Good
9	53.095	15	32.842	38.14	Good
10	61.064	10	44.543	27.06	Good
11	230.161	55	90.563	60.65	V. Good
43	42.728	20	37.504	12.23	Poor
44	21.357	20	14.594	31.67	Good
45	339.195	21	251.162	25.95	Good
46	102.354	16	58.244	43.10	Good
22	36.607	14	21.043	42.52	Good
47	80	14	30.0	62.13	v. Good
48	80	5	77.584	3.02	Poor
28	57.432	10	25.218	56.09	v. Good
29	95.7	14	53.098	44.52	Good

33	22.704	21	12.728	43.94	Good
34	56.1	40	36.607	34.75	Good

Out of 17 vitiligo patients treated with incoherent light, three patients (17.65%) showed very good repigmentation response, eleven patients (64.71%) showed good repigmentation response, and three patients (17.65%) showed poor repigmentation response. There was no excellent or bad repigmentation responses, (See figure 4-5).

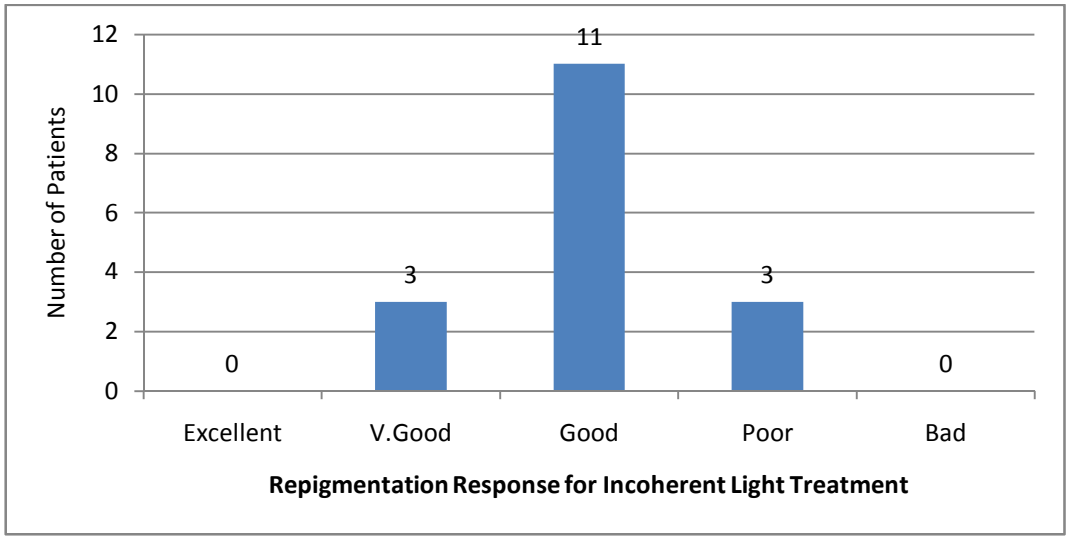

Figure (4-5): Repigmentation Response for Incoherent Light Treatment.

4.6. Repigmentation Response for Laser Treatment:

The total sizes of patches before and after laser treatment for each patient are listed in table 4-5.

Table (4-5): Total Size of Patches before and after Laser Treatment.

Patient S/N	Total size of patches before treatment (mm^2)	No. of sessions	Total size of patches at the end of last session(mm^2)	The percentage of reduction in patches (%)	Treatment response
1	30.632	55	12.006	60.81	v. Good
2	19.17	7	19.17	0.00	Bad
3	41.167	15	23.938	41.85	Good
4	19.475	13	18.375	5.65	Poor
6	12.81	16	7.58	40.83	Good

8	18.11	10	3.142	82.65	Excellent
12	25.596	10	2.67	89.57	v. Good
15	2.5905	22	0.314	87.88	Excellent
16	19.15	20	4.94	74.20	v. Good
17	12.69	20	3.77	70.29	v. Good
18	65.2	21	65.2	0.00	bad
19	48.42	16	38.131	21.25	Poor
23	5.65	7	3.84	32.04	Good
24	12.56	14	5.02	60.03	v. Good
25	22.54	14	8.948	60.30	v. Good
26	31.4	14	14.53	53.73	v. Good
27	12.728	5	7.85	38.33	good
31	49.185	15	16.714	66.02	v. Good
32	15.077	20	2.828	81.24	v. Good
35	57.967	20	15.69	72.93	v. Good
38	70.01	25	29.584	57.74	v. Good
39	74.1682	14	9.4832	87.21	Excellent

Out of 22 vitiligo patients treated with laser, three patients (13.60%) showed excellent repigmentation response, eleven patients (50.00%) showed very good repigmentation response, four patients (18.20%) showed good repigmentation response, two patients (9.10%) showed poor repigmentation response ,and two patients (9.10%) showed bad repigmentation response ,(See figure 4-6).

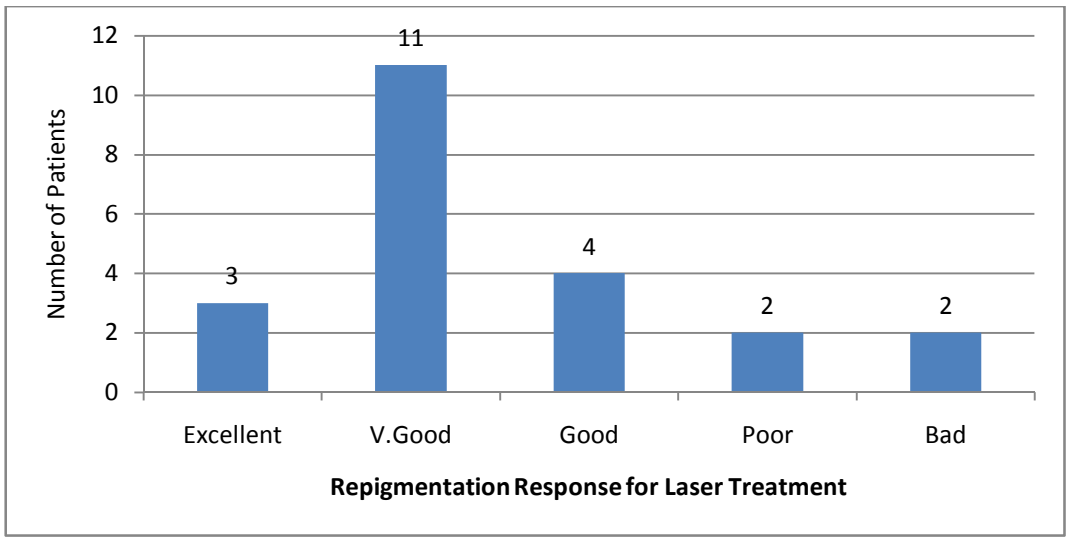

Figure (4-6): Repigmentation Response for Laser Treatment.

Figures (4-7) to (4-12) show photographs for some patients before and after treatment with LLLT.

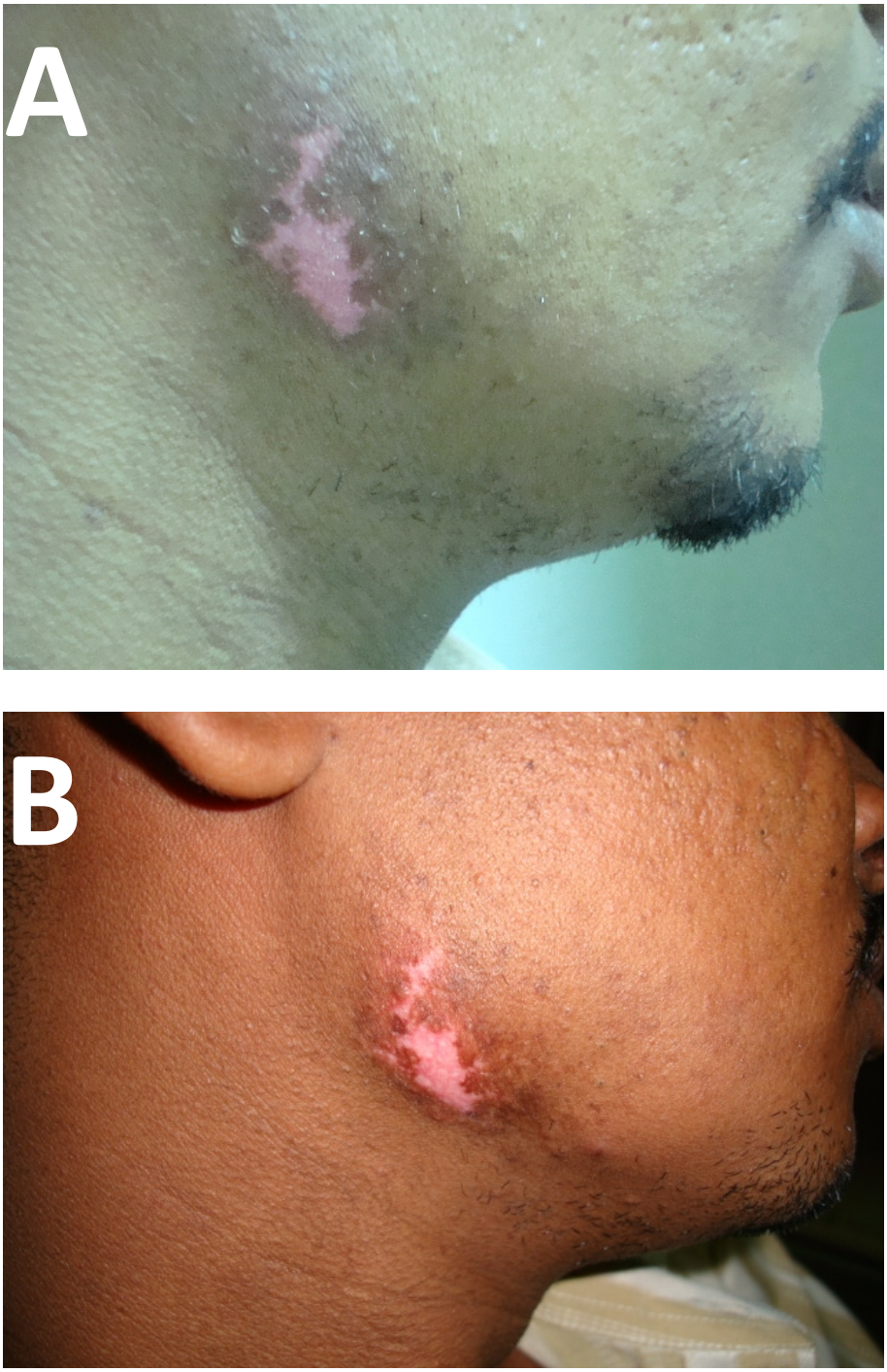

Figure (4-7): a comparison between vitiligo before and after treatment with LLLT in a 28 years old male with focal vitiligo (Pt. No. 27).

A: before treatment.

B: after 5 sessions, fluence 2.4 J/cm^2, exposure time of 10 sec., multi pulse mode, good reduction of vitiligo patches.

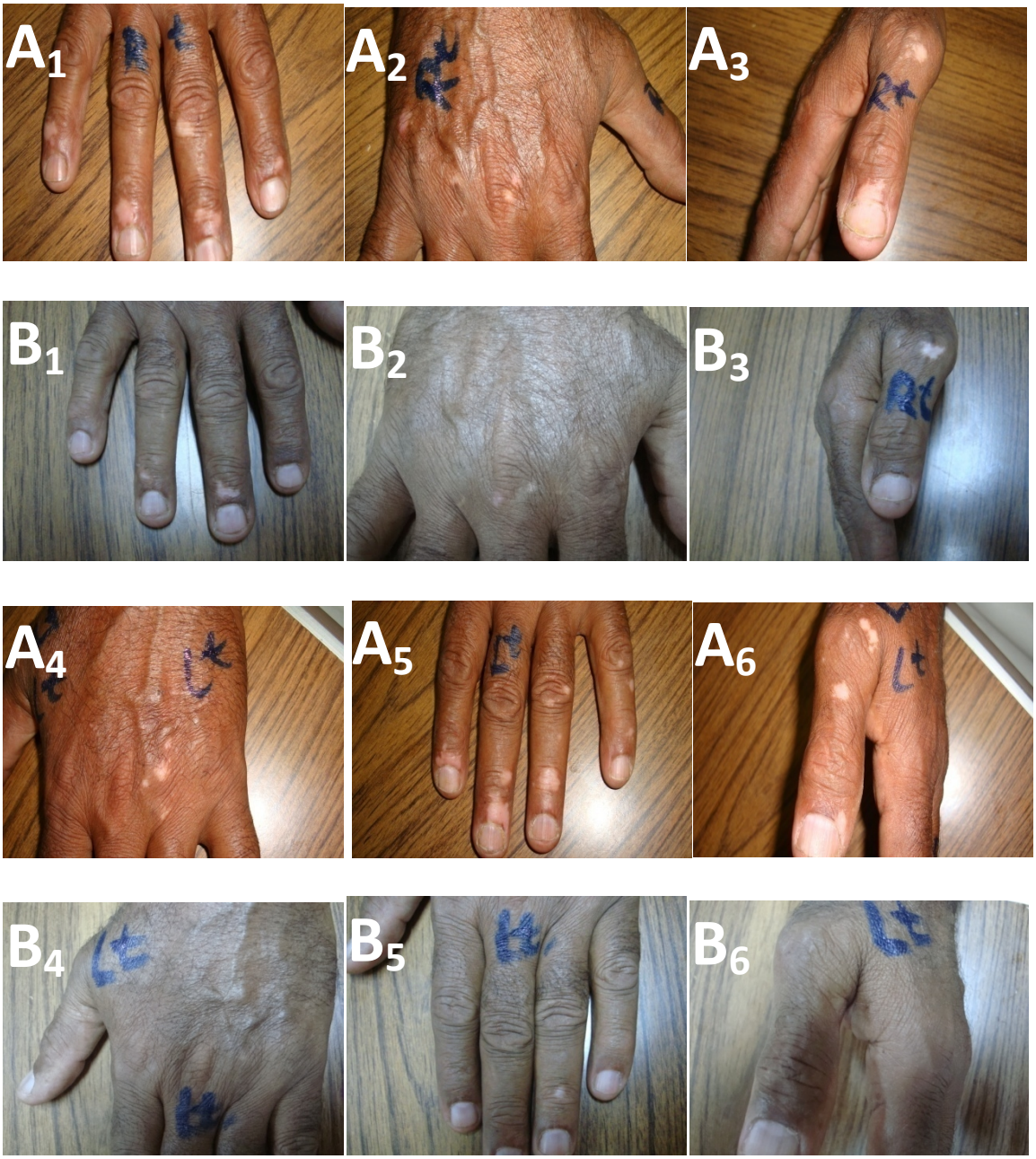

Figure (4-8): figure for Patient No. 39, 56 years old male, who was treated by LLLT for his acral vitiligo in his right, left hands and fingers.

A_1, A_2, A_3, A_4, A_5, and A_6: before treatment.

B_1, B_2, B_3, B_4, B_5, and B_6: after 14 laser treatment sessions, fluence 2.4 J/cm^2, exposure time of 10 sec., multi pulse mode, excellent reduction of vitiligo patches.

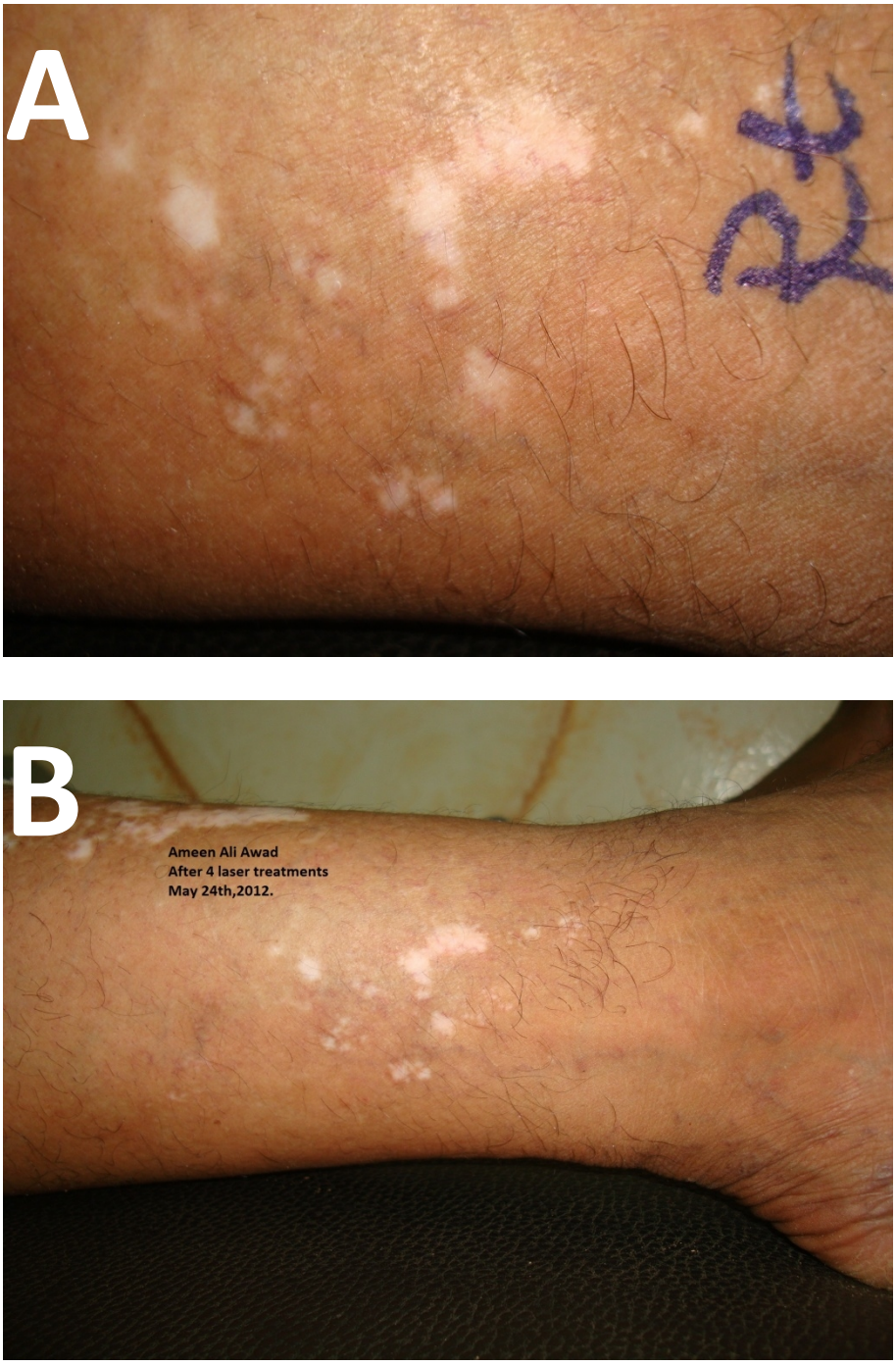

Figure (4-9): a comparison between vitiligo before and after treatment with LLLT in a 30 years old male with segmental vitiligo (Pt. No. 25).

A: before treatment.

B: after 14 sessions, fluence 2.4 J/cm^2, duration of 10 sec., multi pulse mode, very good reduction of vitiligo patches.

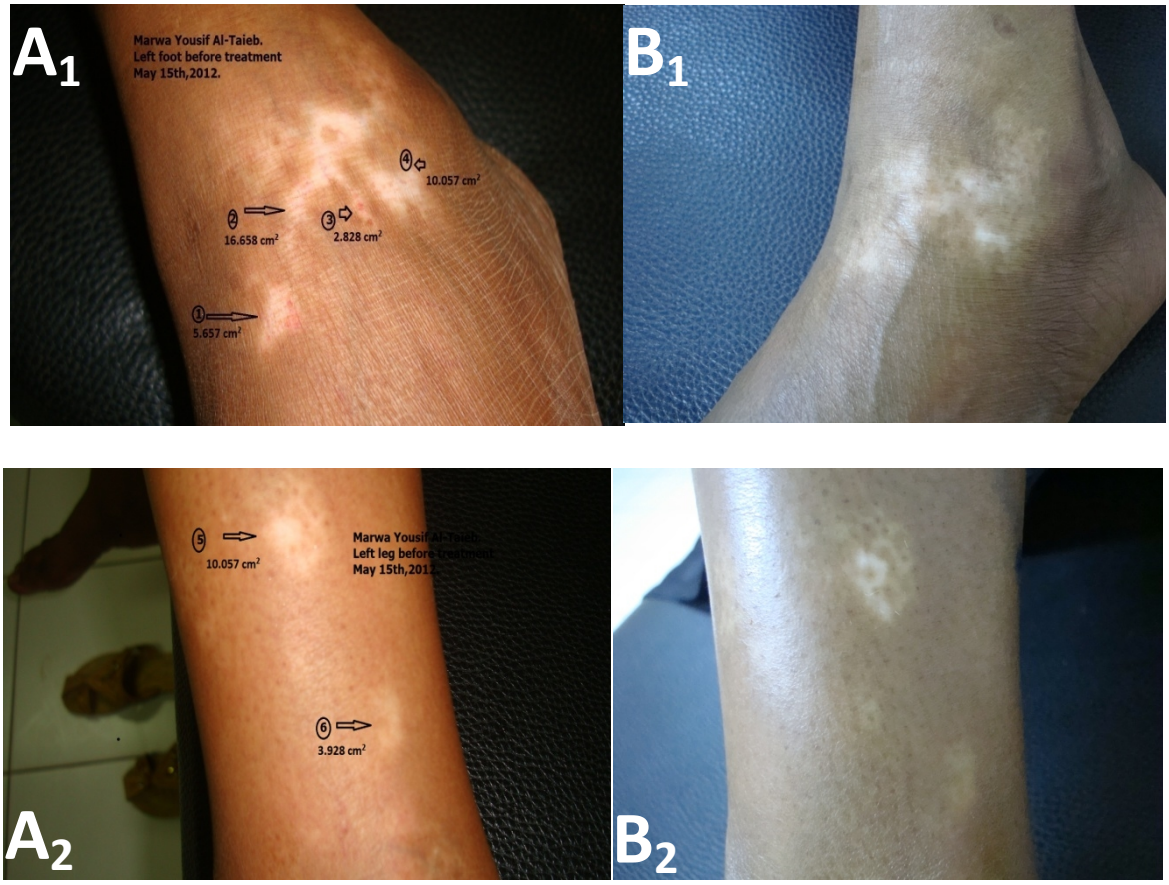

Figure (4-10): a comparison between vitiligo before and after treatment with LLLT in a 23 years old female with acrofacial vitiligo (Pt. No. 31).

A_1 and A_2: before treatment.

B_1 and B_2: after 15 sessions, fluence 2.4 J/cm^2, duration of 10 sec., multi pulse mode, very good reduction of vitiligo patches.

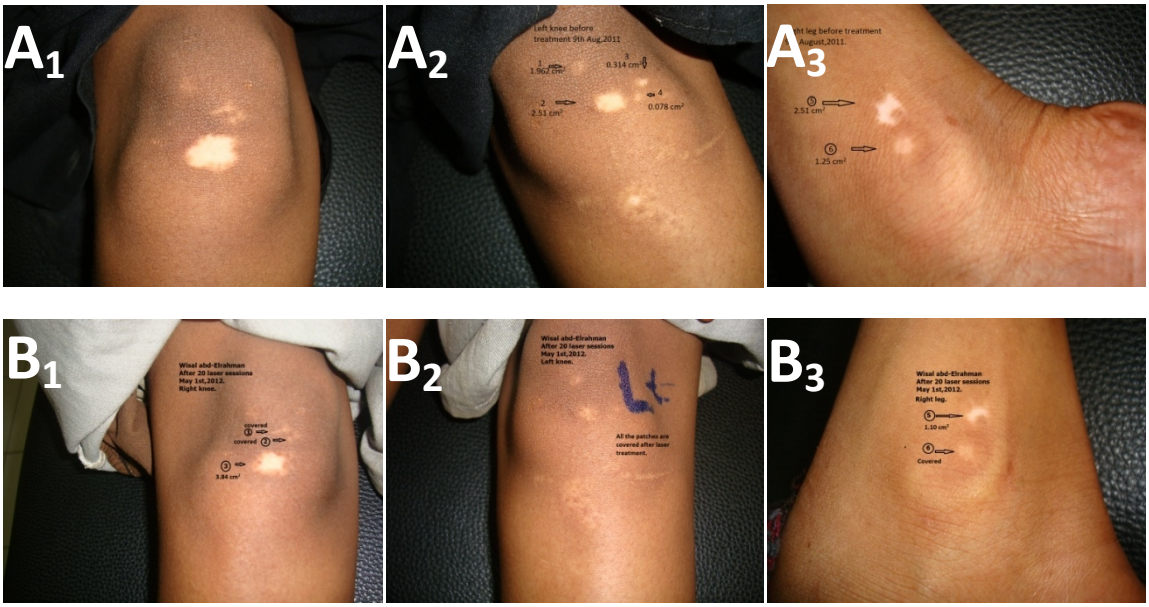

Figure (4-11): a comparison between vitiligo before and after treatment with LLLT in a 20 years old female with acrofacial vitiligo (Pt. No. 16).

A_1, A_2, and A_3: before treatment.

B_1, B_2, and B_3: after 20 sessions, fluence 2.4 J/cm^2, exposure time of 10 sec., multi pulse mode, very good reduction of vitiligo patches.

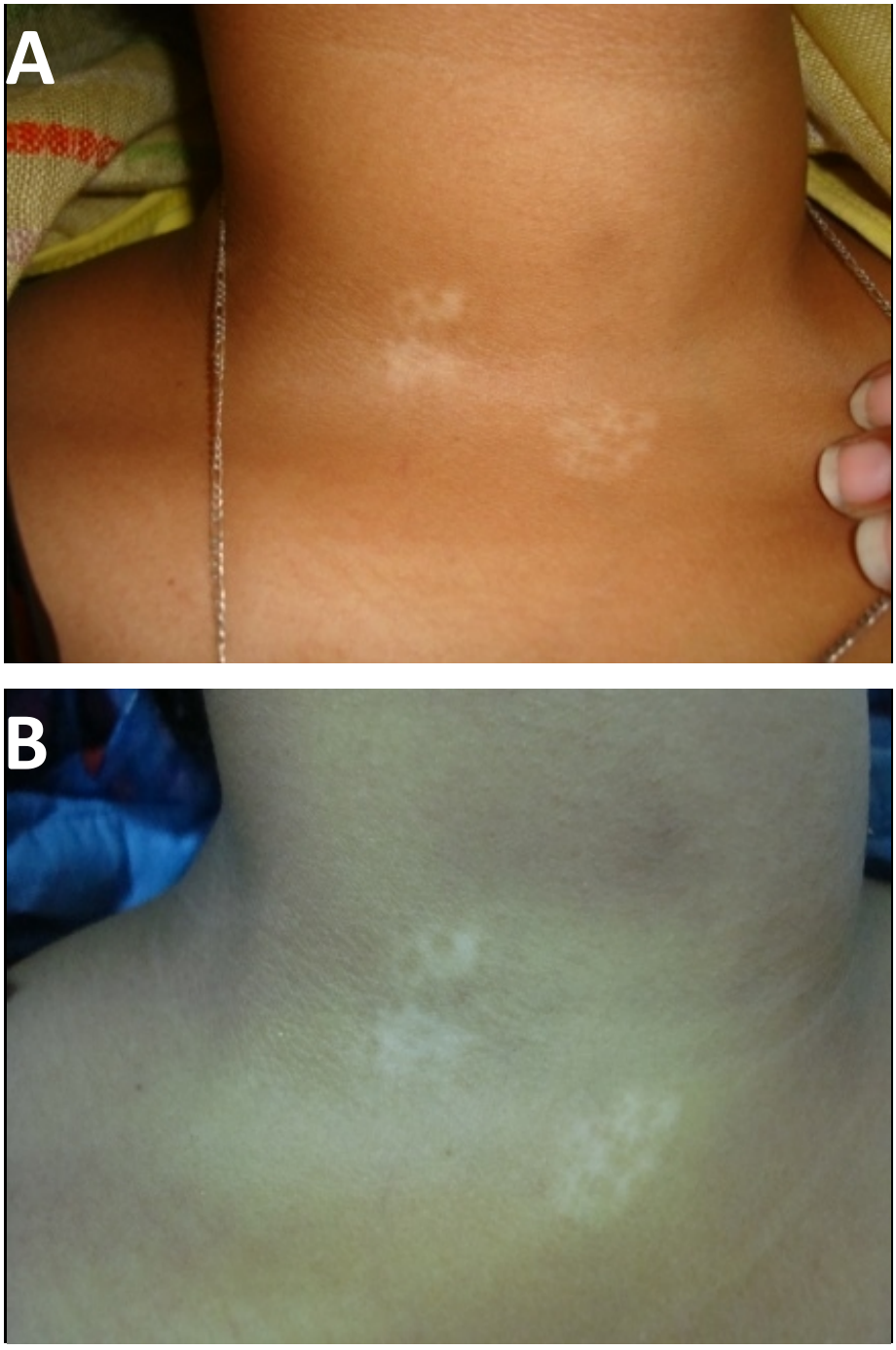

Figure (4-12): a comparison between vitiligo before and after treatment with LLLT in a 23 years old female with generalized vitiligo (Pt. No. 18).

A: Before treatment.

B: after 21 sessions, fluence 2.4 J/cm^2, exposure time of 10 sec., multi pulse mode, bad reduction of vitiligo patches.

Figures (4-13) to (4-14) show photographs for some patients before and after treatment with incoherent light.

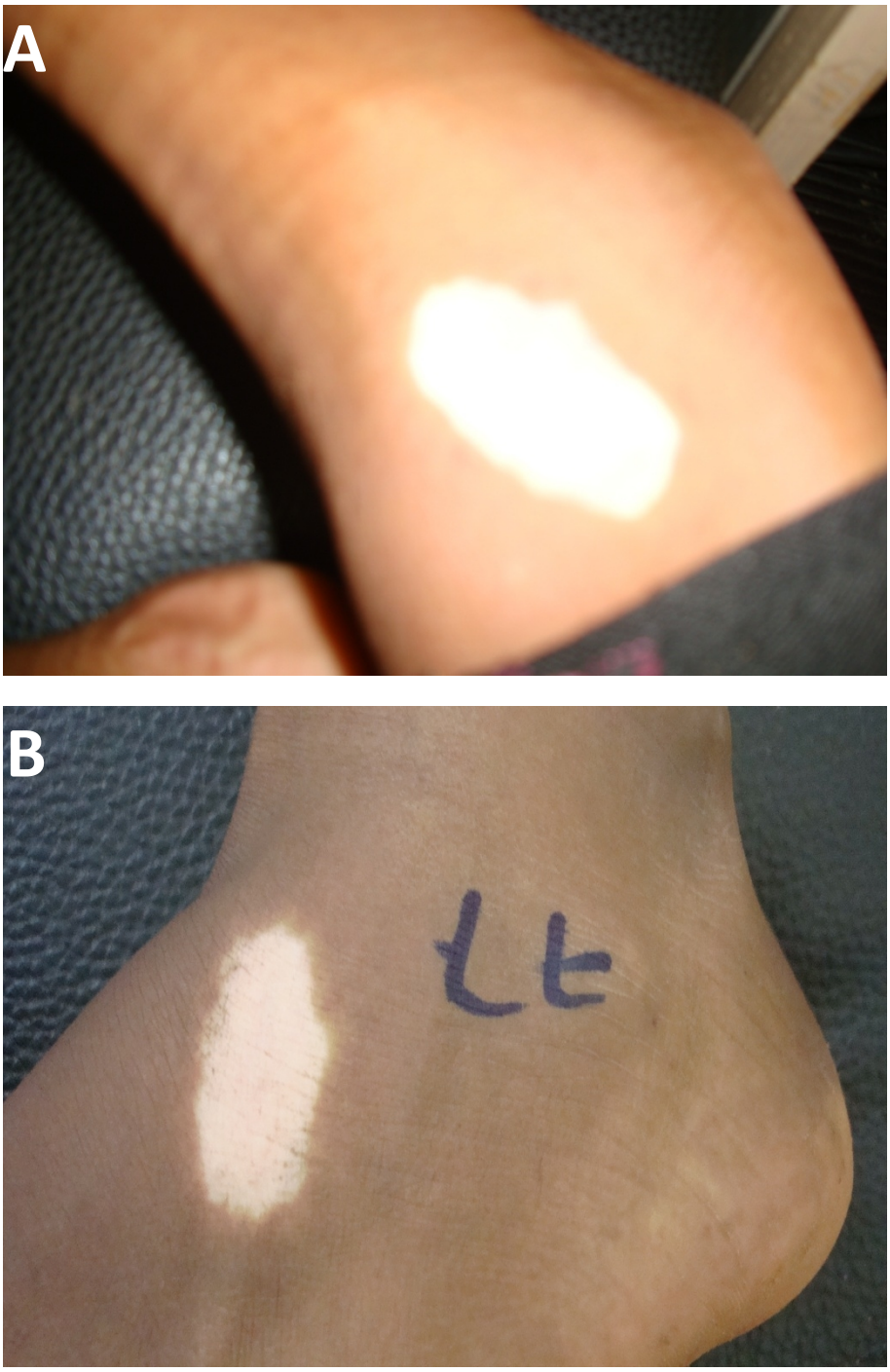

Figure (4-13): a comparison between vitiligo before and after treatment with incoherent light in a 20 years old female with acrofacial vitiligo (Pt. No. 43).

A: Before treatment.

B: after 20 sessions, fluence 2 J/cm^2, exposure time of 20 sec., multi pulse mode, poor reduction of vitiligo patches.

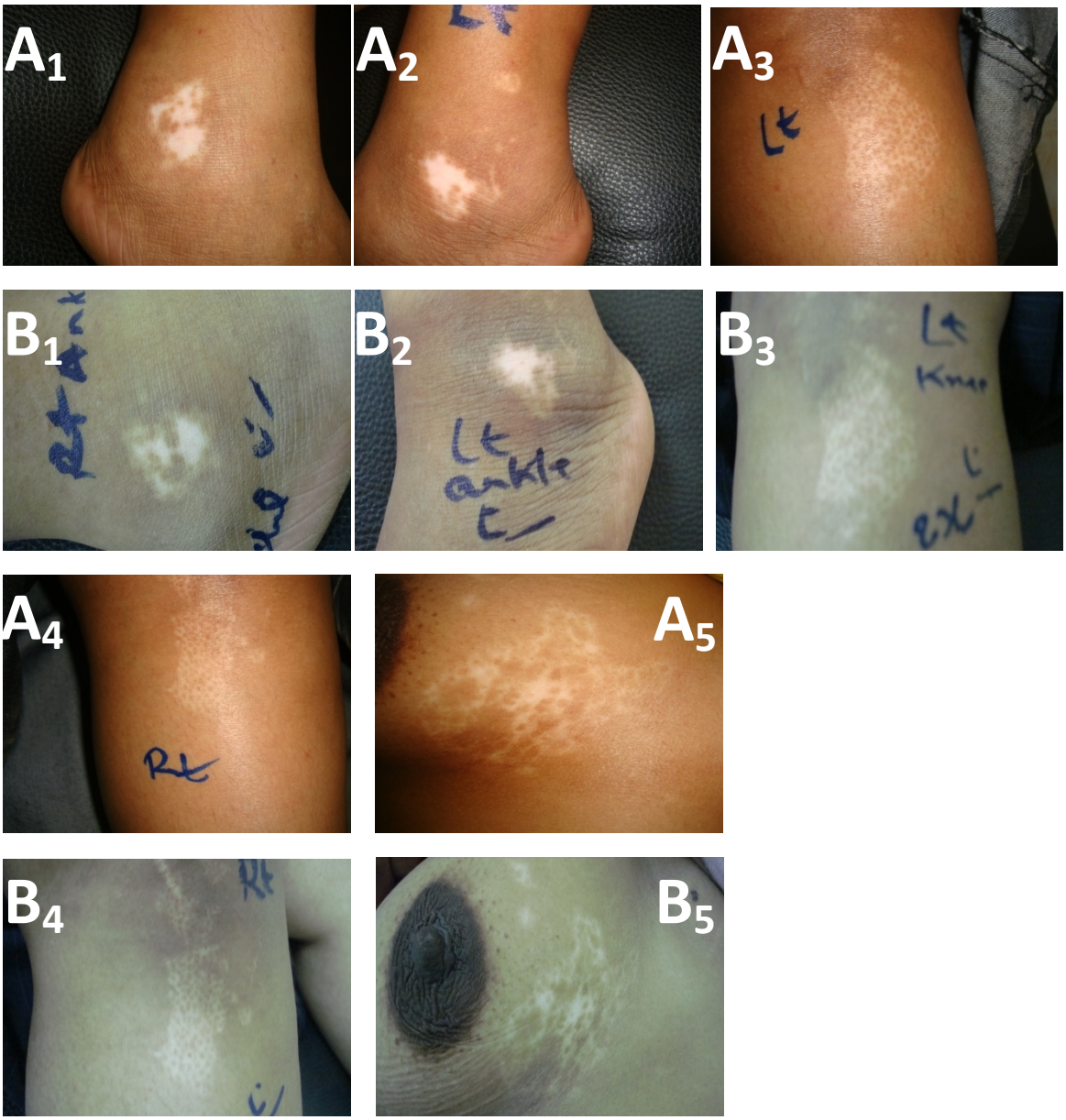

Figure (4-14): a comparison between vitiligo before and after treatment with incoherent light in a 23 years old female with generalized vitiligo (Pt. No. 45).

A_1, A_2, A_3, A_4, and A_5: Before treatment.

B_1, B_2, B_3, B_4, and B_5: after 21 sessions, fluence 2 J/cm^2, exposure time of 20 sec., multi pulse mode, good reduction of vitiligo patches.

4.7. Delayed Repigmentation Response after Stopping the Treatment*:

Delayed repigmentation response for incoherent light and laser treatment means that after the stopping the treatment sessions, some patients showed repigmentation of white patches continued for variable degree and some of them showed complete repigmentation.

The percentage of reduction for patient No.(1) was increased from (60.81%) at the end of last session to (100.00%) at the end of delayed period, for patients No.(4) was increased from (5.65%) to (34.30%), for patient No.(11) was increased from (60.65%) to (92.77%), for patient No.(34) was increased from (34.75%) to (74.10%), and for patient No.(41) was increased from (44.74%) to (81.77%), Table(4-6) and Figure (4-15).

Table (4-6): Delayed Repigmentation Response after Stopping the Treatment.

Patient S/N	Type of treatment	Total number of sessions	The percentage of reduction at the end of last session	Response at the end of last session	The percentage of reduction at the end of delayed period	Response at the end of delayed period	Delayed period after treatment
1	Laser	55	60.81%	V. Good	100.00%	Excellent	32 months
4	Laser	13	5.65%	Poor	34.30%	Good	30 months
11	Light	55	60.65%	V. Good	92.78%	Excellent	24 months
34	Light	40	34.75%	Good	74.10%	V. good	12 months
41	Light	55	44.74%	Good	81.77%	Excellent	32 months

* Those are the only patients who we were able to follow them up after stopping the treatment.

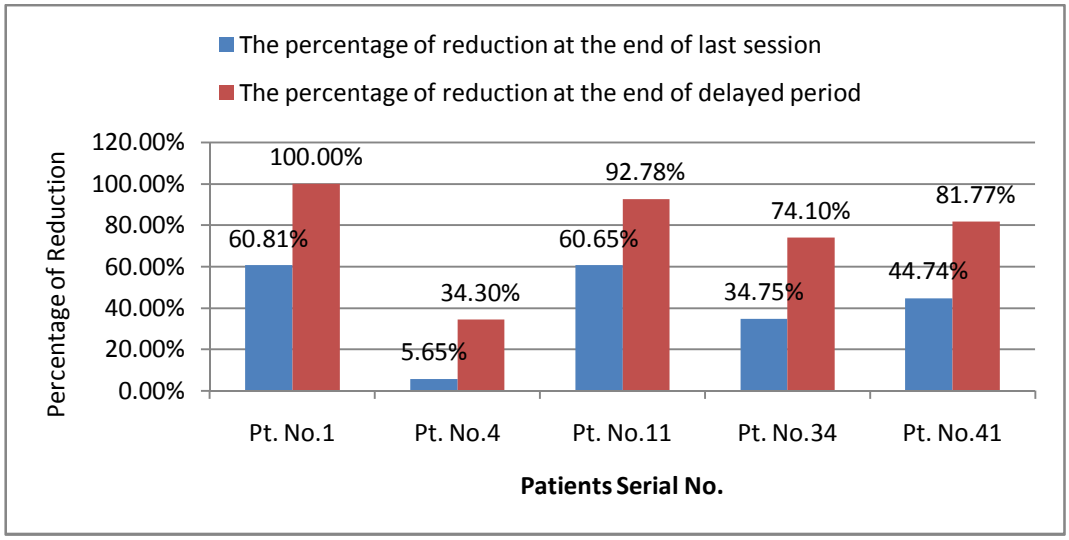

Figure (4-15): The Percentage of Reduction at the End of Last Session and at the end of Delayed Period.

Figures (4-16) to (4-20) show photographs for some patients before and after treatment with incoherent light or LLLT, and the delayed repigmentation response after stopping the treatment.

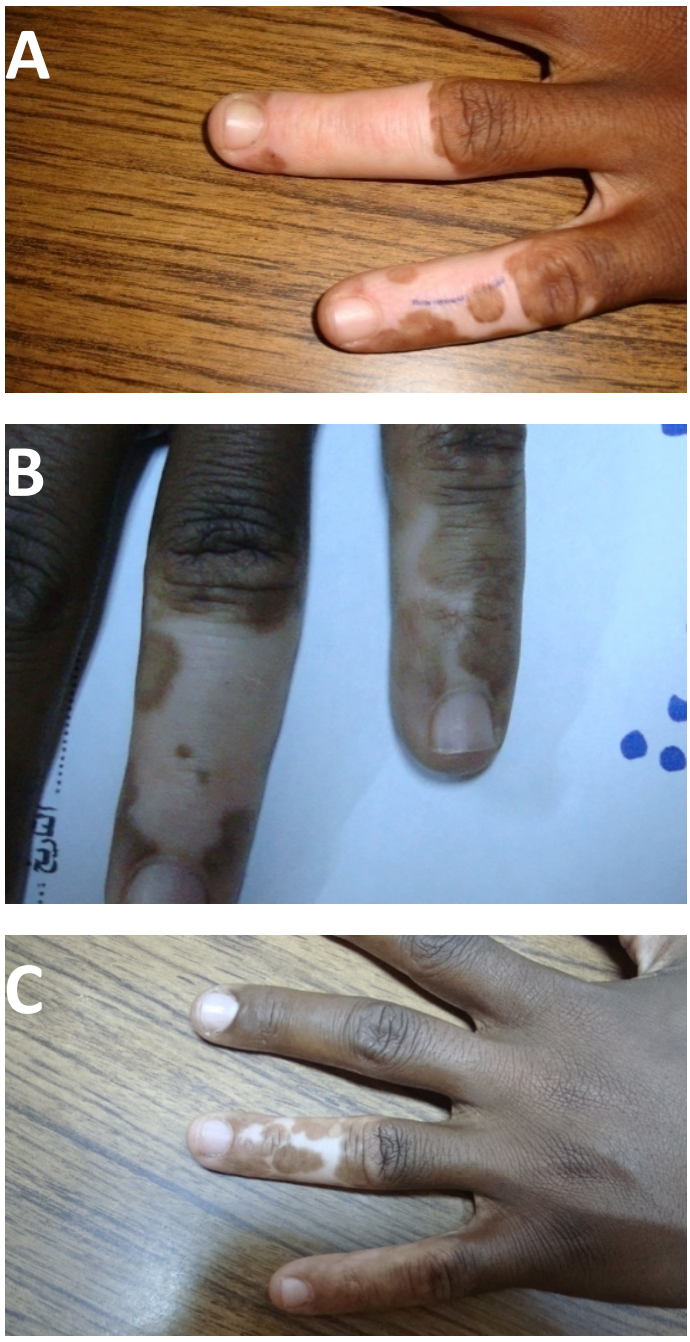

Figure (4-16): photos of patient No. 34, 22 years old male who was treated by incoherent light for his focal vitiligo in his 4th and 5th left hand fingers.

A: before treatment.

B: at the end of 40 treatment sessions which showed good response.

C: after delayed period of 12 months showed progression of repigmentation which is very good response.

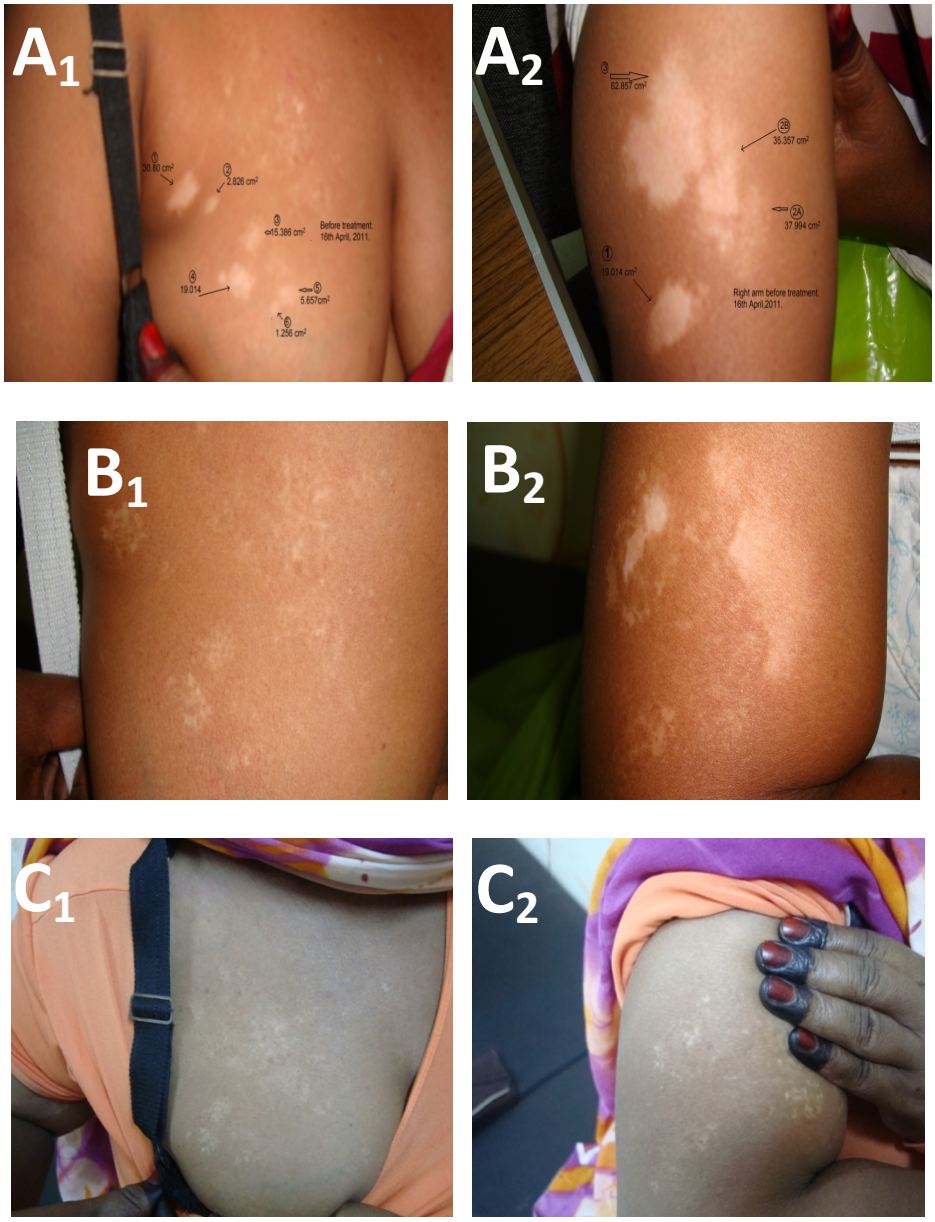

Figure (4-17): photos of patient No. 11, 45 years old female with segmental vitiligo, involved her right side of chest and right forearm who was treated by incoherent light.

A_1: right side chest before treatment, A_2: right forearm before treatment.

B_1 , and B_2: at the end of 55 treatment sessions which showed very good response.

C_1 and C_2: after delayed period of 24 months, which showed progression of repigmentation which is an excellent response.

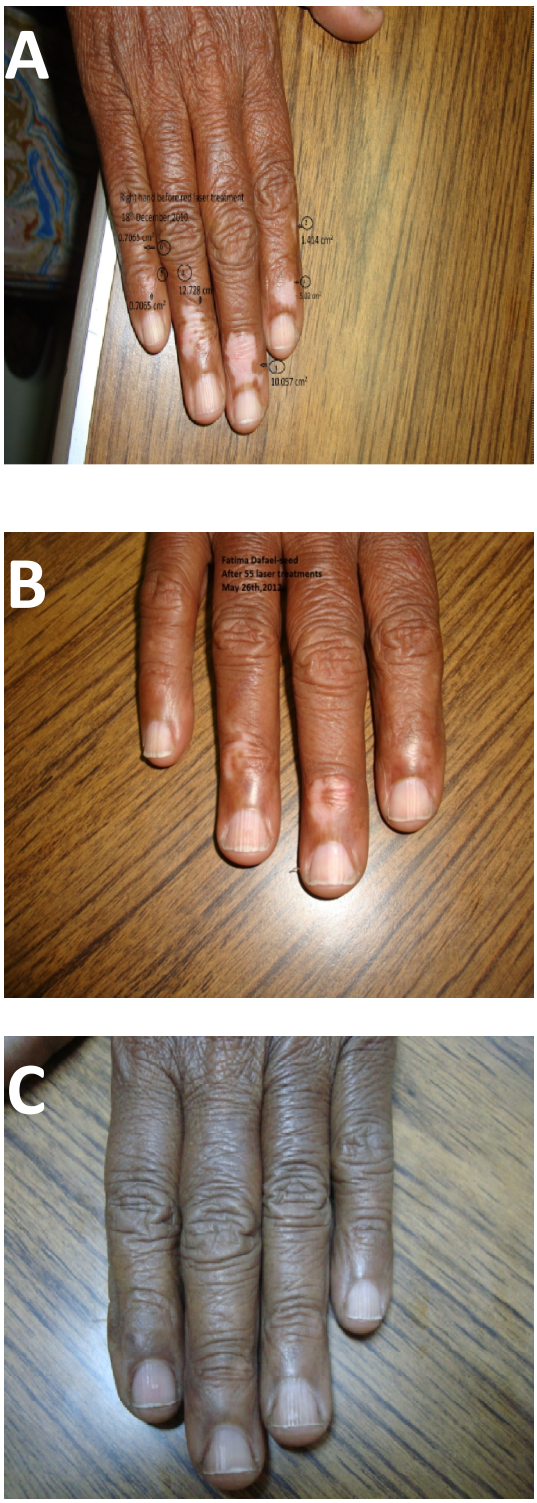

Figure (4-18): photos of patient No. 1, 55 years old female who had acrofacial vitiligo who was treated by LLLT.

A: before treatment.

B: after 55 sessions, with a very good response.

C: after delayed period of 32 months which showed excellent response.

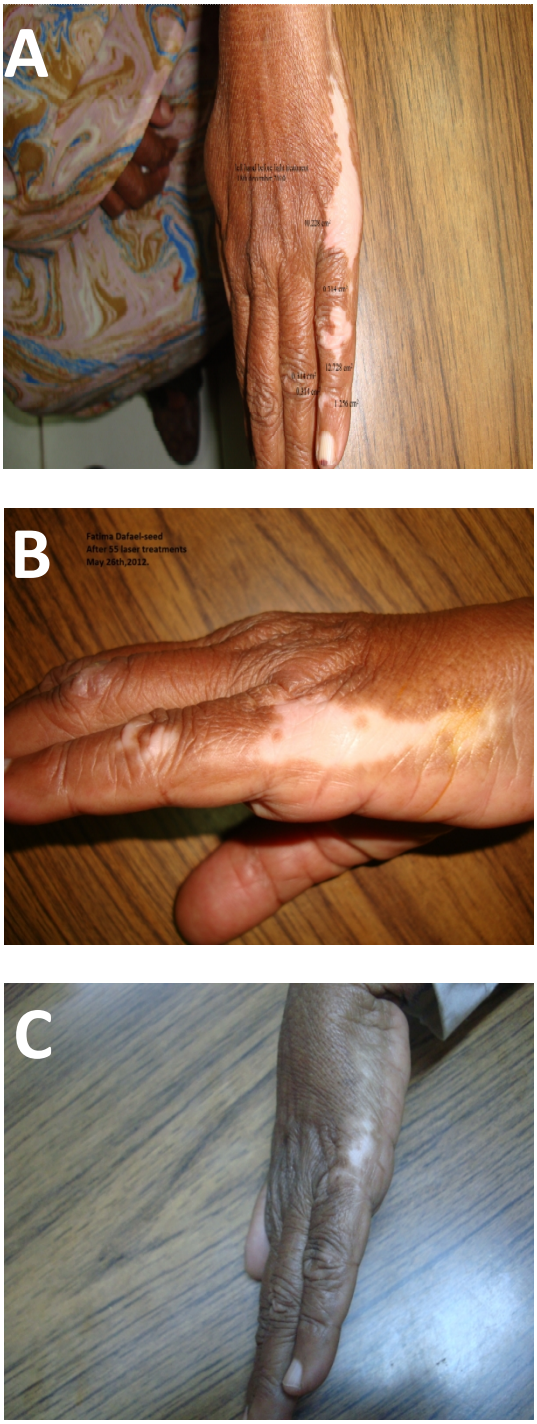

Figure (4-19): photos of patient No. 41, 55 years old female with acrofacial vitiligo who was treated by incoherent light.

A: before treatment.

B: after 55 sessions, showed good response.

C: after delayed period of 32 months showed excellent response.

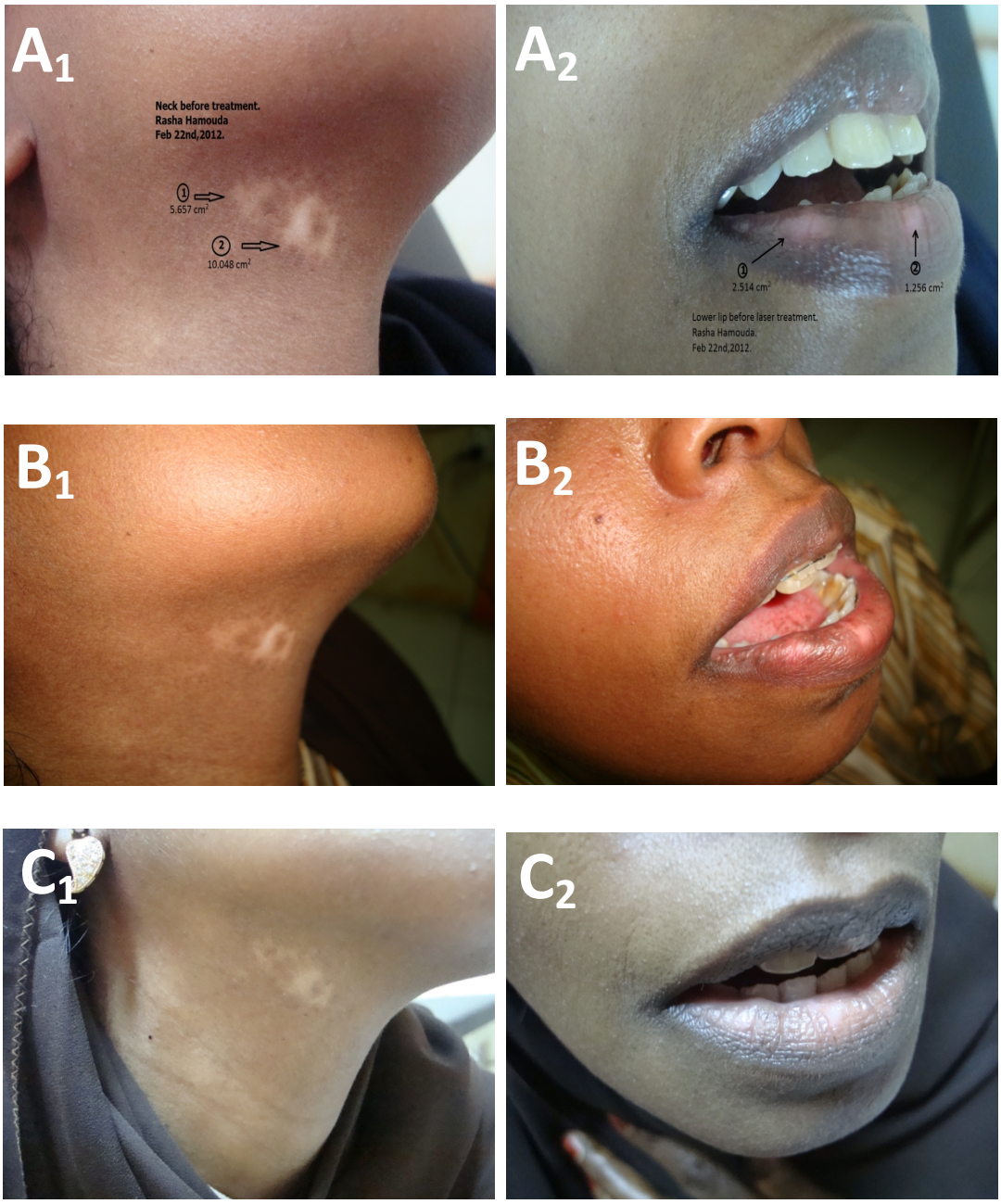

Figure (4-20): photos of patient No. 4, 37 years old female with acrofacial vitiligo who was treated by LLLT.

A_1, and A_2: before treatment.

B_1, and B_2: after 13 sessions showed poor response.

C_1, and C_2: after delayed period of 30 months showed good response.

4.8. Comparison between Incoherent Light Repigmentation Response and Laser Repigmentation Response:

The excellent response was in no patients (0.00%) for incoherent light treatment, while it was in three patients (13.64%) for laser, very good response was achieved in three patients (17.65%) treated with incoherent light, while it was achieved in eleven patients (50.00%) treated by laser. Good response was achieved in eleven patients (64.71%) treated by incoherent light, while it was achieved in four patients (18.18%) treated by laser. Poor response was achieved in three patients (17.65%) treated by incoherent light, while it was achieved in two patients (9.09%) treated by laser. Bad response was achieved in no patients (0.00%) treated by incoherent light, while it was achieved in two patients (9.09%) treated by laser, (See figure 4-21).

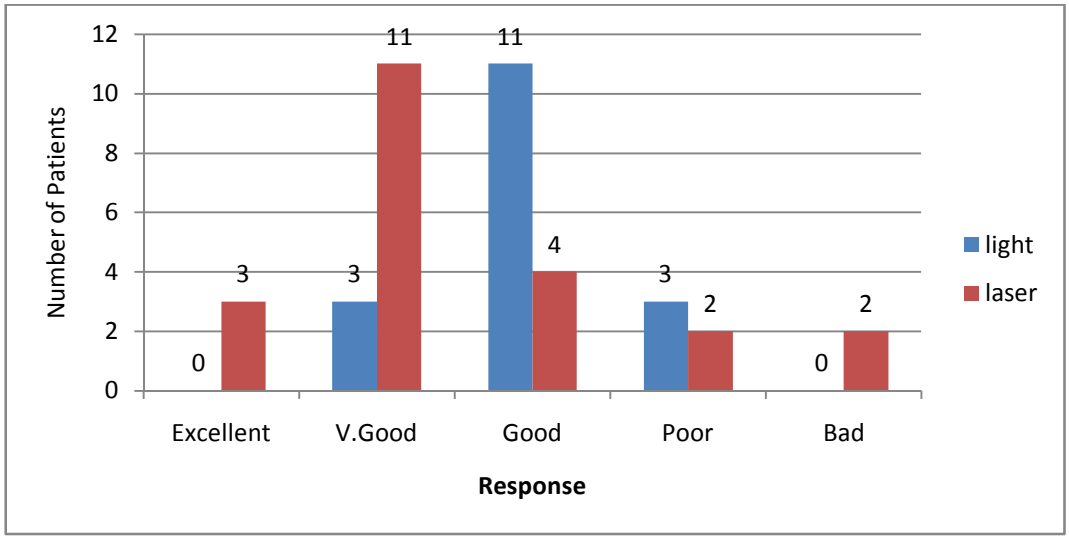

Figure (4-21): Comparison between Incoherent Light Repigmentation Response and Laser Repigmentation Response.

4.9. Incoherent Light and Laser Repigmentation Response According to Age:

The repigmentation response for incoherent light and laser treatments were best in patients whose ages were between 16 and 35 years old, (See table 4-7).

Table (4-7): Incoherent Light and Laser Repigmentation Response According to Age.

Method of treatment	Repigmentation response	Age						Total
		<= 15.00	16.00 - 25.00	26.00 - 35.00	36.00 - 45.00	46.00 - 55.00	56.00+	
Incoherent Light	Excellent	0	0	0	0	0	0	0
	V.Good	0	0	2	1	0	0	3
	Good	0	8	2	0	1	0	11
	Poor	1	1	1	0	0	0	3
	Bad	0	0	0	0	0	0	0
Total		**1**	**9**	**5**	**1**	**1**	**0**	**17**
Laser	Excellent	0	1	1	0	0	1	3
	V.Good	0	5	4	1	1	0	11
	Good	1	0	2	0	1	0	4
	Poor	0	1	0	1	0	0	2
	Bad	1	1	0	0	0	0	2
Total		**2**	**8**	**7**	**2**	**2**	**1**	**22**

4.10. Incoherent Light and Laser Repigmentation Response According to Total Number of Sessions:

A minimum of ten Incoherent light treatment sessions was needed to have very good and good responses, while a minimum of ten laser treatment sessions was needed to have excellent and very good responses. Optimum responses were observed after 14 and 21 incoherent light treatment sessions, and after 14 and 20 laser treatment sessions, (See table 4-8).

Table (4-8): Incoherent Light and Laser Repigmentation Response According to Total Number of Sessions.

Method of treatment	Repigmentation response	Total No. of sessions													Total
		5	7	10	13	14	15	16	20	21	22	25	40	55	
Incoherent Light	Excellent	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	V.Good	0	0	1	0	1	0	0	0	0	0	0	0	1	3
	Good	0	0	2	0	2	1	1	1	2	0	0	1	1	11
	Poor	1	0	0	0	1	0	0	1	0	0	0	0	0	3
	Bad	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Total		**1**	**0**	**3**	**0**	**4**	**1**	**1**	**2**	**2**	**0**	**0**	**1**	**2**	**17**
Laser	Excellent	0	0	1	0	1	0	0	0	0	1	0	0	0	3
	V.Good	0	0	1	0	3	1	0	4	0	0	1	0	1	11
	Good	1	1	0	0	0	1	1	0	0	0	0	0	0	4
	Poor	0	0	0	1	0	0	1	0	0	0	0	0	0	2
	Bad	0	1	0	0	0	0	0	0	1	0	0	0	0	2
Total		**1**	**2**	**2**	**1**	**4**	**2**	**2**	**4**	**1**	**1**	**1**	**0**	**1**	**22**

4.11. Incoherent Light and Laser Repigmentation Response According to Types of Vitiligo:

The best response for patients treated by incoherent light was observed in patients who had focal and generalized vitiligo, while the best response for patients treated by laser was observed in patients who had focal and acrofacial vitiligo, (See table 4-9).

Table (4-9): Incoherent Light and laser Repigmentation Response According to Types of Vitiligo.

Method of treatment	Repigmentation response	types of vitiligo				Total
		Focal vitiligo	Segmental vitiligo	Acrofacial vitiligo	Vitiligo vulgaris (generalized)	
Incoherent Light	Excellent	0	0	0	0	0
	V.Good	1	1	0	1	3
	Good	5	1	1	4	11
	Poor	1	0	1	1	3
	Bad	0	0	0	0	0
Total		**7**	**2**	**2**	**6**	**17**
Laser	Excellent	0	0	2	1	3
	V.Good	3	1	6	1	11
	Good	2	0	2	0	4
	Poor	0	0	1	1	2
	Bad	0	0	1	1	2
Total		**5**	**1**	**12**	**4**	**22**

4.12. Incoherent Light and Laser Repigmentation Response According to Duration of Vitiligo:

All the patients treated by incoherent light with different duration of vitiligo showed the same response, while patients treated by laser who had vitiligo for less than one year showed very good response, better than the other duration groups, (See table 4-10).

Table (4-10): Incoherent Light and Laser Repigmentation Response According to Duration of Vitiligo.

Method of treatment	Repigmentation response	duration of vitiligo								Total
		Less than one year	1-2 years	3-4 years	5-6 years	7-8 years	9-10 years	13-14 years	15-20 years	
Incoherent Light	Excellent	0	0	0	0	0	0	0	0	0
	V.Good	1	1	0	0	1	0	0	0	3
	Good	3	2	2	1	1	0	2	0	11
	Poor	1	0	0	0	1	1	0	0	3
	Bad	0	0	0	0	0	0	0	0	0
Total		**5**	**3**	**2**	**1**	**3**	**1**	**2**	**0**	**17**
Laser	Excellent	0	1	0	1	0	0	1	0	3
	V.Good	4	2	1	1	1	2	0	0	11
	Good	1	1	0	0	1	1	0	0	4
	Poor	0	0	0	1	0	0	0	1	2
	Bad	1	0	0	0	0	0	1	0	2
Total		**6**	**4**	**1**	**3**	**2**	**3**	**2**	**1**	**22**

4.13. Side Effects of the Treatment:

Noticeable adverse events, such as erythema, telangiectasia, and infection were not found in any patient receiving incoherent light or laser treatment sessions, (See table 4-11).

Table (4-11): Side Effects of the Treatment.

Method of treatment	Side effects				Total
	erythema	telangiectasia	Infection	None	
Incoherent Light	0	0	0	17	17
Total	**0**	**0**	**0**	**17**	**17**
Laser	0	0	0	22	22
Total	**0**	**0**	**0**	**0**	**22**

4.14. Discussion:

Forty eight patients diagnosed clinically as vitiligo patients were involved in this study, most of them were between 16-35 years old. The majority of the patients (56.41%) were females. All of them have stable vitiligo before starting the treatment. The treatment was done using incoherent light (fluence 2 J/cm^2, exposure time of 20 sec., multi pulse mode), and laser (fluence 2.4 J/cm^2, exposure time of 10 sec., multi pulse mode). During the treatment, nine of them showed disease activity at distant areas not exposed to light shortly after starting the treatment, so that they were excluded from the study. The activity of vitiligo is a natural course of the disease that can happen to any vitiligo patient with or without treatment.

The duration of vitiligo for the majority of patients (28.21%) was less than one year, while the duration for the other patients ranged from one to twenty years. All of the patients showed disease stability for three months before enrolment in the study. All types of vitiligo were represented in patients involved in the study. The majority of patients received between six and fifteen sessions.

Nearly, all the patients who received incoherent light or laser treatment showed very good initial repigmentation response after the first session. These findings disagree with the study done in 2003 by Yo Hs et al who used He-Ne laser 632 nm , which showed that the initial repigmentaion started after an average of 16 treatment sessions (HS. et al., 2003).

Also our findings disagree with the study done by Wu Cs et al which used He-Ne laser of average fluence 3 J/cm^2, who showed that the initial repigmentation was recorded after an average of 17 sessions (Wu et al., 2008).

The majority of patients treated by incoherent light (64.71%) showed good repigmentation response, while the majority of patients treated by laser

(50.00%) showed very good repigmentation response. Those responses may be explained by the previous studies which documented abnormal physiological changes in vitiligo lesions including abnormal acetylcholine activity, sweat responses, and skin conductance, which were corrected by modulation of the sympathetic function involved in how visible light induces repigmentation (Laila, 2003).

The whole patients who treated by laser showed reduction in surface area of vitiligo patches between 25%-100% which is better than the result obtained by Ataie L who used LLLT with 630 nm GaAlAs laser (20 mW, 1 J/cm^2) and showed reduction of surface area of vitiligo patches between 25%-75% (Ataie, 2011).

In the study done by Yu W-t et al, who used continues wave low energy GaAlAs laser at 635 nm, 3 J/cm^2, (24 sessions), they achieved greater than 25% repigmentation of the patches, which is much less than our study (W-t, Hs and CH-SH, 2010).

Also, in the study done in New York by Suhail M. et al, who used excimer laser 308 nm, 100 mJ/cm^2, they achieved repigmentation response between 50% to 75% of vitiligo patches, which is less than our study (Hadi et al., 2004).

Five patients were followed up between 12 and 32 months after the end of their treatment sessions without receiving any other modalities of treatment of their vitiligo disease during this period. They showed progression of their repigmentation response up to almost total repigmentation of their patches. This delayed repigmentation response is a unique response in our study by using incoherent light and LLLT, which is not found in other studies mentioned above.

Up to our knowledge, there were no previous studies mentioned a delayed repigmentation response of their patients who were treated by other lasers. The continuation of repigmentation after ending the treatment sessions

indicates that our method of treatment have a delayed repigmentation response which might be due to the increase in basic fibroblast growth factor (bFGF) release from both normal keratinocytes and fibroblast around the vitiligo patches and a significant increase in nerve growth factor (NGF) release from keratinocytes. Basic fibroblast growth factor (bFGF) is a putative melanocytes growth factor, whereas nerve growth factor (NGF) is a paracrine factor for melanocyte survival in the skin. Both nerve growth factor (NGF) and basic fibroblast growth factor (bFGF) stimulates melanocyte proliferation and migration from around the normal skin to cover the vitiligo patches (HS. et al., 2003; Wu et al., 2008).

The delayed response noticed in our 5 patients gave us an idea to give a considerable gap between treatment sessions to give a time for melanocyte to multiply and migrate from a normal tissue around the patches to cover the diseased skin.

Therefore, diode laser 675 nm at a fluence of 2.4 J/cm^2 , exposure duration of 10 sec., and a cluster probe containing incoherent light at a fluence of 2 J/cm^2 , exposure duration of 20 sec., may induce a repigmentation of vitiligo patches. These findings agreed with the study done by Wu Cs et al who used He-Ne laser at an average fluence of 3 J/cm^2 who had marked repigmentation (more than 50%) in 60% of patients , following successive treatments, and in the current study more than 50% of patients showed more than 50% repigmentation. In comparison with the same study, there were three patients showed 100% recovery, which is better than our study where one patient had 100% recovery (Wu et al., 2008).

In the current study, excellent repigmentation response was found only in patients received laser treatment. In addition, a very good repigmentation response was noticed more in patients received laser treatment compared to Incoherent light treatment, while a good repigmentation response was seen more in patients received incoherent light treatment compared with laser

treatment. Although excellent and very good repigmentation response were noticed more in patients received a laser treatment, both laser and incoherent light can be used as methods for treatment of vitiligo.

From this study, one can found that during the treatment sessions the repigmentation response showed no difference according to the total number of sessions , while the delayed response was much better in patients received more treatment sessions than those with less treatment sessions.

A minimum of ten Incoherent light treatment sessions was needed to have very good and good responses, while a minimum of ten laser treatment sessions was needed to have excellent and very good responses. Optimum responses were observed after 14 and 21 incoherent light treatment sessions, and after 14 and 20 laser treatment sessions.

All the patients treated by incoherent light with different duration of vitiligo showed the same response, while patients treated by laser who had vitiligo for less than one year showed very good response better than the other duration groups.

4.14.1. Why Incoherent Light and Laser Treatment for Vitiligo were better than Conventional PUVA Therapy?

Incoherent light and lasers emitting in a visible red and infrared light in a low power are non ionizing radiation, which means that they are non carcinogenic radiations and safe to be used in humans.

In contrast, PUVA is used to treat vitiligo, where it has a definite potential to cause skin cancer, including melanomas, because UV light is an ionizing radiation that can cause skin cancer. The risk of developing skin cancer is directly related to the amount of energy administered of UV light (Cole, 2014).

PUVA is well known to cause photo-aging that is unavoidable. While incoherent light and laser used in our study can be used for skin rejuvenation.

If not appropriately monitored, PUVA can produce severe ultraviolet light burns while in this study incoherent light and laser were not causing burns. Occasionally, Oxsoralen used for PUVA can cause nausea so susceptible patients take the drug with food, while no need for Psoralen with incoherent light and laser.

PUVA treatment involves taking psoralen by mouth (orally) or applying it to the skin (topically). This is followed by carefully timed exposure to ultraviolet A (UVA) light from a special lamp or to sunlight. Patients must minimize exposure to sunlight at other times (Hossani-Madani and Halder, 2011). While in our study no need for application of psoralen, and the patients can exposed freely to sun light after the procedure immediately without any problems.

PUVA is time-consuming and care must be taken to avoid side effects, which can sometimes be severe, while incoherent light and laser treatments were not time consuming and no side effects with it.

PUVA increases the risk for cancer of the skin, a risk that includes melanoma, a highly malignant and sometimes fatal form of skin cancer. Patients who receive long-term PUVA treatment should therefore be carefully monitored throughout their lives, while in our procedures which were using non carcinogenic radiation, the patients need not to be carefully monitored throughout their lives.

4.15. Conclusion:

From our study we can conclude that diode laser (omega Xp) with its laser probe 675 nm (fluence 2.4 J/cm^2, exposure time of 10 sec., multi pulse mode) and cluster light probe (fluence 2 J/cm^2, exposure time of 20 sec., multi pulse mode) is very effective and safe in treatment of vitiligo, without showing any side effect or complication.

Both laser and incoherent light repigmentation response were noticed more in patients of age group between 16 -35 years old.

The best response for patients treated by incoherent light were observed in patients who had focal and generalized vitiligo, while the best response for patients treated by laser were observed in patients who had focal and acrofacial vitiligo.

Noticeable adverse events, such as erythema, telangiectasia, and infection were not found in any patients receiving incoherent light or laser treatment sessions.

4.16. Future Work:

The followings can be recommended as future work:

- Diode laser (Omega Xp) with wavelength of 675 nm and 10 mW output power and cluster probe 60 of incoherent light are recommended in the treatment of vitiligo as safe, convenient, and effective method of treatment.
- The gap between treatment sessions should be lengthened for one month to get best delayed repigmentation response.
- From our study, we can recommend the use of an incoherent light for the treatment of focal and generalized vitiligo, while a laser for treatment of acrofacial and focal vitiligo.
- More studies should be done using other LLL with different wave lengths, different pulse modes, and different exposure times to determine the best outcome.

- LLLT with green laser could be compared with the red lasers.
- Combination between cluster probe and LLL could be suggested as a future work.

REFERENCES

REFERENCES

1. Aimbire, F., de Oliveira Ligeiro, A., Albertini, R., Correa, J., de Campos Ladeira, C., Lyon, J., Silva Ja, J. and Costa, M. (2008) *Low level laser therapy (LLLT) decreases pulmonary microvascular leakage, neutrophil influx and IL-1beta levels in airway and lung from rat subjected to LPS-induced inflammation*, 31:189-197.

2. Andreeta, M.R.B., Cunha, L.S., Vales, L.F., Caraschi, L.C. and Jasinevicius, R.G. (2011) *"Bidimensional codes recorded on an oxide glass surface using a continuous wave CO2 laser"*..

3. Ardiç, F., Aktan, S., Kara, C. and Sanli, B. (1998) *High-frequency hearing and reflex latency in patients with pigment disorder*, Am J Otolaryngol. 19(6):365-9.

4. Ashbaugh, D. (1999) *Quantitative-Qualitative Friction Ridge Analysis: An Introduction to Basic and Advanced Ridgeology*, Boca Raton, Fla: CRC Press.

5. Ataie, L. (2011) *Efficacy of lowpoer laser GaA Als (630 nm) in the treatment of vitiligo patients*.

6. Aydogan, K., Turan, O., Onart, S., Karadogan, S. and Tunali, S. (2006) *Audiological abnormalities in patients with vitiligo*, Clin Exp Dermatol. 31(1):110-3.

7. Ball, K.A. (1995) *Lasers: the perioperative challenge*, 2nd ed Book. pp 14–17. St Louis: Mosby-Year.

8. Barton and Fritz (2014) *"Skin Resurfacing". In Charles Thorne. Grabb and Smith's Plastic Surgery*, (7 ed.). Philadelphia: Lippincott Williams & Wilkins. p. 455.

9. Baumann, L. (2007) *Skin ageing and its treatment*, J Pathol.211(2):241-51.

10. Birlea, S., Costin, G. and Norris, D. (2008) *Cellular and molecular mechanisms involved in the action of vitamin D analogs targeting vitiligo depigmentation*, Curr Drug Targets. 9(4):345-59.

11. Birlea, S., Gowan, K., Fain, P. and Spritz, R. (2009) *Genome-Wide Association Study of Generalized Vitiligo in an Isolated European*

Founder Population Identifies SMOC2, in Close Proximity to IDDM8, J Invest Dermatol.

12. Bjordal, J., Couppé, C., Chow, R., Tunér, J. and Ljunggren, E. (2003) *"A systematic review of low level laser therapy with location-specific doses for pain from chronic joint disorders"*, The Australian journal of physiotherapy 49 (2): 107–16.

13. Bjordal, J.M., Lopes-Martins, R.A., Joensen, J., Couppe, C., Ljunggren, A.E., Stergioulas, A. and Johnson, M.I. (2008) *"A systematic review with procedural assessments and meta-analysis of Low Level Laser Therapy in lateral elbow tendinopathy*, 9-75.

14. Brosseau, L., Welch, V., Wells, G.A., de Bie, R., Gam, A., Harman, K., Morin, M., Shea, B. and Tugwell, P. (2005) *"Low level laser therapy (Classes I, II and III) for treating rheumatoid arthritis"*.

15. Burns, D., Breathnach, S., Cox, N. and Griffiths, C. (2004) *Rook's Textbook of Dermatology*, 7th ed. Malden, Mass: Blackwell Science.

16. Cancer orgnization (2014) *Treatments and sidee ffects*, http://www.cancer.org/treatment/treatmentsandsideeffects/complementaryandalternativemedicine/manualhealingandphysicaltouch/cold-laser-therapy.

17. Carlson, B. (1994) *Integumentary, skeletal, and muscular systems. In: Human Embryology and Developmental Biology*, St. Louis, Mo: Mosby. 153-81.

18. Center for Device and Radiological Health (2013) *Radiation-Emitting Product Code*, U.S. Food and Drug Administration.NHN.

19. Chimento, S., Newland, M., Ricotti, C., Nistico, S. and Romanelli, P. (2008) *A pilot study to determine the safety and efficacy of monochromatic excimer light in the treatment of vitiligo*, J Drugs Dermatol. 7(3):258-63.

20. Chow, R., Johnson, M., Lopes-Martins, R. and Bjordal, J. (2008) *Efficacy of low-level laser therapy in the management of neck pain: a systematic review and meta-analysis of randomized placebo active-treatment controlled trials*, Spine.33(4).Suppl:S123-52.

21. Chow, R., Johnson, M., Lopes-Martins, R. and Bjordal, J. (2009) *"Efficacy of low-level laser therapy in the management of neck pain: a*

systematic review and meta-analysis of randomised placebo or active-treatment controlled trials.".

22. Chu, S. and Townes, C. (2003) *"Arthur Schawlow". In Edward P. Lazear. (ed.) Biographical Memoirs*, vol. 83. National Academy of Sciences. p. 202.

23. Cole, G.W. (2014) *What are the advantages and disadvantages of PUVA*, www.medicinenet.com.

24. Do, J., Shin, J., Kim, D., Hann, S. and Oh, S. (2011) *The effect of 308 nm excimer laser on segmental vitiligo: a retrospective study of 80 patients with segmental vitiligo*, Photodermatol Photoimmunol Photomed. 27(3):147-51.

25. Duarte, F. (2003) *Tunable Laser Optics*, Elsevier Academic, New York.

26. Elgoweini, M. and Nour El Din, N. (2009) *Response of vitiligo to narrowband ultraviolet B and oral antioxidants*, J Clin Pharmacol. 49(7):852-5.

27. Enwemeka, C., Parker, J., Dowdy, D., Harkness, E., Sanford, L. and Woodruff, L. (2005) *The efficacy of low-power lasers in tissue repair and pain control: a meta-analysis study*, Photomed Laser Surg. 23(2):177-81.

28. Esfandiarpour, I., Ekhlasi, A., Farajzadeh, S. and Shamsadini, S. (2009) *The efficacy of pimecrolimus 1% cream plus narrow-band ultraviolet B in the treatment of vitiligo: a double-blind, placebo-controlled clinical trial*, J Dermatolog Treat. 20(1):14-8.

29. Ezzedine, K., Diallo, A. and Leaute-Labreze, C. (2011) *Multivariate analysis of factors associated with early-onset segmental and nonsegmental vitiligo: a prospective observational study of 213 patients*, Br J Dermatol. 165(1):44-9.

30. Falabella, R. (2005) *Surgical approaches for stable vitiligo*, Dermatol Surg. 31(10):1277-84.

31. Farajzadeh, S., Daraei, Z., Esfandiarpour, I. and Hosseini, S. (2009) *The efficacy of pimecrolimus 1% cream combined with microdermabrasion in the treatment of nonsegmental childhood*

vitiligo: a randomized placebo-controlled study, Pediatr Derma. 26(3):28.

32. Fongers, A., Wolkerstorfer, A., Nieuweboer-Krobotova, L., Krawczyk, P., Toth, G. and van der Veen, J. (2009) *Long-term results of 2-mm punch grafting in patients with vitiligo vulgaris and segmental vitiligo: effect of disease activity*, Br J Derma. 161(5):1105-1.

33. Fongo, A., Ferraris, E. and Bocca, M. (1966) *Skin tension lines and wrinkles: Anatomoclinical observations*, Minerva Chir. 21(13):627-30.

34. Geusic, J.E., Marcos, H.M. and Van Uitert, L.G. (1964) *"Laser oscillations in nd-doped yttrium aluminum, yttrium gallium and gadolinium garnets"*, Applied Physics Letters 4 (10): 182.

35. Goldberg, D.J. (2005) *Laser Dermatology*, Springer -Verlag. Berlin. Netherlands.

36. Goldinger, S., Dummer, R. and Schmid, P. (2007) *Combination of 308-nm xenon chloride excimer laser and topical calcipotriol in vitiligo*, J Eur Acad Dermatol Venereol. 21(4):504–508.

37. Goldman, M., Shiffman, M., Mirrafati, S. and Lam, S. (2008) *Simplified Facial Rejuvenation*, New York, NY: Springer.47-50.

38. Gordon Gould, R. (1959) *"The LASER, Light Amplification by Stimulated Emission of Radiation". In Franken, P.A. and Sands, R.H. (Eds.) The Ann Arbor Conference on Optical Pumping*, the University of Michigan. p. 128.

39. Grau, C. and Silverberg, N. (2013) *Vitiligo patients seeking depigmentation therapy: a case report and guidelines for psychological screening*, Cutis. 91(5):248-52.

40. Gul, U., Kilic, A., Tulunay, O. and Kaygusuz, G. (2007) *Vitiligo associated with malignant melanoma and lupus erythematosus*, J Dermatol. 34(2):142-5.

41. Hadi, S.M., MPhil, Spencer, J.M. and Lebwohl, M. (2004) *The Use of the 308-nm Excimer Laser for the Treatment of Vitiligo*, New York.983-986.

42. Hadi, S., Tinio, P. and Al-Ghaithi, K. (2006) *Treatment of vitiligo using the 308-nm excimer laser*, Photomed Laser Surg. 24(3):354–357.

43. Halder, R. and Taliaferro, S. (2008) *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-Hil. 72.

44. Hamblin, M.R. (2008) *Mechanisms of Low Level Light Therapy*, Department of Dermatology, Harvard Medical School.

45. Hanel Photonics (2014) *"Laser Diode Market"*.

46. Hann, S.-K. (2004) *Clinical variants of vitiligo. In: Lotti T, Hercogova J. (eds.) Vitiligo: Problems and Solutions*, New York, NY: Marcel Dekker. 159-73.

47. Hélou, J., Maatouk, I., Obeid, G., Moutran, R., Stéphan, F. and Tomb, R. (2014) *Fractional laser for vitiligo treated by 10,600 nm ablative fractional carbon dioxide laser followed by sun exposure*, Lasers Surg Med.Beirut. 46(6):443-8.

48. Hong, S.-B., Park, H.-H. and Lee, M.-H. (2005) *Short-term Effects of 308-nm Xenon-chloride Excimer Laser and Narrow-band Ultraviolet B in the Treatment of Vitiligo: A Comparative Study*, J Korean Med Sci. 20(2): 273–278.

49. Hossani-Madani, A. and Halder, R. (2011) *Treatment of vitiliogo: Advantages and disadvantages*, G Ital Dermatol Venereol. 146(5): 373-95.

50. HS., Y., CS., W., CL., Y., YH., K. and MH., C. (2003) *Helium-neon laser irradiation stimulates migration and proliferation in melanocytes and induces repigmentation in segmental-type vitiligo*, Taiwan.J Invest Dermatol. 120(1):56-64.

51. Huang, Y., Chen, A., Carroll, J. and Hamblin, M. (2009) *"Biphasic Dose Response in Low Level Lightherapy"*, Dose-Response 7 (4): 358–383.

52. Hu, W., Wang, J., Yu, C., Lan, C., Chen, G. and HS., Y. (2007) *Helium -neon laser irradiation stimulates cell prolifration through photostimulatory effects in mitochondria*, J invest Dermatol. 127:2048-2057.

53. Ifflander, R. (2001) *Solid State Laser Material Processing: Fundamental Relations & Technical Realizations*, Springer-Verlag, Berlin, Heidelberg.

54. Institute of Technology (1998) *Laser safety manual*, California.

55. International Electrotechnical Commission (2007) *Safety of laser products - Part 1: Equipment classification and requirements*, (2nd ed.).

56. Jamtvedt, G., Dahm, K.T., Christie, A., Moe, R.H., Haavardsholm, E., Holm, I. and Hagen, K.B. (2007) *"Physical Therapy Interventions for Patients with Osteoarthritis of the Knee: an Overview of Systematic Reviews"*, Physical Therapy 88 (1): 123–136.

57. Jin, Y., Birlea, S. and Fain, P. (2011) *Genome-Wide Analysis Identifies a Quantitative Trait Locus in the MHC Class II Region Associated with Generalized Vitiligo Age of Onset*, J Invest Dermatol. 131(6):1308-12.

58. John, A., Mouzakis, Liu, S. and Cohen, G. (2011) *Rapid Response of Facial Vitiligo to 308nm Excimer Laser and Topical Calcipotriene*, J Clin Aesthet Dermatol. 4(6): 41–44.

59. Karman, G.P., McDonald, G.S., New, G.H.C. and Woerdman, J.P. (1999) *"Laser Optics: Fractal modes in unstable resonators"*, Nature, Vol. 402, 138.

60. Keith, A.K., Avraham, J.S. and Sandy, M. (1997) *Treatment of Vitiligo With the Ultrapulse Carbon Dioxide Laser in Patients Concomitantly Receiving Oral Psoralen Plus UV-A Therapy*, Arch Dermatol. 133(12):1605-1606.

61. Kovacs, S.O. (1998) *Vitiligo*, J Am Acad Dermatol.(1):647-66, 38(5).quiz 667-8.

62. Kripke, M. (1994) *Ultraviolet radiation and immunology: something new under the sun--presidential address*, Cancer Res 54.6102-5.

63. Laila, A. (2003) *Efficacy of low power laser GaAlAs in the treatment of vitiliog patients*,. Gholamerza Esmaeeli Djavid. From the low level laser therapy (LLLT) internet guide selected laser therapy abstracts. Swedish laser medical.

64. Lamberty, B. and Cormack, G. (1990) *Fasciocutaneous flaps*, Clin Plast Surg. 17(4):713-26.

65. Le Poole, I. and Luiten, R. (2008) *Autoimmune etiology of generalized vitiligo*, Curr Dir Autoimmun. 10:227-43.

66. Leal Junior, E., Lopes-Martins, R., Baroni, B., De Marchi, T., Taufer, D., Manfro, D., Rech, M., Danna, V., Grosselli, D., Generosi, R., Marcos, R., Ramos, L. and Bjordal, J. (2008) *Effect of 830 nm low-level laser therapy applied before high-intensity exercises on skeletal muscle recovery in athletes*, Photomed Laser Surg. 26(5):419-24.

67. Lotti, T., Gori, A., Zanieri, F., Colucci, R. and Moretti, S. (2008) *Vitiligo: new and emerging treatments*, Dermatol Ther. 21(2):110-7.

68. Lotti, T., Prignano, F. and Buggiani, G. (2007) *New and experimental treatments of vitiligo and other hypomelanoses*, Dermatol Clin. 25(3):393-400.

69. Luchini, P. and Motz, H. (1990) *Undulators and Free-Electron Lasers*, Claredo Press, Oxford.

70. Maiman, T.H. (1960) *"Stimulated Optical Radiation in Ruby"*, Nature, 187 4736, pp. 493-494.

71. Marjorie, F., Yang, Y., Tuchin Anna, N. and Yoroslavsky (2009) *principle of light skin interactions fundamentals properties of light*, verlog.London.

72. Matz, H. and Tur, E. (2007) *Vitiligo*, Curr Probl Dermatol. 35:78-102.

73. McGovern, T., Bolognia, J. and Leffell, D. (1999) *Flip-top pigment transplantation: a novel transplantation procedure for the treatment of depigmentation*, Arch Dermatol. 135(11):1305-7.

74. McGregor, I. and Morgan, G. (1963) *Axial and random pattern flaps*, Br J Plast Surg. 26:202.

75. McKee, P., Calonje, E. and and Granter, S. (2005) *Disorders of Pigmentation. In: Pathology of the Skin with Clinical Correlations*, Vol 2. 3rd ed. China: Elsevier Mosby; 993-7.

76. Menchini, G., Lotti, T. and Tsoureli-Nikita, E. (2004) *UV-B narrowband micro phototherapy. In: Lotti T, Hercogova J. (eds). Vitiligo: Problems and Solutions*, New York, NY: Marcel Dekker. 323-34.

77. Mester, E., Szende, B. and Tota, J.G. (1967) *"Effect of laser on hair growth of mice"*, Kiserl Orvostud 19: 628–631.

78. Moellmann, G., Klein-Angerer, S., Scollay, D., Nordlund, J. and Lerner, A. (1982) *Extracellular granular material and degeneration of keratinocytes in the normally pigmented epidermis of patients with vitiligo*, J Invest Dermatol. 79(5):321-30.

79. Moore, K. and Persuad, T. (1998) *The integumentary system. In: Before We Are Born: Essentials of Embryology and Birth Defects*, 5th ed. Philadelphia, Pa: Saunders. 481-96.

80. Morris, J. and Gibbins, I. (1997) *Autonomic Innervation of the Skin*, 1st. Informa Healthcare.

81. Moshkovska, T. and Mayberry, J. (2005) *It is time to test low level laser therapy in Great Britain*, Postgrad Med J 81. 436-41.

82. Njoo, M. and Westerhof, W. (2004) *Therapeutic guidelines for vitiligo. In: Lotti T, Hercogova J. (eds.) Vitiligo: Problems and Solutions*, New York, NY: Marcel Dekker. 235-52.

83. Omega company (2006) *Omega laser systems manual. Issue 6 revision 3*, http //www.omegalaser.co.uk.

84. Ongenae, K., Van Geel, N. and Naeyaert, J. (2003) *Evidence for an autoimmune pathogenesis of vitiligo*, Pigment Cell Res. 16(2):90-100.

85. Ortonne, J. (2008) *Vitiligo and other disorders of Hypopigmentation. In: Bolognia, J., Jorizzo, J., Rapin,i R. (eds.)*, Dermatology. Vol 1. 2nd. Spain: Elsevier; 65.

86. Pajvani, U., Ahmad, N. and Wiley, A. (2006) *The relationship between family medical history and childhood vitiligo*, J Am Acad Dermatol. 55(2):238-44.

87. Passeron, T. and Ortonne, J. (2006) *Use of the 308-nm excimer laser for psoriasis and vitiligo*, Clin Dermatol. 24(1):33–42.

88. Passeron, T., Ostovari, N. and Zakaria, W. (2004) *Topical tacrolimus and the 308-nm excimer laser: a synergistic combination for the treatment of vitiligo*, Arch Dermatol. 140(9):1065-9.

89. Patel, C.K.N. (1964) *"Continuous-Wave Laser Action on Vibrational-Rotational Transitions of CO2"*, Physical Review 136 (5A): A1187–A1193.

90. Poblet, E., Jiménez, F. and Ortega, F. (2004) *The contribution of the arrector pili muscle and sebaceous glands to the follicular unit structure*, J Am Acad Dermatol. 51(2):217-22.

91. Polanyi, T. (1983) *Laser physics*, Otolaryngol Clin North Am 16:753.

92. Prost-Squarcioni, C. (2006) *Histology of skin and hair follicle*, Med Sci (Paris).22(2).131-7.

93. Rabe, J., Mamelak, A., McElgunn, P., Morison, W. and Sauder, D. (2006) *Photoaging: mechanisms and repair*, J Am Acad Dermatol. 55(1):1-19.

94. Rashtak, S. and Pittelkow, M. (2008) *Skin involvement in systemic autoimmune diseases*, Curr Dir Autoimmun. 10:344-58.

95. Ribeiro, M.S., Silva, F.D., Araujo, C.E.D., Oliveira, S.F.D., Pelegrini, C.M., Zorn, T.M. and Zezell, D.M. (2004) *Effects of low-intensity polarized visible laser radiation on skin burns: a light microscopy study*, J Clin Laser Med Surg 22.59-66.

96. Rusfianti, M. and Wirohadidjodjo, Y. (2006) *Dermatosurgical techniques for repigmentation of vitiligo*, Int J Dermatol. 45(4):411-7.

97. Santana-Blank, L., Rodriguez-Santana, E. and Santana-Rodriguez, K. (2005) *Photo-infrared pulsed bio-modulation (PIPBM): a novel mechanism for the enhancement of physiologically reparative responses*, Photomed Laser Surg 23. 416-24.

98. Saraceno, R., Nistico, S., Capriotti, E. and Chimenti, S. (2009) *Monochromatic excimer light 308 nm in monotherapy and combined with topical khellin 4% in the treatment of vitiligo: a controlled study*, Dermatol Ther. 22(4):391-4.

99. Sassi, F., Cazzaniga, S. and Tessari, G. (2008) *Randomized controlled trial comparing the effectiveness of 308-nm excimer laser alone or in combination with topical hydrocortisone 17-butyrate cream in the treatment of vitiligo of the face and neck*, Br J Dermatol.

100. Savai, S., Kazemi, B., Esmaeili, M., Fallah, A., Modarresi, A. and Mir, M. (2008) *Effects of low level He-Ne laser irradiation on the gene expression of IL-1 beta,TNF-alpha, IFN-gamma,TGF-beta,bFGF,and PDGF in rat's gingiva*, Lasers Med Sci.23:331-335.

101. Saygun, I., Karacay, S., Serdar, M., Ural, A., Sencimen, M. and Kurtis, B. (2008) *Effects of laser irradiation on the release of basic fibroblast growth factor (bFGF) , insulin like growth factor-1 (IGF-1) , and receptor of IGF-1 (IGFBP3) from gingival fibroblasts.*, Lasers Med Sci.23:211-215.

102. Schallreuter, K., Bahadoran, P. and Picardo, M. (2008) *Vitiligo pathogenesis: autoimmune disease, genetic defect, excessive reactive oxygen species, calcium imbalance, or what else discussion?*, Exp Dermatol. 17(2):139-40; 141-60.

103. Schallreuter, K., Wood, J. and Pittelkow, M. (1994) *Regulation of melanin biosynthesis in the human epidermis by tetrahydrobiopterin*, Science. 263(5152):1444-6.

104. Schuocker, D. (1998) *Handbook of the Eurolaser Academy*, Springer.

105. Schwartz, F., Brodie, C., Appel, E., Kazimirsky, G. and Shainberg, A. (2002) *Effect of helium-neon aser irradiation on nerve growth factor synthesis and secretion in skeletal muscle cultures*, J Photochem Photobio B. 66:195-200.

106. Spritz, R. (2008) *The genetics of generalized vitiligo*, Curr Dir Autoimmun.10:244-57.

107. Sravani, P., Babu, N., Gopal, K., Rao, G., Rao, A. and Moorthy, B. (2009) *Determination of oxidative stress in vitiligo by measuring superoxide dismutase and catalase levels in vitiliginous and non-vitiliginous skin*, Indian J Dermatol Venereol Leprol. 75(3).

108. Steen, W.M. (1998) *"Laser Materials Processing"*, 2nd Ed. New York.

109. Stewen, C., Larionov, M. and Giesen, A. (2000) *"Yb:YAG thin disk laser with 1 kW output power"*, in OSA Trends in Optics and PhotonicsOptical Society of America, Washington, D.C.

110. Taylor, G. and Pan, W. (1998) *Angiosomes of the leg: anatomic study and clinical implications*, Plast Reconstr Surg. 102(3):599-616; 617-8.

111. Toussaint, S. and Kamino, H. (1997) *Noninfectous papular and squamous diseases. In: Elder D, Elenitas R, Jaworsky D, Johnson B Jr.*

Lever's Histopathology of the Skin, Philadelphia, Pa: Lippincot-Raven. 154-5.

112. Townes and Charles, H. (1999) *How the Laser Happened: Adventures of a Scientist*, Oxford University Press. pp. 69-70.

113. Townes and Charles, H. (2008) *"The first laser"*, University of Chicago.

114. Trager, F. (2007) *Principal of Lasers*, Springer Handbook of Lasers and Optics. 583-584.

115. Tumilty, S., Munn, J., McDonough, S., Hurley, D.A., Basford, J.R. and Baxter, G.D. (2010) *"Low Level Laser Treatment of Tendinopathy: A Systematic Review with Meta-analysis"*, Photomedicine and Laser Surgery 28 (1): 3–16.

116. Ueda, Y. and Shimizu, N. (2001) *Pulse irradiation of low-power laser stimulates bone nodule formation*, J Oral Sci 43.55-60.

117. Ueda, Y. and Shimizu, N. (2003) *Effects of pulse frequency of low-level laser therapy (LLLT) on bone nodule formation in rat calvarial cells*, J Clin Laser Med Surg 21.271-7.

118. van den Wijngaard, R., Aten, J. and Scheepmaker, A. (2000) *Expression and modulation of apoptosis regulatory molecules in human melanocytes: significance in vitiligo*, Br J Dermatol. 143(3):573-81.

119. van Geel, N., Ongenae, K. and Naeyaert, J. (2001) *Surgical techniques for vitiligo: a review*, Dermatology.202(2):162-6.

120. van Geel, N., Wallaeys, E., Goh, B., De Mil, M. and Lambert, J. (2010) *Long-term results of noncultured epidermal cellular grafting in vitiligo, halo naevi, piebaldism and naevus depigmentosus*, Br J Dermatol. 163(6):1186-93.

121. Waller, I. (1966) *The Nobel Prize in Physics Presentation Speech*.

122. Weber, M.J. (1991) *Handbook of Laser Science and Technology*, CRC Press, Boca Raton, FL, Ann Arbor, MI, Boston, MA.

123. Whittaker, P. (2004) *Laser acupuncture: past, present, and future*, Lasers Med Sci 19.69-80.

124. Willet, C.S. (1974) *"An Introduction to Gas Lasers"*, Pergamon Press. 407–411.

125. Woodruff, L., Bounkeo, J., Brannon, W., Dawes, K., Barham, C., Waddell, D. and Enwemeka, C. (2003) *The efficacy of laser therapy in wound repair: a meta-analysis of the literature*, J Clin Laser Med Surg. 21(5):249-58.

126. W-t, Y., Hs, Y. and CH-SH, W. (2010) *Noninvasive cutanous blood flow assesment as a reponse predictor for visible light therapy on segmental vitiligo. A prospective pilot study*, British Journal of Dermatology.

127. Wu, X. (2004) *"Ultraviolet photonic crystal laser"*, Applied Physics Letters. 85 (17). 3657.

128. Wu, C., Hu, S., Lan, C., Chen, G., Chuo, W. and Yu, H. (2008) *Low-energy helium neon laser therapy induces repigmentation and improves the abnormalities of cutaneous microcirculation in segmental type vitiligo lesions*, Kaohsiung J Med Sci. 24:180-9.

129. Yang, y., Lin, x., Fu, w., Luo, x. and Kang, k. (2009) *An approach to the correlation between vitiligo and autoimmune thyroiditis in Chinese children*, Clin Exp Dermatol.

130. Yousefi-Nooraie, R. (2008) *Low level laser therapy for nonspecific low-back pain.*.

131. Yu, H., Wu, C., Yu, C., Kao, Y. and Chiou, M. (2003) *Helium-neon laser irradiation stimulates migration and proliferation in melanocytes and induces repigmentation in segmental-type vitiligo*, J Invest Dermatol 120.56-64.

132. Zhang, B., Lin, M., Qi, X., Zhang, R., Wei, Z. and Zhu, J. (2013) *Characterization of circulating CD8+T cells expressing skin homing and cytotoxic molecules in active non-segmental vitiligo*, Eur J Dermatol.

133. Zhang, Y., Song, S., Fong, C.C., Tsang, C.H., Yang.Z. and Yang, M. (2003) *cDNA microarray analysis of gene expression profiles in human fibroblast cells irradiated with red light*, J Invest Dermatol 120.849-57.

APPENDICES

APPENDIX (1)

Sudan University of Science and Technology
College of Graduate Studies

Clinical Evaluation of Using Coherent (Laser) and Incoherent Light Sources in the Treatment of Vitiligo

Questionnaire

By

Nagi Zarif Malati

Date: ……

Individual No.: ……..……..

Name: ……...……..

Phone No.: …….

Personal Data:

1. Age (years):

<=15 ☐ 16 - 25 ☐ 26 - 35 ☐ 36-45 ☐

46 - 55 ☐ 56 and more ☐

2. Sex:

Male ☐ Female ☐

Clinical Data:

1. Duration of Vitiligo:

Less than one year ☐ 1-2 years ☐ 3-4 years ☐ 5-6 years ☐

7-8 years ☐ 9-10 years ☐ 13-14 years ☐ 15-20 years ☐

2. Stage of Vitiligo before Starting the Treatment:

Stable ☐ complete healing and then relapse (unstable) ☐

3. Classification of Vitiligo Type:

Focal vitiligo ☐ Segmental vitiligo ☐ Acrofacial vitiligo ☐

Vitiligo vulgaris (generalized) ☐

4. Any Treatment for Vitiligo in a Period less than Three Months:

Yes ☐ No ☐

Procedures:

1. Type of Treatment:

Incoherent Light ☐ LLLT ☐

2. Total Number of Treatment Sessions:

<=5 ☐ 6-15 ☐ 16-25 ☐ 36-45 ☐ 46 and more ☐

3. Initial Repigmentation Response:

From the first session ☐ No response ☐

4. Repigmentation Response:

Excellent ☐ Very good ☐ Good ☐ Poor ☐ Bad ☐

5. Delayed Repigmentation Response after Stopping the Treatment:

Yes ☐ No ☐

6. Side Effects of the Treatment:

erythema ☐ telangiectasia ☐ Infection ☐ None ☐