Sudan University of Science and Technology

College of Graduate Studies

Comparative Study of The Efficacy of Carbon Dioxide Laser and Low Level Laser Therapy in Treatment of Localized Cutaneous Leishmaniasis

Adissertation submitted in partial fulfillment for the requirement of the higher Diloma in Laser Applications in Medicine

By

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2022-March

بسم الله الرحمن الرحيم

"قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا إِلَّا مَا عَلَّمْتَنَا إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ"

الآية (32) ـ سورة البقرة

Dedication

To my parents,

husband, sons and daughter

To my teachers and colleagues.

To all patients with cutaneous

leishmania, who suffer a lot,

in my beloved Country,

Africa and the

world.

Eman

Acknowledgement

With further gratitude, I would like to thank my supervisor Dr Osman Abd Elmalek for all his helps and great advices, support and efforts.

I would like to thank my supervisor Dr. Sohad Al Wakeel for her advices and support.

Abstract

Lasers have been used for treatment of several skin diseases since 1970, with different results. Recently Lasers have being used for treatment of cutaneous leishmaniasis which is a chronic and resistant skin disease.

In this article treatment of cutaneous leishmaniasis with different lasers types (Carbon dioxide and low level laser therapy) were evaluated and the results are compared.

Methodology: In this comparative meta-analysis study, all available data from online studies using CO_2 laser ,Group A studies (three studies), and LLLT , Group B studies (two studies) , as single treatment modality were collected and re-analyzed and compared.

Results: from available studies patient clinical responsre and complete cure rate we opserved in both laser modalities ,but significantly more with CO_2 laser treatment.Results suggested that one treatment session was enough in all trials using CO_2 laser which was quite less than in LLLT treatment. Side effects and complications were minimal and trivial in both methods.

Conclusion: CO_2 laser treatment for cutaneous leishmaniasis is more effective than LLLT for treatment of cutaneous leishmaniasis.

خلاصة الدراسة

تستخدم اشعة الليزر في علاج عدد من الأمراض الجلدية منذ العام ١٩٧٠، بنتائج متفاوتة. حديثاً تم استخدام الليزر كعلاج لداء الليشمانيا الجلدية التي تعرف بانها مرض مزمن ومقاوم للعلاج.

الهدف من هذا البحث دراسة فعالية علاج داء الليشمانيا الجلدية بواسطة ليزر ثاني أكسيد الكربوني و العلاج بواسطة الليزر منخفض القدرة، ومقارنة العلاجين.

اعتمدت الدراسة علي جمع المعلومات المتاحة من الدراسات السابقة المثبتة المُصدرة عالميا عن علاج داء الليشمانيا بالليزر ثاني اكسيد الكربوني (مجموعة الدراسات أ) والليزر منخفض القدرة (مجموعة الدراسات ب) ومن ثم اعادة تحليل هذه المعلومات واستخلاص النتائج منها على سبيل المقارنة.

خلصت الدراسة الى أن استخدام الليزر ثاني أكسيد الكربوني أفضل من الليزر منخفض القدرة في علاج داء الليشمانيا، حيث انه اكثر فعالية ويحتاج لعدد زيارات علاجية أقل حسب الدراسات التي شملها البحث. كما أن الاعراض الجانبية المصاحبة للمعالجة بالليزر كانت خفيفة و مؤقتة.

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List of Abbreviations

℃	Unit Centigrade
µm	Micrometer
AIDS	Acquired Immuno deficiency Syndrome
BC	Before Crest
CCL	Chronic Cutanious Leishmaniasis
CL	Cutanious Leishmaniasis
Cm	Centimeter
CNS	Central Nervous System
CO_2	Carbon Dioxide
DCL	Disseminated Cutaneous Leishmaniasis
DM	Diabetes Mellitus
DNA	Deoxynucliec Acid
g	Gram
Ga Al As laser	Galminum Alminum Arsinide laser
GIT	Gastrointestinal Tract
HIV	Human immune Defeicency Virus
IL	Intralesional
IM	Intramuscular
IV	Intravascular
Kg	Kilogram
L	Leishmaniasis
LCL	Localized Cutanious Leishmaniasis
LD Bodies	Leishman Donovani Bodies

LLLT	low level laser therapy
MCL	Mucocutaneous Leishmaniasis
mg	Milligram
ml	Millileter
NNN	Nicolle–Novy–MacNeal
OPD	Optical Penetration Depth
PCR	Polymerase Chain Reactio
PHN	Post herpitic neuralgia
Pt	Patient
TRT	Thermal relaxation time
VL	Visceral Leishmaniasis
WHO	World Health Organization

Chapter One

Cutaneous Leishmaniasis and Basic Laser Concepts

1.1. Introduction:

Cutaneous Leishmaniasis is a spectrum of chronic infections caused by the protozoa of the leishmania species. Over twenty species are implicated in human and animal infection (Tony et al ,2004).

Transmission is by the bite of a tiny insect sand fly from the genera Phelepotomus and Lutzomia (Wolff et al,2002)

The disease is world-wide distributed over 98 countries, in South America , Mediterranean , Africa and parts of Asia (Alvar J et al 1997). Epidemics are common in the intertropical temprate regions. Currently exist in Afghanistan, Brazil , India and Sudan. Cutaneous Leishmaniasis (CL) is a complex entity representing a major public health problem in the Eastern Meditrranean region.The World Health Organization reports 1.2 million new case/year of cutanious leishmaniasis (Craig ,2014)

Although it is not a fatal disease; however it may be hardly tolerated by patients for three reasons: first, its particular localization in uncovered areas which makes an esthetic bother for the patient, especially when it lies on the face. Secondly its spontaneous evolution is long (some lesions may require years to heal). Finally, the disfiguring scar that may be caused particularly if it was not well treated (Abderrahman ,2013).

Different medical ,surgical and traditional modalities have been suggested but non is completely effective, and lot of complicatins are usually present.

Recently laser treatment have been introduced in treatment of cutaneous leishmaniasis. Different lasers have been tried including: Carbon Dioxide laser, Neodimun Yitrium Garnet Laser and Low intensive Diode laser, with different results.

In this study results of differents clinical trials using CO_2 laser and low level Diode lasers were studied and their outcome were compared.

1.2 Cutaneous leishmaniasis

1.2.1 Definition:

Cutaneous leishmaniasis is a potentially chronic and disfiguring disease caused by the protozoa of the leishmania species. People with Cutaneous leishmaniasis have one or several long lasting lesions on their skin usually without fever or general symptoms (Craig,2014).

1.2.2 Synonyms:

Old World cutaneous leishmaniasis: Oriental sore, Delhi boil, Bagdad boil , Aleppo boil , Balkan boil, Saldana boil. (Jean et al,1997)

New World cutaneous and mucocutaneous leishmaniasis: chiclero ulcer in Mexico, uta and espundia in Peru, ulcer de Bauru in Brazil, bush or forest yaws in Guyanas(Jean L. Bologonia et al,1997).

In Sudan Al hashara (the insect) and Al Okut (little sister) are common synonyms (A.M.EL et al,2001).

1.2.3 History:

Perscriptions of conspicuous lesions similar to cutaneous leishmaniasis from the seventh century BC, and even earlier texts were found. In Africa ,India and Spain the disease was early described and well known .(Cox,**1996**)

In 1901, Leishman identified the organisms in smears taken from the spleen of a patient who had died from "dum-dum fever" ; Vesciral leishmaniasis(Piero, 2012)

1.2.4 Epidemiology of Cutaneious leishmaniasis :

Cutaneous leishmaniasis principally affects poor populations. Out-breaks can occur anywhere, in both urban and rural areas , and in refugee camps or among internally displaced populations .

The disease burden in the Meditrranean region is 57% of Cutaneous leishmaniasis burden worldwide. Cutaneous leishmaniasis due to leishmania Tropica and L. Major is endemic in 18 countries/territories in the region (Cox,1996).

In Sudan the first cases of CL were reported by Thompson and Balfourv in 1910 in two Egyptian soldiers who had contracted the disease in Egypt.The first indigenous case was described by Archibald in 1911 in a native from Nuba mountains of Kordofan in western Sudan. In the 1970s and 1980s two major epidemics of CL occurred in Northern Sudan .The firist epidemic started in 1976 in Shendi- Atbra area. The second started in 1985 in Khartoum , apparently originating in Tuti (A.M.EL et al,2001).

1.2.5 Mortality and Morbidity:

Localized CL often resolves spontaneously in about 6-9 months without therapy, although some infections persist indefinitely (Shifa ,2014). Most cases of diffuse CL, Post kalazar dermal leishmaniasis, and leishmaniasis recidivans are chronic and resistant to treatment, but are associated with very low mortality rates(Cox ,1996).

1.2.6 Pathophysiology :

The vector: Leishmania parasites are transmitted from a vertebrate host to another by a tiny 2 – 3 mm long insect, the phlebotomine (the genera Phlebotomus in the Old World and Lutzomyia in the New World). The female sandfly typically bites animal or human hosts in the evening or night, commonly on exposed skin. Sandflies live in dark, damp places and can fly only 50 meters from their breeding site. Bites are usually not recalled as being painful (Cox,1996).

Phlebotomos Papatasi is the principal vector of CL in Sudan. Daily bitting activity start at 6:30 pm and reach peak at 7:30 pm (A.M.EL et al,2001).

The reservoirs: Leishmaniasis fall into two catogeries according to the reservoir : anthroponotic CL when humans are the reservoirs of Leishmania, and in the other situations, reservoirs are wild, mainly rodents species, Zoonotic CL(Cox,1996).

There is limited informations about animal reservoir in Sudan but Leishmania has been found in the Nile rodents; Arvicanthis nitoticus (A.M.EL et al,2001).

The causing agent of Cutaneous leishmaniasis is a parasitic protozoa of the phylum Sarcomastigophora, order Kinetoplastida, family Trypanosomatidae (Wolff et al,2002)

Leishmania are dimorphic parasites. In the gut of the sand fly or in culture, they exist in the promastigote form (10 to 20 µm, spindle-shaped, and motile with a single anterior flagellum). In the cells of the host's reticuloendothelial system, leishmania exist in the amastigote form (2 to 6 µm, round/oval, nonmotile, and with a relatively large basophilic nucleus and a smaller rod-shaped kinetoplast of extra-nuclear DNA at the base of the lost flagellum). Amastigotes multiply in the cells of the host essentially in the macrophages. Promastigotes multiply freely in the gut of the sand fly and in culture medium (Wolff et al,2002).

In Sudan L. major zymodeme LONI 1 is the commonest isolated type, but other types are commonly isolated. (A.M.EL et al,2001).

1.2.7 Pathogenesis：

Sandflies inoculate the infective promastigotes when taking a blood meal from the superficial vascular network in the human dermis. Inoculated promastigotes are taken up by histiocytes and newly immigrated monocytes, in which they multiply. Most inoculations do not seem to result in clinical disease as phagocytosis and complement-mediated killing of leishmania parasites results in clearing of the infection. A minority of successful parasite result in localized or disseminated clinical cutaneous leishmaniasis.

After a period of time, which depends on parasite species, size of inoculum, and the host's cellular immune response, a clinical lesion appears. This lesion comprises parasitized macrophages, lymphocytes and plasma cells, with little skin structures. .With time, piecemeal and focal necrosis of parasitized cells is found, probably the result of antibody-dependent cell-mediated immunity. The overlying epidermis becomes hyperkeratotic and breaks down,causing

an ulcer whose surface is covered with a crust composed of hyperkeratotic debris, dried exudate, dead cells, and live and dead parasites.

This activity continues for several months, while the lesion appears clinically static. In other, especially chronic cases, the more classical epithelioid cell, and sometimes giant cell granuloma, develops with relatively little necrosis, but similar epidermal changes. In these cases, parasites are difficult to find (Wolff et al,2002).

1.2. 8 Immunopathology:

Acquired T-cell immunity largely determines both the clinical course and the therapeutic response of leishmaniasis (Elamin et al,2005).

1.2. 9 Clinical Presentation of Cutaneous Leishmaniasis:

Lesions start as erythematous papules that enlarge over a few weeks to form nodules/plaques and often ulcerate and become crusted. The "volcanic" noduloulcerative morphology is characteristic and consists of a painless ulcer with a rolled margin and a necrotic base that is often covered with an adherent crust (Wolff et al,2002).

Other presentations include ice-berg nodules, eczematoid, psoriasiform, erysipeloid, paronychial, verrucous, keloidal and rarely mycetoma-like lesions. Satellitosis, regional lymphadenopathy, localized lymphadenitis, sporotrichoid lymphatic spread and localized hypoesthesia may occur. Secondary bacterial infection is common(Arfanul,Simeenber,2016).

1.2.10 Laboratory Studies:

Experienced health care workers in endemic areas often diagnose leishmaniasis on the clinically with a high degree of accuracy ,alhowever, laboratory confirmation is important before engaging in treatment , which may be systemic ,long duration and may be highly toxic(deLima et al,2005).

1-Skin Smears to demonisterate Leishman Donovani Bodies.

2- Histopathology The hallmark of the disease is the presence of numerous amastigotes within histiocytes (Wolff et al,2002).

3-Culture:Culture(at room temperature) using a biphasic medium such as Novy-MacNeal-Nicolle(Elamin et al,2005)

4- Monoclonal Antibodies: high sensitivity and specificity.

5- Isoenzyme Analysis:allows for species identification.

6- Serology: low sensitivity (antibodies present in low titre)

7- Leishmanin Skin Test (Montenegro test): detects exposure to leishmania without distinguishing between past and active infection.(Singh,2003)

8-Molecular Techniques: high specificity and improved sensitivity over microscopy and culture (Babajev et al 2004).

1.2.11 Differential Diagnosis of Cutaneous Leishmaniasis :

Arthropod bite,pyoderma, Dermatophyte (Majocchi granuloma),Tuberculosis, Atypical mycobacterial infections, deep fungal infections, basal cell carcinoma, squamous cell carcinoma, keratoacanthoma ,Lymphoma , Foreign body granuloma, Discoid lupus and leprosy (Wolff et al,2002).

1.2.12 Diagnosis:

A positive diagnosis of cutaneous leishmania can be suggested, and in most cases confirmed, by the presence of one or more of the following criteria:

1. History of exposure to an endemic area in the previous weeks or months.

2. History of sandfly bites in the previous weeks or months.

3. History of high-risk activities such as sleeping outdoors, jungle or desert trekking.

4. Non-healing chronic nodular, violaceous ulcer for 4–6 weeks or longer.

5.Demonstration of amastigotes in Giemsa-stained smears from infected skin by direct microscopy.

6. Demonstration of intracellular amastigotes in the dermis in skin biopsy.

7. Presence of leishmanial granulomas in the dermis in skin biopsy.

8. Growth of promastigotes in Nicolle–Novy–MacNeal culture medium from lesional specimens.

9. Demonstration of leishmanial DNA by the PCR (Tony et al ,2004).

1.2.13 Management of Cutaneous Leishmaniasis:

Goals:

1- Accelerating healing of skin lesions.

2- Decreasing morbidity.

3- Decreasing risk for local dissemination.

4- Decrease risk of relapse.

5- Decrease risk of human to human transmission (Wolff et al,2002).

General management:

Lesions should be washed, covered by dressing to promote better healing, prevent secondary infection and decrease man to man transmission.

Specific management:

Selection of treatment depend on site , type , size, number of skin lesion/s, duration and evolution of the lesion/s, immunological and general health of the patient.Treatment can be physical , lesional or systemic therapy.

A- Physical therapy:

1.Laser therapy**:** Some studies show that Carbon dioxide laser had provided 94% healing in 1 month, while other studies had tried Neodinum Yag laser and Low level lasers providing good

outcome. Few stusdies had also tried Pulsed Die lasers for cutaneous leishmaniasis (Asilian et al 2006).

2. Thermotherapy: Localized controlled heat (39-40°C),however the procedure is painful and requires local anesthetic (Reithinger et al,2005).

3.Cryotherapy*:* particularly in small non ulcerated lesions. Side effects include hypopigmentation and development of new satellite lesions (Mosleh,2008)

4. Electrotherapy: There is parasiticidal effect of electricity on L.major, both in vitro and in vivo. (Sharquie,1998)

5. Excision: In cases of an isolated, chronic lesion, surgical excision may be indicated (Jean et al,1997).

B- Local/ Lesional drug therapy: Effective in cases of few lesions , without local dissemination or risk of dissemination (relatively benign lesions) , they include:

1- Intralesional injection of pentavalent antimony derivates (PAD): WHO recommends 1-3 ml injection under the edges of the lesion(Urbà González et al,2007). The injection is useful in early non inflamed lesions. Side effects include: infection , burning at the site of injection, itching, inflammation, vasovagal shock due to severe pain and stibio-intolerance(Miranda-Verástegui et al,2005)

2-Imiquimod: 5% cream.The main problem is its high cost (Seeberger J et al,2003)

3-Topical antifungals: 2% miconazole cream and 1%clotrimazole 2% ketoconazole (Miranda-Verástegui et al,2005)

4- Paromomycin ointments: 15% paromomycin sulphate(Lee,Hasbun,2003)

5-Intralesional zinc sulphate 2% (Miranda-Verástegui et al,2005)

6-Intralesional hypertonic sodium chloride:Trials from Iraq gives variable results (Miranda-Verástegui et al,2005)

7- Intralesional metronidazole : Metronidazole 5% solution (Makram et al,2004)

8- In Sudan, there are studies showing the effectiveness of Garlic methanol extracts which shows good results. Neem paste was less effective than garlic, Garad paste was shown to be not effective (Khalid et al,2004).

Topical Leishmanol compound by Sanhory which is formulated mainly from Eugenic acid and Iodine gives a 100% cure rate in treatment of cutaneous leishmaniasis and other chronic wounds(Sanhory,2000)

9-Bees Honey has been shown to keep cutaneous lesions clean, reduce secondary bacterial infection and even reduce the size of some lesions in some studies (Bafghi et al,2008)

C- Systemic Drug Therapy: in localized CL is commonly reserved for complicated cases , such as relapsing disease, failure of topical treatment, presence of more than four lesions , or disease complicated by HIV co-infection (Wolff et al,2002)

1-Pentavalent antimony: sodium stibogluconate and meglumine antimoniate have been used IV or IM. Patients frequently develop gastrointestinal symptoms, arthralgia, headache, abnormal liver function, low platelets and disordered heart rhythm(Tuon et al,2008),.Recently, the rapid emergence of resistance to pentavalent antimonials has been reported (Abdeerhmen et al 2013).

2-Pentamidine : Initial responses are often good but high relapse rates (Abdeerhmen et al 2013).

3-Amphotericin B: may also be used to treat CL leishmaniasis (Wolff et al,2002).

4-Paromomycin: given IM shown comparable results to amphotericin B in some studies(Sundar, Jha,2007),

5-The oral azoles (Wolff et al,2002)

6-Oral rifampicin (Huma,2006), oral zinc sulphate(Sharquie,2001), and azithromycine (Prata et al,2003) have been tried for treatment of CL with limited effects.

1.2.14 Immunization:

Inoculation of live parasite produce long-lasting immunity (Mutiso et al,2010), whereas vaccination with killed parasites or recombinant proteins induces only temporary protection (Yazdanpanah et al, 2006). The main limiting of vaccination is the possibility to develop a lesion similar to that which may occur in real infestation with the same indelible scar, and the restrained efficiency only on Leishmania species used in the vaccine (Yazdanpanah et al, 2006).

1.2.15 Complications of Cutaneous Leishmaniasis:

The major complications of CL are evolvement into Diffuse CL and Chronic CL. Acquired immunodeficiency syndrome (AIDS) and other immunosuppressive conditions increase the risk of visceral dissemination and recurrence after therapy. Other complications include scarring, disfigurement, and social stigma (Wolff et al,2002).

1.2.16 Prevention :

Prevention is difficult for the variety of the reservoirs and vectors , the occurance in poor societies (poor housing,out-door working and sleeping) and increase distruction of the natural reservoir areas leading to more human contact.The preventive treatment is collective and individual, and is based on:eradication of the vector, the eradication of the reservoir and the protection against the bite of sand fly (Jean et al,1997).

1.3 Basic Laser Concepts:

The term laser is an acronym for light amplification by stimulated emission of radiation.

Currently lasers are used in a wide range of applications, in medicine, manufacturing, construction industry, surveying, consumer electronics, scientific instrumentation, and military system.

In 1960, the first functioning laser was developed by Maiman, using a ruby crystal to produce red light of wavelength 694nm, then followed by development of other lasers.Laser and allied technology are developing rapidly becoming widely used in medicine.

New clinical indications are continually being proposed , some of which have been confirmed and others still in the trail stage (Yousif ,Mahdi,2005)

Dr Andre Mester , a professor of surgery Budapest , at Semmelweiss hospital , is often referred to as the grandfather of low level laser therapy (LLLT).

1.3.1 laser system:

Any laser system is composed of:

I.lasing medium:solid , Gas , liquid or semiconductor.

II.Pumping system:optical pumping, electron excitation, chemical reaction or molecule-molecule collision.

III.Optical resonator with one side of 100% reflection and the other side of partial reflection that allow the produced laser beam to pass (figure 1-1).

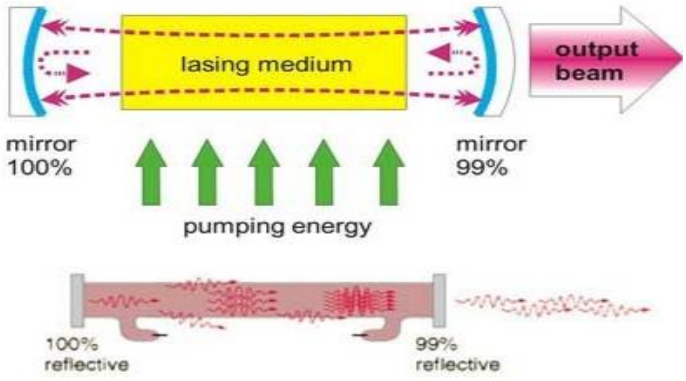

Figure (1-1) Block diagram of Laser system.

1.3.2 Laser Properities:

I. Monochromacity of laser beam: Each laser produces one specific wavelength (figure (1-2)).

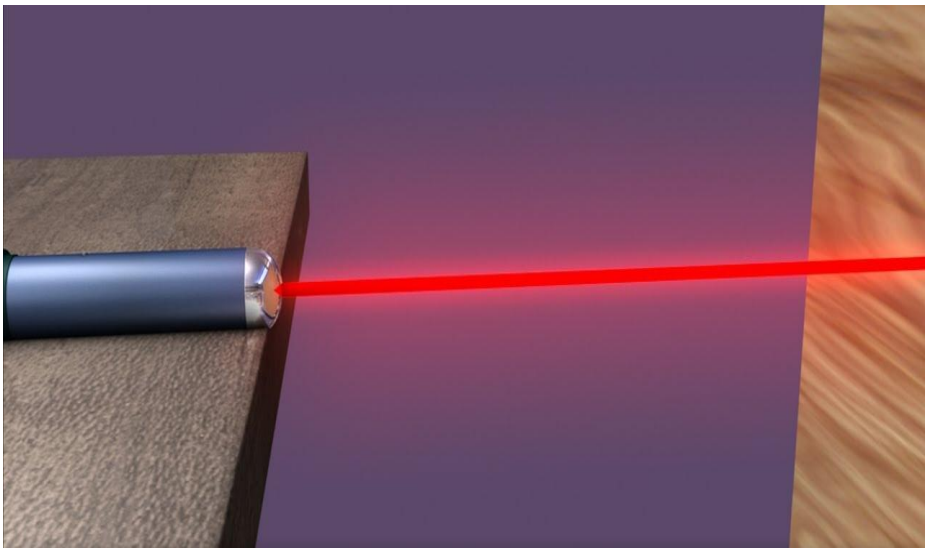

Figure (1-2): photo shows monchromaticity of laser light

II.Directionality of laser beam (figure (1-3)),that mean laser light will not diffuse over distance.

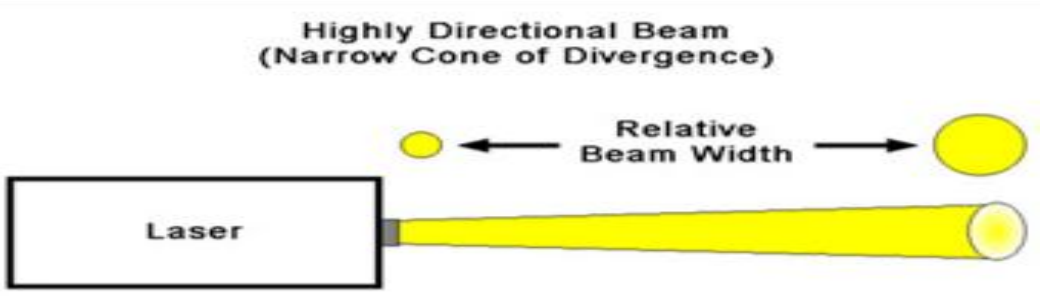

Figure 1-3 : High directionality of laser beam.

III. Beam Coherence of laser light means that all light are parallel and in order of synchronicity. All rays have the same wavelength and start and finish together (in phase) figure (1-4)).

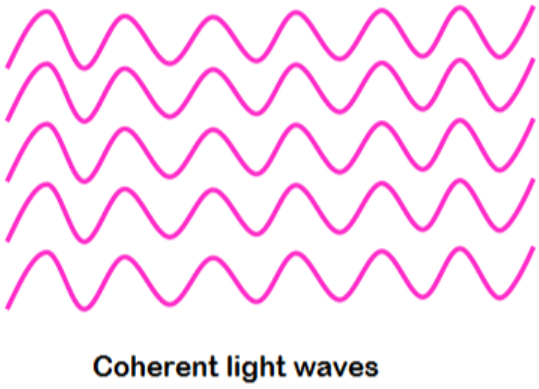

Figure 1-4: Coherence properity of laser light.

1.3.3 Laser Parameters:

Parameters of laser radiation that affect its application in medicine are the length, power, exposure time, beam diameter, power density energy density or fluencies. The variation in these parameters enable the applicator to treat variable range of diseases (Gmbh,2000)

Table 1-1: Laser parameters and its abbreviations.

Parameter	Abbreviations
Wavelength	nm
Power	W
Exposure time	sec
Energy	J
Spot size	cm/ mm
Power density	W/cm2
Energy density	J/cm2

1.3.4 Types of laser:

Laser can be divided in groups according to different criteria: the state of the matter of the active medium, energy level (low intensity (LLLT) and high intensity), risk classification, mode of light delivery: continuous wave mode and pulsed mode (Jan,Lars,2004)

1.3.5 Laser Tissue Interaction :

Light's monochromaticity is responsible for laser selective effect on biologic tissue. Whenever light hits tissues, it can be transmitted, scattered, reflected, or absorbed, depending on the type of tissue and the wavelength (color) of the light. However, light absorption must take place before it can produce a biologic or theraputic effect. A given wavelength of light may be strongly absorbed by one type of tissue , and be transmitted or scattered by another .

The main absorbing components or chromphores in tissues are hemoglobin melanin, water and protein (figure (1-5)) .(Eissa MM,2003)

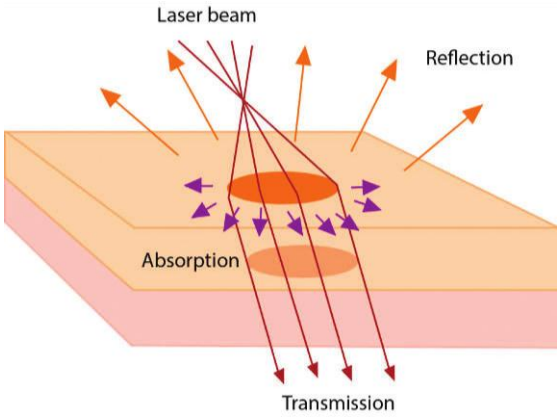

Figure (1-5): Fade of laser beam when it strike a living tissue

When light is absorbed it delivers energy to tissue, and the tissue's reaction depends on the intensity and duration of exposure.These reactions are: photo-mechanical reaction, photo-thermal effect and photo-chemical changes.

In practice, all of these interactions co-exist, although by selecting the proper wavelength, intensity, and pulse duration, any single desired effect can be maximized (Eissa MM,2003)

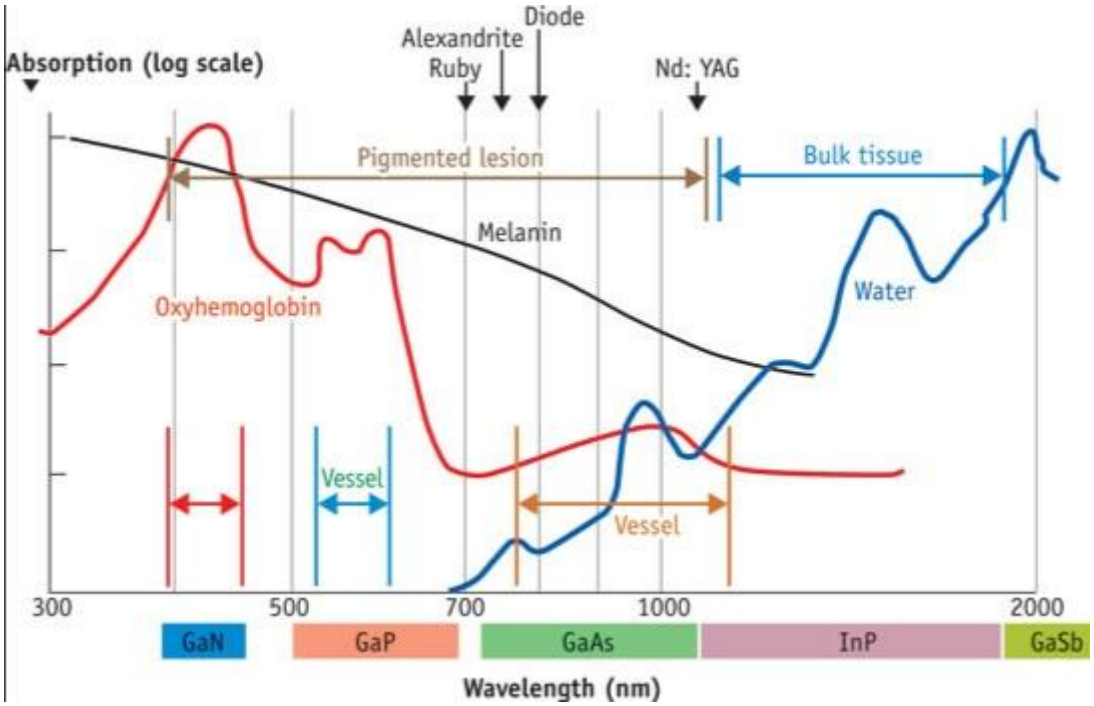

Figure (1-6): Living tissue chromophores and the appropriate laser targeting them.

Laser absorbed with the appropriate tissue exert photo thermal effect in the form of hyperthermia up to 42.5 C, coagulate effect at 60 to 70 carbonization effect up to 159 C, lastly central vibration affect up to 300 C.(figure (1-7)) (Eissa MM,2003).

From periphery to the center the laser exerts it's effect with a zone of hyperthermia, a zone of coagulation, a zone of charring, a zone of vaporization and centrally a zone of ablation. (Eissa MM,2003)

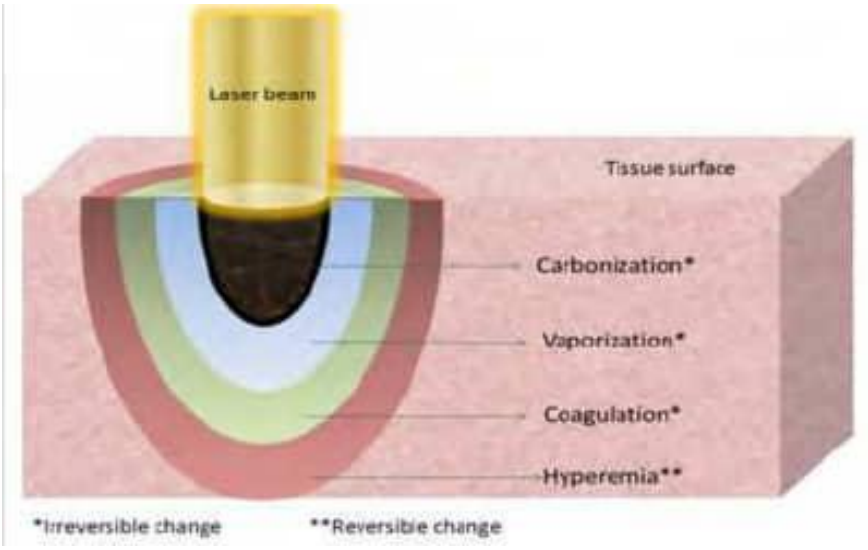

Figure 1-7:Effect of Laser on living tissue.

1.3.6 Laser Applications in Dermatology:

Advances in laser technology have provided dermatologists with more treatment choices and have contributed to improved clinical results .

1.Lasers to resurface the skin: CO_2 laser and Er: YAG laser.

2.Lasers to remove pigmented lesions (eg . Epidermal and dermal pigmented lesions .
Frequency - double -Q - switched Nd : YAG laser , Nd : YAG laser , long pulse ruby laser , and - Q - switched KTP laser .
3.Lasers to remove vascular lesions (eg. Telangiectias, venous lesions, thick port wine stain, and haemangiomas): Pulsed dye laser ,Nd: YAG laser, argon laser and KTP laser.
4.Lasers to remove hair: Nd : YAG laser ,Q - switched Nd :YAG laser and long pulse ruby laser.

5-Lasers to remove tattoos : Q - switched Nd : YAG laser for blue, black , and red tattoos , and Q - switched ruby laser for blue , black , and green tattoos.

6-Lasers to remove superficial masses : eg warts , condyloma , hypertrophic scar , keloid , cyst , xanthelasma , seborrhic keratoses , and epidermal naevi : pulsed dye laser and CO_2 laser.

7-Others uses of lasers:

- Cutaneous leishmaniasis : can be treated by CO_2, LLLT, PDL and Yag laser.
- Psoriasis : Localized and resistant psoriasis has been reported to respond to treatment with both short and long pulse 585 nm photodynamic therapy (PDL) . Treatment is painful and can cause dyspigmentation and scarring.
- Non melanin skin cancers: PDL is used for treatment of non-melanin skin cancers. .
- Herpes simplex : low level laser therapy is effective treatment for oral herpes simplex..
- Low level Laser has an effect both on the acute Herpis zoster and post herpetic neuralgia (PHN). Wound healing: Laser therapy for wound is ideal since it promotes healing and reducing pain.

-Vitiligo: by using Ga AL AS laser or Argon laser. (Eissa MM,2003)

1.3.7 Laser Safety:

laser is a light source that can be dangerous to the people exposed to it through direct exposure, regular or diffuse reflection. Radiations are dangerous for eyes and skin and can cause fire and explosion . Even low power lasers can be dangerous to eyesight . (K.Schroder,Ed,2000)**.**

Laser radiation predominantly causes eye injury via thermal effects on the retina. Sufficient light of wavelength (400-1400 nm) will penetrate the eye-ball and may cause heating of the retina and a transient increase of only 10 ° C can destroy retinal photoreceptors. Infrared and ultraviolet lasers are particularly hazardous, since the body's protective "blink reflex" response only operates if the light is visible (Steve,2006).

Exposure to laser radiation with wavelengths less than 400nm and greater than 1400nm are largely absorbed by the cornea and lens, leading to the development of cataracts or burn injuries. (Michaeal et al,2005)

Maximum permissible exposure is the highest energy density of light source that considered safe.Typically it is set at 10% of the dose that has a 50% chance of creating damage under worst-case conditions although laser beam can damage, cut, or burn skin. (Omega laser system manual,2006)

1.3.8 Risk Classifications:

Since the early 1970s, Lasers have been classified by wavelength and maximum output power into four classes and a few subclasses. The classification categorizes lasers according to their ability to produce damage in exposed people, from class 1 (no hazard during normal use) to class 4 (severe hazard for eyes and skin). (K.Schroder,Ed ,2000)

- Class I:

  Inherently safe ; no possibility of eye damage . (K.Schroder,Ed ,2000)

- Class II

  The blink reflex of the human eye will prevent eye damage, unless the person deliberately stars into the beam for an extended period. A region in the low - power end of Class II where the laser requires in excess of 1000 seconds of continuous viewing to produce a burn to the retina . (K.Schroder,Ed ,2000)

- Class IIIa :

  Lasers in this class are mostly dangerous in combination with optical instruments which change the beam diameter or power density . (K.Schroder,Ed ,2000)

- Class IIIb:

  Lasers in this class may cause damage if the beam enters the eye directly. 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. Protective eyewear is recommended when direct beam viewing of Class IIIb lasers may occur . Lasers at the high power end of this class may also present fire hazard and can lightly burn skin . (K.Schroder,Ed ,2000)

- Class IV:

Lasers in this class may cause severe, permanent damage to eye or skin without being magnified by optics of eye or instrumentation.These are cutting and surgical lasers . (K.Schroder,Ed ,2000)

Laser known to cause other non-beam hazards , fire and high temperatures hazards, chemical hazards ,biological hazards, optical hazards , mechanical hazards , and electrical hazards.(K.Schroder,Ed ,2000)

1.3.9 Contraindications to Laser Therapy:

- Pregnancy: Laser therapy can be used during pregnancy, but better to avoid it, especially a large dose over the abdomen should be avoided. It has been reported that laser coming in contact with acupuncture points may cause abortion. (Jan,Lars ,2004)
- Epilepsy: Pulsed visible light, particularly at pulse frequencies in the 5 to 10 Hz range , can cause epileptic attacks , one should obviously be careful with instruments that use flashing visible light. (Jan,Lars ,2004)
- Thyroid gland: the thyroid gland is a sensitive gland and administrating large doses of laser light over it should be avoided . (Jan,Lars ,2004)
- A concurrent use of photosensitizing drugs. (Jan,Lars ,2004)
- Others conditions which have been reported but no longer considered as contraindication to laser therapy include: Pace maker . (Jan,Lars,2004)
- children: It is now recognized that laser has no effect on the growth plates in children. (Jan, Lars ,2004)
- Cancer is not a contraindication to laser therapy,but avoidance of laser therapy in patients with melanocytic nevus, especially if there is a recent change in it .Laser therapy is not carcinogenic. (Jan ,Lars ,2004)
- Radiation therapy patients: combination of laser therapy and radiation, lead to improving the defense against cancer (Jan ,Lars ,2004).

1.4 low Level Laser Therapy (LLLT):

1.4.1 Difinition:

LLLT composed of light of wavelength in the red and infrared part of the electromagnetic spectrum.Like other lasers it is monochromatic, coherent and polarized light. It penetrate the surface of the skin with no heating effect and no major known side effect. The LLLT biostimulative mechanism works through convertion of laser energy into a chemical energy that promote natural healing and pain relief. (Oron et al,2007)

1.4.2 Physiological Effects of LLLT:

International well-controlled work on LLLT showed a growing knowledge of biochemical mechanism in tissue repair, biomodulation of cell metabolism and motility, collagen production, mast cell chemotactic and degranulation, protein synthesis and fibroblast and macrophage activity and chemotactic effects. Other physiological effects like vasodilatation and angiogenesis, increase serotonin metabolism and acupuncture point stimulation have major therapeutic effects. Therefore LLLT used to enhance healing, increase strength of repaired tissue, reduction of scarring, immune system stimulation, pain relief, resolution of infection and acupuncture point stimulation.(Omega laser manual,2006)

1.4.3 Types of LLLT:

Common low level lasers used in dermatology shown in table (1-2)

Table (1-2) common types of low level laser

Types of Low Level Lasers	**Wavelength**
Argon (Ar)	448-451 nm
Gallium arsenide (Ga-As)	904 nm
Gallium aluminum Arsenide (Ga – AL – AS)	830 nm
Helium – neon (He – Ne)	632 nm
Ruby laser	694 nm

1.4.4 Uses of LLLT in Dermatology:

-Treatment of pain which include: post surgical pain, muscular back pain, cervical or lumbar radiculopathy, tendinitis,oesteoartheritis,dental painful conditions ,frozen shoulder etc

-Collagen synthesis and skin rejuvenation

-Acne treatment

-Inhance tissue proliferation

-Prevent tissue death

-Inhance blood circulation

-Hair growth/regrowth(Rafid,2020)

1.5 Carbon Dioxide Laser :

1.5.1 Difinition:

The Carbon Dioxide (CO_2) laser is a laser system that use carbon dioxide gas as a lasing medium. It emits far infrared light at 10600 nm of the electromagnetic spectrum.

1.5.2 Fundamentals Carbon dioxide laser:

The CO_2 Laser has been developed since 1964, and still is one of the most important laser in dermatological and surgical fields. Because infrared light is invisible the laser is operated with a co-axial low powered (633 nm) helium-neon laser which allows the operator to see where the CO_2 laser beam will be incident on the skin.

The 10600 nm wavelength is difficult to be transmitted by optical fibers, so the beam is delivered along a series of sealed tubes reflecting the beam off a series of of mirrors with rotational articulated couplings .

The original out-put from CO_2 was continuous, but it is now possible to develop CO_2 lasers with pulsed out-puts.With the addition of fractionation of the beam energy into myriad microbeams,

the fractional CO_2 has offered a bridge between the frankly full ablative indications and non ablative skin rejuvenation systems.(Tokuya,Kayoka,2014)

1.5.3 Carbon dioxide laser-tissue interaction:

The CO_2 laser light is strongly absorbed by water which is the major component of most human soft tissues. Light absorption by water is very low throughout the visible and near - infrared spectrum . Absorption start to increase with wavelengths longer than 1200 nm , with the largest peak occurring at approximately 2935 nm . At 10,600 nm the wavelength of currently available CO_2 lasers, the absorptive coefficient is high at over 800 cm – 1.

The response of tissue to CO_2 laser irradiation will be heating up to 100 °C ,the boiling point of water, till vaporization occurs. When heating is rapid all targeted tissue water is vaporized as steam and tissue structures explode outwards owing to the rapid thermal expansion of water, leading shock waves and cavitation effects(Altshuler et al,2001).

A zone of coagulative necrosis 0.6-1.3 mm from irradiated point arrises due to heat diffusion.If the fluence used is insufficient to vaporize tissue rapidly , coagulation , desiccation and carbonization will occur .

Optical Penetration Depth (OPD) is defined as the depth at which the fluence is reduced to 1 /absorbtion co-effietient, or approximately 37 % , of the original value . The OPD for CO_2 laser beam is approximately 20 μm precise tissue ablation is governed by the theory of selective photo thermolysis(Anderson,Parish,1983)

In order to decrease thermal diffusion , sufficient fluence of adequately absorbed wavelength must be delivered in less than the thermal relaxation time (TRT) of the irradiated tissue . TRT is the time required for a given tissue to cool sufficiently from its peak temperature. For the planar geometry of skin ablation, TRT is proportional to the square of the OPD. Thus , for a CO_2 laser , TRT of the epidermis has been calculated to be approximately 1 ms . This value , however , is a topic of debate , as some experimental evidence suggests TRT as high as 20 to 40 ms (Altshuler et al,2001).

At typical settings just above the ablative threshold, CO_2 lasers with 1-ms pulse duration vaporize 30 to 70 um of tissue per pass, essentially removing the epidermis in one pass(Fitzbatrick et al,1999).

1.5.4 Application of Carbon Dioxide Laser in the Dermatology:

-Raised birth marks

-Moles

-Viral warts

-Acne scaring

-Kelloids

-Hypertrophic scars

-Pearly penile papules

-Facial wrinkles and lines

-Freckles, lentigines ,brown spots and melisma

-Rhinophyma

-Actinic keratosis

-Skin Rejuvenation(Anoma,2016)

1.5.5 Complication of Carbon dioxide Laser Therapy:

-Hyper or hypo pigmentation

-Scar

-Infection

- Other hazards peculiar to the CO_2 laser are related to the plume e.g. There are carcinogenic nitrosamines, and some particles are either too small to be filtered by the upper airway or too large to be removed from the alveoli.

1.6 Rationale and Justifications:

With the increase of the international travel, immigration , overseas military exchange and HIV infection leishmaniasis is becoming more prevalent throughout the world.

Treatment of the disease still remains a big challenge due to the natural history of the disease, chronicity, clinical diversity and significant drug toxicity. Hence , searching for safer, cheaper and more effective treatment modalities for the disease arrised as a must.

1.7 Objectives:

1.7.1 General Objectives:

To study the role of laser therapy in treatment of patients with localized cutanious leishmaniasis in studies puplished in Sudan and internationally.

1.7.2 Specific Objectives:

To determine:

- The response of CO_2 laser and LLLT in lesions of localized CL.
- To compare number of sessions needed in CO_2 and LLLT.
- To compare time needed to achieve clinical response in each laser type therapy.
- To access patients tolerability to treatment sessions .
- To access patients satisfaction to outcome
- To detect CO_2 laser and LLLT treatment complications if any.

1.8 Previous Studies:

In 2004, Asilian et al, studied the efficasy of Carbon dioxide (Co_2) laser in the treatment of cutaneous leishmaniasis.

In this study, a CO_2 laser (Sonic 500 machine) was used as a source of a continuous CO_2 laser wave.

Methods: A total of 123 patients (68 female and 55 male) with 183 lesions were treated with the CO_2 laser. The maximum power was 100 W and the pulse width was 0.5-5 s. For the control group, 110 patients (with 250 lesions) were treated with glucantime 50 mg/kg/day for 15 days and, after 15 days of rest, this treatment was repeated (Glucantime Amps, 1.5 g in a 50-mL solution, was used). For follow-up, the patients were visited 1, 3, 4, 8, 12 and 24 weeks after treatment and any complications, recurrences or other wound characteristics were recorded.

Results: Statistical analysis showed that treatment with the CO_2 laser was more effective than treatment with glucantime (P = 0.0007). Complications were also seen less often with the laser treatment than with glucantime and were limited to the ulcer site. The CO_2 laser was more effective in treating cutaneous leishmaniasis than glucantime (1.12 times), had fewer side-effects (4.5% vs. 24%) and resulted in a shorter healing time (1 month vs. 3 months), and treatment could be applied in a single session.

Conclusions: The results of this and previous studies suggest that aser if those providing the treatment are sufficiently experienced. Laser treatment is more cost-effective than other treatments and can be used as first-line therapy for cutaneous leishmaniasis (Asilian et al,2004)

Ofir Arti et al, in 2021 had studied the role of Fractional Ablative CO2 Laser Followed by Topical Application of Sodium Stibogluconate for treatment of cutaneous leishmaniasis to compare the efficacy, safety, associated pain and final cosmetic outcome of fractional carbon dioxide CO_2 laser followed by topical application of sodium stibogluconate vs. sodium stibogluconate injections for the treatment of cutaneous leishmaniasis. A total of 181 lesions (20 patients) were randomly assigned to receive intralesional injections of sodium stibogluconate (control group) or fractional CO_2 laser treatment followed by topical application of sodium stibogluconate (study group). The visual analogue scale (VAS) score of the control group was much higher than that of the study group (6.85 vs. 3.5, respectively, $p < 0.001$). Both the patients

and 2 blinded dermatologists found the final cosmetic outcome to be superior for laser-treated lesions ($p = 0.001$ vs. $p = 0.008$ for controls). Fractional CO_2 laser treatment followed by topical application of sodium stibogluconate is less painful and leads to a better final cosmetic outcome compared with intralesional injections of sodium stibogluconate
(Ofir et al,2021)

In 2015 Ali M. Osman and Nafie A. Almuslet had evaluated CO_2 laser efficacy in the treatment of cutaneous leishmaniasis in a group of 10 Sudanese patients.
The ulcers of 10 randomly selected Sudanese patients, diagnosed as CL patients, were ablated using a 10,600 nm CO_2 laser in fractional mode with a power density of up to 2 W/cm2. The reaction of the patients was observed and their response to the treatment was evaluated 1, 2 and 4 weeks after the laser session. Lesions were photographed and rated by the patient him/herself and an independent blinded evaluator.
Results: The majority of the patients (8 out of 10) had either a complete or very good improvement by the end of follow-up. Pain occurred post-operatively in all patients, while edema, infection and hypo/hyperpigmentation occurred in only two patients. These side effects were trivial and disappeared a few days after treatment.
Conclusion: The fractional CO_2 laser can be used as a good modality for the treatment of CL ulcers (Ali,Nafei,2015).

Efficacy of CO_2 laser for treatment of anthroponotic cutaneous leishmaniasis, compared with combination of cryotherapy and intralesional meglumine antimoniate was studied by
S Shamsi Meymandi,,S Zandi,H, Aghaie and A Heshmatkhah.
Their objectives were to determine the efficacy and safety of CO_2 laser vs combined cryotherapy and intralesional meglumine antimoniate (glucantime) in dry-type CL.
A total of 96 patients were randomly assigned to receive one session of CO_2 laser therapy and 95 patients on combined cryotherapy biweekly with intralesional meglumine antimoniate weekly until complete cure or up to 12 weeks, whichever was earlier. Clinical and laboratory cure, defined as complete re-epithelialization of 100%, complete flattening of induration and negative smear of lesions compared with baseline at weeks 2, 6, 12 and 16, and also at the time of complete cure (week 2, 6, 12 or 16).

Results Of 191 participants, 80 patients with 95 lesions in group A and 80 patients with 95 lesions in group B completed the study. Complete cure was 93.7% (89/95 lesions) in group A and 78% (74/95 lesions) in group B. Complications were similar in the two groups and were limited to the ulcer sites.

They concluded that CO_2 laser was more effective in treating dry-type cutaneous leishmaniasis than combined cryotherapy and intralesional glucantime and resulted in a shorter healing time (6 weeks vs. 12 weeks) with a single treatment session.(S Shamsi et al,2010)

In July 2012 Nafie A. Al-Muslet & Amel Ibrahim Khalid puplished a study aimed to evaluate the effectiveness of Low Level Laser Therapy (LLLT), with specific laser parameters, in the treatment of Cutaneous Leishmaniasis (CL).

Methods: Thirteen patients, clinically and by positive smear diagnosed as cases of CL, were referred from Khartoum Teaching Hospital and were considered as study population. The Treatment was done using diode laser probe with wavelength of 820 nm, followed by cluster probe (assembly of non-coherent and coherent diodes). The dose was: I. Diode laser probe with energy density of 48 J/cm2 for thirty seconds. II. Cluster probe with energy density of 9.6 J/cm2 for two minutes.The distance between the probe and the skin was less than 1cm. The frequency of treatment was three sessions weekly for total of ten sessions.The function of LLLT in this study was to reduce inflammation (anti-inflammatory effect) and accelerate healing. Results:showed that the response was excellent in the majority of treated patient (92.3 %). The complications were minimal and transient. The results proved that LLLT is a successful treatment method for Cutaneous Leishmaniasis and it is easy to perform.(Nafei,Amel,2012)

In November 2020, Karim Khan et al performed a study in University of Malakand .This study comprised of 53 patients (total 123 lesions) with a confirmed diagnosis of CL via positive smear of LD-bodies. The CL lesions were classified in Grade I (i.e., papule of size $\leq$ 1 cm) to Grade V (i.e., vesicle formation, ulceration & superadded infection of size > 4 cm). All the patients were divided into group 1 with low grade (i.e., Grade I and II) CL lesions and group 2 with high-grade disease (i.e., Grade III-V). Red laser light (wavelength = 635 nm) was used for the lesion irradiation, with a light dose of 75 J/cm² and at a low power of 300 mW. The treatment

was divided into four sessions, one session per week. Disease assessment at 10 months follow-up revealed complete response in 91 % and partial response in 9 % patients of group 1, while no response was observed in patients of group 2. LLLT offers a promising treatment modality for patients presenting with early-stage (i.e., Grade I and II) CL lesions.(Karim Khan et al,2020)

Chapter Two

Methodology

2.1 . Study design: Comperative meta-analysis study

2.2. Study Time: January-March 2022

2.3. Inclusion crieteria : On-line puplished Sudanese and International studies using CO_2 laser and LLLT for treatment of cutaneous leishmaniasis**.**

2.4. Exclusion criteria:

- On vitro studies
- Non puplished studies
- Studies used compination medical therapy with laser

2.5. Methods: All data from five available studies that fullfilling the inclusion crieteria , three studying CO_2 laser in treatment leishmaniasis and is reffaired to as Group A in this study, and two studies using LLLT and are refaired to as Group B . Available date regarding number of patients, number of laser treatment sessions, side effects of laser treatment, out come, time needed to achieve clinical response, follow up duration and patient satisfaction were collected and analyzied for each group individually , and then the results for both groups are compared. Statistical analysis was conducted by computer using statistical Package for Social Sciences (SPSS version 20) . The results were presented in tables and figures

Chapter Three

Results, Discussion, Conclusion and Recommendations

3.1Results:

With the increasing incidence of cutaneous leishmaniasis ,and high resistance and toxicity of medical treatment ,and the non-satisfied scaring and cosmotic outcome, laser treatment of cutaneous leishmaniasis gives the last hope for those suffering patients.

3.1.1 Group A studies results:

1ST and 3rd studies used the continuous CO_2 mode while the 2nd study used the fractional mode.

All the three studies had used a one session treatment regemin.

1st study and 2nd study had all patient (100%) complete response in one month, while the 3rd had 91% of patients in a 6 weeks time as shown in figure (3.-1)

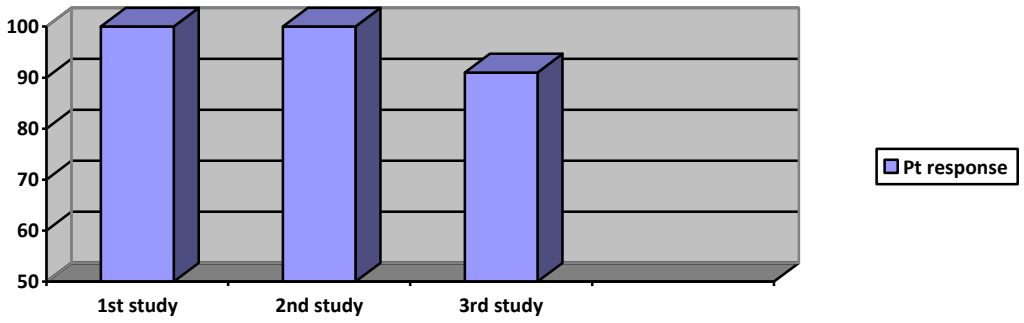

Figure 3-1 : shows the patient response rate in group A studies

All three studies recorded minimal and transient side effects confined at lesion site which were : pain , erythema and hyper-pigmentation as shown in figure (3-2)

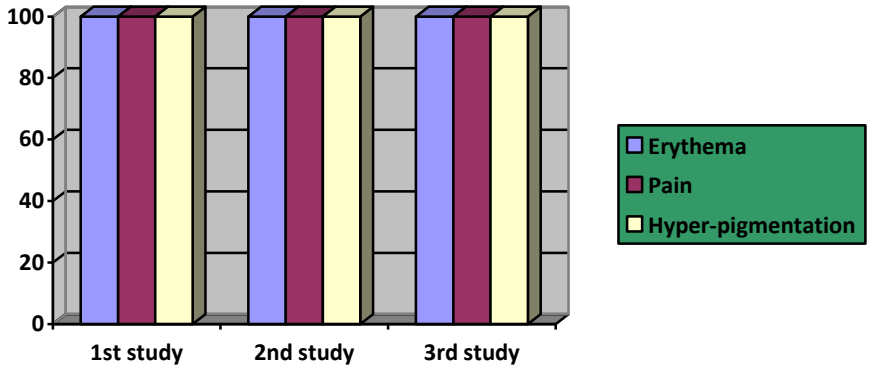

Figure 3-2 : Side effects of laser therapy in Group A studies

3.1.2 Gorup B studies results:

1ST study patient number were 10 while the 2nd study patients were 53.

The 1st study used a protocol of 10 sessions while the second used only four sessions as shown in Table (3-1)

Table 3-1 :Number of Patients and NO of sessions in Group B Studies

	1st study	2nd study
No of patients	10	53
No of sessions	10	4

The first study used a diode laser with 820 nm wave length while the second used 635 nm red laser light.

The 1st study used an energy density of 48 J/cm2 while the 2nd used 75 J/cm2 as shown in Table (3-2)

Table 3-2: comparing different parameters used in Group B studies

Parameter	1ST Study	2nd Study
Laser wave length	820	635
Energy Density	48J/cm2	75J/cm2

Complete response was 92.3 % in the 1st study while is 91% in the second study as shown in (figure 3-3)

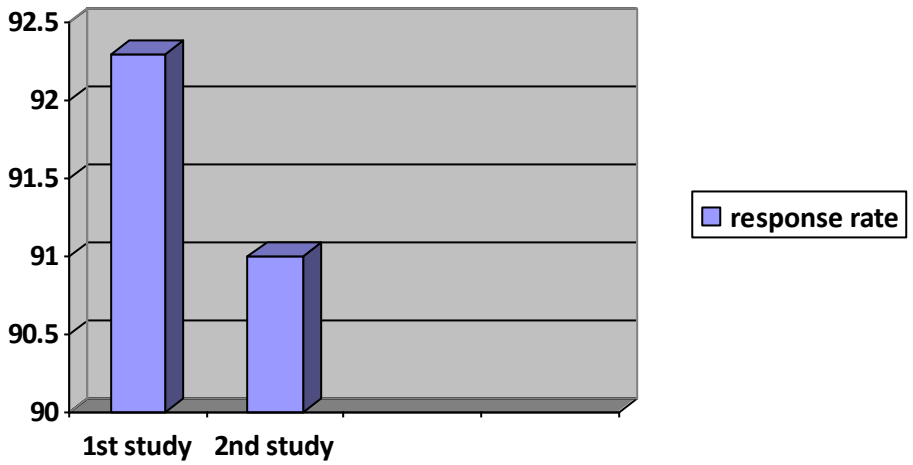

Figure 3-3: patients' clinical response for LLLT

3.1.3 Compararission of Group A and B:

Considering the number of sessions needed in the two groups of studies :

group A (CO_2 laser studies) needed only single session while group B (LLLT studies) needed from 4-10 sessions (7 sessions as average) as shown in figure (3-4)

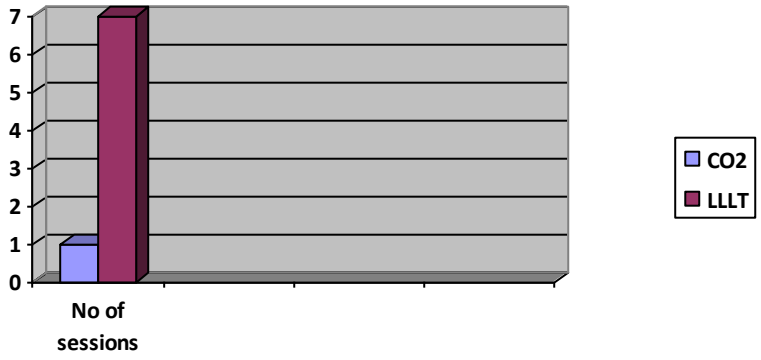

Figure 3-4 Comparision of number of treatment sessions between group A and B laser treatment

The average of patient cure rate in group A was 97.8% while it was 91.6% in group B as shown in figure (3-5)

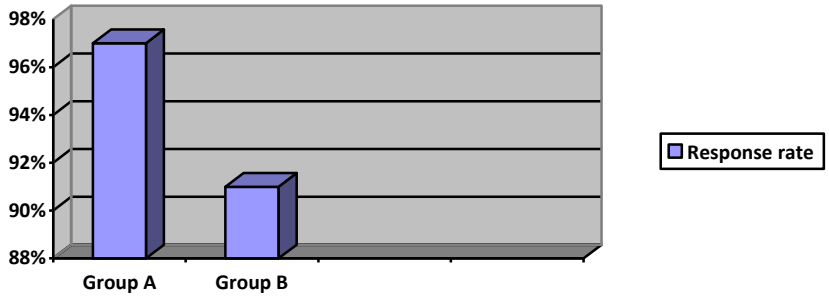

Figure 3-5: Response rate to laser treatment in group A and B studies

3.2 Discussion:

Cutaneous leishmaniasis is still a challenging disease due to chronic course and disfiguring.

Laboratory studies had revealed that leishmania parasites do not multiply within macrophages in temperature more than 39 C in vitro (Saks et al,1989), which arised the idea of using photothermal effect in ablative lasers.While the bio-stimulation and inhansment of immune system arised the idea of using LLLT as treatment modalities in cutaneous leishmaniasis.

When comparing the two mentioned laser treatment modalities, it was evident that both modalities were effective with a high response rate , although CO_2 laser had a superior results than with LLLT.

Other important consideration was the number of treatment sessions, which was only single session in all studies using CO_2 lasser. This had a great impact on patient compliance and make it a good treatment option over other modalities.(Asilan et al,2008)

All studies in both treatment groups recorded side effects, but all were minimal, trivial and localized to treatment site.This when compared to major drug toxicity of conventional medical treatment like cardiotoxicity and nephrotoxicity makes laser therapy ,all modalities, are far better when-ever available. In addission that laser therapy do not require hospital admission and expensive pre-treatment laboratory investigations.

3.3 Conclusion:

- The response and healing of Cutaneous leishmaniasis to CO_2 laser needs less time duration than to LLLT
- Both CO_2 laser and LLLT can be considered as safe treatment modalities for cutaneous leishmaniasis.
- Both laser modalities, CO_2 and LLLT had minimal and trivial side effects that made them good treatment option whenever available.
- CO_2 laser has a better cure rate and fewer treatment sessions making it superior to LLLT.
- Patients have good tolerance and satisfaction as in all studies complete their sessions

3.4 Recommendations:

- Increase orientation of dermatologists, Tropical physicians and other health care providers about laser treatment modalities
- Orientation of population about laser treatment effecasy and safety in cutaneous leishmaniasis
- Further clinical comaritive prospective studies between CO_2 and LLLT with large population are recommended.
- Laser parameters and doses used in treatment should be studied and compared.
- Increase availability of laser devices in Dermatology hospitals and ceters.

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