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Serological Response and PolymeraseChain Reaction to Low Intensity Laser Therapy Versus Polarized Light in Genital Herpes.

Zakaria Mowafy Emam Mowafy[1] Ashraf Hassan Mohamed[1] Hamed Abd Allah Hamed[2] and Eman Abdalla[1]

[1]Physical therapy department for surgery, faculty of physical therapy, Cairo University, Egypt.
[2]Dermatology department, Faculty of Medicine, Cairo University, Egypt.

Abstract : Purpose: to evaluate the efficacy of low intensity laser versus polarized light therapy on serological response and polymerase chain reaction in genital herpes. Methods of evaluation (Measurement of serological tests (IgG and IgM) as well as the polymerase chain reaction). **Methods:-** Forty five patients (15 male and 30 female) suffering from genital herpes simplex infection type II and their ages ranged from 20 to 45 years. They were divided into three groups. Group (A) composed of 15 patients received the LILT for 10 minutes (for the area with cutaneous manifestations) in addition to the topical acyclovir cream 5% twice / day through the treatment period. Group (B) received the BLT for 10 minutes (for the area with cutaneous manifestations) in addition to the topical acyclovir cream 5% twice / day through the treatment period (day after day for 2 months). Group (C) received the topical Acyclovir cream 5% twice / daily throughout the treatment period only. **Results and conclusion:-** Results showed that application both the LILT and BLT in addition to the topical acyclovir cream 5% were effective than application of the topical acyclovir cream 5% alone in improving the genital herpes pain and cutaneous manifestations. But LILT was more beneficial than BLT in improving the genital herpes pain and cutaneous manifestations as evidenced by the highly significant decreases in IgG, IgM and PCR.
Key Words: (Herpes Simplex Virus type II, Serological tests, low intensity laser therapy, Polarized light therapy, Polymerase Chain Reaction and acyclovir cream 5%).

Introduction

Herpes simplex virus (HSV) was recognized as an infectious agent from the last quarter of the 19th century (from 1875), and was the first human virus to be discovered. They are also one of the most commonly studied human viruses due to their ability to cause a range of infections, remain latent in their host, and reactivate to cause lesions at or near the initial site of infection. Herpes viruses are widely distributed in nature and most animal species are susceptible to at least one herpes virus. The following herpes viruses, that have humans as their primary host, have been identified as: herpes simplex virus (HSV-1); herpes simplex virus 2 (HSV-2); human cytomegalovirus (HCMV); varicella-zoster virus (VZV); Epstein-Barr virus (EBV); and Human herpes viruses 6, 7 and 8 (HHV-6, HHV-7, and HHV-8) **Chalmers**[1], **Costanzo et al** [2], **Bethesda**[3], **Bogdanov.** [4].

HSV-1 infection recurs less frequently than HSV-2 infection Herpes simplex is most easily transmitted by direct contact with a lesion or the body fluid of an infected individual. Transmission may also occur through skin-to-skin contact during periods of asymptomatic shedding. Barrier protection methods are the most reliable method of preventing transmission of herpes, but they merely reduce rather than eliminate risk. Oral herpes is easily diagnosed if the patient presents with visible sores or ulcers. Early stages of orofacial herpes and genital herpes are harder to diagnose; laboratory testing is usually required. Twenty percent of the U.S. population has antibodies to HSV-2, although not all of them have a history of genital lesions, **Chalmers**[1], **Bethesda** [3], **Akusjarvi and Stevenin** [5], **Alvine and Hill** [6], **Batterson and Roizman** [8].

Genital herpes can be more difficult to diagnose than oral herpes since most HSV-2-infected persons have no classical symptoms. Further confusing diagnosis, several other conditions resemble genital herpes, including, lichen planus, atopic dermatitis, and urethritis. Laboratory testing is often used to confirm a diagnosis of genital herpes. Laboratory tests include: culture of the virus, direct fluorescent antibody (DFA) studies to detect virus, skin biopsy, and polymerase chain reaction (PCR) to test for presence of viral DNA. Although these procedures produce highly sensitive and specific diagnoses, their high costs and time constraints discourage their regular use in clinical practice. Serological tests for antibodies to HSV are rarely useful to diagnosis and not routinely used in clinical practice, but are important in epidemiological studies. Serologic assays cannot differentiate between antibodies generated in response to a genital versus or an oral HSV infection, and as such cannot confirm the site of infection. Absence of antibody to HSV-2 does not exclude genital infection, because of the increasing incidence of genital infections caused by HSV, **Costanzo et al** [2], **Bogdanov** [4], **Akusjarvi and Stevenin**[5], **Baringer and Swoveland**[7], **Batterson et al** [9].

Physical therapists use low-intensity laser therapy (LILT) to treat patients with chronic inflammatory and fibrotic conditions. The literature on reduction mammoplasty, however, does not mention the use of LILT as a potential treatment for fibrotic breast lumps. Cellular studies support the use of LILT to improve absorption of extracellular fluid, increase neutrophil activity and chemotaxis, increase secretion of macrophage growth factors, enhance DNA synthesis, decrease pain, enhance electron respiratory chain reaction, increase endothelial PGI_2 secretion and degrade fibrin networks. Degradation of clotted blood and enhanced absorption of hematomas by improved circulation were the mechanisms by which LILT improved survival of grafted skin in cases where hematoma had developed, **Georgics et al** [10], **Goldstein and Gilbert** [11], **Blahnik and Rindge** [15].

High dosages of LILT generally have an inhibitory effect on tissue metabolism. Dosages of 8to 32 J/cm^2 are used to treat chronic inflammation and reduce the risk of scar formation in musculoskeletal injuries. To reduce true keloid and hypertrophic scars, 830-nmLILT is applied at a dosage of 30 J/cm^2, in combination with the use of pressure tapes or dressings and steroid injection. Patients usually received LILT twice weekly, and treatment frequency was reduced once they judged that "real improvement" had occurred; total treatment was for periods of up to 1 year. The effect of LILT on scar formation has not been evaluated in a controlled study, and treatment of fibrosis or calcification secondary to hematoma or fat necrosis is not specifically mentioned in the LILT literature, **Karu**[12], **Karu et al** [13], **Bjordal et al** [14], **Mester et al** [16].

Polarized light from low power lasers and non-laser devices has been used as a non-invasive therapy in the treatment of various musculoskeletal disorders, acceleration of wound healing and treatment of skin ulcers. Although the polarized light is known to have numerous photo-biostimulatory effects including cell proliferation, enhanced collagen synthesis, changes to the circulatory system and anti-inflammatory actions, the precise mechanism of its action still remains unclear. The available non-laser optical devices are the Bioptron products which emit a wide beam of polarized, non-coherent, polychromatic, low energy light that contain wavelengths from the visible spectrum (480-700nm) and infrared radiation (700-3400nm); this range provides optimal penetration and stimulation of the tissues without the risk of DNA damage, **Hoeksema et al** [20], **Iordanou et al** [21], **Kubasova et al.** [22].

Bioptron light therapy system has a low energy density (fluency) of an average of 2.4 J/cm^2. Bioptron light reaches the area to be treated with a constant, steady intensity; this energy density has biostimulative effects. With Bioptron light therapy, the energy density dosage can be precisely determined. Furthermore, the effect exerted by light is also defined by its power density. As it is measured at the skin's surface, it varies depending both on the intensity of the light's source and its distance from the area to be treated, the specific power density of Bioptron light is approximately 40 mW/cm^2 at a treatment distance of 10 cm; this is equivalent to an energy density (fluency) of an average of 2.4 J/cm^2 per minute. These properties of Bioptron light allow it

to penetrate the surface of the skin with minimum heating effect, no damage to skin and no known side-effects, **Lubart et al** [23], **Medenica and Lens** [24], **Monstrey et al** [25], **Naja et al** [26.]

Material and Methods

Subjects:

This study was carried out on forty five patients (15 male and 30 female) suffering from genital herpes simplex infection type II and their ages ranged from 20 to 45 years. They were divided into three groups. Group (A) composed of 15 patients received the LILT for 10 minutes (for the area with cutaneous manifestations) in addition to the topical acyclovir cream 5% twice / day through the treatment period. Group (B) received the BLT for 10 minutes (for the area with cutaneous manifestations) in addition to the topical acyclovir cream 5% twice / day through the treatment period (day after day for 2 months). Group (C) received the topical Acyclovir cream 5% twice / daily throughout the treatment period only.

Instrumentation:

In this study the measuring equipment were the State Fax 2100 Equipment (made in USA), that was used for the laboratory assessment of (HSV-II) IgG and (HSV-II) IgM as well as the Step one real time equipment (made in USA), that was used for the laboratory assessment of the polymerase chain reaction (PCR), while the therapeutic equipment were the LASER LTU 904 H, 5 watts Laser Therapy Unit and the Bioptron Compact III polarized light therapy system (PAG-860 manufactured in Switzerland), **Goldstein and Gilbert** [11], **Karu** [12,] **Karu et al** [13,] **Depuydt et al** [17,] **Garrison and Valiant** [18,] **Gigot** [19.]

Procedures

Evaluation:

This method of measurement was conducted by taking blood sample form the patient using 5 cm syringe after sterilization of the area from which the sample was taken then the sample was emptied into plain tube (Blue tip tube) which had the patient number and the No. of measurement then the samples were analyzed by the lab, using state fax-2100 for analysis of HSV-1 IgG and HSV-1 IgM and step one real-time for HSV1 PCR analysis**:** **1-**IgM antibodies appear early in the course of an infection, demonstrating IgM antibodies in patient's serum which indicates recent infection. **2-** IgG antibodies are predominantly involved in the secondary antibody response. They can bind to many kinds of pathogen (viruses, bacteria, and fungi) and protect the body against them by agglutination and immobilization, complement activation, opsonization for phagocytosis and neutralization for these toxins. **3-**The PCR usually consists of a series of 20- to 40 repeated temperature chances called cycles, each cycle typically consists of 2-3 discrete temperature steps (initialization step, denaturation step, annealing step and extension / elongation set) at the end of these steps the amount of DNA target is doubled, leading to exponential (geometric) amplification of the specific DNA. The amount of the virus (viral load) in patients can also be quantified by PCR-based DNA quantization techniques. In molecular biology, the polymerase chain reaction (PCR) is a technique to amplify a single or few copies of a piece of DNA across several orders of magnitude, generating thousands to millions of copies of a particular DNA sequence**.** The method relies on thermal cycling, consisting of cycles of repeated heating and cooling of the reaction for DNA melting and enzymatic replication of the DNA. Primers (short DNA fragments) containing sequences complementary to the target region along with a DNA polymerase (after which the method is named) are key components to enable selective and repeated amplification. As PCR progresses, the DNA generated is itself used as a template for replication, setting in motion a chain reaction in which the DNA template is exponentially amplified. PCR can be extensively modified to perform a wide array of genetic manipulations. Polymerase chain reaction (PCR) are of high specificity and sensitivity can be used clinically, all measurements were conducted before starting treatment (first record) then after 2 months (second final record), [1,3,5,8.]

1- Treatment procedures of the LILT (Group A):

Each patient was placed in comfortable supported supine lying position. The treatment procedure was started within the first 48 hours of appearance of cutaneous manifestations. Both the patient and the therapist wore the protective eye glasses. The Ga-As laser beam was positioned in perpendicular direction to the HSV-II

lesion. The surface of the treated area was treated for 10 minutes by the continuous mode and direct contact method with the laser probe was applied without pressure to avoid the patient's complain of tenderness, 3 times per week for 2 months. This procedure was conducted for patients in the study group (A) with the patient in a comfortable supine lying position[11, 12, 13,14,,15].

2- Treatment procedures of the BLT (Group B):

The treated area was cleaned at first by saline rinse and betadine. BLT device preparation: the plug of the BLT unit was inserted into the main current supply; the on/off switch was switched on. Then set the treatment parameters of BLT. BLT application: point the light beam at the area to be treated, holding the device at right angle (90°) perpendicular to the surface of the treated area and maintaining a distance of 10 cm from the surface of it and applying the BLT for about 10 minutes. Frequency of application: 3 times per week for 2 months. This procedure was conducted for patients in the study group (B) with the patient in a comfortable supine lying position.[17,18,19,20,21.]

Data analysis:

Serological tests (IgG and IgM) as well as the polymerase chain reaction)were measured pre-treatment as a first record and after two months as a second final record in the three groups. Collected data were fed into computer for the statistical analysis; descriptive statistics as mean, standard deviation, minimum and maximum were calculated for each group. The t-test was done to compare the mean difference of the two groups before and after application and within each group. Alpha point of 0.05 was used as a level of significance, **Pipkin** [27.]

Results

As shown in table (1) and figure (1), the mean value of the Immunoglobulin G before treatment was (10.188 ± 2.344) Eu/mL in the first study group (Laser group), while after treatment was (5.112 ± 0.845) Eu/mL. These results revealed a highly significant reduction in the Immunoglobulin G (P < 0.0001). While in the second study group (BLT group), the mean value of the Immunoglobulin G before treatment was (10.189 ± 2.433) Eu/mL, while after treatment was (8.212 ± 1.986) Eu/mL. These results revealed a significant reduction in the Immunoglobulin G (P < 0.001). But in the third group (Acyclovir cream group), the mean value of the Immunoglobulin G before treatment was (10.190 ± 2.441) Eu/mL, while after treatment was (10.122. ± 2.421) Eu/mL. These results revealed non-significant difference in the Immunoglobulin G (P > 0.05).

Table (1): Comparison of the mean values of the Immunoglobulin G in Eu/mL before and after treatment in the three groups

	Before treatment		After treatment		Mean difference	T-value	P.value	Level of significance
	Mean	SD	Mean	SD				
Laser Group	10.188	2.344	5.112	0.845	5.07600	7.89	0.0001	Highly significant decrease
BLT group	10.189	2.433	8.212	1.986	1.97700	2.44	0.022	Significant decrease
Acyclovir cream Group	10.190	2.441	10.122	2.421	0.068000	0.08	0.940	Non-significant

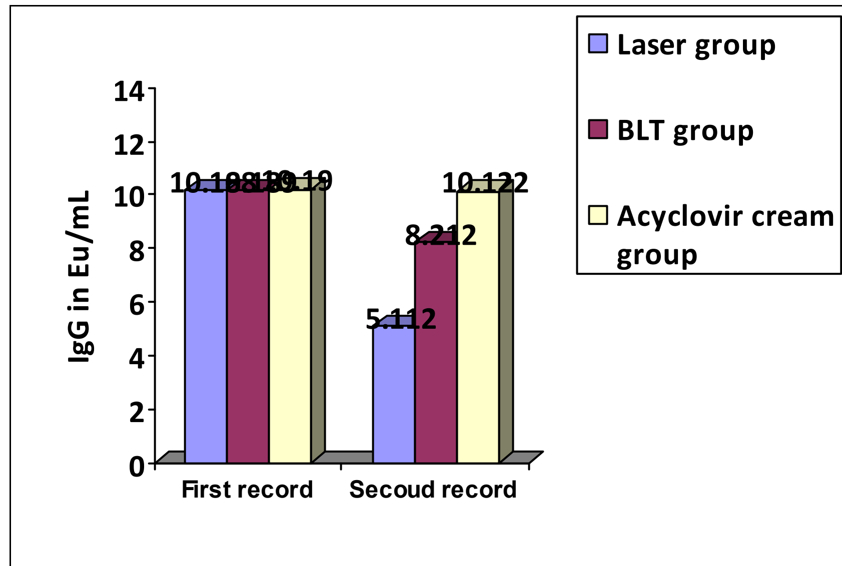

Fig (1): Mean values of the Immunoglobulin G before and after treatment in the three groups.

As shown in table (2) and figure (2), the mean value of the Immunoglobulin M before treatment was (8.185 ± 0.580) Eu/mL in the first study group (Laser group), while after treatment was (3.414 ± 2.305) Eu/mL. These results revealed a highly significant reduction in the Immunoglobulin M ($P < 0.0001$). While in the second study group (BLT group), the mean value of the Immunoglobulin M before treatment was (8.188 ± 0.582) Eu/mL, while after treatment was (7.666 ± 0.440) Eu/mL. These results revealed a significant reduction in the Immunoglobulin M ($P < 0.05$). But in the third group (Acyclovir cream group), the mean value of the Immunoglobulin M before treatment was (8.186 ± 0.578) Eu/mL, while after treatment was (8.182. ± 0.567) Eu/mL. These results revealed non-significant difference in the Immunoglobulin M ($P > 0.05$).

Table (2): Comparison of the mean values of the Immunoglobulin M in Eu/mL before and after treatment in the three groups

	Before treatment		**After treatment**		**Mean difference**	**T-value**	**P.value**	**Level of significance**
	Mean	**SD**	**Mean**	**SD**				
Laser Group	8.185	0.580	3.414	2.305	4.77100	7.77	0.0001	Highly significant decrease
BLT group	8.188	0.582	7.666	0.440	0.522000	2.77	0.01	Significant decrease
Acyclovir cream Group	8.186	0.578	8.182	0.567	0.004000	0.02	0.985	Non-significant

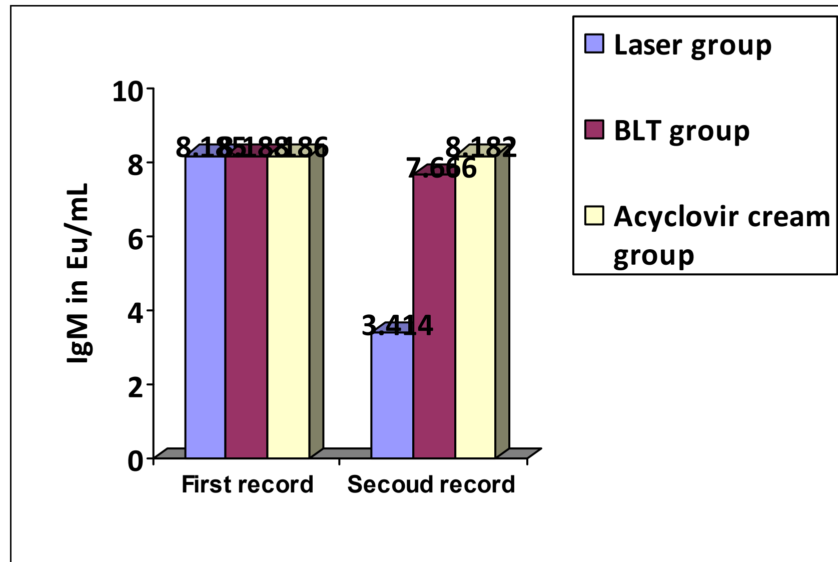

Fig (2): Mean values of the Immunoglobulin M before and after treatment in the three groups.

As shown in table (3) and figure (3), the mean value of the Polymerase Chain Reaction (PCR) before treatment was (0.887 ± 0.361) in the first study group (Laser group), while after treatment was (0.000 ± 0.000). These results revealed a highly significant reduction in the Polymerase Chain Reaction (PCR) ($P < 0.0001$). While in the second study group (BLT group), the mean value of the Polymerase Chain Reaction (PCR) before treatment was (0.934 ± 0.268), while after treatment was (0.643 ± 0.445). These results revealed a significant reduction in the Polymerase Chain Reaction (PCR) ($P < 0.05$). But in the third group (Acyclovir cream group), the mean value of the Polymerase Chain Reaction (PCR) before treatment was (0.898 ± 0.451), while after treatment was (0.884. ± 0.442). These results revealed non-significant difference in the Polymerase Chain Reaction (PCR) ($P > 0.05$).

Table (1): Comparison of the mean values of the Polymerase Chain Reaction (PCR) before and after treatment in the three groups

	Before treatment		After treatment		Mean difference	T-value	P.value	Level of significance
	Mean	SD	Mean	SD				
Laser Group	0.877	0.361	0.000	0.000	0.877000	9.07	0.0001	Highly significant decrease
BLT group	0.934	0.268	0.643	0.445	0.291000	2.17	0.041	Significant decrease
Acyclovir cream Group	0.898	0.451	0.884	0.442	0.014000	0.09	0.932	Non-significant

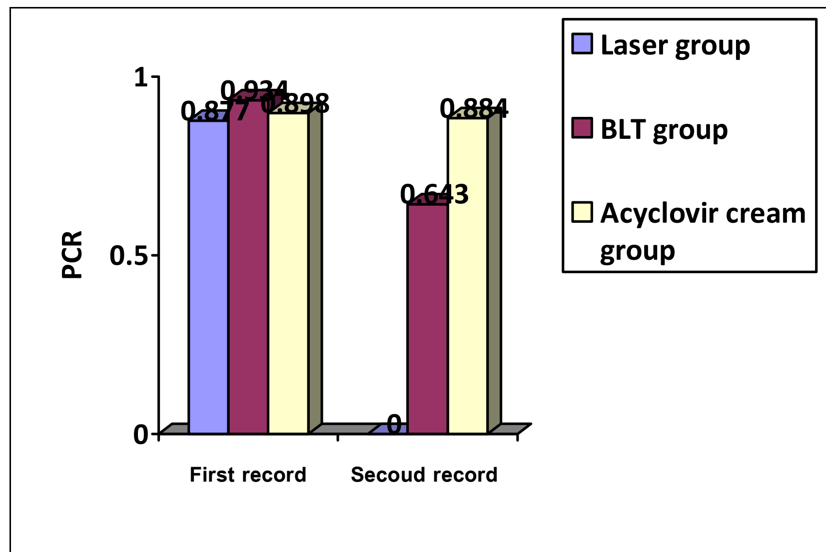

Fig (1): Mean values of the Polymerase Chain Reaction (PCR) before and after treatment in the three groups.

Discussion

Herpes is contagious if the carrier is producing and shedding virus. This is especially likely during an outbreak but possible at other times. There is no cure yet, but there are treatments which reduce the likelihood of viral shedding. An HSV infection on the lips is commonly known as a "cold sore" or "fever blister". The blisters resemble those of chickenpox, an infection caused by another member of the alpha-Herpesviridae subfamily, Varicella Zoster Virus (VZV), also known as Human Herpes Virus 3 (HHV-3). The human herpes viruses all share some common properties. One shared property is virus structure and herpes viruses are composed of relatively large double-stranded, linear DNA genomes encoding 100-200 genes encased within an icosahedral protein cage called the capsids which is itself wrapped in a lipid bilayer membrane called the envelope. This particle is known as the virion,[1, 3,4,5.]

Presently the only mechanism for prevention of mucocutaneous HSV infection is to avoid contact with infected secretions. No proven post-exposure antiviral therapy or vaccines are presently available. In the 1970s, virarabine (an adenine analogue) became the first licensed antiviral**.** Therapeutic treatment for herpes simplex, encephalitis and neonatal infections including virarabine which was soon replaced by acyclovir for the treatment of all herpes virus infections. Now, acyclovir, and its prodrug valacyclovir, as well as peneciclovir and famiciclovir, are the most useful and widely used therapeutics for the treatment of HSV infections. Ocular infections are typically treated with toptrifluorothymidine, with or without concomitant oral acyclovir, valacyclovir, or famiciclovir. Resistance to these drugs is possible through mutations in the thymidine kinase TK gene and HSV DNA polymerase which as a consequence causes these treatments to be inactive,[6, 7, 8,9.]

Laser phototherapy uses radiation both in the visible (400 - 700 nm) and in the near-infrared (700 - 1000 nm) regions of the spectrum, when a photon is absorbed by a molecule, the electrons of that molecule are raised to a higher energy state. This excited molecule must lose its extra energy, and it can do this either by re-emitting a photon of longer wavelength (i.e., lower energy than the absorbed photon) as fluorescence or phosphorescence, or it can lose energy by giving off heat, or it can lose energy by undergoing photochemistry. Photobiological responses are the result of photochemical and/or photophysical changes produced by the absorption of non-ionizing radiation, [11, 12,13.]

Physical therapists use low-intensity laser therapy (LILT) to treat patients with chronic inflammatory and fibrotic conditions. The literature on reduction mammoplasty, however, does not mention the use of LILT as a potential treatment for fibrotic breast lumps. But cellular studies support the use of LILT to improve absorption of extracellular fluid, increased neutrophil activity and chemotaxis, increase secretion of macrophage growth factors, enhance DNA synthesis, enhance electron respiratory chain reaction, increase endothelial PGI_2 secretion and degrade fibrin networks. Degradation of clotted blood and enhanced absorption of hematomas by

improved circulation were the mechanisms by which LILT improved survival of grafted skin in cases where hematoma had developed, [14, 15,16.]

Polarized light from low power lasers and non-laser devices has been used as a non-invasive therapy in the treatment of various musculoskeletal disorders, acceleration of wound healing and treatment of skin ulcers, although the polarized light is known to have numerous photo-biostimulatory effects including cell proliferation, enhanced collagen synthesis, changes to the circulatory system and anti-inflammatory actions, the precise mechanism of its action still remains unclear. The available non-laser optical devices are the Bioptron products which emit a wide beam of polarized, non-coherent, polychromatic, low energy light that contain wavelengths from the visible spectrum (480-700nm) and infrared radiation (700-3400nm); this range provides optimal penetration and stimulation of the tissues without the risk of DNA damage [,17, 18,19.]

Bioptron light therapy device emits light that is polarized, polychromatic, non-coherent and of low energy, the light emitted has a wide range of wavelengths (480-3400nm) and differs from laser light, which is mono-chromatic (of narrow wavelength), coherent, polarized and of high or low energy. Possible risk of burns is present with the laser therapy, while not possible with the Bioptron light therapy. User skills are essential in laser therapy, but not essential with the Bioptron light therapy**.** Higher costs are present with the laser therapy, but not with the Bioptron light therapy, in addition, treatment of large area is available with the Bioptron light therapy, [20, 21,22.]

The biostimulative effects of Bioptron light are the result of synergy between different mechanisms of action as; harmonize the metabolic processes, reinforce the human defence system, stimulate regenerative and reparative processes of the entire organism, promote wound healing and relieve pain or decrease its intensity. The scientific mechanisms underlying various light therapy treatments are still under investigation. However, in general scientists have identified various biological effects that can be initiated and achieved as a result of light stimulation. These include; stimulation of neoangiogenesis, improvement of circulation, increasing the process of phagocytosis, stimulation and activation of ATP production, enhancement of important specific enzymes involved in cell regeneration, increasing the activity of lymphatic system, activation of fibroblast activity and increasing the production of collagen, increasing DNA and RNA production and reducing the excitability of nervous tissue as well as increasing the muscle relaxation [, 23,24, 25,26.]

The findings of the present study showed non-significant differences in the pre-treatment records of IgG, IgM and PCR between the mean values of the three groups.

Results of the first study group revealed a highly significant decrease in the mean values of IgG, IgM and PCR after application of laser, when compared against the pre-application results or when compared against the after-application results of the third study group (Acyclovir cream). Also results of the second study group revealed only significant decrease in the mean values of IgG, IgM and PCR after application of the BLT, when compared against the pre-application results and revealed a highly significant decrease when compared against the after-application results of the third study group (Acyclovir cream). But results of the third study group (Acylcovir cream) revealed non-significant differences in the mean values of IgG, IgM and PCR after application of acylcovir cream alone, when compared against the pre-application results. Results of the first study group (Laser group) revealed a highly significant decrease in the mean values of IgG, IgM and PCR after application of laser, when compared against the after-application results of the second study group (BLT group).

Significant differences showed between the first study and second study groups, between the first study and third study groups, between the second study and third study groups were consistent with those observed and recorded by Georgics et al, 2005; Goldstein and Gilbert, 2004; Karu, 2009; Karu et al., 2006; Bjordal et al., 2004; Mester et al., 2005; Depuydt et al., 2009; Garrison and Valiant, 2006; Gigot, 2009; Hoeksema et al., 2002; Iordanou et al., 2007; Kubasova et al., 2005; Monstrey et al., 2002 and Naja et al., 2009.

Results of this study support the expectation that application of both the LILT and BLT in addition to the topical acyclovir cream 5% were effective than application of the topical acyclovir cream 5% alone in improving the genital herpes pain and cutaneous manifestations. But LILT was more beneficial than BLT in improving the genital herpes pain and cutaneous manifestations as evidenced by the highly significant decreases in IgG, IgM and PCR.

Conclusion

Application of both the LILT and BLT in addition to the topical acyclovir cream 5% were effective than application of the topical acyclovir cream 5% alone in improving the genital herpes pain and cutaneous manifestations. But LILT was more beneficial than BLT in improving the genital herpes pain and cutaneous manifestations as evidenced by the highly significant decreases in IgG, IgM and PCR.

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