Original Article

Detection of Viruses associated with Herpes Labialis Patients in Babylon Province Pre and Post Treatment with Low Power Laser Therapy (Diode Laser)

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Abstract

Background: The secondary or recurrent herpetic infections (RHIs) are caused by herpes simplex virus (HSV) type-1 and type-2. low-power laser therapy 940 nm may consider one of the best and most optimistic and optional local therapeutic and biological options. **Objectives:** To determine both molecular detection and viral load of HSV-1 and HSV-2 by real-time polymerase chain reaction (PCR) pre- and post-diode laser therapy 940 nm for evaluating the laser efficacy. **Materials and Methods:** Thirty patients in age range 6–48 years have participated in this clinical trial study complaining from RHI. Sixty viral swabs were taken for those thirty participant patients including 30 swabs pre and 30 swabs post 3–5 days. Diode laser therapy 940 nm for biostimulation, real-time PCR for accurate detection of both HSV-1 and HSV-2. **Results:** The results in this present study showed mean age range of RHI patients was 25.7 ± 12.1 years with a male ratio more than the female 1.5:1. Overall participants were infected with HSV type-1, whereas 3 (10%) of the participants were noninfected with HSV type-2. There was a significant increase in HSV type-1 PCR threshold cycle (Ct) mean of RHI patients after treatment with diode laser therapy 940 nm which means decreasing in the viral load. **Conclusions:** The real-time PCR technique is a highly efficient, reliable, and rapid technique for accurate diagnosis and viral load measuring of HSV-1 and HSV-2. Low-power laser therapy is utilized to reduce pain levels, change the state of disease from pro-inflammatory to anti-inflammatory, and may reduce the viral load.

Keywords: Diode laser therapy 940 nm, *HSV*-1, *HSV*-2, real-time PCR, recurrent herpetic infection

Introduction

The *Herpesviridae* is divided into three subfamilies, "alpha subfamily" which includes *Herpes simplex virus*-1 (*HSV*-1) "oral-facial type," Herpes *simplex virus*-2 (*HSV*-2) "genital type," and *Varicella-zoster virus*. The "beta subfamily" includes *Human cytomegalovirus*, *Human Herpesvirus*-6, and *Human Herpesvirus*-7, and the "gamma subfamily" includes *Epstein–Barr virus* and *Human herpesvirus*-8.[1]

Both *HSV*-1 and *HSV*-2 are generally responsible for secondary or recurrent herpetic infection (RHI). *HSV*-1, a universal human pathogen, is a double-stranded DNA genome. Upon the primary type of infection, the replication of viruses occurs in the epithelial cells. Then, it enters the sensory neurons by entering the axon terminals then the virus is backward transported to the corresponding sensory ganglia region, usually "trigeminal ganglia," where a latent type of infection is established. In response to the variation of stressors, the latent virus can be reactivated periodically to begin again productive replication followed by the formation of an "infectious virus," which is anterograde transported back to peripheral cells or tissues or else infects further host neuronal cells.[2]

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The *"HSV*-1" evades the host immune response by targeting components such as natural killer cells, complement proteins, major histocompatibility complex/class-I or class-II molecules, and antibodies.[3]

The herpetic lesion usually heals within 10 days, but the virus remains dormant within the "trigeminal ganglion," the virus may sporadically reactivate to make another outbreak of sores within the mouth or lip, the cause is usually *HSV*-1 and occasionally *HSV*-2.[4]

The herpetic infection is typically spread between people by direct nonsexual contact. Attacks can be triggered by fever, sunlight, psychological stress, then finally menstrual period.[5] Worldwide, approximately ninety percent are the global prevalence of *HSV*-1 infection, and in some of the rural areas, the seroprevalence is up to 100% higher.[6]

It should be noted that, currently, there is no therapy to absolutely eliminate *HSV*-1 once an individual is infected.[7]

The conventional type of treatment for "herpes infection" involves either systemic or topical Antiviral medications including "Acyclovir," "Valacyclovir," and "Famciclovir," which are effective drugs for people who complain of "*Herper simplex virus"* infections. All These drugs relieve painful symptoms, block the progression of the lesion, and shorten the course of the disease; also, they restrict viral multiplication.[8] However, those drugs have restricted efficacy in the prevention of lesion recurrence and are not able to cure latent infection.[9]

The secondary or RHI treatment with lasers biostimulation is based either on heat production "high-power lasers" or on the photochemical and photobiological effects of laser light (low-power lasers or "defocused" high-power lasers).[10]

Regarding laser phototherapy "without heating," its effect is based on its capacity to modulate various metabolic processes by the conversion of the laser light energy into useful energy for the biological cell. Visible laser light is absorbed by chromophores part in the respiratory chain of the mitochondrial apparatus, leading to fundamental changes, such as increased production of reactive oxygen species "ROS," synthesis of adenosine triphosphate (ATP), changes in the permeability of cell membrane then finally nitric oxide release. These effects produce several secondary effects; changes in the synthesis of extracellular matrix, increased action potential of nerve cells and new formation of cell capillaries through the release of different growth factors; local effects on components of the immune; also vascular and nervous system; and an increase in levels of intracellular Ca^{2+} and cyclic adenosine monophosphate,[11] which are related to various biological processes such as ribonucleic acid (RNA) and deoxyribose nucleic acid (DNA) synthesis, cell mitosis, and finally protein secretion.[12]

This cascade of biological cellular events accelerates proliferation of the body cells, which promotes cellular healing. The effect of laser phototherapy is based on the physiological state of the cell at the moment of irradiation.[13]

Traditional techniques for *HSV* diagnosis have relied on virus isolation in cell culture, followed by detection by immunofluorescence technology. These methods are difficult, lack sensitivity, are highly dependent on the maintenance of cell culture lines and virus viability, and provide only qualitative results.[14] The development of nucleic acid amplification tests, such as real-time polymerase chain reaction (rT-PCR), for the detection of *HSV* offers an automated diagnostic method with improved sensitivity and specificity.[15]

Materials and Methods

Sixty samples were collected from 30 participant patients suffering from RHI they attended the teaching clinics, College of Dentistry, University of Babylon, and private clinic in Hilla City, Iraq, through extended periods from September 2019 to January 2020. These 60 samples include

1. Thirty viral swabs for those 30 participant patients' pre-diode laser therapy.
2. Thirty viral swabs for the same participant patients' post-diode laser therapy in a period of 3–5 days depending on irregular patient appointment.

Patient name, age, sex, occupation, medical and dental history, episode of RHI per year, family history, and triggering factors were taken.

Inclusion criteria in patient's selection

1. Both sexes.
2. Age 6–48 years.
3. Both herpes labials and RHI of the peri-oral region (extra orally).
4. Patients with all disease stages of RHI.

Patients without any systemic diseases.

Exclusion criteria

1. Pregnant and lactating women.
2. Immunocompromised patients.

The study design was a clinical trial study, and Babylon University, College of Dentistry, gave the ethical approval.

Ethical approval

This study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with patients' verbal and analytical approval before the sample was taken. The study protocol and the subject information and the consent form

were reviewed and approved by a local ethics committee according to document number 18229 on August 23, 2021, to get this approval.

Specimens collection/viral samples

Specimens were prepared in a proper way to avoid any possible contamination from dental clinics, swabs were collected pre- and post (3–5 days)-diode laser therapy from the recurrent herpetic lesion (lips or peri-oral region) by scraping the lesion with sterile lancet after the application of topical anesthesia to get blood swabs from the lesion site by using of viral transport media which contains both cotton swab and medium for preserving the specimen, these samples were placed in a cool box containing ice bags to maintain their viability then preserved in deep freezing under −23°C until being taken to the laboratory examinations to make later the identification for *Herpes Simplex Virus* types 1 and 2, and viral load measured by using real-time polymerase chain reaction.

Diode laser therapy

The low-power laser therapy (Biolase laser) is with the following specifications:

1) Probes with wavelengths 940 nm.
2) Energy density with 10 J/ cm^2 on average, laser device.

The principle of LLLT biostimulation used in treating recurrent herpetic lesions was according to the World Clinical Laser Institute:

A Topical anesthesia on the recurrent herpetic lesions was not used at the time of treatment.
B Uninitiated tip was used, 1.5 W 30 ms/30 ms pulsed (50% duty cycle).
C The entire lesion was painted with covering a 5–6 mm diameter circle.
D Spending as much of the 90 s as close as possible to the lesion.
E If the procedure is still painful, the power increased to 1.80 W for 90 s, if further still painful; the power increased to 2.00 W for another 90 s
F Glycerin lotion was prescribed for the patient after LLLT session for moisturization.

Viral gene-spin viral DNA/RNA extraction kit

Viral DNA Extraction Kit was designed for the rapid isolation of DNA from a variety of sample sources including fresh or frozen serum, plasma, and other cell-free body fluids and virus-infected cell/tissue, viral DNA Extraction Kit uses advanced silica-gel membrane technology for rapid and effective purification of DNA without organic extraction or ethanol precipitation. Furthermore, the buffering conditions were finely adjusted to provide optimum binding of the DNA to the column.

Real-best DNA *HSV*-1/*HSV*-2 real-time PCR

DNA *HSV*-1/*HSV*-2 kit was intended for differential detection of *Herpes simplex virus* types 1 and 2 DNA in clinical specimens (swabs of epithelial cells, tissue fluid, erosive-ulcerative skin lesion) using the method of real-time polymerase chain reaction (PCR) with fluorescence detection of amplified product.

Real-time PCR was based on the detection of the fluorescence produced by a reporter molecule, which increased as the reaction proceeded. The reporter molecule was a dual-labeled DNA probe that was specifically bound to the target region of pathogens' DNA. Fluorescence signal increased due to the separation of fluorescence dye and quencher by Taq DNA-polymerase exonuclease activity during amplification. PCR consists of repeated cycles: temperature denaturation of DNA, primer annealing, and complementary chain synthesis, the device programming is as follows:

Cycle stage 1	50°C—2 min;
Cycle stage 2	95°C—2 min
Cycle stage 3	50 cycles (94°C—10 s; 60°C—40 s);

Statistical analysis

The data were analyzed by the application of the Microsoft Excel program and Statistical Package for Social Sciences version 23. The outcomes of the analysis were arranged in scale variables (means and standard deviation) and categorical variables. Fisher's exact test was used for categorical variables. An independent sample *t* test was used to compare two independent means and a paired *t* test was used in a comparison of two consecutive means. *P*-value of 0.05 or less was regarded as significant.

Results

This study included 30 patients infected with *Herpes Simplex Virus* (diagnosed RHI) presented with a mean age of 25.7 ± 12.1 years. About 6.7% of the patients were in the age group <10 years, 20% in the age group 10–19 years, 36.6% in the age group 20–29 years, 16.7% in the age group 30–39 years, and 20% in age group 40 years and more. Male patients with RHI were more than female RHI patients with a male-to-female ratio of 1.5:1.

The mean period between samples collection was 3.6 ± 0.8 days; 53.3% of samples were taken within 2–3 days and 46.7% of samples were taken within 3–4 days [Table 1].

Regarding male and female patients, the mean of *HSV*-1 PCR Ct of RHI patients was significantly increased after treatment with diode laser therapy (both male and female: $P = 0.01$). While no significant differences were observed in means of *HSV*-2 PCR Ct and of RHI patients after

treatment with diode laser therapy (male: $P = 0.1$; female: $P = 0.3$) as shown in Tables 2 and 3.

Following diode laser treatment, the recurrence of RHI was observed till 6-months recall which was presented in 6.67% of patients, and side effects of treatment were detected in 16.67% of them which was only redness and burning sensation, as shown in Table 4.

All patients were infected with *HSV* type-1, whereas 3 (10%) patients were noninfected with type-2 *HSV*, as shown in Table 5, Figures 1 and 2.

Table 1: Distribution of investigations measures pre- and post-diode laser treatment

Variable	Diode laser treatment		*P* value
	Pre	Post	
	Mean ± SD	Mean ± SD	
HSV-1-PCR (Ct)	23.9±4.0	26.9±2.2	**<0.001**^{*S}
HSV-2-PCR (Ct)	24.9±8.5	25.3±8.6	0.09^{*NS}

S: significant, NS: not significant at *P*-value ≤ 0.05.
*Paired *t* test

Table 2: Distribution of investigations measures pre- and post-diode laser treatment according to different genders

Variable	Pre-laser	Post-laser	*P* value
Male			
HSV-1 PCR (Ct)	23.8±4.2	26.4±2.7	**0.01**^{*S}
HSV-2 PCR (Ct)	23.3±10.7	23.6±10.9	0.1^{*NS}
Female			
HSV-1 PCR (Ct)	24.2±3.7	24.4±0.7	**0.01**^{*S}
HSV-2 PCR (Ct)	27.3±1.2	27.7±0.7	0.3^{*NS}

S: significant, NS: not significant at *P*-value ≤ 0.05.
*Paired *t* test

Table 3: Distribution of investigations measures pre- and post-diode laser treatment according to the period between sample collection

Variable	Diode laser treatment		*P* value
	Pre	Post	
	Mean ± SD	Mean ± SD	
2–3 days			
HSV-1 PCR (Ct)	24.8±4.4	26.6±2.9	0.1^{*NS}
HSV-2 PCR (Ct)	26.4±7.1	26.4±7.0	0.8^{*NS}
4–5 days			
HSV-1 PCR (Ct)	22.9±3.3	27.3±0.5	**<0.001**^{*S}
HSV-2 PCR (Ct)	23.2±9.8	24.0±10.2	**0.007**^{*S}

S: significant, NS: not significant at *P*-value ≤ 0.05.
*Paired *t* test

Table 4: Recurrence and side effects after 6-months' recall of diode laser treatment

Variable	No. of patients	Percentage (%)
Recurrence		
Positive	2	6.67
Negative	28	93.33
Total	30	100.0
Side effects		
Yes	5	16.67
No	25	83.33
Total	30	100.0

Table 5: *HSV* infection types of studied patients

Variable	No. of patients	%
HSV-1		
Positive	30	100.0
Negative	0	–
Total	30	100.0
HSV-2		
Yes	27	90.0
No	3	10.0
Total	30	100.0

Clinical findings

Case 1

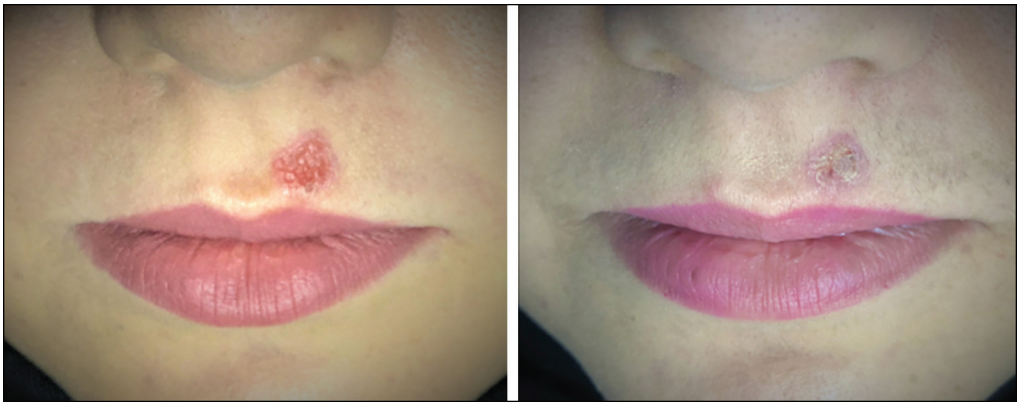

Figure 1: A 6-year-old male with a recurrent herpetic lesion (left: before laser therapy, right: after laser therapy)

Case 2

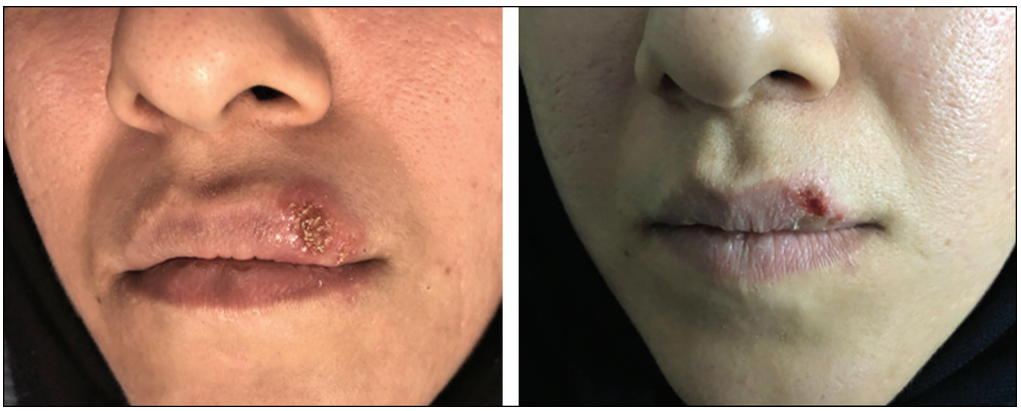

Figure 2: A 28-year-old male with a recurrent herpetic lesion (left: before laser therapy, right: after laser therapy)

Discussion

In the current study, orofacial region is most affected by *HSV*-1 lesions last for approximately 10 days and are accompanied by unspecific symptoms such as malaise, fever, and dehydration.[16]

This study showed that the seroprevalence of *HSV*-1 and *HSV*-2 increases with increased age. From 2005 to 2010, the seroprevalence of *HSV*-1 among patients aged 14–19 years was 30.1% and 63.6% among patients aged 40–49 years. Similar trends exist for *HSV*-2 seroprevalence, with 1.2% of persons aged 14–19 years affected and 25.6% of those aged 40–49 years affected. This finding agrees with the present study. Also, the frequency of *HSV*-1 antibodies is slightly higher in females than in males (33.2% vs 27.1%), these disagree with the current study.[17,18]

Also, the results of this study showed a significant increase in the mean threshold cycle of real-time PCR of *HSV*-1 infected patient (23.9 CT) to (26.9 CT) after treatment with diode laser ($P < 0.001$). This means there was a significant decreasing in the viral load (Ct vice versa viral load) after treatment with diode laser, which occurred due to the immune system sensitization and the multiple biostimulation effects of diode laser therapy (940 nm) and inducing healing process. With regard to the mean Ct of real-time PCR of *HSV*-2 in infected patient, there was a nonsignificant increase pre- and posttreatment *P* value (0.09).

This study agrees with the results of Donnarumma *et al*.[12] that 70% inhibition of *HSV*-1 replication in the viral postadsorption phase and increased production of IL-1β, IL-6, IL-8, and TNFα after diode laser irradiation for 3 min (830 nm). The increased production of these cytokines indicates a better immune response and leads to more rapide healing with less traumatic lesion, since the clinical symptoms of pain, redness, and swelling are caused by the host's anti-inflammatory response.[26-28,31,32]

The HaCaT cells, *HSV*-1 infected and irradiated with infrared laser light, presented a reduction in viral replication, and reduce of pro-inflammatory molecules, speculating that laser light activates immune antiviral response and inhibits virus spreading, this study also comes in accordance with the present study in concerning the diode laser effects on *HSV*-1 infection.[19,30]

The photo biomodulation therapy (PBM) could be a very innovative and noninvasive treatment against such infections.[20,33]

In both protocols, PBM was able to reduce the viral copies either with blue or with NIR wavelengths. Moreover, *ZIKV* virus reduced the cell viability but subsequently observed that PBM increased the vitality in irradiated cells with both PBM protocols, showing levels like those of not infected. These findings agree with the present study which showed a significant decrease in the viral load of *HSV* 1 and *HSV*-2 (sample period 4–5 days) after diode laser therapy (940 nm) of the recurrent herpetic lesion.[19,29,34]

The diode laser of 940 nm facilitates repairing the injured biological tissue, it was demonstrated that diode laser can produce some effects such as biostimulator, analgesic, anti-exudative, antihemorrhagic, anti-inflammatory, antineuralgic, anti-edematous, antispasmodic, and vasodilatory.[20,33]

Also, the importance of findings in the present study is closely related to the need to find a new therapy able to inactivate *HSV* because some viral strains are often resistant to conventional antiviral drugs used in the treatment of herpetic infections. Diode laser treatment has several advantages over conventional treatments: it has a low cost, and its efficacy can be monitored during application and the skin to be treated can be selected without damage to the healthy tissue. These features make this treatment promising in combination with conventional therapy for herpetic infections, the principle of biostimulation promoted by therapeutic lasers was introduced 20 years ago. It was first applied in dermatology, especially supplementing the repair process of skin wounds. Later, it was suggested that biostimulation could also be useful in accelerating the healing of wounds inside the oral cavity.[21,31,36]

Muñoz Sanchez *et al.*[21] reported that LLLT was an effective therapy with no side effects.

Dougal and Lee[13] concluded that the effect of diode laser (1072 nm) on herpes labials was assessed. The results showed that the crust time and healing time for herpes labials decreased in the laser group compared to the control group with no side effects.[13,22]

The treatment of herpes simplex lesion with diode laser reduced the length of recovery time and pain severity and fewer side effects than treatment with acyclovir cream.[22,25,35]

A novel laser protocol for preventive treatment of recurrent herpes labials has shown positive results on decreasing recurrences over a follow-up period of 3 years. These patients used to have 6–12 herpes outbreaks a year, and after the laser preventive therapy, either no recurrence or a great reduction to only 1–2 outbreaks per year during the whole follow-up period was observed.[23,37,38]

The results of this study showed that all recurrent herpetic infected patients were infected with *HSV*-1, whereas 3 (10%) patients were noninfected with *HSV*-2; which are <10 years of age group, this in a compact with the study[24,32] which presented (n = 13,904) 27.1% of persons aged >12 years were seronegative for *HSV*-1 and *HSV*-2; 51.0% were seropositive for *HSV*-1 only, 5.3% for *HSV*-2, and 16.6% for both *HSV*-1 and *HSV*-2 (n = 2861). The seroprevalence of *HSV*-2 was higher in persons with *HSV*-1 antibody.

To the best of our knowledge, there were no previous data or articles related to the measuring of *HSV*-1/*HSV*-2 viral load relatively in blood swabs pre- and post-diode laser therapy of the recurrent herpetic lesion.

Conclusions

Diode laser therapy may be considered a beneficial and biostimulated principle. It decreases *HSV* type-1 and 2 load, limits *HSV* replication and viral spreading from cell to cell, and acts on the host immune response. Also, it acts as preventive measures, which decreased the recurrence rates per year. In the current study, a 6-month recall documented a 93.3% reduction in recurrence.

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Conflicts of interest

There are no conflicts of interest.

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