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Low-Level Laser Therapy for Improvement of In Vitro Fertilization Outcomes in Patients with Recurrent Implantation Failure: A Randomized Clinical Trial

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Abstract

Introduction: Numerous strategies have been investigated for addressing recurrent implantation failure (RIF) and enhancing endometrial receptivity, yet agreement on the optimal intervention remains elusive. Our investigation endeavors to assess the effect of low-level laser therapy (LLLT) on pregnancy outcomes in individuals who have undergone a minimum of three unsuccessful embryo transfer cycles (ET).

Methods: In our randomized single-blinded clinical trial, we enrolled thirty females with a medical history of RIF who were eligible for frozen-thawed embryo transfer (FET). Through a random allocation sequence, the participants were divided into two groups. The LLLT was performed one cycle before blastocyst transfer in 15 cases using a New Age BIOLASER device (New Age Co., Italy) with a 900-milliwatt power output and an 850-nm wavelength. The irradiation sessions were conducted transabdominal on the hypogastric area. The considered outcomes were biochemical pregnancy, identified by a positive blood pregnancy test, and clinical pregnancy, confirmed through visualization of the gestational sac using ultrasonography.

Results: The mean age of the subjects was 34.17 years, and they had undergone three to seven previous embryo transfers. There was no significant difference in basic characteristics between the group undergoing laser treatment and the control group. However, the laser-treated group exhibited elevated rates of both biochemical and clinical pregnancies compared to the control group (46.7% vs. 33.3%; P==0.710 and 33.3% vs. 20.0%; P=0.682 respectively).

Conclusion: To our knowledge, this study represents the first single-blinded randomized clinical trial to assess the effectiveness of LLLT pretreatment in individuals with RIF. The findings propose that LLLT may potentially enhance biochemical and clinical pregnancy rates among RIF patients.

Keywords: Low-level laser therapy (LLLT); Recurrent implantation failure (RIF); In vitro fertilization (IVF), Pregnancy rate; Photobiomodulation.

Introduction

Recurrent implantation failure (RIF) poses a substantial challenge in the field of in-vitro fertilization (IVF). There exist no universally agreed-upon criteria for defining RIF. Still, as per the European Society of Human Reproduction and Embryology consortium, the lack of a gestational sac on ultrasonography imaging at five weeks or beyond after embryo transfer (ET), following either three ET attempts with high-quality embryos or the transfer of 10 high-quality embryos across several transfers, is defined as RIF.[1-5]

Successful implantation of an embryo strongly relies on the receptivity of the endometrium during the implantation window. Increasing evidence suggests that endometrial receptivity and growth are closely related to uterine blood flow.[6] Several approaches have been explored for RIF management and endometrial receptivity improvement. However, achieving a consensus on the most effective treatment remains disputed. Low-dose aspirin, L-arginine, vitamin E, sildenafil, endometrial receptivity array (ERA) and recent presentation of stem cell therapies are suggested interventions.[7-12]

Photobiomodulation therapy, also known as Low-level laser therapy (LLLT), involves the utilization of photons to alter organic activities. This process typically utilizes a red beam or near-infrared laser.[13] The energy emitted during laser exposure is absorbed by intracellular molecules and transformed into metabolic energy. Studies have shown that this process increases cellular ATP levels and leads to increased protein synthesis and the release of cytokines and growth factors, thereby promoting the proliferation of cells.[14,15]

The presentation of LLLT in medicine has sparked

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a revolution in tissue repair and regeneration.[16] Considering the crucial role of endometrial quality in the successful implantation of a fetus,[17] the application of LLLT holds promise for addressing implantation failure despite the advancements in assisted reproductive technologies.[18-21] It is believed that improving uterine arterial blood flow, which influences endometrial growth, can potentially enhance pregnancy outcomes. Several studies have demonstrated that medical interventions to increase endometrial growth and blood flow may improve biochemical and clinical pregnancy rates.[22] We conducted this study to evaluate the effect of LLLT on the outcomes of IVF in infertile patients with a history of RIF.

Materials and Methods

Study Design

This study was accomplished as a single-blinded randomized clinical trial, where the outcome assessor and data analyst were unaware of the group assignments. It included patients attending the IVF clinic of a university hospital in Tehran, Iran. The study population was recruited between May 2023 and September 2023.

Study Population

The study population consisted of 30 infertile women between the ages of 24 and 39 years who had previously undergone at least three ETs with high-quality embryos but had failed to achieve conception and were selected as candidates for frozen-thawed embryo transfer (FET). The exclusion criteria were body mass index (BMI) exceeding 24.9 kg/m^2, smoking, alcohol consumption, prior treatment with cytotoxic drugs, abnormal male fertility factors, hydrosalpinx diagnosed by ultrasonography, abnormalities pertaining to hematology, immune system, hormones, chromosomes, and genetics, adenomyosis, pelvic tuberculosis, and endometriosis, as well as uterine acquired or congenital abnormalities.

Preliminary Evaluations

A single expert radiologist performed preliminary 3D color Doppler transvaginal sonography on either the second or third day of the patient's preceding menstrual cycle. This assessment aimed to investigate the uterus and adnexa for any irregular findings. The assessment of the uterine cavity through hysteroscopy was conducted prior to commencing the treatment cycle if it had not been previously undertaken. Laboratory analyses were carried out to evaluate thrombophilia, hormonal imbalances, antiphospholipid antibodies, and hematological as well as immunological abnormalities in female subjects while karyotyping of couples was also undertaken.

Hormone Replacement Therapy and Endometrial Preparation

For all the participants involved in the FET cycles, hormone replacement therapy was utilized to prepare the endometrium. The protocol consisted of administrating 6 mg/d estradiol valerate starting on the second day of the menstrual cycle. After seven days of treatment if the endometrial thickness was below 8 mm, the dosage was raised to 8 mg/d. Throughout the cycle, progesterone supplementation (400 mg suppository administered twice daily) commenced as soon as the endometrial thickness reached 8 mm.

Treatment Cycle and Randomization

One cycle prior to the planned FET cycle, the patients were randomly divided into two groups using a random allocation sequence generated through a randomized block design with a block size of 4. Group A underwent pretreatment with LLLT, while Group B acted as the control group.

Low-Level Laser Therapy

Low-level laser irradiation was performed using a New Age BIOLASER device (New Age Co., Italy) with a power of 900 mW, frequency of 50/60 Hz, and wavelength of 850 nm. On the basis of the existing literature, we considered the penetration depth of near-infrared (IR) to be 3 mm. The irradiation sessions were conducted transabdominal on the hypogastric area, with an empty urinary bladder. The uterus was gently pushed toward the anterior lower abdominal wall using a vaginal probe to minimize the distance between the laser probe and the uterine body. Six equal sessions of 16 minutes each were administrated on days 2, 4, 6, 8, 10, and 12 of the menstrual cycle. In the next cycle, an embryo transfer cycle was initiated.

The laser source utilized in this study is an AlGaAs diode laser, which operates in continuous wave (CW) mode with semi-polarized output.

The penetration depth of near-infrared (IR) wavelength in various soft tissues, including the uterus, was considered to be at least 3 mm based on the existing literature.[23,24] It is important to note that this value may vary between patients due to such factors as tissue thickness, blood vessel density, and blood concentration. Nevertheless, for the purposes of this study, a conservative estimate of 3 mm penetration depth was adopted. According to the definition of penetration depth, it represents the depth at which 1/e (e = 2.71828) of the incident light intensity is reached.[25] The power reaching a specific depth can be calculated by the following equation:

$$I = I_0 e^{-\frac{t}{t_0}}$$

Where I_0 represents the initial power, t_0 is the penetration depth, and t is the final depth. Considering the input power of 900 mW, the power reaching a depth of 3 cm is approximately 40 mW, which is sufficient for LLLT.

Embryo Transfer Procedure

One or two good-quality blastocysts (Graded as A or B according to embryologic scoring) were transferred under the guidance of abdominal ultra-sonography by a single expert infertility fellow.

Outcome Measures

Biochemical pregnancy was determined by a positive blood pregnancy test conducted two weeks after embryo transfer. Clinical pregnancy was confirmed through ultrasonography visualization of the gestational sac five weeks after embryo transfer.

Statistical Analysis

The analysis of data was conducted by utilizing Statistical Package for Social Sciences 20 (SPSS, SPSS Inc., Chicago). Mean ± standard deviation (SD) was used to describe the data. A threshold of $P < 0.05$ was deemed statistically significant.

For sample size determination, we referred to reference,[26] which reported an implantation rate of 20.3% in the laser group compared to 15.9% in the control group.

Given the specific context of our study, where the maximum number of clients with RIF within one year at the referring infertility clinic is limited to 30 individuals, we adjusted the sample size accordingly. To ensure feasibility within our specific setting, we employed the sample size formula for limited populations. Consequently, the final sample size was determined to be a total of 30 participants, with 15 participants in each subgroup, while maintaining a power of 80%.

To assess the normality of the quantitative variables, we conducted the Kolmogorov-Smirnov test. On the basis of the results, we found that, except for the BMI, the other variables did not follow a normal distribution. Consequently, we employed an Independent T-test to compare the BMI values between the groups, given its normal distribution. For the non-normally distributed variables, we utilized the Mann-Whitney U test for the comparison.

Results

Thirty participants with a mean age of 34.17 years and a past medical history of RIF were enrolled in this study, with 15 cases assigned to each of the laser and control groups. All participants completed the study, and their data were subjected to analysis.

The participants had previously experienced failed ET attempts, ranging from 3 to 7 cycles. No significant difference was detected between the basic characteristics of the patients between the laser group and the control group (Table 1).

All the patients underwent hysteroscopic uterine cavity assessment before commencing the FET cycles, and uterine cavity abnormalities were not detected in any of the participants. No treatment-related side effects or complications were reported in the laser or control groups.

Table 2 displays the biochemical and clinical pregnancy rates of both groups. The laser treatment group showed higher rates of biochemical and clinical pregnancies at 46.7% and 33.3%, respectively, compared to the control group, which had rates of 33.3% and 20.0%, respectively. Nevertheless, the observed difference did not attain statistical significance.

Discussion

The implantation process in a regular menstrual cycle relies on harmonizing various factors within the endometrium. Prostaglandins, adhesion molecules, cytokines, and growth factors play crucial roles in the window of implantation. Research suggests that the levels of growth factors in the endometrium of women who have experienced RIF are lower compared to those of women with normal fertility.[20,27,28]

Several former investigations indicate a potential correlation between endometrial injury and enhanced rates of pregnancies in women with embryo transfer failures.[29-30] However, contrasting studies suggest no discernible impact.[31-32] According to a recent Cochrane database systematic review and meta-analysis, it is still unclear whether endometrial injury improves the

Table 1. Patients' Characteristics in Laser-Treatment and Control Groups

Characteristics	Total N = 30	Laser Group N = 15	Control Group N = 15	*P* Value*
Age (y)	34.17 (4.23)	34.27 (4.30)	34.07 (4.31)	0.967[a]
Previous pregnancy positive	13 (43.3)	7 (46.7)	6 (40.0)	0.500[b]
Duration of infertility (y)	7.7 (4.88)	7.80 (5.08)	7.60 (4.84)	1.000[a]
BMI	25.73 (2.94)	25.49 (3.09)	25.97 (2.86)	0.666[c]
Number of previously failed FET cycles	3.43 (0.85)	3.67 (1.11)	3.20 (0.41)	0.305[a]
Number of previously transferred embryos	6.30 (1.29)	6.60 (1.45)	6.00 (1.06)	0.345[a]
AMH level	4.04 (3.84)	4.01 (4.30)	4.07 (3.46)	0.624[a]

The data are presented by n (%) and mean (standard deviation). **P* value less than 0.05 is considered significant. [a]Mann-Whitney U test, [b]Chi-square, [c]Independent T test.

Table 2. Pregnancy Outcomes in the Two Groups

Pregnancy Rates	Total N=30	Laser Group N=15	Control Group N=15	*P* Value*
Biochemical pregnancy	12 (40)	7 (46.7)	5 (33.3)	0.710
Clinical pregnancy	18 (60)	5 (33.3)	3 (20.0)	0.682

The data are presented by n (%).
**P* value less than 0.05 is considered significant. Fisher Exact test is used.

likelihood of live birth or clinical pregnancy in women who undergo IVF.[33]

LLLT is a well-established method for treating various soft tissue injuries and pain management. This method utilizes light therapy to induce biochemical changes within cells. By stimulating cellular photoreceptors, photons trigger chemical alterations and potentially beneficial biochemical reactions in the body. LLLT, also known as cold laser therapy, has been widely used for pain management for years. This procedure enhances cell metabolism, and as a result of the improved cell metabolism, adenosine triphosphate (ATP) is produced, which promotes cell nutrition and waste removal, which is especially valuable when it comes to the restoration of tissues. Additionally, LLLT enhances vascular activity, which makes it particularly useful in repairing soft tissue and maintaining growth integrity. No severe side effects have been reported after LLLT treatment, so it may be effective for RIF patients by enhancing endometrium blood supply and growth.[34]

In a study conducted by Tsai et al, they employed the helium-neon (He-Ne) laser in RIF-affected women prior to FET. Their approach improved microcirculation and the release of growth factors in the endometrium, thereby enhancing endometrial receptivity. The irradiation process was performed intravenously and involved inserting a plastic laser catheter into a vein. The laser group's clinical pregnancy, implantation, and live birth rates were higher in this non-randomized study. Still, the miscarriage rate was lower, and none of the differences was statistically significant.[26]

In the current study, the treatment group exhibited higher rates of clinical and biochemical pregnancy rates than the control group. However, these differences did not reach statistical significance (Table 2). It is worth noting that the limited sample size may have contributed to this outcome. Nonetheless, the slightly elevated pregnancy rate observed in the treatment group may have practical implications. The outcomes of our investigation align with those reported by Tsai et al[26]; however, it is crucial to note that our methodologies diverge significantly from theirs.

Limitations

Although our study is the first of its kind, one of its limitations was the relatively small sample size, which may affect the statistical power of our analysis. Additionally, we did not investigate the long-term effects of LLLT on IVF outcomes. Therefore, more extensive studies are required to clarify the underlying mechanisms of LLLT and its side effects.

Conclusion

Pretreatment with LLLT might improve biochemical and clinical pregnancy proportions in RIF patients. According to the literature, our study is one of the first single-blinded randomized clinical trials that evaluated the ability of LLLT pretreatment in RIF patients. Still, due to its small sample size, further large, well-designed prospective studies are worthwhile and necessary.

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Authors' Contribution

Conceptualization: Mina Jafarabadi, Mamak Shariat.
Data curation: Mamak Shariat.
Formal analysis: Mamak Shariat.
Methodology: Mamak Shariat and Mina Jafarabadi.
Project administration: Mina Jafarabadi, Yasaman Farbod.
Supervision: Mina Jafarabadi.
Validation: Mina Jafarabadi, Yasaman Farbod, Mamak Shariat.
Writing–original draft: Yasaman Farbod.

Competing Interests

We would like to confirm that all the authors of this manuscript have no conflicts of interest to declare.

Ethical Approval

Our study was approved by the ethics committee of Tehran University of Medical Sciences (IR.TUMS.IKHC.REC.1401.096). All the patients were treated following the Declaration of Helsinki. Informed consent was obtained from all the patients before participation. This study was registered in the Iranian Registry of Clinical Trials [IRCT20100518003950N7].

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