The Egyptian Journal of Hospital Medicine (October 2022) Vol. 89 (2), Page 7237- 7241

Effect of Red Laser on Human Sperm Asthenozoospermia and Abnormal Agglutination Groups

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ABSTRACT

Background: Infertility is a problem in need for solution. Seminal fluid analysis is the first test that needs to be done to study the shape, count, and activity of sperm. Sperm have to move and reach the ovum to initiate fertilization. Many researchers and fertility doctors work hard for this purpose. The first studies clearly indicate that human sperm motility as well as velocity can be improved by laser irradiation

Objective: The aim of our study was to combat infertility by enhancing sperm motility, and also to evaluate the role of laser activity in solving infertility problems.

Materials and Methods: 50 semen samples were selected from infertile patients referred to the" High Institute for Infertility Diagnosis and ART, Al-Nahrain University", through the time span from December 2021 to April 2022. Standard seminal analysis was done to measure sperm concentration, motility, morphology, and agglutination according to WHO 2010 guidelines.

Results: the present work found that the red laser exposure to the samples of asthenozoospermia for 10, 20, and 30 min resulted in a significant increase in the percentage of progressive sperm motility compared to before exposure. However, there was no significant difference in the morphologically normal sperm percentage compared to before the laser exposure.

Conclusion: Sperm motility increased and motility quality showed an improvement in the treated samples, due to low level laser.

Keywords: Laser, Red laser, Sperm, Agglutination, Activation.

INTRODUCTION

Laser is a device that emits light through a process of optical amplification that is based on the stimulated emission of electromagnetic radiation. The term "laser" originated as an acronym for "**L**ight **A**mplification by **S**timulated **E**mission of **R**adiation" [(1)]. The energy of emitted photon (ΔE) must be equal to the energy difference between the two states, the transitions of electrons between energy states or levels accomplished by the following processes, leads to laser production: a- Absorption, b- Spontaneous emission and c- Stimulated emission [(2)].

In medicine, laser uses including laser surgery, laser healing (photobiomodulation therapy), kidney stone treatment, ophthalmoscopy, and cosmetic skin treatments, cellulitis, striae reduction, and hair removal. [(3)]. "Low-level laser therapy" (LLLT) is a photochemical mechanism in which photons from the laser source interact with cells that result in stimulation of the cells or biochemical changes [(4)].

Semiconductor lasers can be used as laser scalpels for precise and efficient cutting. The wound healing will be faster than traditional scalpel cutting. It is also used in selective photo thermal therapy. In this technology use of nano materials to mark tumor cells and then uses semiconductor lasers to generate local high temperature to treat tumors accurately. With one another, in medical treatments such as plastic surgery, ophthalmology, and physical therapy [(5).]

Infertility is a "disease of the reproductive system defined by the failure to achieve a clinical pregnancy after suitable medical time or more of regular (2-3 times per week) unprotected sexual intercourse" [(6)].

The sperm disorders are one of the most important causes of infertility in males, which include low sperm count (oligospermia), low sperm viability (necrospermia), defective sperm morphology (teratospermia), and reduced sperm motility (asthenospermia) [(7)].

A common cause of male infertility is poor sperm motility, since the sperm must travel a long distance to reach and fertilize the ovum, therefore the motility of the sperm is a necessary condition for proper fertilization [(8)].

It is postulated that laser bio stimulation or LLLT has a positive effect on the mitochondria and leads to an increase of ATP synthesis [(9)], which may stimulate the sperm motility of asthenozoospermia samples.

MATERIALS & METHODS

Subjects:

In this study, 50 semen samples were selected of infertile patients referred to High Institute for Infertility Diagnosis and Assisted Reproductive Technologies Laboratory from different infertile men through the period from December 2021 to April 2022. Their ages ranged from 20 to 40 years with a mean of 25 ±5 years.

Received: 15/7/2022
Accepted: 18/9/2022

Semen fluid analysis: All samples were collected in a room near the laboratory by masturbation into a wide mouthed sterile specimen container after an abstinence period of 3 to 5 days. Each sperm sample was analyzed as recommended in the manual of WHO 2010 and 2021. Each sample was divided into two portions, one was used as a control part and one was exposed to different laser doses (10, 20 and 30 min) with wavelength of 650 nm red color.

Sperm Concentration:

The concentration was measured from the mean number of sperms in five high power fields (HPF) with a magnification of X40 objective lens. This number was multiplied by a factor of one million [(10)]. Total sperm count obtained by multiplying the sperm concentration with sample volume.

Sperm concentration (million/ml) = number of sperm in HPF×106

Total sperm count (million/ejaculate) =sperm concentration × volume.

According to sperm concentration guidelines, normal sperm concentration is defined as 15106 sperm/ml or greater (WHO 2010).

pH: pH of the semen has been measured by using pH litmus paper where the paper was immersed in the semen sample for a few seconds resulting in change of the litmus paper colour. The pH of the semen is considered normal when it is slightly alkaline and ranges between (7.2 - 8.0) according to criteria of WHO 2021 and [(11)].

Liquefaction time:

Normal semen sample liquefies within 30 minutes at 37 ˚C, although this usually occurs in a period less than this time. Some samples were induced to liquefy by mechanical mixing or the addition of a volume of culture medium followed by repeated pipetting. The sample was well mixed in the original container before microscopic examination [(12)].

Sperm agglutination:

It refers to the motile spermatozoa stick in such a way (tail-to-tail) (head -to-head), or mixed (tail to head). That means the shape of adhesion either of immotile spermatozoa to each other or of motile spermatozoa agglutination and to mucous strands. For non-sperm cells or debris, it is considered a non-specific aggregation [(13).]

Laser source: The laser light source used in this study was continuous type with an average output power of 128 mW, wavelength 650 nm, and with a beam spot of 4 mm in diameter, as shown in figure (1).

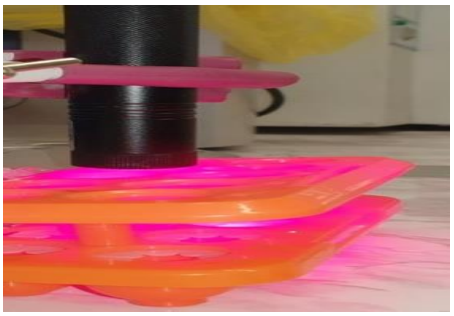

Figure (1) Red laser source used in the experiment
First part: One that was untreated and used as a control volume of (1 ml).

Second part: That was irradiated for (10, 20, and 30 min) in the same manner produce power of 128 mW.
Since diameter of laser spot (D) = 2 mm = 0.2 cm
Radius (r) = 0.1 cm^2
The area (A) = $\pi r2 = 3.14 \times (0.1)^2 = 0.125\ cm^2$
Power density= power W/Area cm^2
Power density= 0.128 W / 0.125 cm^2
Power density= 1.024 W/cm^2

After preparation, laser- (Red laser wavelength 650 nm) treated and untreated samples were examined using microscope.

Ethical consent: An approval of the study was obtained from Baghdad University Academic and Ethical Committee. Every patient signed an informed written consent for acceptance of participation in the study. This work has been carried out in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) for studies involving humans.

Statistical analysis: We used Statistical package for social science (SPSS) in order to get data analysis. Continuous variable was presented as mean ± SD. Independent sample test was used to test a significance of the association of the variable data. Also we used P-value ≤ 0.05 was considered significant value.

RESULTS

Table (1) showed the effects of LLLT on asthenozoospermia parameters after 10, 20, and 30 minutes. There was a significant reduction (p = 0.048) in sperm concentration between those exposed and non-exposed after 10, 20 and 30 minutes. There was a significant improvement in progressive motility (p = 0.045) after 30 minutes. While no-significant difference was observed in non-progressive sperm (p = 0.67), and immotile sperm percentage (p=0.91) after red laser exposure for 10, 20 and 30 minutes compared to before exposure. Moreover, the percentage of morphologically normal sperm (%) didn't significantly (p=1.00) change before and following exposure to the red laser.

Table (1): Effect of red laser exposure on certain sperm function parameters of asthenozoospermic men

Certain sperm parameters		Before activation	Red laser exposure (650 nm)			P value
			10 min	20 min	30min	
Sperm concentration (million/ml)		34.20 ± 4.16 A	30.60 ± 2.62 A	29.33 ± 2.44 A	27.46 ± 2.84 B	0.048 S
Sperm motility (%)	Progressive	24.00 ± 1.36 A	27.26 ± 2.30 AB	28.26 ± 2.95 AB	31.66 ± 3.34 B	0.045
	Non-progressive	43.53 ± 1.76 A	42.00 ± 2.85 A	43.86 ± 2.80 A	39.80 ± 2.75 A	0.67 NS
	Immotile	32.00 ± 2.77 A	30.74 ± 3.06 A	28.53 ± 3.48 A	28.54 ± 4.01 A	0.91 NS
Morphologically normal sperm (%)		45.66 ± 4.72 A	46.76 ± 4.82 A	47.22 ± 4.87 A	45.47 ± 4.80 A	1.00 NS

Effect of red laser exposure on certain sperm function parameters of abnormal agglutinated semen:

The results in table (2) showed a significant (p=0.0448) difference in sperm concentration between control and sperm exposed to 10, 20, 30 minutes of red laser. And this difference was positive which mean that there was an increase in concentration after this laser (650 nm) exposure.

The effect on sperm motility was significantly (p=0.0474) higher when the time of exposure was 30 minutes (39.28 ± 6.65), than that before exposure (29.85 ±2.08). The table also showed non- significant difference (p=0.592) when the results were compared between 10, 20 and 30 min laser exposure. Moreover, there was no significant difference in immotile sperm (p=0.450) between before and after laser exposure.

The percentage of morphologically normal sperm did not change after exposure for different times of irradiation (p=0.439).

A High statistically significant (p=0.0001) reduction was recorded in agglutination percentage after red laser exposure of wavelength 650 nm.

Table 2: Effect of red laser exposure on certain sperm function parameters of abnormal agglutination of semen

Certain sperm parameters		Before activation	Red laser exposure (650 nm)			P value
			10 min	20 min	30min	
Sperm concentration (million/ml)		47.57 ±6.94 A	52.28 ±5.05 AB	52.77 ±4.83 AB	56.00 ±3.45 B	0.0448
Sperm motility (%)	Progressive	29.85 ±2.08 A	35.14 ±3.58 AB	40.42 ±6.70 B	39.28 ±6.65 B	0.0474
	Non-progressive	40.42 ±2.19 A	41.00 ±3.20 A	37.00 ±4.01 A	34.57 ±5.02 A	0.592 NS
	Immotile	29.71 ±1.75 A	27.14 ±4.02 A	22.57 ±3.44 A	25.28 ±2.77 A	0.439 NS
Morphologically normal sperm (%)		55.43 ±6.70 A	53.42 ±6.88 A	54.43 ±6.62 0 A	53.42 ±5.67 A	1.00 NS
Agglutination (%)		24.28 ±3.52 A	6.57 ±1.78 B	4.43 ±0.99 B	4.43 ±0.99 B	0.0001

DISCUSSION

This study may be the consequence of the usual sedimentation of sperm, which happens when dead, immobile sperm and other cells settle to the bottom of a tube as a result of natural gravity. The bulk of immobilized spermatozoa reported benting their heads downward after around five minutes of exposure to gravity before slowly sinking at an average speed of 0.2 µm/s [(14)].

The concentration decreases significantly after 20 mints and more after 30 mins. The results of the current investigation showed that the number of sperms that got laser therapy and transition from immotile or non-progressive movement to progressive has significantly increased. Other research showed that laser irradiation may boost human sperm motility and velocity came to similar conclusions [(15)].

Agglutination in sperm has a negative impact on fertility due to impedance of sperm function parameters, inhibition of fertilization and implantation [(16)]. Agglutination decreased significantly, which means more chances for fertility.

Antisperm antibodies (ASA) reason of sperm agglutination and affect sperm motility, viability and sperm migration in the female reproductive tract [(17).] Antibodies was the main cause of agglutination, which was a defence process caused by the immune system for attacking the sperms as a foreign bodies. So laser turn was a photo thermal sources to dissolve this glutting between sperms and then increase the activity of the sperms by activating the mitochondria, and then increasing the secretion of ATPs which was the main source of cells energy, so the turn of laser was to dissolved the agglutination and released the sperms, that lead to increased sperms numbers, and then activate the sperms (speed).

Limited heating that may induce energy supply determines how fast and how far a spermatozoon moves. Naturally, when the sperm is facing the environment for fertilization of the ovum, capacitation and acrosome reaction processes include energy supply by increased adenosine-5'-triphosphate (ATP) generation are required. The sperm only activates and becomes motile after ejaculation [(18)]. Therefore, laser exposure will increase the ATP production, leading to an increase in the Ca^{+2} influx and in turn, the motility of sperm. The fact that photons have energy can be absorbed at several cellular levels and that the cell organelle with the greatest concentration of chromophores is the mitochondrial membrane, specifically, at the cytochromes of the electronic transport chain. It has been pointed out that the cytochrome is the one that particularly absorbs photons of the laser light wavelength (650 nm) looks like that absorption at end of the breathing chain eases the synthesis of ATP [(19)]. It has been established that ATP is crucial for cells to respond to light and for boosting energy bioavailability [(20)]. The effect of particular light wavelength used in the present study was designed to increase electron transport chain activity, resulting in increased ATP production that leads to increase of sperm motility (activation).

Also, in the current work, we found that an improvement in the progressive motility for asthenozoospermic samples following exposure to red laser light when compared to control samples. Sperm motility improved more in low-quality samples, suggesting that asthenozoospermia may have a deficiency in mitochondrial activity as an underlying cause.

CONCLUSION

Effect of the light used in this project was a CW laser, which was used because pulsed laser is high power. LLLT is designed to increase electron transport chain activity, resulting in an increase in ATP production. This leads to an increase in sperm motility (activation). The outcomes revealed that the sample's motility pattern significantly improved following the treatment course. While the non-treated samples showed a significant increase in the percentage of immotile sperm over time.

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