



Laser Biostimulation Effect on Human Sperm Motility

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(Received 27/4/2021; accepted 24/6/2021)

Abstract: Background: Sperm motility disorder is an important cause of infertility in male, and one of the causes of reduced motility of the sperm is the disorders of the mitochondria because it provides the required energy for sperm motility, Laser biostimulation or low-level laser therapy has a positive effect on the mitochondria and led to increasing the synthesis of ATP. **Method:** Twenty fresh human semen samples were used in this research study, each sample was separated into two portions, one was used as control which is not exposed to the laser beam and the other was irradiated with the wavelength of 410 nm diode laser with an output power of 100 mW and an exposure time of 60 seconds, then the measurement of the progressive motility, non-progressive motility, and the immotile sperm were assessed after 5,15,30 min of irradiation for every control and the irradiated samples. **Results:** the progressive and non-progressive motility of the sperm was significantly increased following irradiation compared to the control samples also the number of immotile sperm was significantly decreed after irradiation. **Conclusion:** We observed that a low-power laser of 410 nm wavelength could cause sperm motility to increase for a short time.

Keywords: Male infertility, Sperm motility, 410 nm diode laser.

1. Introduction

Male infertility is a multi-factor condition that encompasses a variety of disorders and in 50% of the cases, the couple's infertility is caused by a male fertility problem, male infertility can be caused by congenital or inherited genital defects, elevated scrotal temperature, genitourinary disease, genetic defects, endocrine disturbances and immunological cause (Moskvin and Apolikhin, 2018). Also, the disorders of the sperm are one of the most important causes of infertility in males which include low sperm viability (necrostermia), low sperm count (oligospermia), defective sperm morphology (teratospermia), and reduced sperm motility (asthenospermia) (Lesani et al., 2020). Poor sperm motility is a common

cause of male infertility in humans since that the sperm must move a long way to reach and fertilize the ovum, therefore the motility of the sperm is a necessary condition for proper fertilization. (Curi et al., 2003) Spermatozoa motility refers to the ability of the sperm to move; as well, the sperm that is unable to pass through the cervical mucus or penetrating the rigid outer shell of the oocyte if the sperm movement is slow or in a circular motion or both. Severe asthenozoospermia can be caused by specific axoneme ultrastructural defects, mitochondrial defects, lack of dynein arms, a disorganized fibrous sheath, or thick outer fiber with droopy tails, in addition, sperm autoimmunity can be associated with asthenozoospermia. Other moderate-level motility defects have unknown causes. (Siddique et al.,

2011). Low-level laser irradiation is also known as low-level laser therapy (LLLT) in medical terminology, is a form of laser therapy that uses the low-powered laser to cause therapeutic changes, this means it has little effect on biological tissue other than photochemistry, low-level laser therapy works by a photochemical mechanism in which photons from the laser source interact with cells that result in stimulation of them or biochemical changes (Fauzi *et al.*, 2018), and because of its beneficial effect on mitochondria due to the stimulation of the mitochondrial respiratory chain and the synthesis of ATP, low-level laser therapy can be used to increase viability, metabolism, and motility of the sperm cells. This therapy can certainly be useful for preventing the use of certain chemicals in the culture media of spermatozoa and also in promoting the survival and the motility of the sperm cells specifically the following thawing or in largely immotile sperm samples (Zupin *et al.*, 2020).

2. Materials and method

2.1 Sample collection

After routine seminal fluid examination, semen samples of 20 males with lower sperm motility (asthenozoospermia), and ages ranging from (25 to 50) years old, and seminal fluid volume of 2ml or more were collected and then used, after a sexual abstinence period of (48-72) hours, all samples were obtained by masturbation of males into a wide-mouthed sterile test jar in the laboratory. After that, the samples were incubated at 37°C for 30 minutes to be liquefied.

2.2 Laser requirements and the method of irradiation

In this research study, a continuous-mode diode laser with the wavelength of 410 nm and output power of 100 mW, and power density of 0.67 W/cm² was used, each human seminal fluid sample was separated into two portions, one of which was used as control (non-irradiated) and the other of which was irradiated with the laser beam with an exposure time of 60 seconds, in the Eppendorf tube, 1 ml from each liquefied seminal fluid specimen was placed and the laser probe was fixed at a 30 cm distance above the Eppendorf tube.

2.3 Sperm motility analysis

According to a basic motility classification system that is recommended by (WHO 2010) which differentiates spermatozoa with non-progressive motility or progressive motility from those who are immotile. The sperm motility was assessed using the Computer Assisted Semen Analysis (Mira-9000 CASA) system. This system follows WHO (2010) strict criteria for motility patterns and morphometric assessment of human semen. After an incubation period of 5, 15, 30 minutes, the sperm motility of the control and the irradiated samples was measured.

2.4 Statistical analysis

SPSS (v 20) was used to do the statistical analysis. The repeated measure between the irradiated sample and the control was analyzed using ANOVA test, values of $p > 0.05$ were regarded as statically non-significant while values of $p \leq 0.05$ and $< 0.01, 0.001$ were considered significantly different.

3. Results and discussion

As shown in table 1, the results indicate that the progressive motility of the irradiated samples is significantly increased in comparison to the progressive motility of the control sample, also the non-progressive motility of the irradiated sample is significantly increased after 5, 15 min of irradiation in comparison to the non-progressive motility of the control samples as described in table 2, and the number of the immotile sperm is significantly decreased after irradiation with 410 nm diode laser in comparison to the number of immotile sperm of the control samples, as described in table 3.

Since mitochondria supply a portion of the energy needed for the sperm motility, one of the main characteristics of reduced motility of the sperm is caused by impaired integrity of the mitochondrial membrane and compromised function of its sheath as well as the alterations in the functions of mitochondrial respiratory chain enzymes may also impair the sperm motility (Shahrokhi *et al.*, 2020).

Table (1): Results of the progressive motility

Percentage of Progressive motility	Time following irradiation (min)					
	5 min		15 min		30 min	
	Control	irradiated	Control	irradiated	Control	irradiated
Mean $\pm$ SE	20.13 $\pm$ 1.58	27.52 $\pm$ 2.26	16.41 $\pm$ 1.53	22.65 $\pm$ 1.8	14.99 $\pm$ 1.4	21.82 $\pm$ 2.5
P value c vs I	0.01		0.01		0.02	

*c: control, I: irradiated

Table (2): Results of non-progressive motility

Percentage of non-progressive motility	Time following irradiation (min)					
	5 min		15 min		30 min	
	Control	irradiated	Control	irradiated	Control	irradiated
Mean $\pm$ SE	14.46 $\pm$ 0.66	16.93 $\pm$ 0.85	13.09 $\pm$ 0.7	15.3 $\pm$ 0.73	12.96 $\pm$ 1.19	14.71 $\pm$ 1.29
P value c vs I	0.05		0.05		0.09(NS)	

Table (3): Results of immotile sperm

Percentage of immotile sperm	Time following irradiation (min)					
	5 min		15 min		30 min	
	Control	irradiated	Control	irradiated	Control	irradiated
Mean $\pm$ SE	66.67 $\pm$ 2.58	55.74 $\pm$ 2.83	69.01 $\pm$ 1.95	61.02 $\pm$ 2.24	71.86 $\pm$ 2.45	63.61 $\pm$ 3.74
P value c vs I	0.01		0.02		0.03	

Mitochondria are considered to be a possible site for the first effect of light, which results in increased ATP synthesis and regulation of

reactive oxygen species (Farivar, et al, 2014). Mitochondria house the electron transfer chain which includes complex I, II, III, and IV and photon absorption by these complexes induce electrical excited states which can speed up electron transfer reactions, and this result in increased ATP synthesis because more electron transport (Yazdi *et al.*, 2014) found that 830 nm diode laser improve progressive motility depending on both post-exposure time and laser density. (Novikova, Ya.S et al,2013) conducted in vivo experiment on rats by irradiate the testes with a wavelength of 405 and 475 nm for one minute and the number of sessions is 10 days and found that the sperm number and motility is increased after irradiation and has a positive stimulating effect on spermatogenesis.

4. Conclusion:

We observed that a low-power laser of 410 nm wavelength could cause sperm motility to increase for a short period of time. Necessitates for increased ATP production (Hamblin et al, 2006). Flavins and flavoproteins such as Flavin mononucleotide and Flavin dinucleotide are believed to be excited by blue light (400-500 nm), complex II, is a Flavin containing cytochrome (contain FADH2) that absorb blue light, as a result, it's possible that blue light may influence mitochondrial function like red and NIR light (Serrage H ,et al, 2019) . The present study is the first study that used a Serrage H ,et al, 2019) 410 nm diode laser to enhance the motility of the sperm in vitro, and the results of this study agree with many studies that used the laser to improves the motility of the sperm, for instance, (Saeed, Al-Kaisy and Ali, 2014) study the effect of He-Ne laser 632.8nm on the motility of sperm in asthenozoospermia samples in vitro, the results show that progressive motility in treated samples increased significantly while non-progressive motility is not significantly increased also the percentage of non-motile sperms are significantly decreased.

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تأثير التحفيز الحيوي بالليزر على حركة الحيوانات المنوية البشرية

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الخلاصة: الخلل في حركة الحيوانات المنوية هي سبب مهم للعقم عند الذكور، وأحد أسباب انخفاض حركة الحيوانات المنوية هو اضطرابات الميتوكوندريا لأنها توفر الطاقة المطلوبة لحركة الحيوانات المنوية، التحفيز الحيوي بالليزر أو العلاج بالليزر منخفض الطاقة له تأثير إيجابي على الميتوكوندريا ويؤدي إلى زيادة إنتاج (ATP)، الطريقة: تم استخدام عشرين عينة من السائل المنوي البشري في هذه الدراسة البحثية، تم فصل كل عينة إلى قسمين، إحداهما استخدمت كعينة تحكم لم تعرض لشعاع الليزر والأخرى تم تشيعها باستخدام ليزر الداود بطول موجي 410 نانومتر و بطاقة خروج 100 ميغاوات ووقت تعريض 60 ثانية، ثم تم تقييم الحركة التقدمية والحركة غير التقدمية والحيوانات المنوية غير المتحركة بعد 5،15،30 دقيقة من التشيع لكل من عينات التحكم والعينات المشعة. النتائج: تم زيادة الحركة التقدمية وغير التقدمية للحيوانات المنوية بشكل ملحوظ بعد التشيع مقارنة بعينات التحكم، كما ان عدد الحيوانات المنوية غير القابلة للحركة أنخفض بشكل كبير بعد التشيع. الخلاصة: لاحظنا أن الليزر منخفض الطاقة بطول موجة 410 نانومتر يمكن أن يتسبب في زيادة حركة الحيوانات المنوية لفترة قصيرة من الزمن.