Effect of 410 nm Diode Laser Irradiation on Human Sperm Motility in Vitro

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Abstract

Background: Infertility can be defined asan absence of pregnancy after 12 months of regular unprotected sexual intercourse,the most important of the sperm function disorders are reduced sperm motility (asthenospermia). Photobiomodulation have an effect on the decrease of the sperm disorder. **Materials and Methods:** Diode laser with wavelength of 410nm was used to induce the sperm motility in an asthenozoospermia human seminal fluid in vitro,20 fresh seminal fluids samples were used in this study and each sample was divided into two parts, one of them non-irradiated by laser light (control) and the other was exposed to laser irradiation with exposure time of 30 secondsof 410nm diode laser with 0.67 W/cm^2, power density. **Results:** Progressive motility was significantly increase while non- progressive motility was non significantly increased and the immotile spermswere significantly decreased at 5 min following irradiation compared to non-irradiated samples,while at 15,30,45 minfollowing irradiation begin to decreased. We found that low power laser could induce a short term bio stimulation effect on sperm motility.

Key words: *asthenozoospermia, bio-stimulation,laser.*

Introduction

Infertility can be defined asan absence of pregnancy after 12 months of regular unprotected sexual intercourse [(1)].Male infertility factor is a term that involves a variety of different sperm function disorders that can make fertilizing an egg under normal conditions difficult for sperm, problems with male fertility factor due to changes in the quality of the semen as evaluated by the semen analysis, themost important of these are reduced sperm motility (asthenospermia),defective sperm morphology (teratospermia), and low concentration of sperm(oligospermia), semen volume and other seminal markers of prostatic,epididymal and seminal vesicle function are less well associated with infertility[(2)]. the sperm motility is one of the most essential parameters required to determine the reproductive capability of the semen sample because of male with complete lack of motile sperm is sterile since that immotile sperm either it dead or alive cannot penetrate the cervical mucus as well as the fertilizing potential can also be affected by the form of the movement therefore sperm swimming in closed circle cannot easily pass through the uterotubal junction and only sperm with straight motion can fertilize the ova[(3)].also, that in order to accomplish its biological function of delivering the male haploid DNA complement to the oocyte,the sperm cell must reach the fertilization site in the oviduct,therefore the sperm cell must be able to develop a motility pattern during maturation in the testes to reach the oviduct and retain this motility pattern throughout its migration via the reproductive tract of the female,and The ability of the sperm cell to exhibit motion in vivo as well as in vitro is greatly dependent on its capability to produce ATP that in turn is used by the contractile proteins of the flagellum as the dynein ATPase substrate to transform chemical energy into the mechanical function[(4)].Low sperm motility can be caused by genital infection,anti-sperm

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antibodies, inflammation of the accessory sex gland and sperm structural defect, Ultrastructural defects in the sperm midpiece or tail, a condition called immotile cilia syndrome or Kartagener's syndrome can lead to a complete absence of motility[5].Photobiomodulation or low-level laser therapy is the application of light (usually LED or low power laser in the range of 1mW to 500 mW) to pathology to facilitate tissue repair, decrease inflammation and reduce pain[6].And in the field of reproductive research, photobiomodulation can be used to improve viability, metabolism, and motility of the sperm cells because of its positive effect on mitochondria due to the activation of the mitochondrial respiratory chain, and the production of ATP, this therapy can certainly be helpfulfor preventing the use of certain chemicals in the culture medium of spermatozoa and also in promoting the survival and the motility of the sperm cells particularly thefollowing thawing or in largely immotile sperm samples[7].several studies used the laser to enhance the sperm motility ,[8]found that 830 nm diode laser improve progressive motility depending on both post-exposure time and laser density. [9]study the diode laser effect on human sperm motility by comparing two different wavelengths red (635 nm) and infrared (830 nm) with each other and with no laser control group, all sperm motility parameters are significantly increased in both infrared and red with no statically significant difference between two laser group and slightly better results in the infrared group.[10] 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.

Aim of the Study

The main purpose of this study is to evaluate how exposure to continuous wave 410 nm diode laser effect the motility of sperm.

Materials and Method

This study was conducted at the laboratory in the institute of laser for postgraduate study at university of Baghdad during period from February to September 2020, the semen sample of twenty males with decreased sperm motility (Asthenozoospermia), and age between (20-43years old) was selectedthen used after routine semen analysis. After a sexual abstinence time of (48-72) hours, all samples were obtained by masturbation of males into awide-mouthed sterile specimen container. Samples were then incubated to be liquefied at 37^0C for 30 min. After liquefaction, each sample was divided into two parts: one was called control and the second was exposed to the laser beam.

A Continuous-wave 410 nm diode laser with 100 mW output power and laser diameter of 0.15$cm^2$was used in this experiment. Each sample was divided into two portions one called control (non-irradiated) and the second was exposedto the laser beam for 30 seconds. 1 ml of each liquefied sample was put in the Eppendorf tube and the laser probewas positioned at 30 cm distance above the Eppendorf tube. Irradiation was performed from above that mean whole sample was evenly irradiated (the common method for irradiating liquid samples).

Computer Assisted Semen Analysis (Mira-9000 CASA) was used to evaluate the motility of the sperm, this system follows WHO (2010) strict criteria for motility patterns and morphometric assessment of human semen.The motility of the sperm was assessed after 5,15,30,45 min after irradiation, after every measurement the sample was put in the incubator at $37C^0$ to overcome the influence of temperature on the motility of the spermatozoa.

The statistical analysis was carried out by SPSS (v 20). ANOVA test was used to analyze repeated measure between irradiated sample and control. Data expressed as mean±SE. Values of $p>0.05$ were considered statically non-significant while $p\leq0.05$ and $<0.01,0.001$ were considered significantly different, highly significantly different respectively. Estimate of correlation coefficient between different parameters in this study.

Table 1: Results of progressive motility

Percentage of progressive motility	Time following irradiation (min)							
	5 min		15 min		30 min		45 min	
	Control	irradiated	Control	irradiated	Control	irradiated	Control	irradiated
Mean± SE	17.33 ± 1.69	25.06 ± 2.54	15.38 ± 1.58	22.75 ± 1.36	12.27 ± 1.36	19.63 ± 1.75	11.32 ± 1.34	17.05 ± 1.45
P value c vs T *	0.01		0.01		0.01		0.006	

***c: control, T: irradiated**

Table 2: Results of non-progressive motility

Percentage of non-progressive motility	Time following irradiation (min)							
	5 min		15 min		30 min		45 min	
	Control	irradiated	Control	irradiated	Control	irradiated	Control	irradiated
Mean± SE	14.88 ± 1.19	16.84 ± 0.88	12.93 ± 1.19	14.91 ± 1.12	11.26 ± 0.99	14.63 ± 1.07	10.67 ± 1.11	13.36 ± 0.94
P value c vs T	0.19(NS)		0.23(NS)		0.02		0.07(NS*)	

*NS: non-significant

Table 3: Results of immotile sperm

Percentage of immotile sperm	Time following irradiation (min)							
	5 min		15 min		30 min		45 min	
	Control	irradiated	Control	irradiated	Control	irradiated	Control	irradiated
Mean± SE	68.88 ± 2.7	58.22 ± 2.87	71.34 ± 2.29	61.18 ± 2.61	75.43 ± 1.88	66.76 ± 2.47	75.35 ± 2.36	69.1 ± 2.23
P value C vs T	0.01		0.006		0.008		0.06 NS	

Table 4: Results of total motility (progressive and non-progressive motility)

Percentage of (progressive and non-progressive)	Time following irradiation (min)							
	5 min		15 min		30 min		45 min	
	Control	irradiated	Control	irradiated	Control	irradiated	Control	irradiated
Mean± SE	32.66 ± 2.48	42.02 ± 2.76	31.22 ± 2.98	37.77 ± 2.45	24.59 ± 2.21	33.91 ± 2.16	24.64 ± 2.41	31.86 ± 2.57
P value C vs T	0.01		0.09(NS)		0.004		0.03	

Results and Discussion

Diode laser with wavelength 410 nm was used to enhance the motility of the sperm. As shown in the table 1 the progressive motility was significantly increased after laser irradiation, the maximum influence of laser on the progressive motility was at 5 min following irradiation and the progressive motility of control samples was decreased by passing of time while non-progressive motility non-significantly increased after laser irradiation as shown in results in table 2**,** also the number of immotile sperm of irradiated samples was significantly decreased and total motility was significantly increased .

We supposed that the low power laser can enhance the mitochondrial respiratory chain components and increased the energy supply to spermatozoa and this study is the first study that use laser with wavelength of 410 nm to enhance the human sperm motility in vitro. One of the most important causes of reduced sperm motility is characterized by impaired integrity of the mitochondrial membrane and impaired function of its sheath because mitochondria provide apart of energy necessary for the motility of the sperm[11].as light is applied to cells, the first sites of light absorption are mitochondria and the photoreceptors are assumed to be cytochromes, consequently photon absorption induces a sequence of reactions known as cellular signaling pathways which result in ATP synthesis as well as the production of ROS[12]. 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 of more electron transport necessitate for increased ATP production[13]. 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[14].

Conclusion

We found that low power laser could induce a short term bio stimulation effect on the motility of the sperm.

Source of Funding- self

Conflict of Interest –Nil

Ethical Clearance- Before the study began, the institutional ethical and research committee gave their approval.

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