The effect of the low level laser irradiation on the human sperm motility

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Abstract

Sperm motility is known as an effective parameter in male fertility, and it depends on energy consumption. Low-level laser irradiation could increase energy supply to the cell by producing adenosine triphosphate. The purpose of this study is to evaluate the effect of the low-level laser irradiation on the human sperm motility. Fresh human semen specimens were divided into two equal portions and irradiated by 632.8 nm He-Ne laser irradiation with varying doses as: 0 (control) and 12 J/cm^2. At the times of 30 min following irradiation, sperm motilities were assessed by means of computer-aided sperm analysis in all samples. results showed that the irradiating human sperms with low-level 632.8 nm He-Ne laser can improve their progressive motility depending on both laser density and post-exposure time.

تأثير أشعة الليزر منخفض المستوى على حركة الحيوانات المنوية البشرية

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الخلاصة

من المعلوم ان حركة الحيمن هي العامل المؤثر في الخصوبة الذكرية والتي تعتمد على استهلاك الطاقة. ان التعريض لشعاع الليزر واطئ الطاقة من الممكن ان يزيد من الطاقة المجهزة للخلية من خلال إنتاج أنزيم ثلاثي فوسفات الأدنوسين (ATP). الهدف من هذه الدراسة هو لتقييم كيفية تأثير أشعة الليزر واطئ الطاقة على حركة الحيامن البشرية. تم اخذ نماذج من الحيامن البشرية الحية وتقسيمها إلى جزئين متساويين ومن ثم تعريضها إلى الإشعاع باستخدام هليوم– نيون ليزر ذو طول موجي 632.8 نانومتر بجرع مختلفة الجزء الأول أعتبر كمجموعة سيطرة غير معالجة أما الجزء الثاني تم تعريضه بطاقة 12 جول/سم^2 لمدة 30 دقيقة. بعدها تم تقييم حركة الحيامن بواسطة منظومة تحليل الحيامن الإلكترونية المدعمة بالحاسوب لجميع العينات. أظهرت النتائج بان تعريض الحيامن البشرية لهليوم– نيون ليزر واطئ الطاقة يمكن ان يحسن الحركة الأمامية السريعة للحيامن ويعتمد ذلك على كثافة الليزر والفترة الزمنية للتعريض.

Introduction

Spermatozoa travel a long distance to meet and fertilize the oocyte, so sperm motility is a requisite for normal fertilization and it is well known that without assisted reproductive techniques immotile or having low motility sperm never reach the ova and perform fertilization (1). Asthenospermia, or low sperm motility is a frequent cause of male infertility. Many cases of severe asthenospermia are thought to be as a result from fine structural abnormalities of the sperm flagellum (tail). In some instances, these arise as a result of genetic factors (2). These 'primary' aberrations tend to be homogeneous and affect all or most of the sperms in the ejaculate; clearly they are irreversible (3). In other cases, the causes of the sperm tail defects are unclear, although they sometimes

relate to an underlying disorder. This may be due to infection, testicular injury or pathology, or immunological factor. These factors may induce multiple ultrastructural changes in the sperm, which are accompanied by a loss of motility. In general, these 'secondary' or acquired effects are heterogeneous within the sperm sample. In contrast to the genetic defects, these changes may be reversible through treatment of the underlying pathology (2, 4). The medical treatment of infertility is divided into two main categories: specific and non-specific. Specific treatments are used for certain etiologies such as, male accessory gland infection, retrograde ejaculation, and positive antisperm antibody (5). In contrast, empirical medical treatment, also known as non-specific treatment, has been used in men with idiopathic infertility. Empirical medical treatment can also be divided into two categories based on the mode of action: hormonal treatment and antioxidant supplementation (6). However, scientifically acceptable evidence of empirical medical treatment efficacy is limited because of the lack of large, randomized, controlled studies (7). Semen preparation techniques for assisted reproduction, Intrauterine insemination (IUI) and In vitro fertilization (IVF), were developed to separate and/or activate the motile normal morphologically shaped spermatozoa. Leucocytes, bacteria and dead spermatozoa produce oxygen radicals that negatively influence the ability to fertilize the ova. The yield of as many motile, morphologically normal spermatozoa as possible might influence treatment of choice and therefore outcomes (8). Sperms are very sensitive cells and even low-energy visible light has previously been found to cause modulate various processes in their different biological systems. It is accepted that the first step following visible light irradiation is the formation of endogenous cellular photosensitizers that affect the sperm motility and function (9). The He- Ne laser is one of the most economical and commonly used gas lasers on the market. The laser is typically designed to operate in the red at 632.8nm wavelength and its usage in medical application is called low level laser therapy (LLLT) which is a light source treatment that generates light of a single wavelength. Low level laser therapy emits no heat and sometimes called "cold laser". Laser radiation and monochromatic light may alter cell and tissue function. Laboratory studies suggest that irradiation stimulates collagen production, alters DNA synthesis, and improves the function of damaged neurological tissue. In this study, we tried to determine the effects of direct laser irradiation on sperm motility (10).

Materials and Methods

Samples and methods: Semen samples of 100 man were collected from infertility clinics to Alnadear Almoshea Clinical Laboratory were selected during the period from February 2014 to August 2014. All specimens were collected by masturbation at the andrology laboratory into a wide mouthed sterile specimen jar, after an abstinence period of 48-72 hours. The patients were informed that further investigations will be done on their samples for academic purposes and the sample will be discarded using heat after finishing the experience. All the patient information for this study remained confidential. Samples with abnormal colour or that not liquefied or with volume less than 2 millilitre were not included in this study. Following liquefaction, manual semen analysis was performed according to World Health Organization (WHO) guidelines (11) to determine sperm motility. During this study the standard seminal analysis to all samples select samples that fit and exclude samples with azospermia. The samples were divided into two parts, one named untreated (control) and the other will be irradiated using a continuous He-Ne laser model (IFHN05) for 30 minutes. All samples then examined using Computer assisted sperm analysis (CASA) which is a digital system designed for automatic analysis of the sperm concentration, motility, and morphology.

A simple system for grading motility was used that distinguishes spermatozoa with progressive or non-progressive motility from those that are immotile according to the WHO criteria (11). The motility of each spermatozoon is graded as follows:

1. Progressive motility (PR): spermatozoa moving actively, either linearly or in a large circle, regardless of speed.
2. Non-progressive motility (NP): all other patterns of motility with an absence of progression, e.g. swimming in small circles, the flagellar force hardly displacing the head, or when only a flagellar beat can be observed.
3. Immotility (IM): no movement (might be alive or dead).

Statistical package for social science version 20 (SPSS20) was used for data analysis. Continuous variable presented as mean ± SD. A paired Test was used to study the significance difference (P-value <0.05) was considered significant.

Results

Table (1) The effect of low level laser irradiation on the human sperm motility

	Non treated (control)	He-Ne laser irradiated	P value
Progressive motility	**33.6±14.1***	**47.6±15.4**	**0.0001**
Non progressive motility	**22.2±12.9**	**19.4±11.8**	**0.22**
Immotile sperms	**44.1±19.2**	**32.9±16.5**	**0.0005**

***Data presented as mean±sd.**

The results showed that 30 minutes laser irradiation increase the percentage of sperms with progressive motility significantly ($p<0.05$). There was also a significant decrease ($p<0.05$) in the percentage of non-motile sperms in treated samples while the increase in the percentage of non-progressive motile sperms after irradiation was not significant.

Discussion

The present study showed that there was a significant increase ($p<0.05$) in the number of sperms that improve their character of movement from immotile or non-progressive movement to progressive type of movement. The results was in agreement with previous finding that human sperm motility as well as velocity can be improved by He-Ne laser irradiation (12, 13, 14). These results disagreed with (15) who observed a significant decrease in lipid peroxidation and proteins damage post He-Ne laser irradiation which causes a decrease in oxidative stress that may become a threat to cells survival. The movement and speed of a spermatozoon depends upon energy supply. Spermatozoa maintain low energy consumption during storage in the epididymis. These cells are immotile but able to fertilize an egg. Enhanced adenosine-5'-triphosphate (ATP) production becomes critical at the time of fertilization. Motility is activated only upon ejaculation (16). Activation of sperm flagella motility involves both energy metabolism in mitochondria and the motile apparatus of the cells. Mammalian spermatozoa can produce ATP both by anaerobic glycolysis and aerobic respiration (17). (18, 19) concluded that low-intensity red and near-infrared light is acting on cells through a primary photoacceptor: cytochrome C oxidase, the terminal enzyme of the mitochondrial electron transport chain. This evidence implies cytochrome C oxidase absorption, over other possible elements of the electron transport chain. (20) tried to explain the basic mechanism of LLLT implicates cytochrome C oxidase as the primary photoacceptor. They state that once cytochrome C oxidase is stimulated by light, electron transport is accelerated, leading to increased ATP production. (18) thought that at the same time, this photobiostimulation is linked to the generation of reactive oxygen species (ROS) that increased metabolism and then participate to provide energy and intracellular signal transduction. It is well known that even relatively low concentrations of ROS could mediate various intracellular processes, and in sperm cells ROS have a pivotal role in cellular physiology and fertilization capability (21). Regarding immotile

sperms which might be the only choice in some methods of Assisted reproductive techniques (ART) especially Intracytoplasmic sperm injection (ICSI) (22), this study showed that the LLLT might be the future way to testified the viability of the sperms. The immotile sperms are normally found in all seminal samples. It is clinically important to know whether immotile spermatozoa are alive or dead. Sperm vitality, as estimated by assessing the membrane integrity of the cells, may be determined routinely on all samples, but is especially important for samples with less than about 40% progressively motile spermatozoa. A study performed by (23), showed that He-Ne laser radiation (632.8 nm) at a dose of 24 J/cm^2 induced sister chromatid exchange in sheep peripheral mononuclear cells (23). In agreement with (24) who revealed that the oxidative stress due to He-Ne laser irradiation-induced generation of singlet oxygen leads to sub-lethal damage of cells, which may induce better cells repair. In contrast, a non-significant increase in DNA damage compared to control samples was observed by (25) who studied 30 seminal samples treated with 30 second infrared laser pulse of 50mw/cm^2 at 905 nm showing also significant changes in sperm motion kinetics. In addition, a lot of works have been carried out, showing that the low- level laser irradiations is photoprotective and modify the response of cells to ionizing energies such as X and γ- rays, UV light and α- particles that cause cell and tissues damaging (24). An interesting ultra-structural study using electron microscope was done by (26) in which the structure of mitochondria was studied after the irradiation of human lymphocytes with He-Ne laser (wavelength, 632.8 nm; dose, 56 J m^{-2}). Ultrathin sections of the lymphocytes were studied by electron microscopy 1 h after the irradiation. The irradiation resulted in a 20% increase in the number of mitochondrial profiles on the cell section without an increase in their total area. Three-dimensional reconstruction of mitochondria from ultrathin sections through the whole lymphocyte showed that the number of mitochondria was reduced to 9-12 in the irradiated cells compared with 40-45 in the control cells. In the irradiated lymphocytes, 2-4 giant branching mitochondria were also observed among small discrete mitochondria. It was concluded from this study the effects of laser is still a matter of controversy and need further study concentrated on the DNA integrity of sperms after irradiation.

References

1. Curi, S. M.; Ariagno, J. I.; Chenlo, P. H.; Mendeluk, G. R. & Pugliese, M. N. (2003). Asthenozoospermia: analysis of a large population. Arch Androl., 49:343-349.
2. Mobberley, M. A. (2010). Electron microscopy in the investigation of asthenozoospermia. Br. J. Biomed. Sci., 67(2):92-100.
3. Martinez-Heredia, J.; Mateo, S.; Vidal-Taboada, J. M.; Ballesca, J. L. & Oliva, R. (2008). Identification of proteomic differences in asthenozoospermic sperm samples. Human Reprod., 23:783-791.
4. Kang, S. U. & Seo, J. U. (2013). Effect of Varicocelectomy on Male Infertility. Published online.
5. Rittenberg, V. & El-Toukhy, T. (2010). Medical treatment of male infertility. Hum. Fertil., (Camb) 13:208-216.
6. Kohli, R. & Gupta, P. K. (2001). Irradiance dependence of the He-Ne laser-induced protection against UVC radiation in E. coli strains. J. Photochemistry and Photobiol. B: Biol., 69: 161-167.
7. Jung, S. U. & Seo, J. U. (2014). Effect of Varicocelectomy on Male Infertility. Published online.

8. Boomsma, C. M.; Heineman, M. J.; Cohlen, B. J. & Farquha, C. (2007). Semen preparation techniques for intrauterine insemination. Cochrane Database Syst. Rev., 4:45-47.
9. Zan-Bar, T.; Bartoov, B.; Segal, R.; Yehuda, R.; Lavi, R.; Lubart, R. & Avtalion, R. R. (2005). Influence of visible light and ultraviolet irradiation on motility and fertility of mammalian and fishsperm. Photomed Laser Surg., 23(6):549-555.
10. Maiya, G. A.; Kumar, P. & Rao, L. (2005). Effect of low intensity helium-neon (He-Ne) laser irradiation on diabetic wound healing dynamics. Photomed. Laser Surg., 23:187-190.
11. World Health Organization (2010). Laboratory Manual for the Examination of Human Semen and Semen-Cervical Mucus Interaction, 4th ed., Cambridge, UK: Cambridge University Press. P. 60.
12. Fujiwara, A.; Tazawa, E. & Yasumasu, I. (1991). Activating effects of light irradiation at various wavelength on the respiration in sperm of the echiuroid, Urechis unicinctus, in the presence of carbon monoxide. J. Biochem., 109: 486-491.
13. Vesich, I. L. (1994). Some features of rehabilitating action of laser emission on native and cryopreserved human spermatozoa. Probl. Cryobiol., 4: 33-35.
14. Karu, T. I. (2012). Lasers in Infertility Treatment: Irradiation of Oocytes and Spermatozoa. Photomed. Laser Surg., 30(5): 239-241.
15. Stadtman, E. R. (1992). Protein oxidation and aging. Science, 257:1220-1224.
16. Woolley, D. M. (2007). A novel motility pattern in quail spermatozoa with implications for the mechanism of flagellar beating. Biol. Cell, 99(12): 663-675.
17. Ishijima, S. (2011). Dynamics of flagellar force generated by ahyperactivated spermatozoon. Reproduction, 142(3):409-415.
18. Karu, T. I.; Pyatibrat, L. V. & Kalendo, G. S. (2004). Photobiological modulation of cell attachment via cytochrome c oxidase. Photochem. Photobiol. Sci., 3:211-216.
19. Liang, H. L.; Whelan, H. T. & Eells, J. (2006). Photobio-modulation partially rescues visual cortical neurons from cyanide-induced apoptosis. Neuroscience., 139:639-649.
20. Pastore, D.; Martino, C.; Bosco, G. & Passarella, S. (1996). Stimulation of ATP synthesis via oxidative phosphorylation in wheat mitochondria irradiated with helium-neon laser. Biochem. Molec. Biol. Int., 39:149-157.
21. Lamirande, E. D.; Jiang, H.; Zini, A.; Kodama, H. & Gagnon, C. (1997). Reactive oxygen species and sperm physiology. Rev. Reprod., 2 (1): 48-54.
22. Park, Y. S.; Lee, S. H.; Song, S. J. & Jun, J. H. (2003). Influence of motility on outcome of IVF / ICSI with fresh vs. frozen testicular sperm from men with obstructive azoospermia. Firtil. Steril., 80(3):526-530.
23. Ocana-Quero, J. M. I.; Gomez-Villamandos, R.; Moreno-Millan, M. & Valenzuela, J. M. (1997). Detrimental effect of the low power helium-neon laser irradiation on in vitro meiotic maturation of immature bovine oocytes. Lasers Life Sci., 7: 157-166.
24. Kohli, R. & Gupta, P. K. (2003). Irradiance dependence of the He-Ne laser-induced protection against UVC radiation in E. coli strains. J. Photochemistry and Photobiol. B: Biol., 69: 161-167.
25. Firestone, R.; Esfandiari, N.; Moskovtsev, S. I.; Burstein, E.; Videna, G. T.; Librach, C.; Bentov, Y. & Casper, R. F. (2012). The Effects of Low-Level Laser Light Exposure on Sperm Motion Characteristics and DNA Damage. J. Androl., 33 (3): 469- 473.
26. Marnett, L. J. (2000).Oxyradicals and DNA damage. Carcinogenesis, 21:361-370.