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Study the beneficial role of laser irradiation combination with indirect swim-up sperm preparation technique against oxidative DNA damage in infertile men

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Abstract--Background: In order to offer successful assisted reproductive procedures, a variety of in vitro sperm preparation techniques were created to separate normal and motile spermatozoa from other constituents of the sample. Much research on the laser as a sperm motility stimulant has been undertaken, and the results have indicated that the Laser has a good effect on sperm activation in vitro and improves progressive forward movement. Objective: This study is aimed to identify the differences in sperm activation by ISU with or without laser methods and compare them. And detection of oxidative damage to the DNA before and after laser by assessment of the 8-Hydroxydeoxyguanosine (8-OHDG) as a biomarker. Patients and Methods: The current study was conducted on 30 semen samples, divided into two groups (Asthenozoospermia and Normozoospermia individuals), during the period of attendance at the infertility clinic at the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University. From November 2021 until March 2022. Each sperm sample was separated into three portions.

The first sperm in vitro was prepared before activation, the second using the ISU technique, and the third stage the Laser technique and ISU, and the semen concentration of 8-hydroxydeoxyguanosine (8-OHDG) was evaluated before activation and then correlated to sperm characters before and after activation. Results: The seminal fluid characteristics of infertile men after activation, both ISU and laser, resulted in a highly significant increase in mean Sperm (Grade A) Motility % and (Grade B). Motility % (P 0.05), in comparison with before activation, however, the laser resulted in a more significant increase, added to that both ISU and laser resulted in a highly significant reduction in mean (Grade C). Motile Sperm % and (Grade D) Immotile Sperm % (P 0.05) in comparison with before activation, however, the laser resulted in a more significant reduction. Both ISU and laser resulted in a highly significant increase in mean normal sperm morphology in comparison with before activation (P 0.05). And ISU and laser activation methods resulted in a highly significant reduction in 8-OHDG level in comparison to before activation (P 0.05), and laser resulted in a more significant reduction than ISU (P 0.05). Conclusion: 8-Hydroxydeoxyguanosine (8-OHDG) in semen plasma of infertile men was significantly decreased after activation using a laser with ISU.

Keywords---Light Amplification by Stimulated Emission of Radiation (LASER), Indirect Swim Up (ISU), 8-Hydroxydeoxyguanosine (8-OHDG).

1. Introduction

Infertility is a common medical problem in which a couple is considered infertile, if they have not been able to conceive after 12 months of regular unprotected sexual contact, which is an important determinant for the occurrence of pregnancy (Jenkins *et al.*, 2004). Regardless of the cause this condition affects approximately 10-15 % of reproductive-aged groups (Elizabeth *et al.*, 2020). A male-infertility-related factor is identified in half of the infertile couples, along with defective sperm. Male fertility impairment can be caused by a variety of reasons, including congenital, urogenital, infection, malignancies, infections, scrotal illness, endocrine disease, genetics, and/or immunity-related infertility abnormalities. (Jungwith *et al.*, 2018). Male factor infertility accounts for around half of all occurrences of infertility and affects approximately one in every twenty males of reproductive age (defined here as between puberty and 40 years of age) (Zhaku *et al.*, 2019). Male infertility is caused mostly by defective sperm and/or sperm parameters such as low sperm concentration (oligozoospermia), low sperm motility (asthenozoospermia), low sperm vitality (necrozoospermia), aberrant sperm morphology (teratozoospermia), and sperm absence (Aspermia) (Jungwith *et al.*, 2012). and the lack of spermatozoa in the ejaculate (azoospermia). The principle of indirect swim-up, the approach is based on the self-active migration of sperm from a single, pre-washed cell pellet into the cytoplasm after centrifugation. The common method for preparing sperms is the centrifugation and swim-up technique, which results in active and highly sperms but decreases concentration (Hamza *et al.*, 2018). The swim-up principle is the most effective

technique used in IVF laboratories and is selected if the sperm sample has a normal quantity of quality sperm (normozoospermic). The sperms are selected based on their motility and ability to swim out of the seminal plasma using this technique (Natali, 2011). Laser therapy looks to be a safe therapeutic option with a wide range of possible positive outcomes. 8-Hydroxydeoxyguanosine (8-OHDG) is a product of oxidative DNA damage following specific enzymatic cleavage after ROS induced 8-hydroxylation of the guanine base in mitochondria and nuclear DNA (Cooke et al., 2000). The strength of this work lies in the originality of the research design, results, and implications in the human reproduction area, as this is the study to look at the possible influence of laser on human sperm parameters.

Materials and Methods

The current study was conducted on 30 semen samples enrolled in this study. They were attending the infertility clinics at High Institute for Infertility Diagnosis and Assisted reproduction Al-Nahrain University .semen samples were taken from patients and classified into two groups according to the criteria of WHO (1999) for semen analysis; the first group is (15) Asthenozoospermia semen sample and the second group (15) normozoospermia semen sample with normal motility subjects who served as normal infertile volunteers these subjects will be admitted to the institute . after the approval of the ethical committee that must be obtained prior to the initiation of the study. Post-activation of each sample will be divided into two aliquots the first one using a laser at the wavelength (632.8mw), figure 1. combined with indirect swim up, the second using ISU technique alone, then sperm parameters were assessed for these two techniques and the results were statistically analyzed.



Figure 1: The Helium-Neon Laser

8-OHDG biomarker investigation using an enzyme-linked immunosorbent assay:

Using ISU Techniques, this biomarker was evaluated in seminal fluid samples before and after activation with Laser. Based on the biotin double antibody sandwich technique, (Kemal et al., 2018) assayed the Human 8-OHDG biomarker in human serum, blood plasma, and other biological liquid samples. In the current investigation, it is measured in seminal fluid and we read three specimens for each patient; the red-labelled tube indicates seminal fluid before activation and contains 0.5 ml. The color is black The labelled tube indicates seminal fluid stimulated by Laser and contains 1ml. Seminal Fluid stimulated with ISU without Laser is shown by a green-labelled tube. Add 8-OHDG to wells pre-coated with 8-OHDG monoclonal antibodies tagged with biotin to unite with streptavidin – HRP, which forms the immunological complex. After incubation and washing, remove any unbound enzymes and add substrates A and B. With the influence of acid, the solution will become blue and then yellow. The colors of the solution and the concentration of Human 8-OHDG are connected in a good way.

Results

The seminal fluid characteristics of all enrolled infertile men before activation, after ISU, and after LASER activation are shown in table 3.1. Both ISU and LASER resulted in a highly significant decrease in mean sperm concentration in comparison with before activation (p 0.05); however, LASER resulted in a more significant decrease.

In addition, both ISU and LASER resulted in a highly significant rise in mean progressive motile sperm % in comparison with before activation (p 0.05); however, LASER resulted in a more significant rise. Added to that, both ISU and LASER resulted in a highly significant decrease in mean non-progressive motile sperm % and immotile sperm % in comparison with before activation (p 0.05); however, LASER resulted in a more significant decrease. Furthermore, both ISU and LASER resulted in a highly significant rise in mean normal morphology sperm % in comparison with before activation (p 0.05); however, LASER resulted in a more significant rise.

Table 3.1. Seminal fluid characteristics of all enrolled infertile men before activation, after ISU, and after LASER activation.

Total (n=30)	Before activation	After ISU activation	After LASER activation	Before x ISU	Before x LASER	ISU x LASER
Concentration (m/ml)	21.23±4.04	19.07±3.69	18.27±3.71	0.0001 [^]	0.0001 [^]	0.004 [^]
Grade A	14.50±9.32	29.50±16.37	29.80±18.09	0.0001 [^]	0.0001 [^]	0.909
Grade B	31.50±6.58	52.17±18.92	62.87±18.26	0.0001 [^]	0.0001 [^]	0.0001 [^]
Grade C	13.83±5.68	2.67±4.87	0.50±1.53	0.0001 [^]	0.0001 [^]	0.017 [^]

Grade D	40.83±9.01	14.83±16.21	6.83±11.78	0.0001 [^]	0.0001 [^]	0.0001 [^]
Morphology	22.33±3.26	36.07±10.25	40.63±9.96	0.0001 [^]	0.0001 [^]	0.0001 [^]
8-OHDG (ng/ml)	8.116±3.107	6.206±0.739	5.656±1.546	0.004 [^]	0.0001 [^]	0.059
[^] Significant difference between two dependent means using Paired-t-test at 0.05 level.						

The ISU and laser resulted in a highly significant decrease in 8-OHDG level in comparison to before activation (p 0.05), and the laser resulted in a more significant decrease than ISU and laser (p 0.05). Regarding the normozoospermia group, there was a highly significant decrease in the mean 8-OHDG level following the use of either laser activation in comparison to ISU activation (p 0.05). Regarding the Asthenozoospermia group, both ISU and laser resulted in a highly significant decrease in 8-OHDG level in comparison to before activation (p 0.05); and laser resulted in a more significant decrease than ISU and laser (p 0.05).

Table 3.2. The level of (8-OHDG) in infertile men enrolled in the current study before activation and after activation using indirect swim-up and laser categorized into normozoospermia and Asthenozoospermia groups

Characteristic	Total n = 30	Normozoospermia n = 15	Asthenozoospermia n = 15	p
8-OHDG before activation				
Mean ±SD	7.849±2.015	8.84±4.21	7.39±1.06	0.221
Range	(4.576-22.117)	(4.576-22.17)	(5.954-9.727)	
8-OHDG after ISU				
Mean ±SD	6.206±0.739	6.16±0.50	6.25±0.94	0.735
Range	(4.498-8.412)	(5.06-6.65)	(4.498-8.41)	
8-OHDG after LASER				
Mean ±SD	5.656±1.546	5.79±1.84	5.52±1.23	0.638
Range	(2.131-9.220)	(2.13-9.22)	(2.131-7.127)	
p	0.05	0.05	0.05	
#Significant difference between two independent means using Students-t-test at 0.05 level.				

Regarding the normozoospermic group, there was a highly significant decrease in mean 8-OHDG level following the use of either laser activation in comparison to ISU activation (p 0.05), figure2. Regarding the Asthenozoospermia group, both ISU and laser resulted in a highly significant decrease in 8-OHDG level in comparison to before activation (p 0.05); and laser resulted in a more significant decrease than ISU and laser (p 0.05).

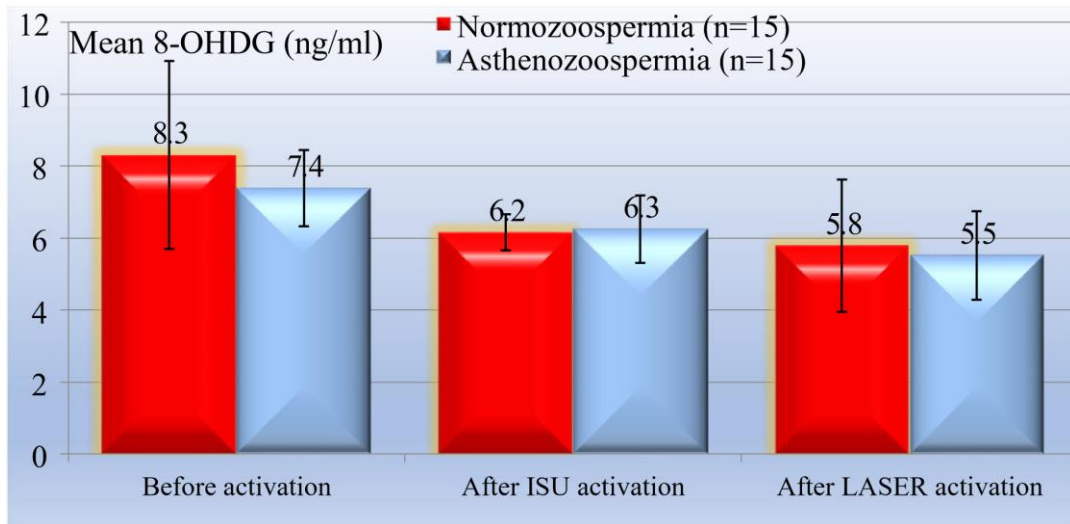


Figure.2: Bar chart showing the comparison of % change in 8-OHDG after indirect swim up and after laser in all patients, in normozoospermia group and in Asthenozoospermia group

Discussion

Techniques for preparing sperm are an essential part of assisted reproductive technology (Kadhim et al., 2017). Meanwhile, enhanced sperm parameters increased sperm fertilization ability and ART results (Kadhim et al., 2017). The objective of sperm separation techniques is to treat spermatozoa in vitro in order to increase their functionality, i.e. motility, and to create a protective environment with the aim of maintaining or improving their functional ability for successful fertilization (Kadhim et al., 2017). After the exclusion of seminal plasma, an increase in the percentages of sperm motility and progressive sperm motility is considered a normal response for sperm activity because it contains dead sperm, leukocytes, epithelial cells, debris, and microbial contamination that produce many oxygen radicals that can negatively influence sperm functions (Kadhim et al., 2017). Low-level laser therapy (LLLT) is a highly successful physical therapy process that is used in many sectors of medicine, including obstetrics and gynaecology, andrology, and urology, and it is indicated as an important component of the difficult treatment of infertility. According to a review of the research, LLLT is beneficial in treating male infertility. Lasers have a significant influence since they improve spermatozoa survival, motility, and movement speed. Laser therapy can help with prostatitis, and vasculitis can help with infiltrative exudative changes as well as improving reproductive and ovulatory function (Gerber et al., 2008). According to the findings of this study, both ISU and LASER are effective in reducing semen concentration and improving sperm motility; however, LASER is superior to ISU in improving sperm quality and Morphology and significantly improving Grade A sperm motility % and improving Grade B motile sperm %; both methods reduce Grade C motile sperm % and Grade D immotile, and both methods reduce round cell count and leukocytes. on the other hand, it was proved that laser irradiation (at wavelength 632.8 nm) of active live human sperm enhanced motility and speed (Vesich, 1994; Lenzi et al.,

1989). The laser was superior to the indirect swim-up method in upgrading the percentage of progressively motile spermatozoa and upgrading the percentage of normal morphology sperm. Recently, there is increasing evidence suggesting that oxidative sperm DNA damage is closely associated with impaired sperm function and male infertility. The biomarker 8-hydroxydeoxyguanosine (8-OHDG) is thought to be a specific and sensitive indicator of oxidative DNA damage. Han-Ming Shen et al. (1999). This study aimed to assess oxidative damage by assessing the amount of this biomarker before and after activation using ISU and laser techniques in two groups of patients: Asthenozoospermia and Normozoospermia. The production of 8-Hydroxydeoxyguanosine (8-OHDG), a major biomarker of oxidative DNA damage, is widely regarded as a key biomarker of oxidative DNA damage and plays an essential role in the aetiology of many disorders (Ames et al., 1993). According to several research, the amount of antioxidants in sperm is directly related to the quantity of 8-OHDG in the sperm (Fraga et al., 1991). Many more articles and/or studies have pointed to this correlation.

Conclusion

8-Hydroxydeoxyguanosine (8-OHDG) in semen plasma of infertile men was significantly decreased after activation using LASER with ISU.

DECLARATIONS Conflict of Interest

The authors declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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