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Influence of Non-Thermal Plasma (DBD) On Infertility Male Semen with Low Sperm Motility and Dna Damage

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Abstract:
Non-thermal plasma(Dielectric barrier discharge) has many uses including living tissue sterilization, inactivation of the bacteria, excimer formation, angiogenesis, and surface treatment. This research aim is to use cold plasma as a tool to search the effect of the dielectric barrier discharge system at room temperature on human sperm motility and DNA integrity. This work was performed on 60 human semen samples suffering from low motility; each sample was prepared by centrifugation method, then each semen sample was divided into two sections, the first section is before significant exposure to the plasma system (DBD) and the second section is after treatment with the DBD system at ambient temperature. Before and after exposure to non-thermal Plasma (DBD), DNA integrity and sperm motility were assessed, patients suffered from asthenospermia has a high level of DNA fragmentation than fertile male, (24.16±4.14) $p<0.001$ for untreated and after treatment the semen slide with dielectric barrier discharge the percentage decreases to (9.16±1.76) $p>0.006$, and the comet slide was (35.44±4.15) then the percentage decline to (19.86 ±2.44) these results have shown that cold plasma improves sperm motility and decreases from DNA damage in patients with medium and high level of DNA damage**.**

Keywords: DBD, DNA, Non-Thermal Plasma, Sperm motility.

تأثير البلازما غير الحرارية (DBD) على العقم عند الذكور المصابين بانخفاض حركة النطف وتلف الحمض النووي

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الخلاصة:
تم في هذا البحث دراسة تأثير استخدام منظومة البلازما الباردة من نوع تفريغ حاجز الجهد الكهربائي (DBD)عند درجة حرارة الغرفة على حركة النطف البشرية وسلامة الاحماض النووية فيها. تم استخدام 60 عينة من السائل المنوي البشري لمرضى يعانون من ضعف حركة النطف ، تم تحضير عينات السائل المنوي بوساطة جهاز الطرد المركزي بعدها تم تقسيم كل عينة الى قسمين: قبل وبعد التعريض لمنظومة تفريغ حاجز

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الجهد الكهربائي ((DBD، أذ تم فحص حركة النطف وسلامة الاحماض النووية و تبين ان المرضى الذين يعانون من ضعف حركة الحيوانات المنوية لديهم مستوى عالي من تحطم الاحماض النووية مقارنة بالأشخاص الطبيعيين اذ ان نسبة الضرر في ال DNA كانت (24.16±4.14) p <0.001 ليس لها تأثير معنوي قبل التعريض لمنظومة البلازما , وبعد تعريض شريحة السائل المنوي لمنظومة تفريغ حاجز الجهد الكهربائي (DBD) قلت النسبة الى (9.16±1.76) p>0.006 لها تأثير معنوي وفي شريحة قياس فحص المذنب كانت النسبة (35.44±4.15). وبعد تعريض الشريحة الى منظومة ال (DBD) اصبحت ±19.86) (2.44. أظهرت النتائج ان التعريض لمنظومة البلازما الباردة زادت من الحركة الفعالة للنطف وقللت من ضرر تحطم المادة الوراثية في المرضى الذين لديهم ضرر متوسط وعالي من تحطم المادة الوراثية.

الكلمات المفتاحية: بلازما التفريغ الكهربائي، حركة النطف، الاحماض النووية.

INTRODUCTION

Infertility is officially defined as a condition during which, after 12 months of unprotected sex, a couple of reproductive age desiring a child cannot conceive. Infertility is one in all the foremost common diseases and affects couples between 17% and 25% [1,2]. Infertility is sometimes caused by infertility within the male factor, which generally accounts for 40% –50 percent of all cases [3,4]. The causes of male infertility including both quantitative impairment of spermatogenesis caused by primary testicular failure, ductal obstruction and disturbances of hypothalamic- pituitary as well as qualitative sperm defects such as abnormal sperm morphology or motility. Currently, these known factors represent 40% of patients in the andrology clinic [5]. As such, asthenozoospermia is one of the main causes of decreased fertility or men's infertility [6]. Therefore, the cause of infertility could also be linked to irregular sperm DNA in infertile men with normal semen parameters [7,8]. Sperm integrity DNA and the quality of sperm is one of the foremost important factors within the laboratory for in vitro fertilization (IVF) [9,10,11]. The percentage of male infertility was between the ages of 20 and 30, and the options of treatment mostly concentrate on improving the quality of sperm. Sperm chromatin structure assay (SCSA) techniques, alkaline and neutral comet and terminal deoxnucleotidyl (TUNEL) assay are the foremost commonly used methods for detecting DNA integrity in individual spermatozoa [12-16].

In this study, cold plasma was done to assess the result of cold plasma on human sperm motility and DNA integrity in asthenospermia patients. The use of thermal plasma is limited in medical and biological fields due to its high temperature (2000 K up to 10,000 K). This temperature will damage the tissue and cause cauterization. On the other hand, non-thermal plasma works at room temperature, and it is suitable for its high efficiency for the health sector [17]. In studies which are carried on sperms of animals showed that Non- thermal plasma increases the motility, viability and functional integrity of the sperm membrane [18], as well as Non thermal plasma (Dielectric barrier discharge), has many uses including: living tissue sterilization, inactivation of the bacteria, excimer formation, angiogenesis and surface treatment [19]. Plasma usually is an ionized gas that incorporates particles of charge (electrons, ions, and molecules). The word ionized refers to the life of a free-electron or more but does not refer to an atom or molecule. Plasma has free charging particles where the positive and negative charges store each other roughly at the macroscopic stage [20]. The Non thermal plasma (DBD) system contains two flat metal electrodes, which are surrounded by the material of dielectric. the gas used passes between the two electrodes and is ionized for the generation of non-thermal plasma. the electrode one is an electrode of high voltage because the need to the discharge for generation of non-thermal plasma, the high voltages of Alternative Current (AC) normally power DBD's with kHz frequencies, the consumption of power varies from 10 to 100 W, and the other an electrode of grounded nature [21,22]. This paper aims to examine the effects of non-thermal plasma DBD on the exposed semen samples

suffered from low sperm motility. During this research, dielectric barrier discharge was wont to attain the best exposure time. DBD plasma generated the plasma utilized in this study.

MATERIALS AND METHODS

Experience design

Seminal fluid was collected from 20-49 years old infertile males, samples collected at the hospital of Kamal Al Samuray (the laboratories of infertility and test tube baby). Sixty samples of semen were collected, after three days of abstinence, the samples were put in a clean, wide-mouthed jar. Routine semen analysis was performed manually after half-hour of liquefaction at 37°C incubator. In line with World health organization(WHO) [23], the concentration of sperm, Sperm motility, the agglutination of sperm, the morphology of the sperm assessment was performed. Semen samples were chosen for experimental use, with poor sperm motility. The dielectric barrier discharge created from this device was applied to samples of semen as shown in Figure 1. Working with 2.45 GHz frequency, 175 Volt applied voltage, after that, the samples formulated by the technique (swim-up) by adding 5ml flushing medium to the original semen sample and mixing. Centrifugation was conducted at 3500 RPM for 5minutes. The supernatant was extracted, by adding a flushing medium and incubating for a half-hour, a swim-up was done. After that, the samples were split into two parts, the part one is before exposure to non-thermal plasma (DBD), therefore the second part is after treatment with a non-thermal plasma device. At ambient temperature. The time of exposure to the dielectric barrier discharge was (160 sec), and sperm motility must be checked before and after exposure to the non-thermal plasma device by microscope. Then, Acridine orange test (AOT) and single-cell gel electrophoresis test evaluated the damage in cell DNA.

Comet Assay and Acridine Orange Test

Comet Assay could be a single cell gel electrophoresis assay (SCGE) for easy evaluation of the breakdown of cellular DNA from the vital cells. Molten agarose was combined with the cells of sperm before being added to the slides of the comet. These embedded cells were then added with Lysis buffer and alkaline solution, and this treatment would result in relaxation and denaturant DNA. to tell apart intact DNA from fragmented DNA, the horizontal chamber was used to electrophoresed the samples, then dried, stained with DNA dyes and analyzed by the fluorescent microscope. The measurement of the comet tail is employed to work out the extent of DNA fragmentation. The measurement of comet tail is usually accustomed to estimate the degree of DNA damage by calculating the distance between the resulting tail and the comet head. Twenty samples were randomly selected to estimate comet cells. And by the Acridine Orange test stated by Tejada et al (1984) [24], sperm smear was then prepared and dried, sperm fixation in menthol-glacial carboxylic acid (3:1) was performed overnight at room temperature. After some minutes of drying, then stain for 5 minutes with Acridine orange (AO) (0.19 mg / mL, pH 2.5). by adding 10 mL of stock solution to 40 mL of 0.1 M acid and a pair of 2.5 mL of 0.3M Na2HPO4, 7 H2O, the staining solution was prepared and maintaining a solution at room temperature. Later, rinsed by H_2O, coverslip placed and shown by the fluorescence microscope. Green fluorescent sperm heads had healthy DNA integrity and diminished DNA integrity with orange-red staining.

RESULTS AND DISCUSSION

Acridine Orange Test

Human sperm motility and DNA integrity is modified by exposure to non-thermal plasma and this motility modification and DNA damage reduced dependent on exposure times. The results indicate that the non-thermal plasma (DBD) device had a good effect on the integrity of human sperm DNA by acridine orange test on the prepared sperm for age (20 to 35 <), as shown in Table 1. Results were calculated in the control work environment (before using

DBD technique), and exposed to non-thermal plasma (DBD) at a fixed time (160 sec) after the semen samples were prepared. Its standard deviation measured the mean value. Before exposure to non-thermal plasma (DBD), the DNA fragmentation ratio of PS was 24.16± 4.14 percent for samples between 20-25 ages, and DNA fragmentation decreases to 9.16 ±1.76 percent when treated with cold plasma and from 26-30 age was 23.333±5.165% before treatment with DBD system, and after treatment it became10.41± 2.05% followed from 31-35 < in the control work environment (before cold plasma therapy), it was 25.83 ± 4.70%. Once exposed to DBD technique, the decreases in DNA fragmentation to 12.83 ± 2.34 percent at constant 160 secs, thus findings of DNA fragmentation ($P<0.05$) is significant in 26-30 and 31-35 age.

Table 1-Acridine orange test with cold plasma (DBD) for DNA fragmentation percent of the prepared sperm

Age (Year)	DNA fragmentation (%) (mean±SD)	
20-25	Before treated	A 24.16±4.14
	After treated with DBD	B 9.16±1.76
P-value		0.024
LSD		6.25
26-30	Before treated	A 23.3335±.165
	After treated with DBD	B 10.412±.05
P-value		0.009 *
LSD		4.07
31-35<	Before treated	A 25.834±.70
	After treated with DBD	B 12.832±.34
P-value		0.006 *
LSD		5.11

Note: stars for significant reading at $p<0.05$

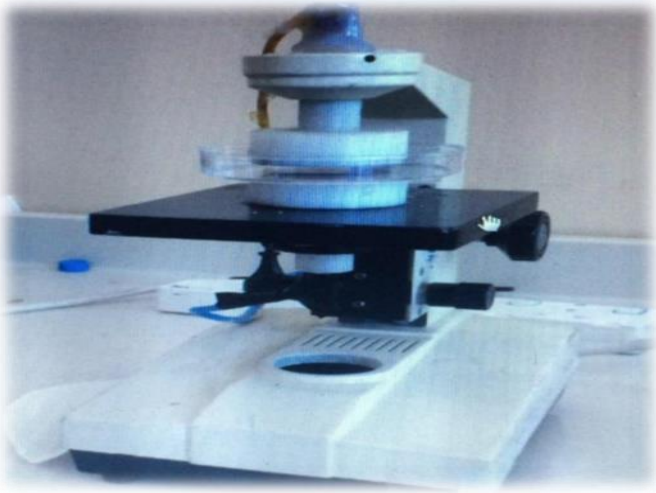

Figure 1- DBD-system photograph

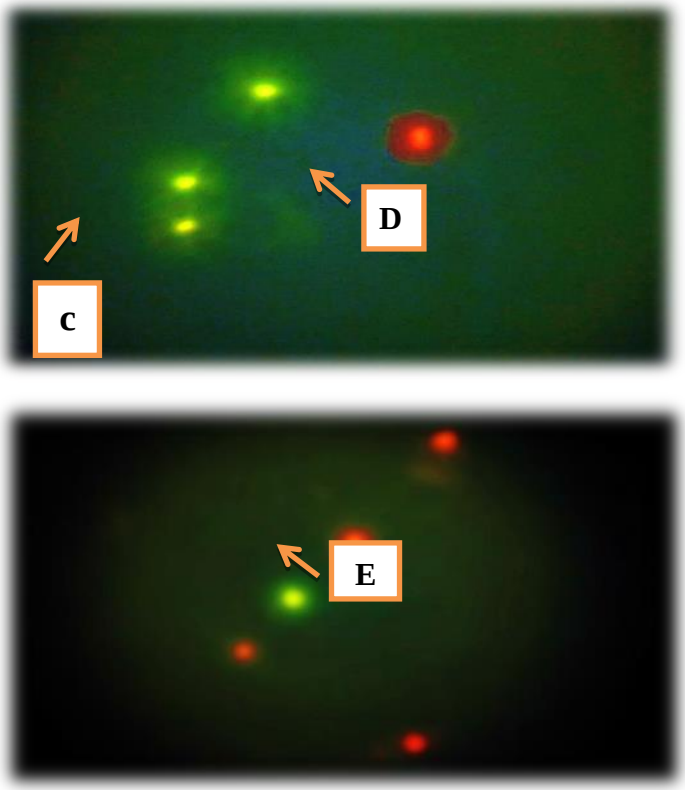

Figure 2- sperm stained with DNA dye (Acridine orange). (C): green normal (D): red abnormal. (E) spermatozoa from Patients suffering from infertility**.**

Comet assay

Table 2. displayed the results of Comet assay for high, medium, and low damage in DNA [HD, MD, LD, ND] before and after treatment with non–thermal plasma (DBD) at room temperature for prepared sperm (PS) at a fixed time (160 sec); with a percentage of DNA damage; the percentage of No DNA damage before treatment with cold plasma was 14.19 ± 2.16 %; and increased to 26.08± 3.38 %; After exposure to barrier discharge system. Low DNA damage was 21.23±3.07 %, rising to 33.26±4.26 %, after prepared semen has been treated with silent discharge. Medium DNA damage was 28.84 ± 3.36 percent, decreasing to 21.22± 2.94 %, after exposure to non–thermal plasma. High DNA damage (before treatment with DBD) was 35.44 ±4.15%, decreasing to 19.86 ± 2.44%. The variations in these three percentages were substantial where the ($P<0.05$) was observed, but they are not significant. the percentage of low DNA damage. So the desired effect was found in the group of HD and MD when compare the treated with the untreated group and not effective in ND before and after treatment respectively. And also in LD group it was 21.23±3.07, 33.26± 4.26 before and after treatment respectively.

The results of both tests comet assay and Acridine Orange Test gave relatively similar predictive values for DNA fragmentation, although rapid fading of fluorescence, and heterogeneous staining of slides [25,26]

Table 2-The Comet percentage of prepared sperm at room temperature, before and after exposure to barrier discharge

A: means. no damage at NO; B: means low damage at LD; C: means medium damage at MD; D: means high damage at HD

	No damage % (meanS±D)	Low damage % (mean±SD)	Medium damage % (meanS±D)	High damage % (meanS±D)
Before treated	A 14.19± 2.16	A 21.23±3.07	A 28.84±3.36	A 35.44±4.15

(diseased untreated with cold plasma)				
After treated with DBD	C 26.08± 3.38	C 33.26± 4.26	B 21.22± 2.94	B 19.86± 2.44
P-value	0.017	0.046	0.033	0.023
LSD	3.48	5.12	4.62	5.29

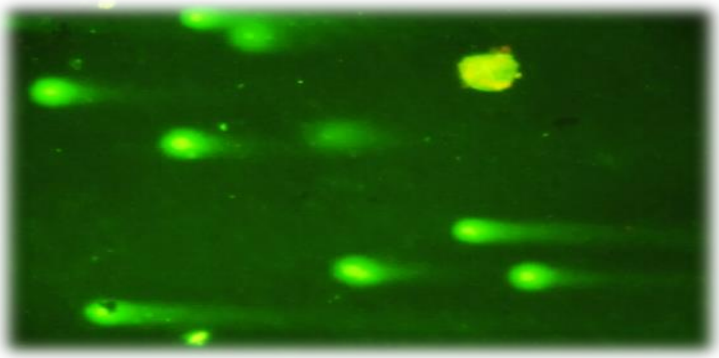

Figure 3-Sperm field after comet assay using and after cold plasma (DBD) pretreatment**.**

CONCLUSION

Based on our results, we conclude that the discharge of the dielectric barrier is significantly correlated with the fragmentation of DNA sperm. Reducing DNA fragmentation and improving sperm motility at higher doses (longer exposure times), Further studies are recommended to find out the role of non-thermal plasma in treating male infertility.

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