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Influence of cold plasma on sperm motility and oxidative stress in patient with asthenospermia

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This paper sheds light on the influence of cold plasma on sperm motility and oxidative stress. This study conducted on patients with asthenospermia. Non-thermal argon plasma needle at atmospheric pressure was constructed. The experimental setup was based on a simple and low cost electric component that generates a sufficiently high electric field at the electrodes to ionize the argon gas which flows at atmospheric pressure. Non–thermal Argon plasma needle effect can improve the motility of some human sperm and this will lead to improvement in the quality of semen and in the potential of sperm fertilization. This prospective study carried on 60 semen samples, each sample was prepared by swim - up technique and divided into 2 parts: part (1) is before exposure to plasma needle, and part (2) is after plasma system treatment at room temperature. The sperm motility was assessed before and after exposure to plasma system. The results of semen samples showed that plasma needle has significantly increased the sperm motility as well as improved the motility of sperms and DNA integrity.

Keywords: Cold plasma, Sperms motility, DNA fragmentation, Anti-oxidant

INTRODUCTION

In physics plasma is considered the fourth state of matter next to solids, liquids and gases. In fact, the past ten years have resulted in significant developments of non-thermal plasma for various applications in medicine (Moisan and Zakr zewski, 1991). The plasma is on average a neutral ionized gas composed of positive charged ions, electrons, and neutral particles (Alyaa et al., 2018). There are two types of plasma: thermal and non-thermal or cold atmospheric plasma. Thermal plasma has electrons and heavy particles (neutrals and ions) at the same temperature. Cold Atmospheric Plasma (CAP) is said to be non-thermal because it has electrons at a hotter temperature than the heavy particles that are at room temperature (Clotilde et al., 2013; Mazhir et al., 2018). Recent studies showed that CAP can exert beneficial effects when applied selectively in certain pathologies with minimal toxicity to normal tissues. The rapid increase in new investigations and development of various devices for CAP application suggest early adoption of cold plasma as a new tool in the biomedical field. This review explores the latest major achievements in the field, focusing on the biological effects, mechanisms of action, and clinical evidence of CAP applications in areas such as skin disinfection, tissue regeneration, chronic wounds, and cancer treatment and sperm motility (Gay-Mimbrera et al., 2016; Mazhir et al., 2016). Male fertility depends on different factors, including sperm count, motility and morphology. According to studies, the mentioned factors are considerably vulnerable to free radicals (Amini et al., 2009). Free radicals are oxygen molecules containing one or more unpaired electrons. hyper activation and oocyte-sperm fusion, elevated rates of ROS can lead to low sperm motility, high sperm DNA damage, lipid peroxidation of sperm membranes

and reduced oocyte-sperm fusion. Therefore Oxidative stress is the main cause of male infertility in %30 to %80 of cases (Kefer et al., 2009).

In this study atmospheric plasma needle applied on human sperm which have Low spermatozoal motility.to showed influence on sperms motility and oxidative stress. Cold plasma has proved their effectiveness to be used in bio-medical applications such as sperm parameters and histopathological changes of test is in mice (Al-Ahmed et al., 2018).

The present study indicated that cold plasma (needle) decreased the oxidative stress, furthermore a reverse association has been observed between the quantity of ROS detected in the semen and the percentage of motile sperm (Aitken et al., 2012). DNA damage in mature sperm can be induced by immature sperm, because of high levels of ROS production by them, and this damage would be produced after spermiation from the seminiferous tubules to the epididymis and after ejaculation (Gil-Guzman et al., 2001). The sperm DNA can be damaged by ROS either directly or through the activation of sperm caspases and endonucleases which might lead to initiation of apoptosis (Jena 2012).

MATERIALS AND METHODS

The study was carried out in the Biotechnology Research Center laboratories, Al-Nahrain University, and Kamal Al Samuray Hospital laboratories (Test tube baby and infertility laboratories) from November 2017 to June 2018. on 60 human semen samples ; the mean age was 20-49 years , smoker and non-smoker, alcoholic and not alcoholic, no history of chronic drug intake, no chronic disease like hypertension ,diabetes mellitus and varicocele and have normal semen analysis according to WHO (WHO, 2010).

Experimental design:

Sperm motility :

The samples were obtained from men after a minimum 3-7 days of abstinence and ejaculated into a clean, wide-mouthed container. After liquefaction for 30 minutes at 37 C° incubator, routine semen analysis was performed manually according to WHO [10] to assess sperm concentration, sperm motility, sperm agglutination, sperm morphology. Semen samples were with Low spermatozoal motility (Asthenozoospermia) parameters were selected for experimental use.

semen sample is prepared by swim -up technique. Then each semen sample was divided into 2 parts, one part is before exposure to plasma jet (needle) and the other part after plasma system treatment. At room temperature. the semen samples was exposure to plasma jet (needle) for constant time (120sec) . before and after exposure to plasma jet system the semen samples must be examine the motility of sperms in grade A,B,C, and D to determine the sperms motility and determining Antioxidant test before and after exposure to plasma needle .

Anti-oxidant:

Malondialdehyde

MDA Quantification of lipid peroxidation is essential to assess oxidative stress in pathophysiological processes. Lipid peroxidation forms Malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE), as natural bi-products. Measuring the end products of lipid peroxidation is one of the most widely accepted assays for oxidative damage. MDA Microplate Assay Kit provides a convenient tool for sensitive detection of the MDA in a variety of samples. The MDA in the sample is reacted with Thiobarbituric Acid (TBA) to generate the MDA-TBA adduct. The MDA-TBA adduct can be easily quantified colorimetrically (λ = 532 nm).

Sample preparation

1. For cell and bacteria samples

Collect cell or bacteria into centrifuge tube, discard the supernatant after centrifugation, add 1 ml Assay buffer for 5 × 10^6 cell or bacteria, sonicate (with power 20%, sonication 3s, intervation 10s, repeat 30 times); centrifuged at 8000g 4 °C for 10 minutes, take the supernatant into a new centrifuge tube and keep it on ice for detection.

Reagent	Sample
Sample	100 µl
Dye Reagent	200 µl
Mix, put it in the oven, 90 °C for 30 minutes, then put it on ice, centrifuged at 10000g, 25 °C for 10 minutes. Add the supernatant into the microplate.	
The supernatant	200 µl
Record absorbance measured at 532nm, 600nm.	

2. For tissue samples Weigh 0.1 g tissue, homogenize with 1 ml Assay buffer on ice, centrifuged at 8000g 4 °C for 10 minutes, take the

supernatant into a new centrifuge tube and keep it on ice for detection.

3. For serum or plasma samples Detect directly.

Assay procedure

Warm Reagent I on 37 °C for 30 minutes before use.

Add following reagents into the microplate:

Reagent	Blank	Standard	Sample
Sample	--	--	80 µl
Standard	--	80 µl	--
Distilled water	80 µl	--	--
Reaction Buffer	80 µl	80 µl	80 µl
Substrate	40 µl	40 µl	40 µl
Mix, wait for 10 minutes, record absorbance measured at 412 nm.			

Calculation

According to the protein concentration of sample

Glutathione (mmol/mg) = **(**$C_{Standard}$ × V_{Sample}) × (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / (V_{Sample} × $C_{Protein}$)

= (OD_{Sample} - OD_{Blank}) / ($OD_{Standard}$ - OD_{Blank}) / $C_{Protein}$

According to the weight of sample

Glutathione (mmol/g) = (CStandard × VSample) × (ODSample - ODBlank) / (ODStandard - ODBlank)/ (VSample × W/ VAssay)

= (ODSample - ODBlank) / (ODStandard - ODBlank) / W

C Protein: the protein concentration, mg/ml;

W: the weight of sample,g;

C Standard: the Standard concentration, 1 mmol/ml;

V Sample: the volume of sample, 0.08 ml;

V Assay: the volume of Assay buffer, 1 ml.

RESULTS AND DISCUSSION

Sperm motility:

Table 1 showed that the sperm motility percentage is affected by treated with cold plasma jet (needle) at room temperature. Prepared sperm, for age from 20-49 at constant time 120 sec for all age.

the percentage of RP motility(grade A) before exposed to plasma needle was 4.71+1.04 %, it increases to18.46+3.27% after treated with plasma jet (needle) at room temperature for age 20-25, and for (grade B) the percentage of motility before plasma jet treatment, was14.05+3.37% it increases to25.73+2.89% after exposed to plasma(needle) . Therefore , the difference in these two types of movement was significant ($P < 0.05$).The mean percentage of NP motility(grade C) before exposure to plasma (needle) was 27.33+5.18%, it decreases after treatment at room temperature to 22.72+4.08% **,** The mean percentage of immotility (IM) grade D before plasma jet treatment was 54.11+9.33% it decreases the % to 33.26+8.52% after exposed to cold plasma (needle) . The results showed that the plasma jet (needle) decreased but not increased the % of IM, Also the difference was significant ($P < 0.05$) in the decrement by plasma needle.

While for age from 26-30 , the percentage of RP motility(grade A) before exposure time to plasma needle was5.79+1.58 %, after treatment with plasma needle it increases to17.54+2.59 % and the value of (grade B) at control 15.13+2.86% .When exposed to plasma needle, sperm motility increased to 26.81+5.03% ,While for prepared semen , the percentage of NP motility(grade C) before treatment was28.41+3.18 %, after exposed to plasma system the motility reached to 21.80+4.36%, Also there was decreases in the percentage of IM, (grade D) before exposure was 51.19+9.27%, and after treatment with cold plasma (needle) decreases the % to 34.34+5.66%. The results showed that the cold plasma decreased but not increased the % of IM and the difference in these two types of motility was significant ($P < 0.05$). Also, There was significant ($P < 0.05$) in the decrement by plasma jet.

The mean percentage of rapid progressive motility (RP) before plasma needle treatment for age 31-35>(grade A) was 5.49+1.65%, exposure to plasma increased it to19.24+2.17 % , followed by(grade B) was13.83+2.39 % , plasma jet exposure reached the value to 26.51+3.27%, and for prepared semen , The mean percentage of non-progressive motility (NP) grade C before exposed was 27.11+3.51 %, plasma needle treatment decreased the % to 20.5+3.19 %, followed by the mean percentage of immotility (IM) grade D before treatment with plasma needle was 53.89+9.66 %, by treatment it decreases the % to34.04+4.26%.

Table (1): Sperm motility of prepared sperm before exposure to plasma needle and after cold plasma treatment.

Age (Year)		Motility A (mean+SD)	Motility B (mean+SD)	Motility C (mean+SD)	Motility D mean+SD)
20-25	**Before treated**	A 4.71+1.04	A 14.05+3.37	A 27.33+5.18	A 54.11+9.33
	After treated with plasma	B 18.46+3.27	B 25.73+2.89	B 22.72+4.08	B 33.26+8.52
P-value		0.015	0.017	0.026	0.052
LSD		5.23	3.34	4.65	6.37
26-30	**Before treated**	A 5.79+1.58	A 15.13+2.86	A 28.41+3.18	A 51.19+9.27
	After treated with plasma	B 17.54+2.59	B 26.81+5.03	B 21.80+4.36	B 34.34+5.66
P-value		0.024	0.036	0.022	0.043
LSD		4.18	3.09	5.11	5.34
31-35<	**Before treated**	A 5.49+1.65	A 13.83+2.39	A 27.11+3.51	A 53.89+9.66
	After treated with plasma	B 19.24+2.17	B 26.51+3.27	B 20.5+3.19	B 34.04+4.26
P-value		0.031	0.023	0.031	0.019
LSD		4.03	6.14	5.26	5.94

Capital Letters A and B for comparison between before and after treated with plasma needle. *P* <0.05 significant

The results showed that the cold plasma decreased but not increased the % of IM and the difference between two types of movement was significant where the (*P* < 0.05). and this decrement in the type of (IM) by plasma jet was significant.

Anti-oxidant :

Table (2) showed the results of antioxidants before treated with cold plasma jet (needle) and after exposure at room temperature for the prepared (PS) at constant time (120 sec), The percentage Glutathione of the prepared sperm (PS) before exposed to cold plasma (needle) at room temperature was2.35+1.29 % and When treated with cold plasma, the Glutathione increases to 4.08+1.46 % Therefore , results of Glutathione were significant (P<0.05). The percentage of Malondialdehyde before exposed was 7.93+2.49%, plasma needle treatment decreased the % to 5.62+2.07%. The results showed that the plasma jet decreased but not increased the % of Malondialdehyde, therefore, there was no significant difference in percentage of Malondialdehyde.

Table (2) antioxidant percentage of prepared semen before and after treated with plasma jet system (needle) at room temperature.

Groups	GPX mmol/mg	MDA nmol/ml
Before treated	A 2.35+1.29	A 7.93+2.49
After treated with plasma	B 4.08+1.46	B 5.62+2.07
P-value	0.042	0.037
LSD	1.28	1.14

Capital Letters A and B for comparison between before and after treated with plasma needle.
P <0.05 significant

CONCLUSION

From these results it was concluded that using argon plasma needle may exert beneficial effects in the preservation and improvement of sperm motility and DNA integrity. More studies are recommended to find out fertilization point of the sperm.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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AUTHOR CONTRIBUTIONS

SNM designed the plasma system. SNM and MSH and worked on the Physical part. HIA and MSH worked on biological part. All authors reviewed and approved the final version.

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