ATLANTIS PRESS
Advances in Health Sciences Research, volume 38
Proceedings of the 1st International Ninevah Conference on Medical Sciences (INCMS 2021)

Mitochondrial Number in Oligo and Azoospermia Male Patients

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ABSTRACT

Male infertility causes present in about 50% of the infertility cases, the standard quantity and quality of semen ejaculated during each coitus averages about 2-4 milliliters and every milliliter of seminal fluid contains contain about 100-120 million sperm/ ml. When the number of sperm in each milliliter drops below 20 million; the person is probably having infertile problem. Forty-five semen samples were collected from males with ages ranged from 25 to 45 years. The samples were divided into three groups each consists of 15 males. The first group represent control group included 15 apparently health fertile subject. The second group contain 15 patients with oligospermia with sperm count ≤20 million sperm / ml. The results of this table display there were significant variation in count between control group (54 ± 19.7) in comparison to oligo group (9.2 ± 7.49) and the p ≤0.000. there is a significant variation in active motility between control group (37.6 ± 15.1) in comparison to oligo group (18.0 ± 10.1), we can note a variation in normal morphology between control group (69.2 ± 8.0) and oligo group (60 ± 9.6) but it is less than the variation between count and the active motility as we mentioned above, whereas the p-value≤ (0.01). There was significant reduction in both seminal pyruvate and Lactate in Azoospermia patients in comparison to control group p<0.01 figure 1 and 2 respectively. In conclusion; Mitochondrial copy number significantly related to fertility status in males

Keywords: *mitochondria copy number, infertility,* Azoospermia, *oligospermia.*

1. INTRODUCTION

Infertility is a world-wide heath problem affecting up to 15% of married people in the world (Dohle, et al., 2020). Infertility is the inability to have conception after one year of intercourse despite of unprotected sexual activity (Jungwirth et al., 2017).

Male infertility causes present in about 50% of the infertility cases, the standard quantity and quality of semen ejaculated during each coitus averages about 2-4 milliliters and every milliliter of seminal fluid contains contain about 100-120 million sperm/ ml. When the number of sperm in each milliliter drops below 20 million; the person is probably having infertile problem. Mitochondria are micro-organelle that responsible for many biological function as generating energy, Ca homeostasis and apoptosis. The number and shape of mitochondria vary according to the cell types, as the number increase in cells that required high energy like muscles and sperm flagella (Perchec et al. 1995; Gronczewska et al.,2019).

Sperms contain mitochondria copy number less than in oocyte in human cells. The Oxidative phosphorylation proposed to be crucial determinant of the motility of sperms (Singh Rajender a.et al ,2010). Previous studies propose that fertilizing capacity related to characteristics of progressive motility of sperms and the sperms motility depending on the content of ATP (Christen et al. 1987; Perchec et al. 1995; Gronczewska .et al ,2019).

Extracellular pyruvate consumed by sperms is turn to lactate even under aerobic conditions. This reaction is catalyzed by Lactate dehydrogenase (LDH) within the final step of carboxylic acid fermentation which associated with production of NAD+ from NADH,

which is important for the rapid glycolysis. The aim of this work was to study the changes in mitochondrial copy number between patients have oligo and Azoospermia in Mosul.

2. MARTIALS AND METHODS

Forty-five semen samples were collected from males with ages ranged from 25 to 45 years. The samples were divided into three groups each consists of 15 males. The first group represent control group included 15 apparently health fertile subject. The second group contain 15 patients with oligospermia with sperm count ⩽20 million sperm / ml. The third group contain patients with azoospermia. The experiment carried out from August 2019 to January 2021 under ethical approval No. in 2020. Semen samples were handled using CASA method (Computer Assisted Semen Analysis). The Casa devices maintain a temperature of 37, because sperm motility is susceptible to temperature, sperms concentration and the characteristics of Motility can be assessed in undiluted semen. Sperm motility can be evaluating in samples with sperm concentrations between 2 × 106 per ml and 50 × 106 per ml (Garrett et al., 2003). The levels of pyruvate and L- Lactate were determined according cayman assay kit (Egras, et al., 2002, chung, et al., 2006) (Galdden .L.B. et al ., 2004 , passarella ,S.de. et al., 2008).

Sperm DNA isolated according Add Bio kit then DNA concentration determined by Nano-drop system samples contain 100 ng/µl. The qPCR method has been used to detect accurate quantification of ND1 copy number gene expression

Gene	Sequence	Size
Homo sapiens GAPDH	F CGGGTCTTTGCAGTCGTATG R CTGTTTCTGGGGACTAGGGG	168
Homo sapiens mt-ND1	F ATTATCGCCCCAACCCTCTC R GCTCGTAGGGCTCCGAATAG	191

Data were present as mean ± SD. ANOVA and paired t-Test were used to define the degree of significance at p⩽ 0.05. Person correlation was used to study the correlation between the measured parameters.

3. RESULTS

The results of this table display there were significant variation in count between control group (54 ± 19.7) in comparison to oligo group (9.2 ± 7.49) and the p ⩽ 0.000. there is a significant variation in active motility between control group (37.6 ± 15.1) in comparison to oligo group (18.0 ± 10.1), we can note a variation in normal morphology between control group (69.2 ± 8.0) and oligo group (60 ± 9.6) but it is less than the variation between count and the active motility as we mentioned above, whereas the p-value⩽ (0.01). On the other hand, there is no significant variation between control group and oligo group. when we compare the volume between these two groups, whereas the p-vale⩽ (0.7), as show in Table (1).

Table 1. Seminal fluid analysis results for fertile and Oligospermia

Parameters	Control	Oligo	*p*-value
Volume (ml)	3.6 ± 1.29	3.4 0.9	0.7
Count million/ml	54 ± 19.7	9.2 7.49	0.000***
Active progressive %	37.6 ± 15.1	18.0 10.1	0.000***
Normal morphology%	69.2 ± 8.0	60 9.0	0.010*

In table (2) below, we can note there are zero value in p-value when we compare between control group (54 ± 19.7) and Azoospermia (0) in case of count, active, and normal morphology, and there is a significant variation in case of volume between these two groups, and the p ⩽ 0.000.

In table (3), there were highly significant reduction in count, sperm activity, volume and morphology between oligo group and azoospermia. were oligo=9.2 ± 7.49, Azoospermia=0 respectively at p ⩽ 0.000. Activity of sperm percentage also showed significant re oligo=18.0 ± 10.1, Azoospermia =0) p⩽ (0.000) and volum(oligo =3.4 ± 0.9, Azoospermia= 2.1 ± 0.4) and the p-value ⩽ (0.000), but , ther s zero p-value when we compare between the two group in case of normal morphology as shown in the table (3).

Table 2. Seminal fluid analysis results for fertile and Azoospermia

parameters	Oligo	Azoospermia	p-value
Volume(ml)	3.4 ± 0.9	2.1±0.4	0.000
Count million/ml	9.2 ± 7.49	0	0.000
Active progress %	18.0 ± 10.1	0	0.000
Normal morphology%	60 ± 9.6	0	0.000

Table 3. Seminal fluid analysis results for Oligo and Azoospermia

parameters	Control	Azoospermia	*p*-value
Volume (ml)	3.6 ± 1.29	2.1 ± 0.4	0.000***
Count (million/ml)	54 ± 19.7	0	0.000
Active progressive (%)	37.6 ± 15.1	0	0.000
Normal morphology(%)	69.2 ± 8.0	0	0.000

There was significant reduction in both seminal pyruvate and Lactate in Azoospermia patients in comparison to control group p<0.01 figure 1 and 2 respectively.

4. DISCUSSION

The corner stone for evaluation of the man fertility remains semen fluid analysis; and the conventional parameters given most importance have been the concentration, progressive motility, and normal morphology of sperm in the ejaculate (Lewis, 2007).

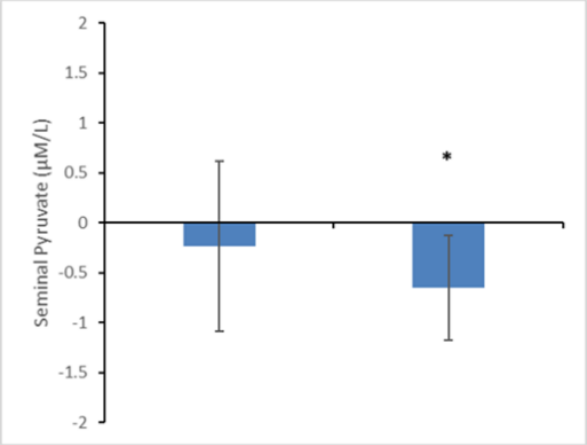

Figure 1. Normalized Seminal Fluid Pyruvate in both Oligo and Azoospermia

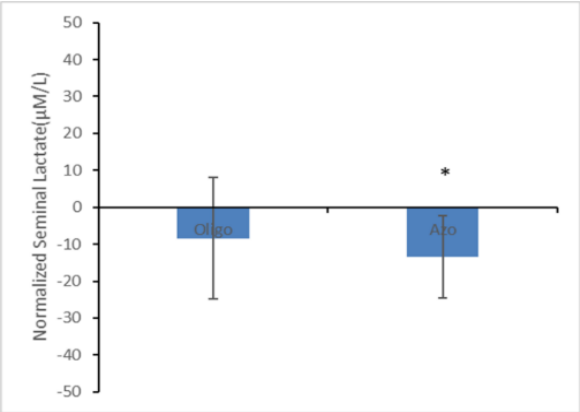

Figure 2. Normalized Seminal Fluid Lactate in both Oligo and Azoospermia

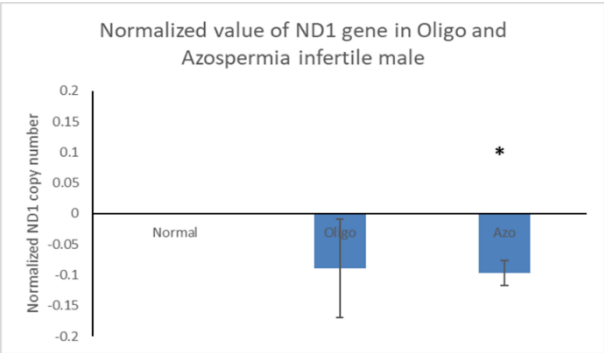

Figure 3. Normalized value of ND1 gene in Oligo and azoospermia in infertile male

Although it gives some quantitative and qualitative information about the sperm sample, recent insight into the molecular biology and genetics of the sperm cell have demonstrated that concentration, morphology and motility alone are not the only grounds upon which sperm should be evaluated (Marchesi and Feng, 2007).

This study designed to explain the correlation between mitochondrial copy number and infertility, the results of this work showed that in both oligospermia and azoospermia groups; mitochondrial copy number significantly reduced than the copy number and control groups. This finding goes with other researchers that found that mtDNA copy number associated with low sperm count, motility, concentration and morphology (May-Panloup et al., 2003; Song and Lewis, 2008; Zhang et al., 2016) (Wu, H., et al.,2019). Also Song and his group, indicate that significant decrease in mtDNA integrity were observed in the spermatozoa samples from patients with abnormal semen parameters compared to the patients with normal semen parameters. The integrity and copy number of mtDNA were significantly correlated with sperm count Song, G. J.et al (2008).

The probability of the presence of mtDNA in the azoospermia is likely to residual contamination of somatic cells in the final sperm population could affect the accurate quantification of sperm mtDNA measures. According to Chen et al., Comparing with normozoospermia, patients and oligoa then azoospermia had a significant decrease in cell-free mtDNA copy number (Chen, Y. et al., 2018).

Also, in the present study, seminal lactate content in the control group was higher than it is in oligo and Azoospermia groups and that goes with the finding of Mumcu et al., who reported that the level of lactate was significantly decreased in patients with OAT compared to the controls. reported decreased levels of lactate and citrate, in oligozoospermic patients compared to the normo and azoospermia men (Mumcu et al, 2020).

5. CONCLUSION

In conclusion; Mitochondrial copy number significantly related to fertility status in males. There was significant reduction in both seminal pyruvate and Lactate in Azoospermia patients in comparison to control group.

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