Effect of Peroxiredoxin 6 On Azthenozoospermic Patients

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Abstract

Background: Idiopathic male infertility may be caused by oxidative stress Due to its antioxidant effects, Peroxiredoxins are scavengers and modulators for Reactive Oxygen Species Peroxiredoxins are considered as antioxidant enzymes. Aim of study:Assessing the level of Prdx6 in the semen plasma of Azthenozoospermic patients and its correlation with different sperm parameters including (concentration, count, morphology, and motility). Subjects, and Methods:The data included 50 semen samples collected by masturbation from subjects with age ranging from 21 to 64 years. The samples were placed in a 37 °C incubator in order to fully liquefy the samples for semen analysis, and macroscopic and microscopic examinations were conducted according to World Health Organization (2010). The samples were divided into four groups: Normozoospermia, oligozoospermia, asthenozoospermia and combined defect group, this was based on the semen characteristic of the patients. The semen was z supernatant in an eppendorf tube to freeze. After collecting all the samples, Prdx6 in semen plasma was assessed by ELISA kit assay. **Results**: There were no significant correlations (Table 2) between peroxiredoxin-6 with seminal fluids parameters of normozoospermia, asthenozoospermia, oligozoospermia and combined groups except;There was a significant negative correlation between progressively motile sperms and peroxiredoxin-6 (r= -0.623 & p=0.023) in asthenozoospermia group. **Conclusion**: Asthenozoospermia is major cause in male infertility. A significant negative correlation between progressively motile sperms and peroxiredoxin-6 in the asthenozoospermia group was found. The level of Peroxiredoxin-6 was higher in asthenozoospermia group in comparison with the other groups.

Keywords: Reactive oxygen species, Infertile men, Peroxiredoxin 6.

Introduction

Infertility is characterized as the ability of woman and man to attain a conceive following a year from engaging in consistent, sexual relation without any contraceptive (Alazzawi 2023); on the other hand, the American Society for Reproductive Medicine (ASRM) adds to the definition, a period of 12 months or more in women aged 20-34 years, or 6 months or more in women aged 35 years or older, (Taha, 2022).

Infertility due to males is represents approximately fifty percent of infertility cases (Leslie et al. 2020). Furthermore, the incidence of male subfertility is on the rise, becoming a prevalent global problem that affects a significant proportion of the population, surpassing the prevalence of other common diseases (Hussein, 2023).

Reactive oxygen species are oxidative agents that exert a significant influence on the functional aspects of spermatozoa (Aitkem, 2020). They are a fundamental constituent of biological systems and maintain a state of equilibrium. Oxidative Stress arises when Reactive oxygen species overcome the protection of the semen antioxidant, resulting in cellular harm to the sperm (Showell et al., 2014). Mammalian spermatozoa are vulnerable to Oxidative Stress due to the lack of antioxidant protection mechanisms (Bumanlag, Scarlata, and O'Flaherty, 2022).

The tail of the sperm is between 40 to 50 micrometers, nearly 10 times longer than the head, the tail is responsible for providing movement ability for the sperm. The tail also contains all the sperm's cell movement apparatus. The sperm neck region generates waves that passes down distally in a whiplash style (Durairajanayagam, D. *et al.* 2015).

Asthenozoospermia happens when the number of progressively mobile sperms to the total number of sperms multiply by 100% is below the WHO guideline specified level. may arise as a result of various contributing factors such as: abnormal semen liquefaction, bacterial infections, genetic defects, and potentially unidentified molecular-level alterations, such as proteomic changes, are potential factors that can contribute to this condition Nowicka-Bauer and Nixon (2020).

Peroxiredoxin 6 is an intracellular phospholipase A2 (referred to as aiPLA2) that operates independently of calcium ions (Ca2+). It is primarily found in the cytosol, lysosomes, and lysosomal-related organelles. Engagement in these activities is imperative for the prevention of lipid peroxidation (O'Flaherty, 2019).

The plasma membrane of spermatozoa have a plenty of polyunsaturated fatty acids, which are responsible for organizing the spermatozoa membrane permeability and spermatozoa fluidity, therefore oxidation of these polyunsaturated fatty acids impact the fluidity of the spermatozoa membrane union events e.g. sperm mobility acrosome reaction, sperm-ovum interaction and binding capacity (Safarinejad, M.R. *et al.* 2010).

There is a negative correlation between sperm mobility and lipid peroxidation. Reactive oxygen species lead to fragmentation of mitochondrial DNA, causing reduction of energy and ATP quantity available, hindering the spermatozoa mobility (Wagner, 2018).

Subjects and Methods

The Subjects

The research analyzed 50 men semen, collected from patients seeking treatment at the infertility clinics located at the High Institute for Infertility Diagnosis and Assisted Reproductive Technology affiliated with Al-Nahrain University. The research was conducted from November 2022 to April 2023. Patients' age ranged from 21 to 64 years old, following the necessary approval from the ethical committee prior to the commencement of the research.

Inclusion criteria: No major systemic illness and With primary or secondary infertility

Exclusion criteria:

1- The criteria excluded from consideration in this study are azoospermia and oligoasthenoteratospermia.
2- Background of previous male infertility caused by trauma, mumps infection, male gonadal disorders, and surgical interventions.
3- Cases of chemotherapy and mutations, alcohol consumption, prostatic infections, varicocele, Diabetes, chronic and infectious diseases.

Study design:

The collection of semen samples was conducted, and subsequent seminal fluid analysis was done with respect to WHO (2010) guidelines. A volume of one milliliter of the semen sample was subjected to centrifugation at a consistent speed and duration (3000 revolutions per minute for 10 minutes). Following centrifugation, the supernatant was carefully separated from the pellet. The

supernatant, was then transferred into an eppendorf tube for freezing. Subsequently, all collected samples were assessed for the presence of Prdx6 in the semen plasma using an ELISA kit assay. The obtained results were then correlated with various seminal fluid parameters.

Methods

Semen Collection:

Samples of semen were collected after a period of sexual abstinence between 3 to 7 days, as recommended by WHO (2010), the individual masturbated in a private room adjacent to the laboratory. The act of masturbation was performed directly into a sterilized wide-mouth container, which was provided by the laboratory. Subsequently, the collected samples were placed in an incubator at a temperature of 37°C, allowing for complete liquefaction prior to analysis. After complete liquefaction, a skilled technician conducted both macroscopic and microscopic analysis of the semen (WHO, 2010). Based on the findings of the semen evaluation, the participants were categorized into four distinct groups: normozoospermic, asthenozoospermic, oligozoospermic, and males with combined defects.

Results

Classification of patients involved in the studied groups

The participants included in the current study were categorized into four distinct groups depicted in Figure (1). Group 1 consisted of 13 patients, accounting for 26% of the total, who exhibited normozoospermia. The second group comprised 19 patients, representing 38% of the total, who presented with asthenozoospermia. Group 3 consisted of 7 patients, making up 14.0% of the total, who displayed oligozoospermia. Lastly, Group 4 included 11 patients, accounting for 22.0% of the total, who exhibited combined defects.

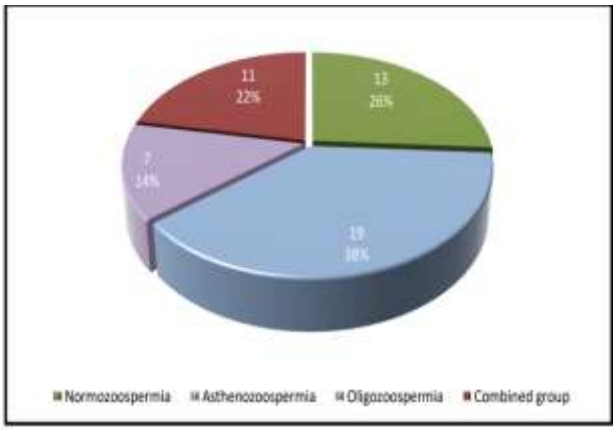

Figure (1) Classification of patients

Table (1):Comparison of seminal fluids peroxiredoxin-6 between the studied groups.

Groups	Peroxiredoxin-6 Mean ± SE
Normozoospermia group	197.0 ± 13.64
Asthenozoospermia group	235.3 ± 12.75
Oligozoospermia group	216.7 ± 18.18
Combined group	213.7 ± 13.78
p value	0.229 ∀ NS

Parameters	Peroxiredoxin-6

Table (2): Correlations between peroxiredoxin-6 with seminal fluids parameters

Sperms count (x10^6/ml)	R	0.394
	p value	0.096
Sperms concentration	R	0.009
	p value	0.972
Progressively motile sperms %	R	-0.657
	p value	0.002
Non-Progressively motile sperms %	R	-0.164
	p value	0.503
Immotile sperms %	R	0.237
	p value	0.328
Morphologically normal sperm %	R	0.374
	p value	0.115
Round cells	R	-0.044
	p value	0.861
Liquefaction time (min)	R	0.389
	p value	0.100
DFI %	R	0.208
	p value	0.394

No significant correlations were observed between peroxiredoxin-6 with seminal fluid parameters of normozoospermia, asthenozoospermia, oligozoospermia, and combined groups, as indicated in Table (2), except; negative significant correlation between progressively motile sperms and peroxiredoxin-6 (r= -0.623 & p=0.023) in asthenozoospermia group.

Discussion

The purpose of this research was to examine the influence of peroxiredoxin 6 on seminal parameters. The 50 adult males involved were categorized into 4 classes based on seminal fluid analysis parameters. In which the asthenozoospermia group was the largest group. This can be explained according to Saraswat, M. et al. (2017) study, in which they found a high percentage (24%) of infertile men present with isolated asthenozoospermia, influenced by multiple causes including genetic, varicocele, and infection. While (Asghari, 2017) said (asthenozoospermia) is one of the most common types and is responsible for about half of cases of male infertility. A lot of infertility cases have an unknown cause and are classified as idiopathic (Saraswat, M. et al., 2017).

One of the major causes of infertility in men, Asthenozoospermia, is distinguished by low sperm mobility. Low sperm mobility can happen because of: endocrine dysfunction, physical factors, abnormal semen liquefaction chemical factor, anti-sperm antibodies, varicocele and ultrastructural abnormalities. (Shen, S. et al. 2013).According to the result of the current study, it was observed that there is no statistically significant association, except for a negative significant correlation between progressively motile sperms and peroxiredoxin-6 (r= -0.623 & p=0.023) in asthenozoospermia group.

Against to Ozkosem, B. et al. (2015) results, in which they found that Spermatozoa from male with infertility with high DNA damage and low sperm motility have lower levels of PRDX6.This is may be due to our results being on humans, while their study was on animals. Also, the same result in a Canadian study, relating decreased levels of PRDX1 and PRDX6 with a decrease in sperm mobility and increased DNA damage; their Spermatozoon samples were taken from infertile male with clinical varicosity or idiopathic infertility (Ozkosem, B. and O'Flaherty, C. 2012).

This may be explained due to the varicosity in their patient samples while we excluded those cases from our study. Finally, Gong, S. et al. (2012) found that thiol oxidation ratio of PRDXs (thiol-oxidized PRDX/total PRDX) correlated negatively

with both the total mobility of sperm and progressive mobility of sperm. Thiol oxidation ratio of PRDXs is inversely proportional with sperm lipid peroxidation and, sperm DNA fragmentation, which is the same result of this current study.

References

❖ Aitken, R.J., 2020, January. The male is significantly implicated as the cause of unexplained infertility. In Seminars in reproductive medicine (Vol. 38, No. 01, pp. 003-020). Thieme Medical Publishers, Inc..

❖ Asghari, A., Marashi, S.-A. and Ansari-Pour, N. (2017) 'A sperm-specific proteome-scale metabolic network model identifies non-glycolytic genes for energy deficiency in asthenozoospermia', Systems Biology in Reproductive Medicine, 63(2), pp. 100–112. Available at: https://doi.org/10.1080/19396368.2016.1263367.

❖ Bumanlag, E., Scarlata, E. and O'Flaherty, C. (2022) 'Peroxiredoxin 6 Peroxidase and Ca2+-Independent Phospholipase A2 Activities Are Essential to Support Male-Mouse Fertility', Antioxidants, 11(2), p. 226. Available at: https://doi.org/10.3390/antiox11020226.

❖ Durairajanayagam, D. et al. (2015) 'Sperm Biology from Production to Ejaculation', in G.L. Schattman, S.C. Esteves, and A. Agarwal (eds) Unexplained Infertility. New York, NY: Springer New York, pp. 29–42. Available at: https://doi.org/10.1007/978-1-4939-2140-9_5.

❖ Du Plessis, S.S. et al. (2015) 'Contemporary evidence on the physiological role of reactive oxygen species in human sperm function', Journal of Assisted Reproduction and Genetics, 32(4), pp. 509–520. Available at: https://doi.org/10.1007/s10815-014-0425-7.

❖ Gong, S. et al. (2012) 'Low Amounts and High Thiol Oxidation of Peroxiredoxins in Spermatozoa From Infertile Men', Journal of Andrology, 33(6), pp. 1342–1351. Available at: https://doi.org/10.2164/jandrol.111.016162.

❖Hussein, E. (2023) Seminal Plasma Coenzyme Q10 Level and Sperm DNA Integrity in Relevance to Sperm Parameters in Infertile Men. Al- Nahrain University.

❖Leslie, S.W., Soon-Sutton, T.L. and Khan, M.A. (2022) Male Infertility. StatPearls Publishing, Treasure Island (FL). Available at: http://europepmc.org/books/NBK562258.

❖Nowicka-Bauer, K., Lepczynski, A., Ozgo, M., Kamieniczna, M., Fraczek, M., Stanski, L., Olszewska, M., Malcher, A., Skrzypczak, W., and Kurpisz, M. K. (2018). Sperm mitochondrial dysfunction and oxidative stress as possible reasons for isolated asthenozoospermia. Journal of Physiology and Pharmacology. 69 (3) p 403-441.

❖Ozkosem, B. et al. (2015) 'Advancing age increases sperm chromatin damage and impairs fertility in peroxiredoxin 6 null mice', Redox Biology, 5, pp. 15–23. Available at: https://doi.org/10.1016/j.redox.2015.02.004.

❖Ozkosem, B. and O'Flaherty, C. (2012) 'Detrimental Effects of Oxidative Stress on Spermatozoa Lacking Peroxiredoxin 6', Free Radical Biology and Medicine, 53, p. S86. Available at: https://doi.org/10.1016/j.freeradbiomed.2012.10.345.

❖O'Flaherty, C. (2019) 'Orchestrating the antioxidant defenses in the epididymis', Andrology, 7(5), pp. 662–668. Available at: https://doi.org/10.1111/andr.12630.

❖O'Flaherty, C. (2018) 'Peroxiredoxin 6: The Protector of Male Fertility', Antioxidants, 7(12), p. 173. Available at: https://doi.org/10.3390/antiox7120173
❖Russe Alazzawil (2023) The Effect of N-Acetyl Cysteine (NAC) on Hyperviscous Semen. Al-Nahrain University.

❖Safarinejad, M.R. et al. (2010) 'Relationship of omega-3 and omega-6 fatty acids with semen characteristics, and anti-oxidant status of seminal plasma: A comparison between fertile and infertile men', Clinical Nutrition, 29(1), pp. 100–105. Available at: https://doi.org/10.1016/j.clnu.2009.07.008.

❖Saraswat M, Joenväärä S, Jain T, Tomar AK, Sinha A, Singh S, et al. Human Spermatozoa Quantitative Proteomic Signature Classifies Normo- and Asthenozoospermia *. Molecular & Cellular Proteomics. 2017 Jan 1;16(1):57–72.

❖Shen, S. et al. (2013) 'Comparative proteomic study between human normal motility sperm and idiopathic asthenozoospermia', World Journal of Urology, 31(6), pp. 1395–1401. Available at: https://doi.org/10.1007/s00345-013-1023-5.

❖Showell, M.G. et al. (2014) 'Antioxidants for male subfertility', Cochrane Database of Systematic Reviews [Preprint], (12). Available at: https://doi.org/10.1002/14651858.CD007411.pub3.

Taha (2022) Evaluation of Hyaluronan Binding Assay (HBA) as Predictive Marker of Intrauterine Insemination Outcome in Relevance to Sperm Preparation Techniques in Iraqi Infertile Couples. Al-Nahrain University.

Wagner, H., Cheng, J.W. and Ko, E.Y. (2018) 'Role of reactive oxygen species in male infertility: An updated review of literature', Arab Journal of Urology, 16(1), pp. 35–43. Available at: https://doi.org/10.1016/j.aju.2017.11.001