Original Article

Heat Shock Protein 70 Biomarkers in Sperm and Its Association on Oligoasthenozoospermia, and Asthenozoospermia in Male Infertility

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Abstract

Background: The heat shock protein (Hsp70) is a protein that helps cells survive in harsh conditions and is involved in a variety of cellular biological processes. **Objectives:** To study the relationship between the levels of (Hsp70) in semen plasma of infertile (oligoasthenozoospermia (OA), asthenozoospermia (AZ) and fertile men and the effect of an increase and decrease on fertility. **Materials and Methods:** The study was conducted on 119 semen samples, 31 men with (AZ), 28 men with (OA), whereas another 60 healthy controls were diagnosed, semen samples were collected from Iraqi patients who attended infertility clinics for infertility diagnosis and assisted reproductive technologies in Karbala for the period between November 2021 and May 2022. **Results:** The mean (Hsp70) in the patient group were significantly higher than in the control group ($P < 0.001$). In comparison to (AZ) groups with (OA) found a significant difference in the mean level of (Hsp70) between (AZ) groups and (OA) groups (Hsp70) levels showed a negative significant correlation with the concentration, progressive cell and non-progressive cell, and a positive significant correlation with the sample volume, viscosity of semen, $P < 0.05$. **Conclusion:** The total level of Hsp70 in the semen of men is higher in infertile men (AZ) and (OA), whereas we find lower levels in the semen of fertile men. A negative relationship was also found between the level of Hsp70 with semen concentrations and sperm motility and a positive relationship with the volume of the semen sample and its viscosity.

Keywords: Asthenozoospermia, heat shock protein 70, normozoospermia, oligoasthenozoospermia

Introduction

Infertility, according to the World Health Organization, is the inability to conceive while engaging in frequent, unprotected sexual activity for a year or longer.[1] The ages of the man and woman, the presence of pathological changes in the reproductive organs, the presence of men who exhibit infertility that is attributed to female factors (about 1/3 of infertility), and the diagnosis of conditions such as oligo menorrhea or amenorrhea are all factors that affect a couple's fertility.[2] Male factor infertility is frequently characterized by anomalies on sperm analysis, such as low motility and nonexistent or low sperm counts. However, the diagnostic power of the male investigative instruments currently in use is constrained, and it is likely that this understates the true prevalence of male variables in infertile couples.[3] The genetic quality of the oocyte and sperm, the embryo's capacity to implant, and the endometrial receptivity are some of the elements that affect whether or not a pregnancy occurs. The majority of biological systems depend on the maintenance of equilibrium. Reactive oxygen species (ROS) offer physiological contributions at optimum quantities, but soon become destructive if left unchecked. Oxidative harmony within the male reproductive system is essential to fertility.[4] About 50% of infertility cases have overlapping male and female variables, whereas the

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percentage of male factors is similar to the percentage of female factors. Idiopathic infertility is still a significant issue in all undiagnosed cases of male infertility.[5,6] Male infertility has several major, well-established causes, including the lack of spermatozoa (azoospermia), a lack of spermatozoa in general (oligozoospermic), a lack of sperm in general (asthenozoospermia), a lack of sperm in general (necrozoospermia), and abnormal sperm morphology (teratozoospermia).[7] Semen analysis, which is carried out at any infertility laboratory, is the first step in determining the status of semen samples.[8] However, sperm protein expression, absence, or presence in sperm cells or seminal plasma has also been linked to male factor infertility. As a result, it is necessary to examine some sperm proteins linked to male infertility, such as Hsp70, in addition to routine semen or sperm examination (Hsp70). Male infertility may also occur from some of these sperm proteins being under-expressed, poorly expressed, or possibly over-expressed. The heat shock proteins family which includes both eukaryotic and prokaryotic living things includes heat Hsp70.[9] Hsp70 is a key player in plasma sperm defense against cellular stress, particularly heat stress, by enhancing cells' tolerance to environmental changes and pathologic situations.[10] Families of the Hsp70 gene appear to be important sperm biological membranes molecules involved in motility, capacitation, and fertilization.[11]

Materials and Methods

The study was conducted on 119 semen samples, 31 men with (AZ), 28 men with (OA), while another 60 healthy controls were diagnosed, semen samples were collected from Iraqi patients who attended infertility clinics for infertility diagnosis and assisted reproductive technologies in Karbala for the period between November 2021 and May 2022. The review participants in this study were chosen from a Karbala infertility clinic and Miley was excluded. The following are included in the exclusion criteria: genetic disorders, microbial infections, stimulants (alcohol, drugs), age 20 or greater, use of antibiotics, nonsteroidal anti-inflammatory drugs, corticosteroids, vitamins, dietary supplements, inflammation of the genitourinary tract in the previous six months, and surgery. Men who had children (one or two children (where they were considered as the control group. Hsp70 Status in sperm plasma was measured by Sandwich-ELISA method.

Statistical analysis

All participants' questionnaire responses were put on a data sheet and given a serial identity number. Errors were prevented by using multiple entries. The Statistical Package for the Social Sciences, version 28.0, was used to create the data analysis for this project (IBM, SPSS, Chicago, Illinois). On the participant data from each group, descriptive statistics were run. Mean standard deviation was used to illustrate values for continuous variables. The Shapiro test was used to examine the data distribution. Odds ratios and a 95% confidence interval range, which were computed using a no conditional logistic regression, were used to estimate the connection between the components that had been examined. Analytical statistical tests were used to confirm that there were significant variations in categorical variables between the parameters. Results of every hypothesis test with a two-sided *P*-value of 0.05 or lower were deemed statistically significant.

Ethical approval

The study was conducted in accordance with the ethical standards set forth in the Declaration of Helsinki. Before sampling, it was performed with the patient's oral and analytical consent. The local ethics committee checked and approved the study protocol, subject information, and permission form pursuant to Document 3326 (containing the number and date December 5, 2021) for this approval.

Results

Infertility and fertility groups' average ages were 32.2 ± 5.76 and 31.07 ± 5.59 years, respectively. When compared to men who were fertile, there were no statistically significant differences between infertile men who had (OA) and (AZ) as shown in Table 1. In the fertility and infertility groups had mean body mass indexs of 27.52 ± 5.10 and 29.25 ± 5.75, respectively; although there was a little increase in the mean mass index, there were no appreciable variations among infertile males as shown in Table 1.

The results in Table 2 indicated that there was a significant difference in the mean level of (Hsp70) in patient's groups compared to control group. The mean level of Hsp70 was 47.66 ± 7.10 in patients' groups whereas it was 40.17 ± 1.94 in healthy control.

The results in Table 3 indicated that there was a significant difference in the mean level of (Hsp70) in (OA) groups compared to control group. The mean level of Hsp70 was 50.02 ± 7.39 in (OA) groups whereas it was 40.17 ± 1.94 in healthy control.

Table 1: Sociodemographic differences between infertile men and the control group

Parameter	Normozoospermia (*N* = 60)	Patient (*N* = 59)	*P*-value
	Mean ± SD	Mean ± SD	
Age	32.2 ± 5.76	31.07 ± 5.59	0.27 [NS]
BMI	27.52 ± 5.10	29.25 ± 5.75	0.139 [NS]

BMI, body mass index

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Table 2: Seminal plasma Hsp70 indicators in the control and infertile groups, findings

Parameter	Control ($N = 60$)	Patient ($N = 59$)	*P*-value
	Mean ± SD	Mean ± SD	
Heat shock protein 70	40.17 ± 1.94	47.66 ± 7.10	**<0.001[S]**

Table 3: Seminal plasma Hsp70 indicators in the control and (OA) groups, findings

Parameter	Control ($N = 60$)	Oligoasthenozoospermia ($N = 28$)	*P*-value
	Mean ± SD	Mean ± SD	
Heat shock protein 70	40.17 ± 1.94	50.02 ± 7.39	**<0.001[S]**

Table 4: Seminal plasma Hsp70 indicators in the control and (OA) groups, findings

Parameter	Control ($N = 60$)	Oligoasthenozoospermia ($N = 28$)	*P*-value
	Mean ± SD	Mean ± SD	
Heat shock protein 70	40.17 ± 1.94	50.02 ± 7.39	<0.001 [S]

The results in Table 4 indicated that there was a significant difference in the mean level of (Hsp70) in (AZ) groups compared to control group. The mean level of Hsp70 was 45.52 ± 6.21 in (AZ) groups whereas it was 40.17 ± 1.94 in healthy control.

The results in Table 5] indicated that there was a significant difference in the mean level of (Hsp70) in (OA) groups compared to (AZ) group. The mean level of Hsp70 was 50.02 ± 7.39 in (OA) groups whereas it was 45.52 ± 6.21 in (AZ).

The study additionally looked at the effects of the (AZ) and (OA) groups' semen viscosity characteristics on the biomarkers. According to the semen viscosity, patients were classified into three groups (low, moderate, and high viscosity). Table 6 compares the amounts of biomarkers in the groups that were statistically significant in (Hsp70).

The correlation between biomarkers and independent variables including (basic semen characteristics among the infertile men patients group.

A significant positive-correlation was demonstrated between Hsp70 levels with sample volume and non-moving sperm cell%, while the concentration, progressive cell and non-progressive cell demonstrated a negative significant correlation as shown in Table 7.

Table 5: Difference between biomarker mean level in (AZ) and (OA)

Parameter	Asthenozoospermea ($N = 31$)	Oligoasthenozoospermia ($N = 28$)	*P*-value
	Mean ± SD	Mean ± sD	
Heat shock protein 70	45.52 ± 1.94	50.02 ± 7.39	0.008[S]

Table 6: Difference between biomarker mean level in (AZ) and (OA) groups in term semen viscosity characteristic

Biochemical parameters	Viscosity ($N = 119$)			*P*-value
	Low $N = 79$	Moderate $N = 22$	High $N = 18$	
Heat shock protein 70	41.89 ± 4.54*	47.04 ± 6.84*	48.75 ± 8.62*	<0.001[S]

Table 7: Correlations of the biochemical parameters independent variables

Variables	Heat shock protein 70
Sample Volume	r = 0.4
	p = <0.001[S]
Concentration	r = -0.3
	p = <0.001[S]
Progressive cell	r = -0.5
	p = <0.001[S]
Non-progressive cell	r = -0.2
	p = 0.012[S]
Nonmoving sperm	r = 0.5
	p = <0.001[S]

Discussion

Older men have a deterioration in testicular function and metabolism as a result of age-related morphological changes in the testis, such as a reduction in the quantity of germ cells, Leydig cells, and Sertoli cells, as well as structural changes, such as the constriction of seminiferous tubules. Primary hypogonadism develops as a result of the gradual decline in free and total testosterone concentrations that occurs as men age. The control of the Hypothalamus-Pituitary-Adrenal axis also varies with age in men. The buildup of ROS in aging male germ cells leads to oxidative stress and sperm DNA damage. Furthermore, the apoptosis rate rose in the aged testes.[12]

Obese men may experience reduced fertility due to poor semen quality, endocrinopathy, aromatization activity, psychological and thermal consequences, sleep apnea, leptin, and small toxins, in addition to sexual dysfunction.[13]

The protective role of Hsp70 in reducing cell-related oxidative stress may be the reason for the elevated levels

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of Hsp70 in the semen of infertile subjects (OA), (AZ) as shown in Table 2. Hsp70 is present in plasma in relatively low amounts, but when levels of oxidative stress exceed the physiological limit concentration can be greatly increased. As a result, raising it can be used to prevent ROS or free radicals from damaging or killing sperm cells, as noted in one studies.[14–17]

Plasma levels of Hsp70 are relatively low, but when oxidative stress exceeds the physiological limit, the level of Hsp70 can rise dramatically. Therefore, it can be raised to stop reactive oxygen species or free radicals from causing distortion or death of sperm cells. Tables 3 and 4 show that the percentage of protein was increased in (AZ) and (OZ) cases, and the following research supported this result.[18,19]

The rate of viscosity increase is closely related to the increase in oxidative stress of the semen of men, as previously stated in a study.[20] Since the amount of antioxidants is inversely related to Hsp70, there is an increase in the amount of protein with an increase in viscosity, and this results has been confirmed by several studies.[21,22]

According to the studies[23,24] Hsp70 is a family of housekeeping proteins that are constitutively expressed in cells to control a variety of cellular pathways, including translocation, transcription, translation, and signal transduction, when cells are under stressful conditions, such as when they are exposed to heat, hypoxia, and oxidative stress. And since any negative exposure to semen cells leads to the release of heat shock protein in greater quantities than it is in the normal state as shown in Table 7, where the relationship of shock protein is inverse with the normal and direct concentrations of sperm. With a deficiency and absence of sperm, as confirmed by the following study[25] and a positively correlation with sample volume and non-moving sperm and this result was confirmed by study.[26]

Conclusions

It can be deduced that levels of Hsp70 that are higher than normal physiological levels are associated, at least in part, with male infertility because the (Hsp70) of semen from infertile men enrolled in the current study was higher than that reported in fertile men in previous studies. The highest mean (Hsp70) was observed in (OA) and the lowest was reported in (AZ). The Hsp70 showed a negative significant correlation with the concentration, progressive cell and non-progressive cell, and positive significant correlation with the sample volume, semen viscosity, and non-moving sperm cell.

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Conflicts of interest

There are no conflicts of interest.

References

1. Zegers-Hochschild F, Adamson GD, de Mouzon J, Ishihara O, Mansour R, Nygren, K, *et al*. International Committee for Monitoring Assisted Reproductive Technology (ICMART) and the World Health Organization (WHO) revised glossary of ART terminology, 2009. Fertil Steril 2009;92:1520-4.
2. World Health Organization, Department of Reproductive Health and Research. WHO Laboratory Manual for the Examination and Processing of Human Semen. 5th ed. Geneva, Switzerland: WHO; 2010.
3. Bieniek JM, Drabovich AP, Lo KC. Seminal biomarkers for the evaluation of male infertility. Asian J Androl 2016;18:426.
4. Máté G, Bernstein LR, Török AL. Endometriosis is a cause of infertility. Does reactive oxygen damage to gametes and embryos play a key role in the pathogenesis of infertility caused by endometriosis? Front Endocrinol 2018;9:725.
5. Lotti F, Maggi M. Sexual dysfunction and male infertility. Nat Rev Urol 2018;15:287-307.
6. Tunçdemir M, Yenmiş G, Tombultürk K, Arkan H, Soydaş T, *et al*. NFKB1 rs28362491 and pre-miRNA-146a rs2910164 SNPs on E-cadherin expression in case of idiopathic oligospermia: A case–control study. Int J Reprod Biomed 2018;16:247.
7. Jodar M, Ventura SA, Oliva R. Semen proteomics and male infertility. J Proteomics 2017;162:125-134.
8. Aboutorabi R, Mazani F, Rafiee L. Evaluation of CD52 positive sperms in subfertile human semen samples: Is there any relationship with main semen parameters? Adv Biomed Res 2014;3:124.
9. Ma F, Luo L. Genome-wide identification of Hsp70/110 genes in rainbow trout and their regulated expression in response to heat stress. PeerJ 2020;8:e10022.
10. Hussein R, Abbas LH, Rayhaan S, Fadhil HA, AL-Mousawi ZR The impact of adding melatonin and other antioxidants on post-thaw human sperm quality during cryopreservation. Med J Babylon; 2023;20:18-23.
11. Hiyama G, Matsuzaki M, Mizushima S, Dohra H, Ikegami K, Yoshimura T, *et al*. Sperm activation by heat shock protein 70 supports the migration of sperm released from sperm storage tubules in Japanese quail (*Coturnix japonica*). Reproduction 2014;147:167-78.
12. Gunes S, Hekim GNT, Arslan MA, Asci R. Effects of aging on the male reproductive system. J Assist Reproduct Genetics 2016;33:441-54.
13. Katib A. Mechanisms linking obesity to male infertility. Central Eur J Urol 2015;68:79.
14. Aworu AM, Chioma AB, Elekima I, Brown H, Bartimaeus EAS. Pattern of Some Sperm Proteins, Anti-oxidants, Testosterone, and Prostate-Specific Antigen of Infertile Males with Sperm Cell Deformities in Port Harcourt, Rivers State. Asian J Res Med Pharm Sci 2022;11:36-47.
15. Ferlin A, Speltra E, Patassini C, Pati MA, Garolla A, Caretta N, *et al*. Heat shock protein and heat shock factor expression in sperm: Relation to oligozoospermia and varicocele. J Urol 2010;183:1248-52.
16. Agarwal A, Hamada A, Esteves SC. Insight into oxidative stress in varicocele-associated male infertility: Part 1. Nat Rev Urol 2012;9:678-90.
17. Waheed MM, El-Bahr SM. Al-Haider AK. Influence of seminal plasma antioxidants and osteopontin on fertility of the Arabian horse. J Equine Vet Sci 2013;33:705-9.
18. Krisfalusi M, Miki K, Magyar PL, O'Brien DA. Multiple glycolytic enzymes are tightly bound to the fibrous sheath of mouse spermatozoa. Biol Reproduc 2006;75:270-8.
19. Gavriliouk D, Aitken RJ. Damage to sperm DNA mediated by reactive oxygen species: Its impact on human reproduction and the health trajectory of offspring. The male role in pregnancy loss and embryo implantation failure; 2015. p. 23-47.
20. Schallmoser A, Bakjaji F, Königsberger S, John J, Färber C, Schmidt E, *et al*. Effect of mild α-chymotrypsin treatment of highly viscous semen samples on fertilization rates. Transl Androl Urol 2021;10:448.

21. Siciliano L, Tarantino P, Longobardi F, Rago V, De Stefano C, Carpino A. Impaired seminal antioxidant capacity in human semen with hyperviscosity or oligoasthenozoospermia. J Androl 2001;22:798-803.
22. Harchegani AB, Rahmani H, Tahmasbpour E, Shahriary A. Hyperviscous semen causes poor sperm quality and male infertility through induction of oxidative stress. Curr Urol 2019;13:1-6.
23. Agarwal A, Hamada A, Esteves SC. Insight into oxidative stress in varicocele-associated male infertility: Part 1. Nat Rev Urol 2019;9:678-90.
24. Nixon B, Bromfield EG, Cui J, Iuliis GND. Heat shock protein A2 (HSPA2): Regulatory roles in germ cell development and sperm function. Adv Anat Embryol Cell Biol 2017;222:67-93.
25. Green KI, Nwachuku EO. Seminal analysis as a tool to determine the infertility prevalence among men reported to infertility clinic in Port Harcourt. Asian J Med Health 2018;11:1-6.
26. Colagar HA, Karimi F, Jorsaraei AGS. Correlation of sperm parameters with semen lipid peroxidation and total antioxidants levels in astheno- and oligoasheno- teratospermic men. Iranian Red Crescent Med J 2013;15:780-5.

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